InSiGHT

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O1 - AN ASPIRIN A DAY? THE (STILL) SECRET WEAPON AGAINST COLORECTAL CANCER IN LYNCH SYNDROME

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Background and aims

Lynch syndrome (LS) is the commonest cause of hereditary colorectal cancer (CRC). Aspirin is proven to reduce the incidence of CRC in LS. The New Zealand Familial Gastrointestinal Cancer Service (NZFGCS) manages all known LS families in New Zealand (NZ) and provides updated clinical information to patients. We conducted a survey of aspirin use in gene positive LS patients (MLH1, MSH2, MSH6, PMS2 and EPCAM) in NZ to assess both the awareness of the protective effect of aspirin and the prevalence of aspirin use in this high-risk population.

Method

There are 975 known gene positive LS patients in NZ managed through the NZFGICS aged between 18 and 75 years. They were all invited to participate in the survey via email or hardcopy. Data was collated and analysed using Excel.

Results

502 of 975 (51.5%) gene positive LS individuals completed the survey. Of those 290 (57.8%) were female and 212 (42.2%) male. The majority of patients 74.3%, were over 45 years, 26.9% patients were over 65 years.

Of all who responded 256 people (51.0%) were aware of the potential chemopreventive benefits of aspirin; in 105 of those patients (41%) this was due to education from the NZFGCS.

119 (23.7%) of the total patients were taking regular aspirin. This increased to 25.8% in those patients with a first degree relative with CRC and increased further to 32.6% in those patients with a personal history of CRC. The most common dose of aspirin taken was 100mg (86.6%). Only 4% were taking 600mg aspirin daily.

Of the 119 patients taking aspirin, 88 (72.2%) were doing so for LS chemoprophylaxis and 31 (25.6%) for cardiovascular or cerebrovascular disease. The mean duration of aspirin use was 5.6 years, median was 4 years and range 1 month to 30 years.

Of the 88 patients taking aspirin as chemoprophylaxis, 43 (48.8%) were due to recommendation from NZFGCS, 35 (39.8%) due to recommendation from other medical professionals and 11 (12.5%) due to own research including social media and Facebook groups.

Of the 383 patients currently not taking aspirin, 48 (12.5%) had previously taken aspirin regularly. The most common reason given for stopping aspirin was due to side effects in 18 patients (37.5%).

Conclusions

Although the beneficial effect of aspirin has been known for over a decade, only just over half of LS patients in NZ were aware of the potential benefits of aspirin in reducing risk of CRC.

Overall, only 23.7% of gene positive LS patients were regularly taking aspirin. Having a first degree relative with CRC increased this rate to 25.8% and having a personal previous diagnosis of CRC increased this significantly to 32.6%.

There is still significant need for ongoing targeted patient education about this important aspect of colorectal cancer prevention in LS in NZ.

O2 - ESTABLISHING A DEEP MUTATIONAL SCAN FOR MSH6 MISSENSE VARIANT CLASSIFICATION

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Background and aims

Lynch Syndrome is a dominantly inherited colorectal and gynecological cancer predisposition syndrome caused by loss-of-functio n variants in genes encoding DNA mismatch repair (MMR) factors. MSH6 is one of the key MMR factors implicated in Lynch Syndrome and is notable in particular for its high penetrance for endometrial cancer risk. Actionability of clinical MSH6 testing is limited by the burden of missense variant interpretation as demonstrated by MSH6 missense variants in the NCBI ClinVar database, nearly all of which (2745/2802, 98.0%) are variants of uncertain significance (VUS) or have conflicting interpretations. To address this unmet clinical need, we applied deep mutational scanning (DMS) to systematically generate functional data to support variant classification.

Method

We have established a DMS-based platform to systematically test missense variants across MSH6, and here we describe proof-of-principle experiments targeting a 50-residue segment of MSH6 (codons 1054-1103). We generated a saturation mutagenesis library representing all 950 distinct missense variants within this region and introduced these one at a time into human HAP1 MSH6 knockout cells. These cells are then treated with the nucleotide analog 6-thioguanine, which selects against intact MMR activity, to deplete neutral MSH6 variants and enrich for pathogenic variants, resulting in a loss of function (LoF) score to quantify MMR activity.

Results

Consistent with known functional constraint in this region, 18.8% of missense variants have a deleterious LoF score, especially around the critical p.R1076 residue, and among proline substitutions in regions of secondary structure **(Figure 1)**. The LoF scores of this pilot tile were 100% concordant with known clinical pathogenic (6/6, Figure 1 yellow box) and benign (15/15, Figure 1 green box) classifications, and are well correlated with effects observed at the equivalent residues in the binding partner and distant paralog MSH2 in a previous DMS study from our group **(Figure 2)**.

Conclusions

These experiments demonstrate the feasibility of deep mutational scanning of all possible MSH6 missense variants, toward their prospective interpretation.

Figure 1.

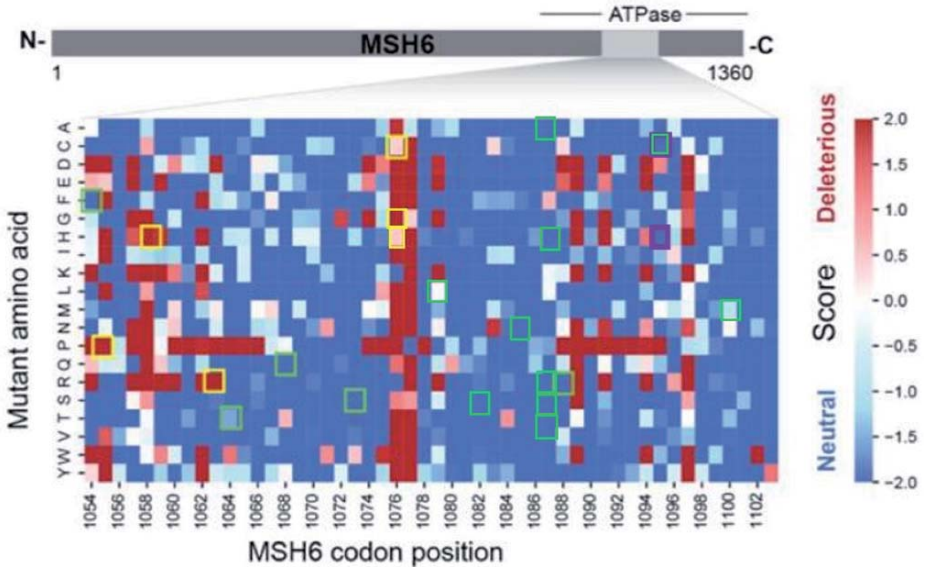
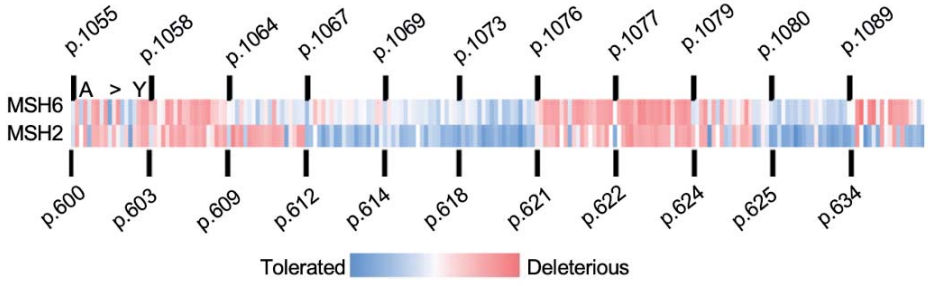


Figure 2.



O3 - CHARACTERIZATION OF THE MUTATIONAL LANDSCAPE OF COLORECTAL TUMORS FROM INDIVIDUALS WITH ADENOMATOUS OR SERRATED POLYPOSIS

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Background and aims

Individuals with adenomatous (AP) or serrated polyposis (SP) are suspected to be predisposed for colorectal tumor development. In routine diagnostics germline pathogenic variants in known causative genes are identified in about 50% of AP and a minority of SP cases, thus many individuals remain without a genetic diagnosis. Within the EU project Solve-RD (No.779257), the ERN GENTURIS aims at molecularly characterizing colorectal tumors from individuals with unexplained AP/SP to extend biological insights and identify potential clues for underlying germline causes.

Methods

Whole-exome sequencing was performed on DNA from 215 formalin-fixed paraffin-embedded gastrointestinal tumors (115 adenomas; 81 serrated polyps, 19 carcinomas) from 124 patients with AP/SP. Somatic variants were called and annotated using an in-house pipeline. Tumor mutational burden (TMB), mutation spectra, and mutational signatures were analyzed and compared to control datasets. Driver gene analysis was based on the COSMIC Cancer Gene Census list (n = 736). Additional analysis of a CpG-island methylator phenotype (CIMP) was done for SP tumors.

Results

99% of all tumors had a low TMB (< 10 mut/Mb) and were microsatellite stable. Driver gene analysis showed that *APC/CTNNB1* and *BRAF* mutations were almost exclusively present in AP (86%) and SP (81%) tumors, respectively. Notably, all three traditional serrated adenomas carried *APC* mutations. *APC* mosaicism was found in 30% (11/37) of AP patients (≥ 2 sequenced tumors). Further recurrent (likely) pathogenic somatic mutations were identified in *AMER1, KMT2C, LRP1B*, and *THRAP3* (25% of *APC/CTNNB1*-mutated tumors) and in *AKAP9* and *BRCA2* (15% of *BRAF*-mutated tumors). Overall, 30% of SP tumors were CIMP-high. *De novo* signature extraction revealed a significantly lower contribution of the clock-like signature SBS1 in *APC/CTNNB1*-mutated tumors and a significantly higher contribution of the normal colon tissue signature SBS89 in *BRAF*-mutated tumors compared to sporadic colorectal tumors.

Conclusion

Distinct driver genes including novel, recurrently mutated potential driver genes characterize tumors in AP/SP individuals. APC mutational mosaicism explains a substantial portion of AP patients. Signature analysis indicates that *BRAF*-mutated tumors are molecularly more similar to normal colon tissue than *APC/CTNNB1*-mutated tumors. Overall, molecular tumor profiling provides further insights into mechanisms of molecular tumorigenesis.

Keywords

Polyposis, hereditary colorectal cancer, tumorigenesis, molecular profiling, Tumor Mutational Burden (TMB); CpG-island methylator phenotype (CIMP).

O4 - CANCER RISK IN HAMATOMATOUS POLYPOSIS SYNDROMES: A FOCUS ON JUVENILE POLYPOSIS AND PEUTZ-JEGHERS

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Background and aims

Hamartomatous polyposis syndromes (HPS) comprise a group of heterogeneous disorders, including Juvenile Polyposis Syndrome (JPS) and Peutz-Jeghers Syndrome (PJS). Currently, only limited data exist on the risk of malignancy associated with these conditions. Our study aims to investigate the management strategies and cancer risk in patients with HPS.

Methods

Records of HPS patients between 2000 and 2023 were retrospectively analysed. Patients were categorised according to the pathogenic variants (PVs): JPS (SMAD4 or BMPR1A PVs), PJS (STK11 PVs) and those without mutations (no PVs detected on SMAD4, BMPR1A and STK11 genes). Kaplan-Meier curve were generated to study Cancer-free survival (C-FS) and Gastrointestinal Cancer-free survival (GC-FS).

Results

A total of 66 patients with HPS were enrolled. Of them, 35 (53%) were JPS, 23 (34%) were PJS, and 8 (12%) were without PVs on SMAD4, BMPR1A and STK11 genes. Overall,

the median number of polyps was 13(8-22), with 18(10-32) exhibiting hamartomatous histology and 10(8-11) adenomatous histology. Genotypes did not influence histology and polyps' number. At baseline, 6(9%) HPS patients have cancer without significant differences between the genotypes (p>0.99). At a median follow-up of 54(16-153) months, 3(4%) HPS patients developed cancer. The 10-y C-FS was similar between JPS and PJS (92% vs. 80%, p=0.10). During surveillance, 8(12%) of HPS patients developed polyps with high-grade dysplasia; however, none experienced gastrointestinal cancer during the follow-up period.

Conclusions

Despite existing literature, in HPS patients under surveillance, cancer risk is low and does not differ between JPS and PJS. Regular endoscopic surveillance contributes to managing gastrointestinal cancer risk in HPS patients.

O5 - FAMILIAL UVEAL MELANOMA AND OTHER TUMOURS IN 25 FAMILIES WITH MONOALLELIC GERMLINE MBD4 VARIANTS

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Background and aims

Monoallelic germline pathogenic variants (PV) in *MBD4* are involved in the predisposition to uveal melanoma (UM)[1]. UM developed in this context show a specific mutational signature with good response to immunotherapy[2]. Monoallelic tumour PV in *MBD4* have also been reported in cerebral tumours, breast cancers (BC) and myxofibrosarcomas[3]. Carriers of biallelic germline PV in *MBD4* develop adenomatous polyposis and acute myeloid leukaemia at high frequencies[4]. We aimed to explore tumour spectrum in monoallelic germline PV carriers.

Methods

12 carriers of a monoallelic germline PV in *MBD4* with UM have been identified in previous projects[1]. We sequenced *MBD4* in the 289 patients treated for UM at Institut Curie since July 2021 and for the 3240 patients who underwent genetic analyses in the context of a suspected predisposition to BC[5].

Results

25 carriers of a monoallelic germline PV in *MBD4* were identified, 18 with UM and 7 with BC. Among patients with UM, 4 had at least one documented other choroidal naevus and one had one naevus and 2 independent UM of the right eye at ages 20 and 26, developed on pre-existing naevi. Two patients also presented another cancer: a papillary thyroid carcinoma and an *in situ* BC. Another patient reported colorectal polyps. Several types of cancers were reported among relatives, including 10 BC, a colorectal cancer at age 39, and another UM in a proband's sister who also carried the familial *MBD4* PV. Among patients with BC, one had bilateral BC and another also developed an ovarian clear cell carcinoma and a cutaneous melanoma. The *MBD4* PVs co-segregated with tumours in 2 families with BC aggregation and a sarcoma. Moreover, we found a positive association between BC and *MBD4* PVs comparing with a control population (Non-Finnish European non-cancer females in gnomAD version 2.1.1, p=0.0281).

Conclusions

Monoallelic germline PVs in *MBD4* are associated with multiple and familial UM. They are also associated with other tumours, especially BC and possibly some sarcomas. Carriers' identification is crucial to precise the tumour spectrum and estimate tumour risks, in order to define their surveillance and treatment.

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O6 - EARLY ONSET COLORECTAL MORTALITY TRENDS IN ARGENTINA, 1997-2020. IS IT TIME TO REDUCE THE AGE OF SCREENING IN AVERAGE-RISK ADULTS? FIRST REPORT IN LATIN AMERICA

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Background and aims

CRC incidence and mortality have been increasing among adults < 50yo in several countries since the 90´s. Because of this, the North American guidelines recommend, since 2021, to begin CRC screening in average-risk adults at 45yo instead of 50yo. In June 2022 our NCI published guidelines for organized CRC screening in average-risk people, in which it suggested, for the meantime, not to screen for CRC people between 45-49yo. We conducted a CRC mortality trend retrospective study in adults >20yo in Argentina during 1997-2020.

Method

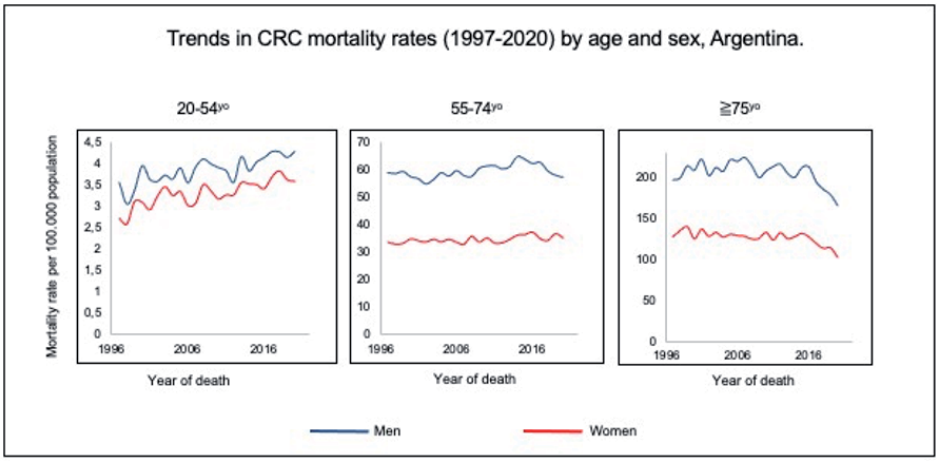
CRC mortality rates per 100000 population (MR) from 1997 through 2020 were obtained from the argentine Health Ministry for decedents who were aged 20years or older, as reported by the Vital Statistics System of Health Information (DEIS). DEIS mortality data are based on the underlying cause of death reported on death certificates filled in all 24 provinces, reflecting more than 95% of deaths. Deaths were identified by ICD-10 codes C18 to C20. First, we compared CRC MR in people 20-54yo vs 55-74yo and vs ≥75yo. Secondly, we analyzed CRC MR in people 20-54yo according to sex an age groups. Lastly, we compared CRC MR in people 20-54yo vs. ≥55yo with a rate ratio.

Results

During 1997-2020, CCR MR in people 20-54yo increased constantly, from 3.16 in 1997 to 3.95 in 2020, with a 25% increase in the percentage variation (PV). A very slight CCR MR decrease was observed in people between 55-74yo: from 46.11 to 45.25, with a 1.86% negative PV. In those ≥75yo, a more accentuated decrease was observed: from 154.72 to 128.01, with a 17% negative PV. When adults 20-54yo were analyzed by age groups and by sex, the greatest increase in CRC MR was observed in women (from 1,26 in 1997 to 2,17 in 2020, 72% positive PV) and men (from 1,68 in 1997 to 2,66 in 2020, 58% positive PV) aged 30-39yo. Finally, when comparing CRC MR evolution over time in individuals ≥55yo vs. 20-54yo, we observed that the rate ratio decreased from 20.81 in 1997 to 15.01 in 2020.

Conclusions

Based on these new epidemiological data about early onset CRC mortality in Argentina, we think we should rediscuss which is the ideal age to begin CRC screening in our country. Importantly, this is the first report about this topic in Latin America, which added to others from the United States, Canada, Europe, China and Australia, we can definitely conclude that the early onset CRC rise is a global phenomenon.



O7 - DETECTION OF EARLY GASTRIC CANCER DURING ENDOSCOPIC SURVEILLANCE IN CDH1 AND CTNNA1 PATHOGENIC VARIANT CARRIERS: HIGHER YIELD WITH TARGETED THAN WITH RANDOM BIOPSIES

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Background and aims

Hereditary diffuse gastric cancer (HDGC) is caused by germline pathogenic variants (PV) of the *CDH1* or *CTNNA1* genes, which play an important role in orientation and adhesion of gastric mucosal cells. The current guideline advises carriers of HDGC to undergo either a prophylactic gastrectomy or annual gastroscopic surveillance with extensive targeted and random biopsies in an expert center. Although most PV carriers develop multiple T1a diffuse gastric cancer (T1a-DGC) lesions, only 30-40% develops diffuse gastric cancer (DGC) >T1, which indicates an indolent behavior of the ubiquitously present T1a-DGC lesions.

The aim of this study was to evaluate the yield of DGC with targeted and random biopsies during annual surveillance endoscopy in *HDGC*.

Method

A retrospective cohort study of all PV carriers who underwent at least one surveillance endoscopy was performed in two HDGC expert centers in the Netherlands.

Results

104 *CDH1* and 27 *CTNNA1* PV carriers (57 men, 40 (17-84) yrs) from 46 families, underwent 398 surveillance endoscopies. The average follow up was 4,3 (±4,3 yrs). Only 3 advanced

gastric cancers (>T1) were found, all at baseline endoscopy. No DGC >T1 was identified in 272 patient years of follow-up. DGC was identified during endoscopy in 47 (36%) carriers; in 27/131 (21%) by targeted biopsies only, in 12/131 (9%) by random biopsies only, and in 8/131 (6%) in both random and targeted biopsies. DGC was detected in 83/1187 targeted biopsies (7%), whereas 32/5526 (0,6%) random biopsies revealed DGC. Seventy-one carriers underwent a prophylactic total gastrectomy. At least one T1a-DGC lesion was found during pathological examination of the gastric mucosa in 63 patients (89%) using the Swiss roll technique. DGC had been identified by endoscopic biopsies in 37 of these 63 (59%) DGC positive specimens. All missed lesions in 26 patients were T1a DGC lesions.

Conclusion

In our expert centers DGC was identified by an extensive endoscopic surveillance protocol in 36% of all HDGC carriers. Missed lesions were all T1a-DGC. No advanced DGC has been missed. No interval advanced cancers were seen. During endoscopy only a low number of DGC were detected through random sampling; all of these were T1a-DGC lesions. These findings, combined with the knowledge that T1a-DGC lesions often display an indolent behavior and as such their clinical relevance is doubtful, we plea for a focus on signs of (early) invasion during endoscopy. This demands a critical reappraisal of random biopsy sampling in the HDGC guideline which will be updated in 2024/2025.

O8 - GASTRIC EPITHELIUM AND PATIENT-DERIVED GASTRIC ORGANOIDS FROM BRCA1/2 CARRIERS HARBOR INCREASED PROLIFERATION AND DNA DAMAGE

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Background and aims

Recent evidence suggests carriers of a pathogenic germline variant (PGV) in *BRCA1* or *BRCA2* have increased gastric cancer risk, however, the specific mechanisms underlying gastric carcinogenesis in *BRCA1/2* PGV carriers remain largely unknown. Herein we aim to elucidate potential avenues of gastric carcinogenesis amongst *BRCA1/2* PGV carriers.

Methods

Gastric body and antrum biopsies were collected from *BRCA1/2* PGV carriers (n=5 for both *BRCA1* and *BRCA2*) as well as from individuals that tested negative for a *BRCA1/2* PGV (control, n=5). Biopsies were used to generate patient-derived gastric organoids (PDGOs), where organoid number, size, and morphology were measured at various timepoints post initiation after single cell digestion and standardized seeding. Biopsy tissue and corresponding PDGOs were also embedded and immunolabeled to evaluate for DNA damage.

Results

PDGO number, size, and morphology were measured at 10-, 15-, and 20-days post initiation (Figure 1). Compared to controls, the number of PDGOs formed from both the antrum and body of *BRCA2* PGV carriers were increased at all timepoints **(Figure 1A-B)**. While PDGOs from *BRCA1* PGV carriers did not form more organoids than controls, PDGOs from the gastric body of *BRCA1* PGV carriers grew significantly larger than those derived from *BRCA2* PGV carriers and controls **(Figure 1C).** No differences in size were observed among PDGOs derived from the gastric antrum **(Figure 1D)**. There were no differences in PDGO morphology as measured by sphericity **(Figure 1E-F)**.

The prevalence of DNA damage was assessed via immunolabeling for established DNA damage markers 53BP1 and γ-H2AX. There is increased nuclear localization of 53BP1 and γ-H2AX in biopsy tissue from the gastric body of *BRCA1/2* PGV carriers compared to controls, particularly for BRCA2 PGV carriers **(Figure 2)**. Similar results were obtained for biopsies of the gastric antrum as well as from gastric PDGOs (images not shown).

Conclusion

Herein we show that PDGOs from *BRCA1/2* PGV carriers exhibit a growth advantage compared to PDGOs from individuals without a known germline genetic alteration. Furthermore, the prevalence of DNA damage in gastric biopsy tissue and PDGOs is increased for *BRCA1/2* PGV carriers. Taken together, our data suggests that the gastric epithelium of *BRCA1/2* PGV carriers harbors increased proliferation and DNA damage, which may contribute to gastric carcinogenesis and increased gastric cancer risk amongst *BRCA1/2* carriers.

Figure 1. Patient-derived gastric organoids (PDGOs) generated from biopsies of the gastric body and gastric antrum. Number of PDGOs generated from gastric body biopsies (A) and gastric antrum biopsies (B). PDGO size as measured by area (μm2) from biopsies of the gastric body (C) and gastric antrum (D). PDGO morphology as measured by sphericity from biopsies of the gastric body (E) and gastric antrum (F). n = 5 patients per group. * = statistically significant difference ($p \leq 0.05$) at the indicated timepoint.

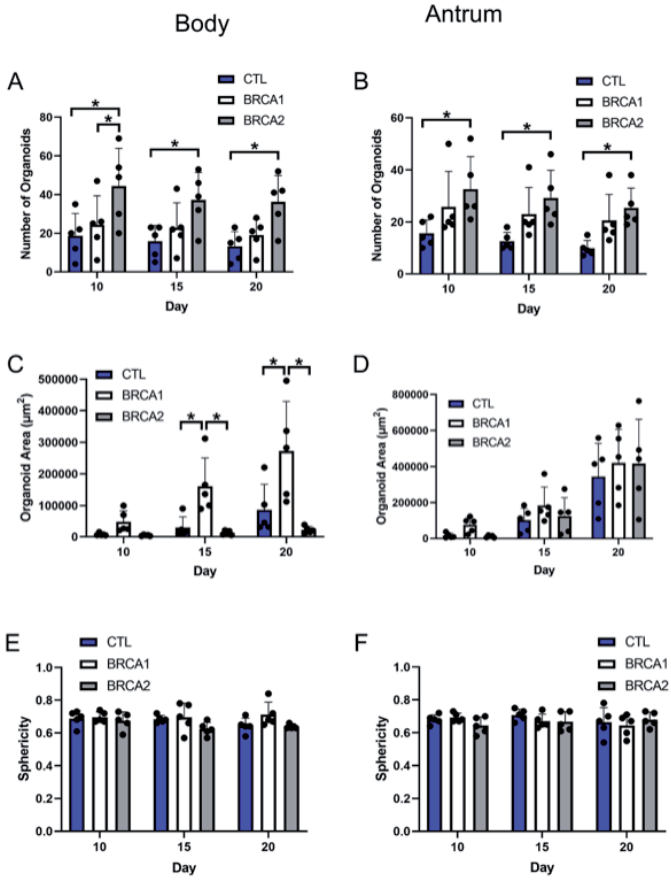
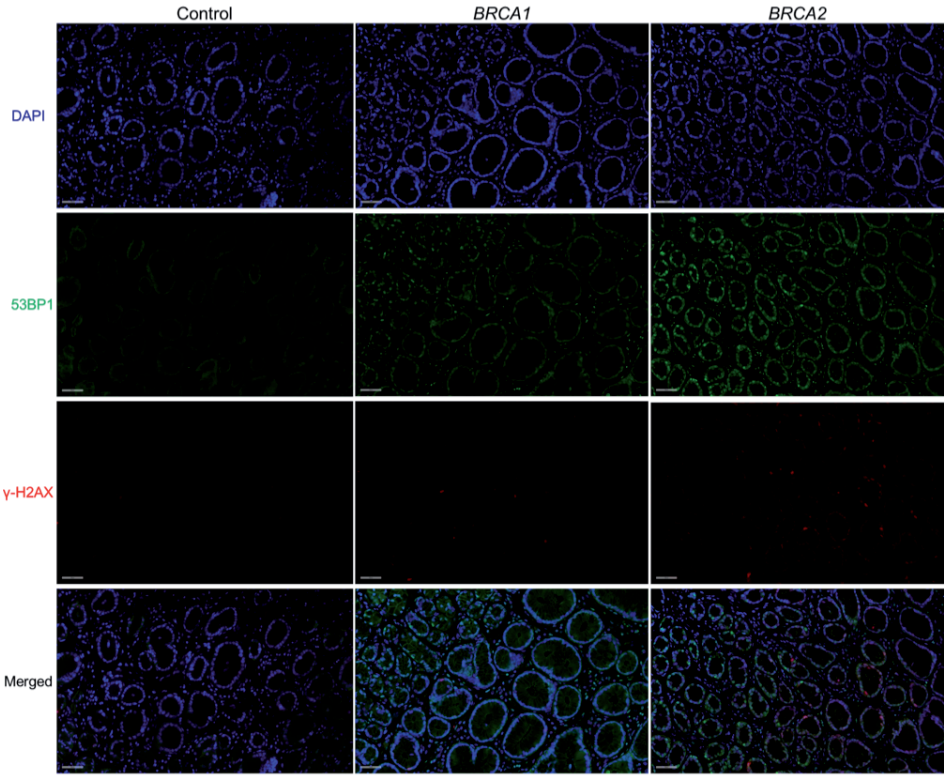


Figure 2. Prevalence of DNA damage in biopsies of the gastric body as assessed via expression of DNA damage markers 53BP1 and γ-H2AX.



O9 - LYNCH SYNDROME (LS) PATIENTS WITH INFLAMMATORY BOWEL DISEASE (IBD) HAVE SIGNIFICANTLY HIGHER INTESTINAL NEOPLASIA RISK THAN LS PATIENTS WITHOUT IBD

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Background and aims

Inflammatory bowel disease (IBD) and Lynch syndrome (LS) are both risk factors for colorectal cancer (CRC), however it is unclear if those with LS+IBD have compounded cancer risk or if they would benefit from more intensive surveillance. Our primary aims were to compare the prevalence of CRC in those with LS+IBD vs LS alone, and evaluate factors associated with CRC. Secondary outcomes included describing prevalence and risk factors for colorectal polyps, gastric, small bowel, and gynecologic cancer.

Methods

We performed a retrospective cohort study from the national Epic Cosmos Expertly Determined De-Identified data set (EDDI), which includes >210 million patients from 205 academic and community centers in the United States. EDDI provides anonymized patient- and encounter-level data based on pre-specified discrete data fields, medication orders, diagnostic codes, and billing codes. We included those with a billing, encounter, or problem-list diagnoses of LS from 1/1/2020 to 7/1/2023. We collected sociodemographics, comorbidities, medications, family history and history of neoplasia. Cohort characteristics and neoplasia outcomes were compared using Chi-square tests. Multivariable regression analysis was performed to evaluate factors associated with neoplasia.

Results

Of 24,584 patients with LS, 71.5% were female and 86.4% were white. Genotypes included *MLH1* (4.9%), *MSH2/EPCAM* (6.8%), *MSH6* (10.9%), *PMS2* (10.4%) and unspecified (66.9%). 568 (2.3%) LS patients had comorbid IBD. Compared to LS patients without IBD, LS patients with IBD were less likely to be Hispanic/LatinX (3.9% vs 5.8%, p< 0.0001), more likely to have a personal history of primary sclerosing cholangitis (0.5 % vs 0.02%, p< 0.0001), and be prescribed aspirin (6.5% vs 4.4%, p=0.0235). LS+IBD patients had a higher prevalence of CRC (7.7% vs 4.9%, p =0.003), colorectal polyps (39.8% vs 30.8%, p< 0.0001), and small bowel cancer (3.5% vs 1.2%, p< 0.0001), but there was no significant difference in gynecologic or gastric cancer **(Table 1)**. After multivariable adjustment, IBD (OR 1.55, 1.10-2.18), *MLH1* genotype (OR 1.54, 1.21-1.97), increasing age (for age 65-74, OR 4.15, 2.40-7.17), Hispanic ethnicity (OR 1.38, 1.09-1.75) and active/former smoking status (OR 1.33, 1.15-1.54) were independently associated with CRC **(Table 2).** IBD, genotype,

increasing age and were also associated with colorectal polyps and small bowel cancer, with increased BMI and aspirin use also being associated with colorectal polyps.

Conclusions

There is a significantly higher prevalence of CRC, small bowel cancer, and colorectal polyps among those with LS+IBD vs LS alone. IBD, *MLH1/MSH2/EPCAM* genotype, and increasing age are independent risk factors for neoplasia, suggesting that LS patients with these risk factors may benefit from more intensive surveillance.

Table 1: Cohort Characteristics and neoplasia outcomes in Lynch syndrome patients with and without comorbid Inflammatory Bowel Disease (IBD)

	Lynch with IBD (n = 568)	Lynch without IBD (n = 24016)	p*
Genotype			**0.008**
MLH1	35 (6.2%)	1181 (4.9%)	
MSH2/EPCAM	52 (7.9%)	1632 (6.8%)	
MSH6	41 (7.2%)	2640 (11.0%)	
PMS2	55 (9.7%)	2505 (10.4%)	
Unspecified	385 (67.8%)	16058 (66.9%)	
Sex			**<0.0001**
Male	205 (36.1%)	6788 (28.3%)	
Female/Unknown	363 (63.9%)**	17228 (71.7%)***	
Age (years)			0.386
<18	1 (0.2%)	149 (0.6%)	
18-29	37 (6.3%)	1329 (5.5%)	
30-39	68 (12.0%)	3199 (13.3%)	
40-49	112 (19.7%)	4466 (18.6%)	
50-64	178 (31.3%)	8052 (33.5%)	
65-74	111 (19.5%)	4631 (19.3%)	
≥75	62 (10.9%)	2190 (9.1%)	
BMI			0.252
<18.5	2 (0.3%)	152 (0.6%)	
18.5-24.9	93 (16.4%)	4344 (18.1%)	
25.0-29.9	157 (27.6%)	6848 (28.5%)	
≥30.0	314 (55.3%)	12435 (51.8%)	
Unknown/Unavailable	2 (0.3%)	237 (0.1%)	
Race			0.580
White	497 (87.5%)	20745 (86.4%)	
Black	33 (5.8%)	1339 (5.6%)	
Asian	17 (3.0%)	739 (3.1%)	
Other/Unknown	21 (3.7%)	1193 (5.0%)	
Hispanic or LatinX Ethnicity	22 (3.9%)	1396 (5.8%)	**<0.0001**
Prescribed or reported aspirin	37 (6.5%)	1065 (4.4%)	**0.0235**
Active or former smoker	122 (21.5%)	4514 (18.8%)	0.118
Primary Sclerosing Cholangitis	3 (0.5%)	5 (0.02%)	**<0.0001**
Family history of colorectal cancer	82 (14.4%)	3776 (15.7%)	0.439
Neoplasia Outcomes			
Colorectal cancer	44 (7.7%)	1183 (4.9%)	**0.003**
Colorectal polyp(s)	226 (39.8%)	7402 (30.8%)	**<0.0001**
Gynecological cancer	76 (13.4%)	3251 (13.5%)	0.963
Gastric cancer	12 (2.1%)	283 (1.2%)	0.068
Small bowel cancer	20 (3.5%)	293 (1.2%)	**<0.0001**
Cancer of duodenum	*9 (45%)*	*183 (62.4%)*	
Cancer of jejunum	*6 (30%)*	*55 (18.8%)*	
Cancer of ileum	*5 (25%)*	*55 (18.8%)*	

BMI: Body Mass Index

*Chi-square test; **Unknown Sex = 0; ***Unknown Sex = 5

Table 2: Multivariable logistic regression analysis evaluating factors associated with neoplasia in Lynch Syndrome Patients.

	CRC (N=1227) aOR (95% CI)*	P	Colon Polyps (N=7628) aOR (95% CI)*	P	Small Bowel Cancer (N=213) aOR (95% CI)*	P
No IBD (n=24,016)	Reference		Reference		Reference	
IBD (n=568)	1.55 (1.10,2.18)	**0.011**	1.46 (1.21, 1.76)	**<0.0001**	2.66 (1.58,4.47)	**0.0002**
Genotype						
Unspecified (n=16,444)	Reference		Reference		Reference	
MLH1 (n=1,216)	1.54 (1.21,1.97)	**0.0005**	1.43 (1.25,1.64)	**<0.0001**	1.89 (1.18,3.04)	**0.008**
MSH2/EPCAM (n=1,684)	1.17 (0.92,1.48)	0.189	1.38 (1.22,1.55)	**<0.0001**	1.85 (1.22,2.79)	**0.004**
MSH6 (n=2,681)	0.56 (0.43,0.72)	**<0.0001**	1.21 (1.10,1.34)	**<0.0001**	0.63 (0.38,1.03)	0.068
PMS2 (n=2,560)	0.57 (0.44,0.74)	**<0.0001**	1.01 (0.92,1.12)	0.800	0.76 (0.47,1.22)	0.260
Age Group (years)						
≤ 29 (n=1,516)	Reference		Reference		Reference	
30-39 (n=3,267)	2.91 (1.66,5.10)	**0.0002**	1.82 (1.48,2.24)	**<0.0001**	3.02 (0.70,13.10)	0.140
40-49 (n=4,578)	3.50 (2.02,6.05)	**<0.0001**	2.65 (2.17,3.24)	**<0.0001**	3.04 (0.72,12.87)	0.131
50-64 (n=8,230)	3.80 (2.22,6.52)	**<0.0001**	3.52 (2.90,4.27)	**<0.0001**	5.81 (1.42,23.69)	**0.014**
65-74 (n=4,742)	4.15 (2.40,7.17)	**<0.0001**	4.01 (3.28,4.89)	**<0.0001**	10.29 (2.52,42.01)	**0.001**
>75 (n=2,252)	3.99 (2.26,7.01)	**<0.0001**	3.78 (3.05,4.65)	**<0.0001**	11.67 (2.81,48.35)	**0.0007**
BMI						
<24.9 (n=4,591)	Reference		Reference		Reference	
25.0-29.9 (n=7,005)	0.70 (0.32,1.52)	0.362	1.81 (1.11,2.97)	**0.018**	1.27 (0.17,9.18)	0.813
30.0+ (n=12,749)	0.77 (0.36,1.68)	0.518	2.07 (1.27,3.39)	**0.004**	1.27 (0.18,9.14)	0.812
Unknown/Unavailable (n=239)	0.26 (0.07,0.90)	0.033	0.36 (0.18,0.72)	**0.004**	N/A	N/A
Ethnicity						
Not Hispanic/LatinX (n=23,166)	Reference		Reference		Reference	
Hispanic/LatinX (n=1,418)	1.38 (1.09,1.75)	**0.007**	0.91 (0.80,1.04)	0.174	1.21 (0.72,2.03)	0.470
Prescribed or reported aspirin						
No (n=23,482)	Reference		Reference		Reference	
Yes (n=1,102)	1.06 (0.81,1.40)	0.661	1.19 (1.04,1.37)	**0.001**	1.47 (0.93,2.31)	0.095
Active or former smoker						
No (n=19,948)	Reference		Reference		Reference	
Yes (n=4636)	1.33 (1.15,1.54)	**0.0001**	0.93 (0.87,1.01)	0.086	1.30 (0.97,1.73)	0.075
Primary sclerosing cholangitis						
No (n=24,576)	Reference		Reference		Reference	
Yes (n=8)	N/A	N/A	1.02 (0.19,5.39)	0.983	N/A	N/A

aOR: adjusted Odds Ratio; CI: Confidence Interval; CRC: Colorectal Cancer; EoCRC: Early age Onset Colorectal Cancer; BMI: Body Mass Index
**Regression model adjusted for IBD diagnoses, Lynch Genotype, Age Group, Highest BMI, Ethnicity, Aspirin, Smoking, Primary Sclerosing Cholangitis diagnosis*

O10 - COLORECTAL ADENOMA AND CANCER INCIDENCES AND SURVIVAL IN *PATH_MMR* CARRIERS UNDERGOING SURVEILLANCE COLONOSCOPY

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Background and aims

In addition to the accelerated adenoma-carcinoma pathway (1), carriers of pathogenic variants of the *MMR* genes (carriers) have multiple mismatch repair-deficient (dMMR) crypts from which colorectal cancer (CRC) may develop (2). The host immune system may control dMMR crypts, and immunotherapy illustrates that the immune system may remove MSI cancers. Colonoscopy in carriers does not reduce overall CRC incidence in carriers (3). We examined our theory that colonoscopy may reduce CRC incidence in carriers with colorectal adenomas (CRA) (2).

Methods

We estimated cumulative incidences of CRA and CRC by *MMR* genetic variant in the Prospective Lynch Syndromes Database.

Results

Among carriers where the required details were reported, 2,444/3,574 (68%) had never had a CRA (Group 1) and 1,130/3,574 (32%) had one or more CRA(s) removed (Group 2); findings that are similar to those reported in normal populations (4-6). Annual incidences of both CRC and CRA increased with age. *Path_PMS2* carriers had less CRAs than the others ($p<0.01$), and only a few at any age and with any genetic variant had multiple CRAs **(Figure 1)**. In total, 236 carriers had prospectively diagnosed CRC, of which 139 (56%) were from Group 1 and 110 (44%) from Group 2. Mean ages at first CRC diagnosis in Groups 1 and 2 were 46.7 and 50.2 years, and 10-year survival after CRC was 81% and 88% ($p>0.05$), respectively. CRC

incidences from 25 years onwards were equal in both groups for *path_MLH1* and *path_MSH2* carriers **(Figure 2)**. Numbers of *path_MSH6* and *path_PMS2* carriers were insufficient for calculating CRC incidences. Our results are in conflict with a previous report describing a limited number of carriers and seemingly supporting our theory, but they considered neither CRC nor CRA incidences by age, and did not report life-time cumulative incidences (7).

Conclusions

The incidences of CRAs in LS carriers and CRC patients are similar to that in the wider population and most CRCs in *path_MLH1* and *pathMSH2* carriers are likely to arise from dMMR crypts, rather than through accelerated carcinogenesis in CRAs. Further data is required to clarify the situation in *path_MSH6* and *path_PMS2* carriers.

Keywords

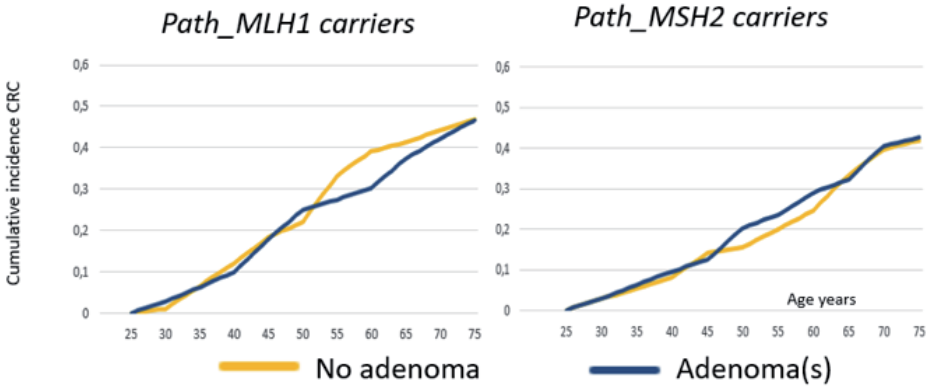
Lynch syndromes, colorectal cancer, adenoma, colonoscopy.

References

[1] PMID 1624160
[2] PMID 37821984
[3] PMID 36182917
[4] PMID 33579203
[5] PMID 36178673
[6] PMID 22985608
[7] PMID 36964717

Figure 1
Pie-charts showing total number of CRAs by genetic variant and ordinary least squares regression on number of CRAs by genetic variant and age.

Figure 2
Cumulative incidences of CRC in path_MLH1/MSH2 carriers with and without CRA(s).



O11 - LYNCH SYNDROME-RELATED NEOANTIGENS PREDICTION AND VALIDATION FOR A DENDRITIC-CELL BASED CANCER PREVENTION VACCINE

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Background and aims

Lynch syndrome (LS), caused by germline mutations on DNA mismatch-repair genes (MMR) predisposes to colorectal and endometrial cancer (CRC, EC) amongst other tumors. Although CRC prevention is effective, no strategies exist for most LS-related tumors. Ex-vivo generated and tumor-antigen-loaded dendritic cell (DC) vaccines have been used in cancer immunotherapy; however, their full therapeutic potential would likely be as a preventive approach in high-risk cancer patients. LS is a paradigmatic model for its limited and predictive mutational spectrum in repetitive DNA sequences termed microsatellites (MS). We aimed to identify and validate frameshift derived neopeptides (FSDN) to develop a cancer preventive DC-based neoepitope vaccine.

Methods

Search and selection of LS-related coding MS (cMS) mutations and prediction of neoantigens with high coverage on common HLA class I and II alleles (pVACbind; pVACtools v2.0.1). Sequencing and analysis of the presence of FSDN mutations on colorectal adenomas (CrAD), EC and CRC samples from LS patients, non-LS MS instability (MSI) tumor sequences and in RNA and DNA sequences from tumor cell lines (mutational signature analysis, HLA-typing, neoantigens prediction (pVACseq)). *In vitro* analysis of the synthetic neoantigens immunogenicity on tissue infiltrating lymphocytes (TILs) from LS CrADs, CRCs and normal mucosa by IFNγ ELISPOT assays, flow cytometry. Detection, expansion, and characterization of neoantigen-specific CD8+ T-cells.

Results

98 neoepitopes from 53 coding-MS-containing genes were prioritized (53 HLA-I and 45 HLA-II restricted). *In silico* analysis showed that ≥1 neoantigen-related mutations are found in all analyzed CrADs (31), EC (8) and CRC (52) LS samples and in 18-84% non-LS MSI tumors.

Additionally, we found the FSDN mutations in DNA (66% cMS) and cDNA (69.8%) from MSI tumoral cell lines. *In vitro* analysis showed that 71% tested FSDN gave a positive IFNy response in at least one LS patient (n=9). FSDN-specific T cells were able to be detected and isolated from CrAD and normal colonic mucosa for further characterization. Based on the results we prioritized a set of 24 FSDN that constitute the vaccine.

Conclusions

Our predicted neoepitope set has an optimal coverage among LS patients in terms of HLA alleles, associated cancers and prevalence, and is capable of inducing an IFNy inflammatory response in LS individuals. A phase Ib clinical trial will start on 2024 to determine the safety and efficacy of the autologous DC-based vaccine in LS.

O12 - SYSTEMATIC REVIEW OF THE PHENOTYPIC CANCER SPECTRUM IN NTHL1-ASSOCIATED TUMOUR SYNDROME EMPHASIZES THE NEED TO IDENTIFY MORE FAMILIES TO ASSESS CANCER RISKS

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Background and aims

Germline biallelic pathogenic variants in *NTHL1* cause an extraintestinal and intestinal polyposis syndrome. We aimed to systematically review the literature for all individuals with *NTHL1*-associated tumour syndrome (NATS) to provide a complete overview of phenotypic spectrum.

Method

A systematic review of literature across Medline, Embase and Web of Science was carried out including clearly defined individuals with germline biallelic *NTHL1* (likely) pathogenic variants (LPV/PV) and relevant clinical phenotypes. Covidence used for text screening, followed by citation search to find additional studies. Quality assurance was performed using a modified JBI tool. Specific identifiers such as age, gender, type of variant and unique pedigrees were used to remove duplicates. Collected data from the families were analyzed for common trends and patterns in clinical attributes.

Results

We identified 20 studies reporting 71 individuals from 49 families with *NATS*. In total, 13 different (L)PVs were found across *NTHL1*. *NTHL1* (NM_002528.7) c.244C>T, p.(Gln82*) was the most common PV with 91.8% (45/49) families carrying at least one allele. However, 44.9% of families (22/49) carried (L)PVs other than *NTHL1* c.244C>T, p.(Gln82*). 83.1% of individuals (59/71) developed at least one malignancy (average age of diagnosis at 52.6 years [range 24-76]). Colorectal cancer (CRC) was most common malignancy (36/71 individuals; 50.7%; average age of diagnosis was 52.6 years [range 31-73]), followed by breast cancer (23/71 individuals; 32.4%; average age 55.4 years [range 37-76]). Other observed cancers

include skin cancer (14/71; 19.7%), gynecological cancer (6/36;16.7%), urinary tract cancer (8/71; 11.3%) and Hematological cancer (6/71; 8.5%). Colorectal polyps were the most common premalignancy followed by meningiomas affecting 88.7% (63/71) and 15.5% (11/71), respectively. In total, 69.0% (49/71) of individuals were ascertained because of the diagnosis of CRC or breast cancer.

Conclusion

(L)PVs occurred throughout *NTHL1,* emphasizing testing the entire gene in routine diagnostics. High numbers CRC and breast cancer developed in the third decade of life, supporting existing surveillance for premalignancies in the second decade. High ascertainment risk in CRC and breast cancer highlights need to identify new families to complete cancer risk estimated in NATS. The InSiGHT *NTHL1* consortium invites anyone with new NATS cases to join and contribute, please contact alan.gao@mh.org.au for details.

Keywords

NTHL1-associated tumour syndrome (NATS), polyposis, colorectal cancer, breast cancer, gastroenterology, oncology.

O13 - DISCOVERY OF RECESSIVE EFFECT OF HUMAN POLYMERASE DELTA PROOFREADING DEFICIENCY THROUGH MUTATIONAL ANALYSIS OF POLD1-MUTATED NORMAL AND CANCER CELLS

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Background

Constitutional heterozygous pathogenic variants in the exonuclease domain of *POLE* and *POLD1*, which affect the proofreading activity of corresponding polymerases, cause a cancer predisposition syndrome (polymerase proofreading-associated polyposis), characterized by increased risk of gastrointestinal polyposis, colorectal cancer, endometrial cancer and other tumor types. The connection between the disruption of polymerase proofreading activity and cancer development is through an increase in the somatic mutation rate.

Methods

We studied an extended family with multiple members heterozygous for the pathogenic *POLD1* variant c.1421T>C p.(Leu474Pro). Genome sequencing was performed to evaluate

mutational patterns of patient-derived fibroblasts colonies and of de novo mutations obtained by parent-offspring comparisons. Exome sequencing data from tumors developed by patients with the hereditary cancer syndrome were also analyzed.

Results

Heterozygous *POLD1* L474P just subtly increases somatic and germline mutation burden in non-tumor tissues. In contrast, tumors developed in individuals with a heterozygous mutation of the exonuclease domain of *POLD1*, including L474P, have extremely high mutation rates (>100 mut/Mb) associated with tumor mutational signature SBS10d. To explain these observations, also common to other *POLD1* exonuclease domain pathogenic variants, we show, for the first time, that *POLD1* proofreading deficient tumors developed in the context of the hereditary cancer syndrome require somatic inactivation of the wildtype allele in the target tissue (e.g. colon or endometrial epithelium), usually through loss of heterozygosity, resulting in the elimination of all exonuclease-proficient copies of the gene.

Conclusion

These results provide strong evidence that POLD1 pathogenic variants have a recessive effect on mutation rate in somatic cells.

O14 - THE NEW NATIONAL ENGLISH BOWEL CANCER SCREENING PROGRAMME FOR LYNCH SYNDROME COLONOSCOPIC SURVEILLANCE

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Background and aims

Quality assurance, timeliness and access to colonoscopy for people with Lynch syndrome (LS) has been variable and largely institution-based, with variable population ascertainment in England. The National Health Service (NHS) Bowel Cancer Screening Programme (BCSP) delivers high quality colonoscopy to the average risk population, from colonoscopists who have undergone high-level accreditation. The NHS BCSP for LS was launched in July 2023 and seeks to address these issues, by incorporating this programme within the existing population screening programme framework designed to deliver high quality care.

Method

LS was defined as individuals carrying a germline pathogenic/likely pathogenic variant in mismatch repair genes or EPCAM, and only such individuals are eligible for this programme. Comprehensive retrospective diagnoses of LS in England (since the 1990s) were ascertained from all 17 regional genetics services and St Mark's LS service, and an interactive portal was established to prospectively identify new diagnoses. The National Disease Registration Service (NDRS) developed a national registry of eligible individuals. An existing national screening IT framework designed to support the population average risk BCSP was adapted to incorporate disease specific clinical pathway information, and a screening protocol following existing national UK guidelines was applied[1]. Nationally standardised training for national BCSP teams was delivered to over 2000 staff from 64 national screening centres in early 2023, including colonoscopists, pathologists, specialist screening practitioners (SSP) and administrative staff. Patient representation was provided to support programme development from national charities including Lynch syndrome UK and Bowel Cancer UK.

Results

Invitations to the new programme were sent for prospective and retrospective cohorts from July 2023. In total 8471 eligible individuals were identified, of whom approximately 1000

were not yet age eligible but would be invited later in life. By early December 2023, 4111 people with LS have been invited by the 5 National Screening Hubs, and 2813 have attended a pre-procedure consultation with an SSP, and 1019 colonoscopies have been performed. 300 patients have opted out of the new programme and in most cases chosen to continue surveillance with their existing providers. A backlog of >1000 patients overdue colonoscopy with their previous providers is being managed via a 'smoothing' process prioritising those with higher-risk genotypes and/or with the longest delays with their previous providers. Approximately 100 new LS diagnoses are being added to the NDRS prospective portal each month.

Conclusions

This programme includes complete ascertainment of the diagnosed national LS population in England, ascertained without requirement for referral. Individuals with LS now have access to high-quality, timely colonoscopy through an accredited programme which is quality assured along the entire pathway. By June 2024 we will be able to share updated figures about progress in this novel nationally coordinated programme.

1. Monahan KJ, Bradshaw N, Dolwani S, et al. Guidelines for the management of hereditary colorectal cancer from the British Society of Gastroenterology (BSG)/Association of Coloproctology of Great Britain and Ireland (ACPGBI)/United Kingdom Cancer Genetics Group (UKCGG). Gut 2020;**69**:411–44. doi:10.1136/gutjnl-2019-319915

O15 - YIELD OF MULTIGENE PANEL GERMLINE GENETIC TESTING AMONG THOSE WITH ADVANCED COLORECTAL ADENOMAS

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Background and aims

Advanced adenomas (AAs) [≥1cm, villous, or high-grade dysplasia (HGD)] are precursors to colorectal cancer (CRC) and although guidelines support multi-gene panel testing (MGPT) in those with CRC, it is unclear if MGPT should be performed in individuals with CRC precursors. Our aim was to assess the yield of MGPT among those with AAs and asess factors associated with pathogenic/likely pathogenic variants (PV/LPV).

Methods

Individuals age ≥18 with at least one adenoma ≥10mm, or with HGD or villous architecture were identified between January 2011 to June 2023 from a statewide healthcare system including 13 endoscopy centers. Individuals with an established hereditary GI cancer syndrome, known PV/LPV in CRC gene, personal history of CRC, inflammatory bowel disease, or prior MGPT were excluded. For those who provided informed consent, telehealth genetic counseling and commercial 84-91 MGPT was offered. Demographics, medical/family history, and colonoscopy/pathology data were gathered from the electronic health record and participant surveys. Wilcoxon rank sum test, Pearson's Chi-squared test, and Fisher's exact test were used to assess factors associated with PV/LPV. NCT04160832.

Results

Of 842 individuals with an AA over the study period, 167 consented and completed MGPT **(Table 1)**. Overall, median age was 50, and majority were female (52.7%) and White (92.8%). Indications for colonoscopy were screening (39.5%; 21.5% for family history of CRC & 15.4% average risk), polyp surveillance (15.0%) and diagnostic (34.1%). AAs were based on size only (29.3%), histology only (9.0%) and both size and histology (34.7%). Anatomic distribution of AAs was predominantly rectum (26.1%) and left colon (40.0%), compared with right colon (33.9%). At least one PV/LPV was found in 24 patients (14.4%); however, when excluding

MUTYH carriers and the *CHEK2* c.470T>C variant, 17/167 (10.1%) had a clinically actionable PV/LPV, with three (1.8%) in a mismatch repair (MMR) gene **(Table 2).** Having a first degree relative with CRC was significantly associated with PV/LPV (72.7% vs 27.1%, p=0.011). Only 11.8% (2/17) with a clinically actionable PV/LPV had a PREMM5 score ≥2.5. At least one variant of uncertain significance (VUS) was found in 45.5% (76/167) and 15.0% (25/167) had more than one VUS. Of the 108 total VUS' found, 10 were in MMR genes and 13 in other CRC genes (2 *APC*, 3 *CHEK2*, 3 *POLD1*, 2 *POLE*, 1 *PTEN*, 1 *SMAD4*, 1 *STK11*).

Conclusions

In this study, 10% of patients with AAs had a clinically actionable PV/LPV in a cancer predisposition gene, with 1.8% in mismatch repair genes, and the majority in genes not typically associated with CRC risk. Only 12% of those with a PV/LPV met current criteria for MGPT. Yield was higher among individuals with a family history of CRC. These data can guide clinicians on expected yield of MGPT in those with AAs.

Table 1. Cohort Characteristics and comparison between those with and without pathogenic or likely pathogenic variant.

Baseline Characteristics	Overall, N = 167[1]	PV/LPV, N = 24[1]	No PV/LPV, N = 143[1]	p-value[2]
Age at colonoscopy	50.0 (42.0, 53.0)	50.0 (38.8, 54.0)	49.0 (43.0, 53.0)	>0.9
<50	83 (49.7%)	11 (45.8%)	72 (50.3%)	0.7
≥ 50	84 (50.3%)	13 (54.2%)	71 (49.7%)	
Sex				0.8
Female	88 (52.7%)	12 (50.0%)	76 (53.1%)	
Male	79 (47.3%)	12 (50.0%)	67 (46.9%)	
White/Caucasian	155 (92.8%)	23 (95.8%)	132 (92.3%)	>0.9
Black/African American	7 (4.2%)	1 (4.2%)	6 (4.2%)	>0.9
American Indian/Alaskan Native	2 (1.2%)	0 (0.0%)	2 (1.4%)	>0.9
Asian/Asian American	3 (1.8%)	0 (0.0%)	3 (2.1%)	>0.9
Missing	4 (0.6%)	0 (0.0%)	4 (0.7%)	>0.9
Hispanic or Latino Origin	16 (9.6%)	1 (4.2%)	15 (10.5%)	0.5
Daily aspirin use	30 (18.0%)	6 (25.0%)	24 (16.8%)	0.2
Current Smoker	13 (7.8%)	2 (8.3%)	11 (7.7%)	0.7
No Alcohol	49 (29.3%)	6 (25.0%)	43 (30.1%)	0.3
Diabetes	38 (22.8%)	6 (25.0%)	32 (22.4%)	0.8
No Exercise	27 (16.2%)	5 (20.8%)	22 (15.4%)	0.7
PREMM5 score				0.11
< 2.5	85 (94.4%)	14 (87.5%)	71 (95.9%)	
2.5-5	4 (4.4%)	1 (6.3%)	3 (4.1%)	
> 5	1 (1.1%)	1 (6.3%)	0 (0.0%)	
Unable to calculate*	77	8	69	
FDR with CRC	21 (35.6%)	8 (72.7%)	13 (27.1%)	**0.011**
Colonoscopy indication				
Screening	66 (39.5%)	9 (37.5%)	57 (39.9%)	0.8
Surveillance	25 (15.0%)	7 (29.2%)	18 (12.6%)	0.058
Diagnostic	57 (34.1%)	5 (20.8%)	52 (36.4%)	0.14
AA Characteristics				
Size only (≥15mm)	49 (29.3%)	7 (29.2%)	42 (29.4%)	>0.9
Histology only (Villous or HGD)	15 (9.0%)	3 (12.5%)	12 (8.4%)	0.5
Size + Histology	58 (34.7%)	8 (33.3%)	50 (35.0%)	0.9
HGD	5 (3.0%)	1 (4.2%)	4 (2.8%)	0.5
Size + Histology + HGD	23 (13.8%)	3 (12.5%)	20 (14.0%)	>0.9
HGD with size >= 20mm	33 (19.8%)	4 (16.7%)	29 (20.3%)	0.8
Polyp size (mm)	20.0 (14.0, 30.0)	20.0 (11.5, 30.0)	20.0 (15.0, 30.0)	0.6
Polyp location				0.7
Left colon	66 (40.0%)	9 (37.5%)	57 (40.4%)	
Rectum	43 (26.1%)	8 (33.3%)	35 (24.8%)	
Right colon	56 (33.9%)	7 (29.2%)	49 (34.8%)	

[1]Median (IQR); n (%)

[2]Wilcoxon rank sum test; Pearson's Chi-squared test; Fisher's exact test

*Unable to calculate due to no first- or second-degree relative with a Lynch Associated Cancer

Table 2. Participants with pathogenic and Likely pathogenic variants.

Age at Polyp Diagnosis	Polyp Location	Polyp Size	Polyp Histology	PREMM5 Score	Gene	Variant	Variant type
31	Sigmoid	24mm	TVA w/ HGD	*	*MSH2*	c.2275G>T	Truncating
32	Sigmoid	20mm	TA	6.5	*MLH1*	c.793C>T	Substitution
45	Rectum	40mm	TSA	0.9	*PMS2*	Deletion (Exons 9-10)	Deletion/truncating
49	Ascending	5mm	TA w/ HGD	2.8	*BRCA1*	c.68_69del	Frameshift/truncating
38	Sigmoid	10mm	TA	2.2	*SDHB*	Deletion (Exon 8)	Deletion
52	Cecum	20mm	TVA	*	*SDHC*	c.397C>T	Truncating
55	Transverse	12mm	TVA	*	*CHEK2*	c.1368dup	Frameshift/truncating
59	Ascending	30mm	TA w/ HGD	0.6	*ATM*	c.5932G>T	Truncating
39	Sigmoid	14mm	TVA	1.1	*ATM*	c.7913G>A	Truncating
41	Sigmoid	12mm	TVA	*	*RAD51D*	c.620C>T	Substitution
49	Rectum	90mm	TVA w/ HGD	0.8	*BRIP1*	c.1871C>A	Truncating
52	Cecum	20mm	TA	0.4	*BRIP1*	c.2255_2256del	Deletion/truncating
27	Descending	10mm	TVA	*	*MITF*	c.952G>A	Substitution
37	Rectum	7mm	TVA	2.1	*POT1*	c.124+2T>C	Splice donor
50	Sigmoid	7mm	TVA	0.7	*MITF*	c.952G>A	Substitution
54	Rectum	50mm	TVA	*	*MITF*	c.952G>A	Substitution
54	Descending	15mm	TA	0.5	*FH*	c.1431_1433dup	Duplication
					NTHL1	c.859C>T	Truncating
51	Rectum	30mm	TA	0.8	*MUTYH carrier*	c.700G>A	Substitution
53	Ascending	6mm	TVA	0.6	*MUTYH carrier*	c.536A>G	Substitution
50	Rectum	22mm	TA	*	*MUTYH carrier*	c.1187G>A	Substitution
54	Transverse	20mm	TVA	0.9	*MUTYH carrier*	c.1187G>A	Substitution
54	Rectum	30mm	TVA	1.7	*CHEK2*	c.470T>C	Substitution
56	Transverse	25mm	TVA	1.3	*CHEK2*	c.470T>C	Substitution
29	Rectum	30mm	TVA	*	*CHEK2*	c.470T>C	Substitution

TVA: tubulovillous adenoma; TA: tubular adenoma; TSA: traditional serrated adenoma; HGD: High-Grade Dysplasia
*Unable to calculate due to no first- or second-degree relative with a Lynch Associated Cancer
Gray shading indicates variants not currently considered to be clinically actionable

O16 - PREVALENCE OF DNA MISMATCH REPAIR (MMR) GENE MOSAICISM AND SPECIFIC INTRONIC PATHOGENIC VARIANTS IN PEOPLE WITH MMR-DEFICIENT CANCERS

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Background and aims

People with Lynch syndrome have an increased risk of colorectal (CRC), endometrial (EC) and sebaceous skin (SST) cancers. Despite advances in next-generation sequencing, the detection of germline pathogenic variants in the DNA mismatch repair (MMR) genes remains challenging, including detecting low level mosaic MMR variants or deep intronic variants. We aimed to determine the prevalence of MMR mosaicism and specific intronic MMR pathogenic variants.

Method

Previous matched tumour-normal multigene panel sequencing of 137 suspected Lynch syndrome/Lynch-like cases identified 122 CRC-, EC- or SST-affected cancers with biallelic somatic MMR-deficiency and 15 single somatic MMR mutation cases. In these cases, MMR mosaicism was assessed by ultra-deep sequencing of DNA from blood (n=126), normal colonic mucosa (n=44), and saliva/buccal (n=13) (57 cases had multiple DNA sources tested). Ultra-sensitive digital droplet polymerase chain reaction (ddPCR) was used to confirm mosaicism. Blood DNA from 106/137 cases were tested for six previously reported pathogenic intronic variants using Sanger sequencing.

Results

The study included 137 participants, of which 17.5% (24/137) of the individuals had a second primary cancer (87.5%, 21/24 in the Lynch syndrome spectrum) and 53.3% (73/137) had a first degree relative with a Lynch spectrum cancer. In 4/137 of the cases, the somatic MMR mutation identified in the MMR-deficient tumour was found in an alternate DNA source from that person, suggesting mosaicism. We confirmed a likely *de novo,* mosaic case with the pathogenic variant *MSH6:*c.1135_1139del p.Arg379* identified in all three germ layers (variant allele fraction - CRC: 20.2%, EC: 10.1%, normal colonic mucosa: 5.3%, saliva: 3.5% and blood: 1.6%) in a woman with MSH6-deficient CRC and EC using ddPCR technology. ddPCR testing of the three remaining cases is currently underway. None of the six known intronic variants were identified in the 106 biallelic or single somatic MMR mutation cases tested.

Conclusions

In 137 cases with biallelic or single somatic MMR-deficiency, the prevalence of MMR gene mosaicism was < 3% while none of the known intronic pathogenic variants were identified. Our findings do not support routine testing for MMR mosaicism, although testing may be indicated in cases with multiple primary cancers. Although previously reported intronic variants weren't seen in our cohort, the need to look for de novo intronic variants is still warranted.

Keywords

Colorectal cancer, DNA mismatch repair deficiency, mosaicism, intronic pathogenic variant (PV), next-generation sequnencing, suspected Lynch syndrome.

O17 - CAN GENOMIC FEATURES OF ADENOMAS BE USED TO IDENTIFY BIALLELIC MUTYH AND NTHL1 CARRIERS?

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Background and aims

Tumours from hereditary colorectal cancer (CRC) and polyposis gene carriers exhibit specific genomic features relevant for diagnosis. Applying this approach to pre-malignant adenomas could enable earlier intervention and improve the effectiveness of CRC prevention strategies. The aim of this study was to assess the utility of genomic features from CRCs and adenomas from *MUTYH*- and *NTHL1*-associated polyposis syndrome carriers.

Method

Whole-exome sequencing was performed on FFPE tissue and matched blood-derived DNA from 82 individuals comprising 6 adenomas and 12 CRCs from 8 biallelic *MUTYH* carriers, 9 adenomas and 2 CRCs from 5 biallelic *NTHL1* carriers and a reference group of DNA mismatch repair proficient, non-hereditary adenomas (n=27) and CRCs (n=26) from 46 individuals. Derived genomic features included COSMIC v3.2 tumour mutational signatures (TMS), tumour mutation burden (TMB), neoantigen load and hotspot somatic mutations with each assessed for differences between adenomas and CRCs in carriers and for their ability to discriminate carriers from non-carriers.

Results

Adenomas from biallelic *MUTYH* carriers were significantly different from non-hereditary adenomas, exhibiting high TMS SBS18+SBS36 (mean±standard deviation, 40±21 vs 0±1, p-value=2x10-11, t-test), increased TMB (3.3±2.8 vs 1.0±0.7, p-value=5x10-4) and elevated neoantigen load (2.9±3.5 vs 0.3±0.4, p-value=4x10-3). Adenomas from biallelic *NTHL1* carriers were enriched for TMS SBS30 (49±16 vs 1±3, p-value=7x10-17) and TMB (3.4±2.5 vs 1.0±0.7, p-value=7x10-5) compared with non-hereditary adenomas. There was no significant difference between these features in adenomas and CRCs in both *MUTYH* and *NTHL1* biallelic carriers **(Figure 1)**. Specific somatic hotspot mutations in *KRAS* were enriched in adenomas from both biallelic *MUTYH* carriers (*KRAS*: c.34G>T p.G12C (3/6 vs 2/27, p-value=3x10-2, Fishers exact test) and biallelic *NTHL1* carriers (*KRAS*: c.35G>A (3/9 vs 2/27, p-value=8x10-2) compared with non-hereditary adenomas.

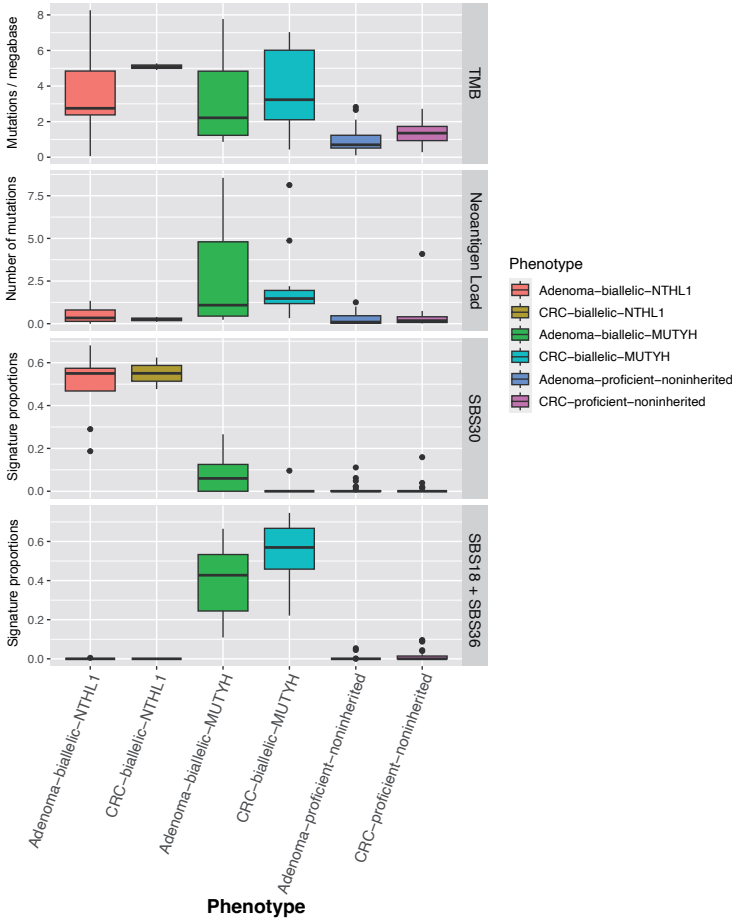
Conclusions

Our study demonstrated that base-excision repair tumour mutational signatures and TMB in adenomas were associated with the *MUTYH* and *NTHL1* recessive polyposis syndromes and were present at comparable levels to CRCs from the same syndromes. Therefore, testing adenomas for syndrome-related genomic features including *KRAS* somatic hotspots may improve the identification of carriers and present opportunities for CRC prevention and variant classification.

Keywords

MUTYH, NTHL1, colorectal cancer, tumour mutational signatures, adenomas, hotspot mutations.

Figure 1. Boxplots displaying the distribution of TMB, neoantigen load, TMS SBS30 and TMS SBS18+SBS36 by phenotype. Abbreviations: TMB, tumour mutation burden; TMS, tumour mutational signature; SBS, single base substitution.



O18 - THE ROLE OF GENOTOXIC GUT BACTERIA IN CRC TUMOURIGENESIS IN LYNCH SYNDROME

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[3] *Lynch syndrome (LS), colorectal cancer (CRC), gut bacteria, pks+ Escherichia coli (pks+ E.coli), Fusobacterium nucleatum (Fn), Enterotoxigenic Bacteroides fragilis (ETBF) to the abstract.*

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Background and aims

Differences in the gut microbiome may underlie the variability in colorectal cancer (CRC) development in people with Lynch syndrome (LS). We aimed to determine the prevalence of genotoxic gut bacteria, *pks*$^{+}$ *Escherichia coli* (*pks*$^{+}$ *E.coli*$^{+}$), all *E.coli*$^{+}$ (*pks*$^{+/-}$), Enterotoxigenic *Bacteroides fragilis* (ETBF) and *Fusobacterium nucleatum* (*Fn*) in CRCs (including synchronous and metachronous CRCs) and adenomas from people with LS in association with clinicopathological and molecular features.

Method

A total of 360 LS cases from the Australasian Colon Cancer Family Registry comprising 386 CRCs, 93 adenomas, and 197 normal colonic mucosa (NCM) DNA samples were tested using qPCR. LS intra-tumoural and intra-adenoma bacteria prevalence were compared to prevalences in CRC from 1,352 non-LS, population-based cases.

Results

The prevalence of *pks*$^{+}$ *E.coli*$^{+}$, all *E.coli*$^{+}$, ETBF and *Fn* in LS CRCs was 15.4%, 34.7%, 6.3% and 61.5%, respectively. *Pks*$^{+}$ *E.coli*$^{+}$ (*P*=0.02, OR=1.6 [95%CI=1.1-2.4]), all *E.coli*$^{+}$ (*P*< 0.01, OR=2.7 [1.9-3.8]) and *Fn* (*P*< 0.01, OR=20.1 [14.0-29.0]), but not ETBF (*P*=0.9, OR=0.96 [0.54-1.67]), were enriched in LS CRCs compared with non-LS CRCs. All *E.coli*$^{+}$ was associated with older CRC diagnosis age in *MSH2* (*P*=0.02) and *MSH6* (*P*=0.04) carriers. The presence of *pks*$^{+}$ *E.coli*$^{+}$, ETBF and *Fn* was not associated with CRC diagnosis age, sex or affected MMR gene (*P*>0.05).

The prevalence of *pks*$^{+}$ *E.coli*$^{+}$, all *E.coli*$^{+}$, ETBF and *Fn* in LS adenomas was 7.0%, 35.2%, 2.5% and 21.9%, respectively. Compared to LS CRC, *Fn* was found less in LS adenoma (P< 0.01, OR=0.17 [0.09-0.31]). No association was found between bacterial presence and diagnosis age, affected MMR gene or sex (P>0.05) in adenomas.

CRCs from people with LS who developed multiple primary CRCs were enriched with all *E.coli*$^{+}$ (P=0.02, OR=1.74 [1.1-2.77]) and *Fn* (P=0.01, OR=1.73 [1.12-2.69]) when compared with CRCs from LS cases who had solitary CRC. The presence of *Fn* was concordant in 70% of the synchronous CRCs, with 90% of them having concordant *Fn* presence in synchronous CRCs and NCM from the resection margin, suggesting a widespread colonic infection of *Fn*.

Conclusions

pks^{+} *E.coli*$^{+}$, all *E.coli*$^{+}$ and *Fn* were enriched in CRCs from patients with LS. *Fn* and *E.coli* infection may have a specific role in multiple CRC development in LS. Future studies should determine the risk of CRC, including risk of multiple CRCs in LS carriers presented with these genotoxic bacteria to inform personalised clinical management.

O19 - EARLY ONSET COLON AND RECTAL CANCER MORTALITY IN LYNCH SYNDROMES CARRIERS SUBJECTED TO SURVEILLANCE COLONOSCOPY

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Background and aims

Colon and sigmoid-rectal cancer before 50 years of age (EOCC and EORC) are gaining attention (1). Similar to our previous report on survival and mortality following gyneacological cancer in carriers of pathogenic MMR (*path_MMR*) carriers (2), we now report survival and mortality by gene at different ages for colon and sigmoid rectal cancer in *path_MMR* carriers.

Methods

The results represent the consequences of surveillance with colonoscopy and treatment that was undertaken mostly before immunotherapy became available. For calculations the previously reported PLSD data set (3) was used. Potential overdiagnosis through colonoscopy surveillance (4) is adjusted for when calculating prospective incidence and survival/mortality from the same series. Compliant with methods previously detailed (2,3) we calculated cumulative incidences in specific age groups, the corresponding 10-year survival, mortality as 1-survival by group, and 10-year mortality as cumulative incidences in the specific age groups multiplied by 10-years survival in these groups.

Results

The results are detailed in **Table 1** and **Figure 1**. By genetic variant, there was no difference in survival between sexes (p>0.1). Overall mortality was 3% for EOCC prospectively diagnosed in male *path_MLH1* carriers and 2% in female *path_MLH1* carriers, and 2% in *path_MSH2* carriers. For carriers of both *path_MLH1* and *path_MSH2* overall mortality was <= 1% for EORC. Incidences of EOCC between 25 and 49 years of age in *path_MSH6* and *path_PMS2* carriers were 6% and 0%, respectively, with an insufficient number of EOCCs for stratification on age and for estimating survival. EORCs were less frequent, having incidences of 2% and 4%, but with lower survival, resulting in 0% and 1% EORC related mortality in *path_MLH1* and *path_MSH2* carriers, respectively. There was no EORC in *path_MSH6* or *path_PMS2* carriers.

Conclusions

Despite high cancer incidences, death after EOCC and EORC was infrequent (0-3%) in *path_MLH1* and *path_MSH2* carriers because of high survival rates. In *path_MSH6/PMS2* carriers cancer death were lower for both EOCC and EORC because of low cancer incidences.

References

1 PMID 36549470
2 PMID 33257847
3 PMID 37181409
4 PMID 36182917
5 PMID 37821984

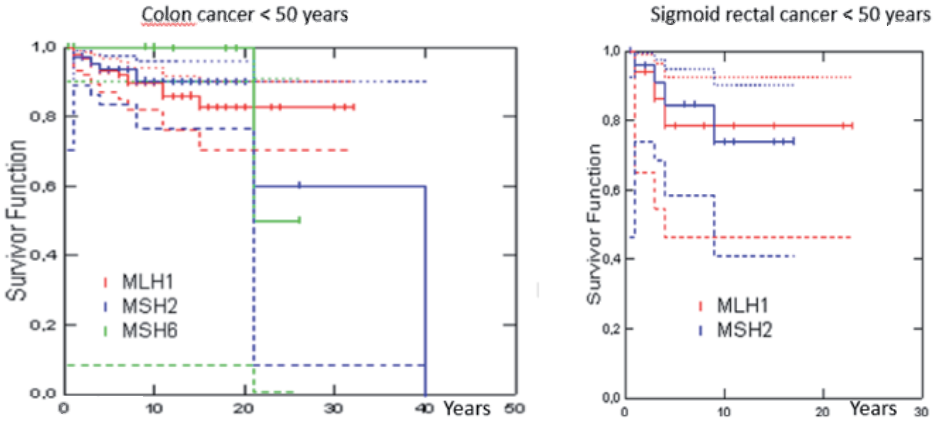
Keywords

Lynch syndromes, early colon cancer, early rectal cancer, mortality, age.

Table 1. Number of observation years in carriers without colon or sigmoid rectal cancer including first to last ages in group, number of prospective carriers with cancer diagnosed including first to last ages in group, cumulative cancer incidences, and survival and mortality in the different age groups in *path_MLH1* and *path_MSH2* carriers subjected to colonoscopy.

	Colon cancer						
	Carrier ages in years	Obser-vation years	Number carriers diag-nosed	Cumulative incidence first to last age included in group	10 years overall survival	10_years morta-lity	Overall mortality calculated as cumulative incidence multiplied with 10-years mortality in group
Male *path_MLH1*, carriers	25-39	3506	37	0.14	0.86	0.14	0.02
	40-49	2443	57	0.21	0.93	0.07	0.01
	50-64	1901	38	0.24	0.78	0.22	0.05
	25-49	5949	94	0.32	0.90	0.10	0.03
	25-64	7850	132	0.48	0.86	0.14	0.07
Female *path_MLH1*, carriers	25-39	4054	26	0.08	0.86	0.14	0.01
	40-49	3222	30	0.09	0.93	0.07	0.01
	50-64	3025	53	0.24	0.78	0.22	0.05
	25-49	7276	56	0.16	0.90	0.10	0.02
	25-64	10301	109	0.36	0.86	0.14	0.05
Path_MSH2 carriers, both sexes combined	25-39	6,131	33	0.08	0.95	0.05	0.00
	40-49	4,839	53	0.10	0.87	0.13	0.01
	50-64	5,197	79	0.21	0.87	0.13	0.03
	25-49	10,970	86	0.17	0.90	0.10	0.02
	25-64	16,167	165	0.35	0.89	0.12	0.04
	Sigmoid and rectal cancer						
path_MLH1, carriers, both sexes combined	25-49	17,102	18	0.02	0.78	0.22	0.00
	25-64	9,352	18	0.05	0.69	0.31	0.01
Path_MSH2 carriers, both sexes combined	25-49	13,231	26	0.04	0.74	0.26	0.01
	25-64	8,053	33	0.10	0.65	0.35	0.04

Figure 1


Colon cancer < 50 years
Survivor Function
1,0
0,8
0,6
0,4
0,2
0,0
MLH1
MSH2
MSH6
0
10
20
30
40
50
Years
Sigmoid rectal cancer < 50 years
Survivor Function
1,0
0,8
0,6
0,4
0,2
0,0
MLH1
MSH2
0
10
20
30
Years

O20 - OPTIMIZING MAINSTREAMING OF GENETIC TESTING IN PARALLEL WITH OVARIAN AND ENDOMETRIAL CANCER TUMOR TESTING: HOW DO WE MAXIMIZE OUR IMPACT?

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Background and aims

Although germline genetic testing (GT) is universally recommended for all patients with ovarian cancer (OC) and certain patients with endometrial cancer (EC), testing rates remain low with marked inequities. Our center performs GT in parallel with tumor somatic testing via a targeted sequencing assay (MSK-IMPACT) and initiates this process in clinic with the primary oncologist (mainstreaming) rather than referring patients to a separate genetics service. We seek to study our processes to optimize GT uptake in patients with gynecologic cancers.

Method

We performed a quality improvement study to evaluate our GT processes within gynecologic oncology clinics. All patients with newly diagnosed OC/EC presenting to clinic were identified for GT and tracked in a REDCap database by a trained study team. Clinical information and data on testing rates and logistics, including reasons for declining GT, were abstracted by the study team who met regularly to review and analyze data qualitatively for recurrent themes.

Results

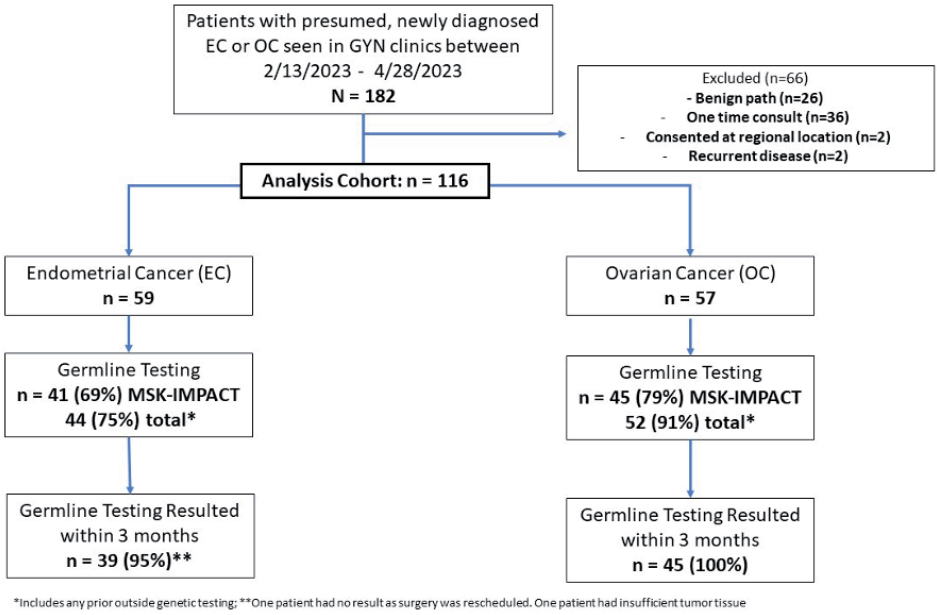
From 2/13/2023 to 4/28/2023, 116 patients with newly diagnosed OC (n=57) and EC (n=59) treated at our center were included, **Figure 1**. GT was performed in 52 (91%) patients with OC (7 prior outside GT and 45 MSK-IMPACT) and in 44 (75%) patients with EC (3 prior outside GT and 41 MSK-IMPACT). Testing results were available within 3 months for 100% of patients with OC and 95% of patients with EC with 1 patient having surgery delayed and another having insufficient tissue. Reasons for declining GT included language barriers, financial and privacy concerns, and patients being overwhelmed at the initial visit. In review of our processes, we found resources concentrated at the initial visit and little follow-up to encourage GT at subsequent points of care, leading to missed opportunities by the medical team. Notably, 4 mismatch repair deficient (MMR-D) ECs were observed amongst the 18 patients without GT via MSK-IMPACT. Of these, 2 (50%)

tumors underwent methylation analyses, with 1 having MLH1 hypermethylation and 1 an indeterminate result, **Figure 2**.

Conclusions

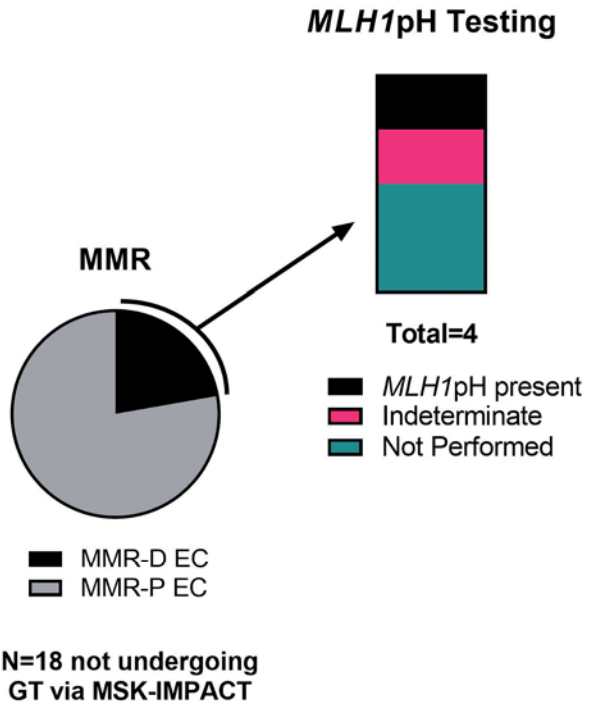
A mainstreaming approach that couples somatic and germline testing resulted in high testing rates for patients with OC and EC; however, Lynch Syndrome may still be missed. Processes that encourage GT at multiple points of care and allow self-directed, multilingual digital consenting may improve uptake and should be investigated in future, prospective studies.

Figure 1.



*Includes any prior outside genetic testing; **One patient had no result as surgery was rescheduled. One patient had insufficient tumor tissue

Figure 2.


*MLH1*pH Testing
MMR
Total=4
*MLH1*pH present
Indeterminate
Not Performed
MMR-D EC
MMR-P EC
N=18 not undergoing
GT via MSK-IMPACT

O21 - RISK OF CANCER AND SECONDARY SURGERY FOLLOWING COLECTOMY WITH ILEORECTAL ANASTOMOSIS AND PROCTOCOLECTOMY WITH ILEAL POUCH-ANAL ANASTOMOSIS IN FAMILIAL ADENOMATOUS POLYPOSIS.

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Background and aims

Patients with familial adenomatous polyposis (FAP) undergo (procto)colectomy with ileorectal anastomosis (IRA) or ileal pouch-anal anastomosis (IPAA) to prevent colorectal cancer (CRC).

After surgery, patients remain at risk of developing adenomas and cancer in the retained rectum or pouch. Therefore, this study aims to compare the long-term risk of cancer following IRA or IPAA in FAP patients.

Methods

We performed an international multicenter historical cohort study of FAP patients undergoing colectomy with IRA or proctocolectomy with IPAA from 1990 to 2022. The proportion of patients developing cancer following surgery was estimated using the Kaplan Meier method.

Results

(Procto)colectomy was performed in 673 patients (53.9% female); 359 (53.3%) had IRA and 314 (46.7%) IPAA. Median age at IRA and IPAA was 22 and 27 years, and median follow-up was 12 and 15 years, respectively. The overall incidence of rectal and pouch cancer following IRA was 2.0%, after IPAA 1.0%. The estimated 10- and 20-year cancer incidence after IRA vs IPAA were 1.2% vs 0.4% and 2.1% vs 0.9%, respectively (log-rank $p = 0.23$). Reoperation was performed in 38 (10.6%) patients with an IRA and 24 (7.7%) patients following IPAA. The most common indication was severe polyposis not amenable to endoscopic management in both groups. Endoscopic follow-up data was available in 589 patients (87.5%). The median number of lower surveillance endoscopies per year was 1 versus 0.7 in the IRA and IPAA group, respectively ($p < 0.001$). In the IRA group, the median number of polypectomies per year was 2.6 and in the IPAA group 0.1 ($p < 0.001$).

Conclusions

During the last three decades, the risk of cancer in the rectum or pouch after (procto) colectomy in FAP was low. This might be due to an improved selection of the type of (procto) colectomy and frequent endoscopic surveillance including polypectomy.

O22 - OBJECTIVE AND SUBJECTIVE LIFE EXPECTANCY IN LYNCH SYNDROME

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Background and aims

Lynch syndrome (LS) is a genetic condition caused by DNA mismatch repair gene mutation. Despite prophylactic surgeries and advancements in cancer surveillance, mortality among individuals with LS may still be elevated due to surveillance noncompliance, missed lesions, and hard-to-prevent forms of cancer. Individuals with LS may also have subjective beliefs of increased mortality risks, which can affect their life decisions and quality of life. The aim of this study was to estimate mortality in individuals with LS.

Methods

Individuals at 50% risk to inherit LS were registered at the Netherlands Foundation for the Detection of Hereditary Tumors and had no cancer before mutation analysis. The median age at mutation analysis was 41 years, with a median follow-up of 17 years. We estimate mortality in a sample of 1030 mutation-positive and 569 mutation-negative individuals, and in a matched sample of unaffected individuals from the general Dutch population. To estimate effects on subjective mortality beliefs, we conduct a survey among mutation-positive individuals and match it with a nationally representative survey of the Dutch population.

Results

Mutation-positive individuals face a significant increase in mortality compared to mutation-negative individuals (HR: 1.51, 95% CI, 1.10-2.15). The predicted median lifespan reduction is four years (83 vs. 87 years). The increase in mortality is larger among men (HR 1.72, 1.08-2.72) than women (HR 1.38, 0.84-2.27) and among carriers of an MLH1, MSH2 or EPCAM mutation (HR 1.91, 1.27-2.86) than carriers of an MSH6 or PMS2 mutation (HR 1.35, 0.68-2.68). Compared to the matched general population, mortality is not significantly increased among mutation-positive individuals (HR: 1.10, 0.88-1.39), while it is significantly lower among mutation-negative individuals (HR: 0.71, 0.56-0.92). This latter finding might reflect sample selection among tested individuals. Mutation-positive survey respondents believe that they are 7.3% to 9.3% less likely to live to the age of 75, compared to the general population. This translates into an 2.4 years shorter subjective life expectancy.

Conclusions

Despite cancer surveillance and prophylactic surgeries, Lynch syndrome may still be associated with a significant increase in mortality among mutation-positive individuals compared to mutation-negative ones, especially among men. Mutation-positive individuals may have subjective beliefs of increased mortality risks.

O23 - THE YIELD OF ARTIFICIAL INTELLIGENCE (GI GENIUS) IN LYNCH SYNDROME –A RANDOMIZED TANDEM-COLONOSCOPY TRIAL

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Background and aims

Lynch syndrome (LS) patients are at high risk of colon cancer despite regular endoscopic surveillance, probably related to small and flat polyps that are often overlooked. Artificial intelligence (AI)- assisted colonoscopy was shown to increase the adenoma-detection rate (ADR) in the general population but there is a paucity of data regarding LS. Also, there is a wide phenotypic heterogeneity between carriers. Therefore, this study aimed to investigate the incremental detection rate of polyps using AI- assisted colonoscopy compared with high-definition white-light endoscopy (HD-WLE).

Methods

We performed a tandem colonoscopy trial in which HD-WLE was performed first on all patients and either AI- assisted colonoscopy or HD-WLE was performed second, with a 1:1 allocation ratio. Patients with LS ≥ 18 years with a pathogenic germline variant in one the MMR-genes were eligible.

Results

Between Nov-2022 and Nov-2023, 100 LS patients were enrolled in the study, 39% of them were male, with a median age of 47. The predominant pathogenic variants were *MSH2* (54%) and *MSH6* (31%) due to founder mutations. Twenty two percent of patients and 31% had previous colon cancer and adenomatous polyps, respectively. 22% had their first colonoscopy, while 34%, 35% and 18% had previous colonoscopy at 12 months, 13-24 months and 25-36 months intervals, respectively. The findings of colonoscopies are presented in **Table 1**. In the HD-WLE arm (N=50), an additional 2 missed adenomas (1 in ascending colon, 1 in recto-sigma) were detected, compared to 6 additional adenomas (4 in the descending colon, 2 in the ascending colon) in the AI-assisted colonoscopy arm (N=50) [ADR - 4% vs. 12%, $p = 0.127$]. All the missed polyps were sessile and non-advanced adenomas. The median withdrawal time did not differ significantly between HD-WLE and AI (10.9 vs. 11.1 min.; $p = 0.73$).

Conclusions

AI- assisted colonoscopy may improve the ADR in LS patients, particularly in detecting small adenomas. Its impact on interval colon cancer and mortality should be further evaluated.

Keywords

Lynch syndrome, artificial intelligence, colonoscopy .

COI

There is no conflict of interests to the authors.

Table 1. Polyp number and detection rates in the study population

	Whole cohort N = 100	Group 1 HD-WLE / HD-WLE N = 50	Group 2 HD-WLE / AI N = 50	P value
First colonoscopy				
- Adenomas detected, n	28	17	11	
- Adenoma detection rate,% (95% CI)	23 (15.3-29.6)	26 (18.4-33.8)	20 (13.7-27.3)	0.08
- Advanced neoplasia detected, n	4	2	2	
- Advanced neoplasia rate, % (95% CI)	4 (2.2-6.0)	4 (2.2-6.0	4 (2.2-6.0)	1
- Polyps detected, n	79	40	39	
- Polyp detection rate, % (95% CI)	45 (36.2-55.5)	46 (37.1-56.2)	44 (35.1-54.4)	0.86
Second colonoscopy				
- Adenomas detected, n	8	2	6	
- Adenoma detection rate,% (95% CI)	8 (2.3-12.2)	4 (1.4-8.3)	12 (7.4-16.4)	0.12
- Advanced neoplasia rate, %	-	-	-	
- Polyps detected, n	12	4	8	
- Polyp detection rate, % (95% CI)	12 (8.2-15.8)	8 (5.3-13.3)	16 (11.2-21.3)	0.09

O24 - DEVELOPMENT OF ABDOMINAL DESMOID TUMOURS AFTER COLECTOMY AND ILEORECTAL ANASTOMOSIS VERSUS PROCTOCOLECTOMY AND ILEAL POUCH-ANAL ANASTOMOSIS IN FAMILIAL ADENOMATOUS POLYPOSIS

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Background and aims

Desmoid tumours (DT) are an important cause of morbidity and mortality in patients with familial adenomatous polyposis (FAP). DT development might be related to the type and approach of colectomy. We aimed to compare DT development after colectomy with ileorectal anastomosis (IRA) and proctocolectomy with ileal pouch-anal anastomosis (IPAA).

Methods

We performed an international historical cohort study in FAP patients who underwent IRA or IPAA between 1961 and 2020. The primary outcome was the incidence of abdominal DT (either mesenteric, retroperitoneal or abdominal wall). Patients with a DT diagnosis before or at colectomy were excluded. Time to DT was considered censored at an eventual secondary proctectomy after IRA. We used multivariable Cox regression modelling to adjust for potential confounders.

Results

We analysed data from 852 patients: 514 after IRA and 338 after IPAA (median follow-up 21 and 16 years, respectively). DTs were diagnosed in 64 IRA patients (12%) and 66 IPAA patients (20%). The cumulative DT incidence at 5 and 10 years was 7.5% and 9.3% after open IRA and 4.7% and 10.9% after laparoscopic IRA. These estimates were 13.6% and

15.4% after open IPAA and 8.4% and 10.0% after laparoscopic IPAA. The post-operative risk was significantly higher after IPAA ($p < 0.01$) in multivariable analysis, while approach did not significantly influence the risk.

Conclusions

The risk of developing an abdominal DT was found to be significantly higher after IPAA than after IRA. Postoperative DT risk should be taken into account when choosing between IRA and IPAA in FAP.

O25 - GASTRIC AND DUODENAL CANCER IN INDIVIDUALS WITH LYNCH SYNDROME: A NATIONWIDE COHORT STUDY

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Background and aims

Lynch syndrome increases the risk of gastric cancer (GC) and duodenal cancer (DC), particularly in individuals with *MLH1* and *MSH2* pathogenic variants (PVs). To provide further insight into whether, and from what age, esophagogastroduodenoscopy (EGD) surveillance may be beneficial, we evaluated the cumulative incidence and tumour characteristics of GC and DC in a large nationwide cohort of Dutch LS individuals.

Method

Clinical data of LS individuals registered between 1989-2021 at the Dutch Hereditary Cancer Registry were matched with pathology reports filed between 1989-2021 by the Dutch Pathology registry. Cumulative incidences of GC and DC were estimated for high-risk (*MLH1, MSH2* and *EpCAM)* and low-risk (*MSH6 and PMS2)* PVs using competing risk methodology.

Results

Among 1002 individuals with high-risk and 765 individuals with low-risk PVs, 29 GCs (1·6%) and 39 DCs (2·2%) were diagnosed. Cumulative incidence of GC and DC under the age of 50 was very low (≤1%) for all individuals. At age 70 and 75, cumulative incidence for high-risk PVs was 3% [95% CI 1%-5%] and 5% [95% CI 3%-8%] for GC and 5% [95% CI 3%-7%] and 6% [95% CI 3%-8%] for DC, respectively. Low-risk PVs had 1% [95% CI 0%-

2%] and 1% [95% CI 0%-2%] cumulative incidence of GC and 1% [95% CI 0%-1%] and 2% [95% CI 0%-4%] of DC at age 70 and 75, respectively. Primary tumour resection was performed in 62% (18/29) of GCs and 77% (30/39) of DC cases. Early-stage GC, defined as TNM stage I, was found in 32% (9/28) of GCs. Early-stage DC, defined as TNM stage I-IIa, was found in 39% (14/36) of DCs.

Conclusion

Individuals with *MLH1, MSH2,* and *EpCAM* PVs have an increased risk of developing GC and DC at the age of 70 years, but this risk is negligible before the age of 50 years. The age of onset of surveillance, the yield of GC and DC during EGD surveillance, and its cost-effectiveness should be subject of future studies.

O26 - KRAS-G12C: A NEGLECTED BIOMARKER FOR IDENTIFYING MUTYH-ASSOCIATED POLYPOSIS PATIENTS

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Background and aims

MUTYH-associated polyposis (MAP) is an underdiagnosed recessive syndrome that predisposes individuals to colorectal cancer (CRC). It exhibits a remarkably variable phenotype with patients presenting from none to hundreds of adenomatous polyps. Biallelic loss of *MUTYH* causes a specific pattern of somatic mutations, often leading to the presence of *KRAS*-p.G12C mutations in CRC. Despite of previous studies suggesting the use of *KRAS*-G12C as a marker for identifying MAP patients, widespread adoption of reflective *MUTYH* germline testing in these patients has not occurred. This study aims to investigate the utility of the detection of *KRAS*-G12C in two scenarios: to identify carriers of germline pathogenic variants (GPVs) in *MUTYH* gene and to classify germline variants of uncertain clinical significance (VUS) in *MUTYH*.

Method

From 7,623 Brazilian CRC patients tested for *KRAS* mutations, we identified 242 (3.2%) cases with the p.G12C mutation. *KRAS*-G12C CRC patients were screened for the 5 most frequent *MUTYH* GPVs in the Brazilian population using targeted amplicon sequencing. Full *MUTYH* sequencing was performed for identified monoallelic carriers. Additionally, we evaluated the frequency of detection of *KRAS*-G12C in adenomas and CRC tissues from 8 MAP patients and 2 patients with *MUTYH* VUS.

Results

Among the 98 patients with *KRAS*-G12C tested so far, 12 (12.2%) had at least one GPV in

MUTYH. Out of these, 8 were MAP (biallelic carriers) and 4 were monoallelic carriers. The MAP detection rate in patients under 60 years old was 15% (7/47). GPVs were associated with an earlier age of CRC onset ($p< 0.02$) and the presence of polyps ($p=0.05$). Regarding the second aim, we identified *KRAS*-G12C in 100% (4/4) of CRC and 27% (3/11) of adenomas from MAP patients. Adenomas/CRC from two suspected MAP patients harboring a *MUTYH* VUS (p.Pro273Arg and p.Lys422Met) combined with a pathogenic *MUTYH* variant revealed the presence of *KRAS*-G12C in the p.Pro273Arg carrier, supporting its classification as likely pathogenic (PP3_strong, PM2_supp, PP4).

Conclusions

In summary, the high detection rate of GPVs in *MUTYH* among CRC patients with *KRAS*-G12C indicates that the presence of this mutation should be considered a biomarker for guiding the diagnosis of MAP, enabling appropriate follow up, surveillance and preventive measures for affected individuals. The high frequency of *KRAS*-G12C in MAP CRC and adenomas can be useful for classifying VUS in *MUTYH*.

O27 - CAPTURERNA-SEQ AS A SUPPLEMENT TO DNA GERMLINE TESTING TO INCREASE THE DIAGNOSTIC YIELD OF HEREDITARY TUMOR SYNDROMES

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Background and aims

Hereditary tumor syndromes contribute to 5-10% of cancer cases, necessitating accurate identification of causal genetic variants for effective patient management. The diagnostic yield of whole-exome sequencing (WES), the gold standard for molecular diagnostics, ranges from 15-25% in these syndromes. To improve diagnostic yield, high-throughput functional studies, such as RNA-seq, play a crucial role in reclassifying variants of uncertain significance (VUS) and unveiling pathomechanisms like aberrant splicing, particularly when initial variant detection fails. Here, we developed CaptureRNA-seq, a targeted RNA-seq approach to improve the diagnostic yield of hereditary tumor syndromes.

Methods

We developed a cost-efficient, high-throughput RNA-seq approach to analyze RNA phenotypes where we complement polyA mRNA capture with enrichment of 49 cancer-associated genes from PAXgene-derived RNA samples.

Result

We achieved ultra-high coverage sequencing data of ~3,500X mean target coverage and, on average, 80% of exons covered with >50 read depth. In almost 80% of 24 positive controls for aberrant splicing, we detected a difference of >20% in the PSI (percent-spliced in) score compared to 23 negative controls. A total of >100 cases have been investigated by RNA-seq, all of which had been previously analyzed by NGS exome sequencing without revealing a clear pathogenic variant. Of these cases, 97 carried a VUS, while 4 showed no initial variants of concern. RNA-seq helped to reclassify 7 VUS to (likely) pathogenic variants. Among these reclassified cases, 5 variants affected an intronic splice site, and 2 were missense variants outside of obvious splice-relevant regions. For one case with no initially reported variants, RNA-seq identified the use of a cryptic exon in the APC gene, guiding the subsequent analysis of the affected intronic region.

Conclusion

RNA-seq can provide clinically relevant information on VUS and help establish a clear diagnosis for otherwise unsolved cases, e.g., through the detection of aberrant splicing. Cases with no initial variant may also be elucidated by targeted analysis of high-relevance genes. Overall, we were able to reclassify ~7% of cases that could not be molecularly solved through DNA exome sequencing. Thus, the diagnostic yield is significantly improved by RNA-seq, and patient care can be optimized based on a confirmed diagnosis of hereditary cancer syndromes.

Keywords

RNA-seq, hereditary tumor syndromes, VUS, splicing, diagnostics.

O28 - DIFFERENCES IN ADENOMA AND POST-COLONOSCOPY COLORECTAL CANCER DETECTION BETWEEN LYNCH SYNDROME CARRIERS WITH AND WITHOUT A PREVIOUS COLORECTAL CANCER

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Background

Colorectal adenomas are the main precursor lesions for colorectal cancer (CRC) in Lynch syndrome (LS). Adenoma detection and resection reduces post-colonoscopy CRC (PCCRC), but PCCRC rates in LS are still a clinical problem. CRC risk factors remain poorly studied and it is still unclear if patients with a previous CRC are at a higher risk.

Aim

Describe and compare the adenoma, advanced adenoma (AA) and PCCRC detection rates in individuals with LS with and without CRC.

Methods

Multicenter retrospective study including LS carriers under colonoscopy surveillance (≥ 2 colonoscopies). We described and compared the adenoma, AA, and PCCRC detection rates in patients with CRC (previous or at first colonoscopy) and healthy carriers (HC). We performed logistic regression and Cox regression analysis adjusted by confounders and the percentage of remnant colon (i.e. 100%=intact colon; 50%=hemicolectomy; 0%=no colon).

Results

We included 1,201 LS carriers, 308 (25.7%) patients with previous CRC, and 893 (74.3%) HCs **(Table 1)**. Patients with a previous CRC had a median remnant colon of 50% (IQR 50-90). Patients with previous CRC were more frequently more males, *MLH1* carriers and less frequently *MSH6* and *PMS2* carriers **(Table 1)**; additionally, they were significantly older at the beginning of surveillance [51 vs 40 years, $p<0.0001$]. Univariate analysis showed a higher AA detection in patients with CRC (24% vs 15%, p=0.001), however, multivariable analysis adjusting for age, sex, gene and remnant colon only showed a higher incidence of PCCRC (11.4% vs 5.4%; $p=0.001$). 10-year adenoma, AA and PCCRC cumulative detection rates in patients with previous CRC and HC were: 58.8% vs 61.8% ($p>0.05$); 32.2% vs 20.4% ($p=0.003$) and 13.5% vs 7.9% ($p=0.005$), respectively. Multivariate Cox regression analysis adjusted by sex, gene and age at first colonoscopy, showed that adenoma detection was significantly lower in patients with a previous CRC [OR: 0.64 (0.51-0.78)] with no differences in AA, nor PCRC incidence **(Figure 1)**.

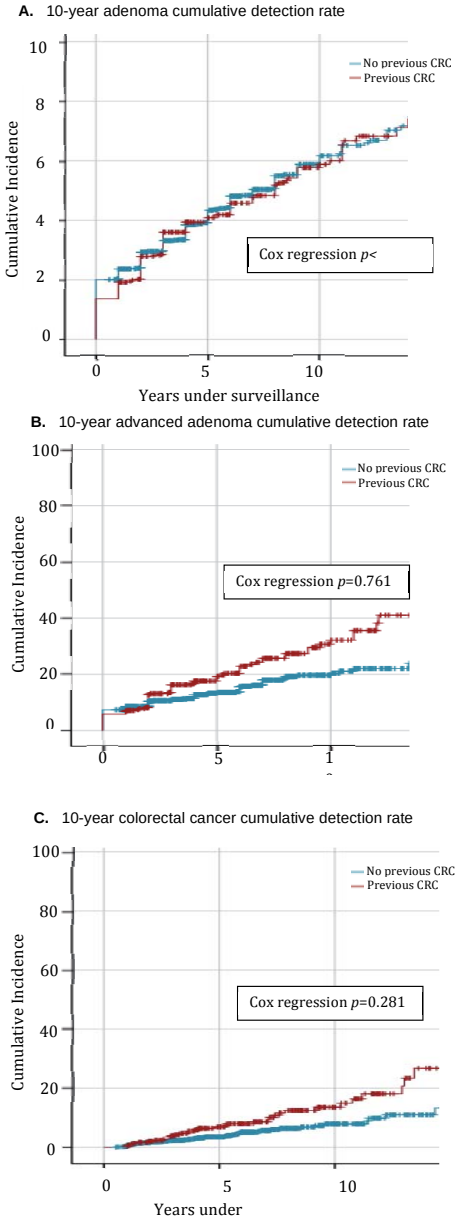
Conclusions:

PCCRC incidence is increased in patients with LS with a previous CRC. Cumulative adenoma detection seems to be lower in patients with previous CRC probably due to a shorter colonic remnant. However, they display similar AA and PCCRC cumulative detection rates. These results suggest that patients with previous CRC are at higher CRC risk and should be surveyed more intensively.

Table 1. Cohort characteristic compared between carriers with and without previous colorectal cancer (CRC).

	Total	CRC before first colonoscopy	No CRC before first colonoscopy (HC)	*P* Value univariate analysis		*P* Value multivariate analysis [OR (95%CI)]
N (%)	1201	308	893			
Females (%)	708 (59%)	139 (45.1%)	569 (63.7%)	**.000**		**.000 [.337 (.250-.454)]**
MLH1	435 (36.2%)	150 (48.7%)	285 (31.9%)	**.000**	**.000**	**.000 [1.97 (1.4-2.75)]**
MSH2	415 (34.6%)	99 (32.1%)	316 (35.4%)	.331		
MSH6	256 (21.3%)	44 (14.3%)	212 (23.7%)	**.000**		**.001 [.491 (.32-.76)]**
PMS2	95 (7.9%)	15 (4.9%)	80 (9%)	**.020**		**.011 [.43 (.23-.83)]**
Age first colonoscopy	43 (35-53.5)	51 (43-59)	41 (32-52)	**.000**		**.000 [1.07 (1.06-1.09)]**
N of colonoscopies	3 (2-6)	3 (1-6)	3 (2-6)	.617		
Length of follow-up (y)	5.45 (3.05-8.46)	5.95 (3.41-9.32)	5.34 (2.96-8.15)	.095		
Time between colonoscopies	12.6 (11.78-17.98)	12.59 (12.09-15.92)	13 (12.13-20.85)	.051		
Polyps	761 (63.4%)	208 (67.5%)	553 (61.9%)	.086		
Adenomas	565 (47%)	155 (50.3%)	410 (45.9%)	.186		
Advanced adenomas	209 (17.4%)	74 (24%)	135 (15.1%)	**.001**		.667
AA >10 mm	135 (11.2%)	45 (14.6%)	90 (10.1%)	**.036**		
HGD	60 (5%)	17 (5.5%)	43 (4.8%)	.649		
Villous	53 (4.4%)	17 (5.5%)	36 (4%)	.264		
AA HGD	111 (9.2%)	38 (12.3%)	73 (8.2%)	**.039**		
<10 mm	61 (5.1%)	24 (7.8%)	37 (4.1%)	**.016**		
Villous	46 (3.8%)	16 (5.2%)	30 (3.4%)	.168		
AA Villous	107 (8.9%)	39 (12.7%)	68 (7.6%)	**.010**		
<10 mm	57 (4.7%)	22 (7.1%)	35 (3.9%)	**.029**		
Non-advanced adenomas (<10mm LGD)	481 (40%)	121 (39.3%)	360 (40.3%)	.787		
Serrated lesions	380 (31.6%)	97 (31.5%)	283 (31.7%)	1		
Hyperplastic polyps	313 (26.1%)	76 (24.7%)	237 (26.5%)	.548		
Sessile Serrated lesions	63 (5.2%)	12 (3.9%)	51 (5.7%)	.239		
PCCRC	83 (6.9%)	35 (11.4%)	48 (5.4%)	**.001**		**.032 [1.32 (1.05-2.86)]**

Figure 1. 10-year cumulative detection rate in LS patients with a previous CRC and HC.



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O29 - PREVALENCE OF PATHOGENIC GENETIC VARIANTS IN GASTRIC CANCER PATIENTS ASCERTAINED THROUGH MULTIGENE PANEL TESTING

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Background and aims

Gastric cancer (GC) is the fourth leading cause of cancer-related deaths worldwide. The role of pathogenic/likely pathogenic variants (PV/LPVs) in cancer predisposition genes is not well understood and only studied in small cohorts. We aimed to determine the prevalence of PV/LPVs in GC patients undergoing germline genetic testing (GGT) via multi-gene panel testing (MGPT) at a large commercial laboratory.

Methods

Retrospective review of MGPT (>10 genes) in GC patients at a large commercial laboratory (Invitae Corp.) from March 2015 to July 2023 was performed. GC histologic subtypes included diffuse, intestinal or unspecified type, as provided by ordering clinicians. Association of patient characteristics with positive GGT results was assessed with logistic regression with a significance threshold of $p < 0.05$.

Results

In total, 3,706 GC patients underwent GGT **(Table 1)**. Overall, 495 (13.4%) patients were found to carry ≥1 PV/LPVs, including 29 patients with 2 PV/LPVs and 3 patients with 3 PV/LPVs. GGT was negative in 1,890 (51.0%) patients, 1,199 (32.4%) had a variant of uncertain significance and 121 (3.3%) carried a single PV/LPV in a gene associated with autosomal recessive inheritance. PV/LPVs were identified in 38 genes **(Figure 1)** of which 77.7% (385/495) were in a gene previously associated with GC, primarily the homologous recombination repair genes (*BRCA1, BRCA2, PALB2* and *ATM*, 34.9%), Hereditary Diffuse Gastric Cancer genes (*CDH1* and *CTNNA1*, 19.6%) and mismatch repair genes (*MLH1, MSH2, MSH6, PMS2* and *EPCAM*, 17.4%). Males were more likely to carry a PV/LPV than females (OR 1.4, 95% CI 1.1-1.7) and having a personal history of another cancer increased the odds of carrying a PV/LPV (OR 1.3, 95% CI 1.0-1.7). Age, number of genes tested and histology were not significantly associated with PV/LPV.

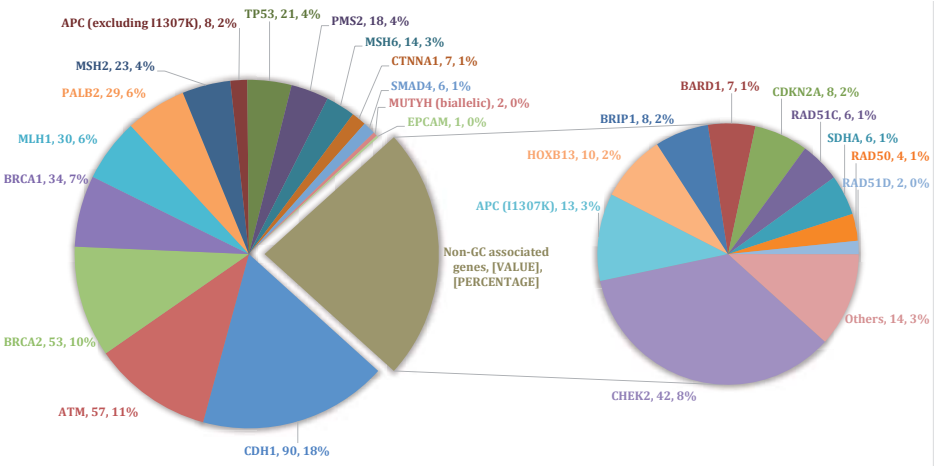
Conclusions

In this large study of GGT in GC patients, the prevalence of PV/LPVs in cancer associated genes was 13.4%, higher than previous estimates of 3-5%. PV/LPVs were predominantly in genes associated with GC. Male sex and history of other cancers were associated with identification of a PV/LPV. Limitations include incomplete histology data, varying panel size, and ascertainment bias as patients may have had additional personal/family history that prompted GGT. These results support consideration of GGT in all GC patients as yield of PV/LPVs is similar to other cancer types for which guidelines recommend universal genetic testing.

Table 1: Baseline characteristics of the study population.

Parameter	All gastric cancer patients (N=3,706)
Age at testing, years, median (range)	58 (14-90)
Sex, n (%)	
Male	1,704 (46.0)
Female	2,002 (54.0)
Self-reported ancestry, n (%)	
Ashkenazi Jewish	51 (1.4)
Asian or Pacific Islander	358 (9.7)
Black or African American	345 (9.3)
Hispanic	598 (16.1)
Multiracial	314 (8.5)
Native American	24 (0.6)
White	1,738 (46.9)
Other	14 (0.4)
Unknown	264 (7.1)
Histologic subtype, n (%)	
Diffuse gastric cancer	420 (11.3)
Intestinal type	45 (1.2)
Unspecified	3,241 (87.4)
Number of genes analyzed, n (%)	
10-19	413 (11.1)
20-29	219 (5.9)
30-39	139 (3.8)
40-49	1,148 (31.0)
50-79	190 (5.1)
80+	1,597 (43.1)
Personal history of cancer other than gastric, n (%)	
None	2,818 (76.0)
Brain	15 (0.4)
Breast	381 (10.3)
Colorectal	508 (13.7)
Genitourinary	228 (6.2)
Gynecologic	204 (5.5)
Hematologic	123 (3.3)
Hepatobiliary	59 (1.6)
Lung	54 (1.4)
Pancreas	89 (2.4)
Sarcoma	28 (0.8)
Skin	102 (2.8)

Figure 1. Distribution of pathogenic genetic variants in the study population.
Others (1 each) - *AXIN2, BAP1, CASR, FANCM, FLCN, GREM1, NF1, NTHL1, PTEN, RB1, SDHB, SDHC, TERT, VHL*.



O30 - FAMILIAL SERRATED POLYPOSIS SYNDROME IN THE GENETICS OF COLONIC POLYPOSIS STUDY

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Background and aims:

People with Serrated Polyposis Syndrome (SPS) and their first-degree relatives (FDRs) have a higher risk of developing colorectal cancer (CRC), but evidence that FDRs develop SPS is limited. The aim of this study was to characterise the phenotype of familial SPS.

Methods

People with polyposis that met the WHO criteria for SPS (2010 or 2019) were recruited across Australia to the Genetics of Colonic Polyposis Study. To consolidate the WHO 2010

and 2019 criteria, SPS phenotypes were defined as *P1* (2010 criteria 1 or 2019 criteria 1), *P2* (2010 criteria 3 or 2019 criteria 2), and *P3* (2010 criteria 1 & 3 or 2019 criteria 1 & 2). *P1* is characterised as ≥5 serrated polyps proximal to the sigmoid colon (or rectum), with ≥2 being ≥10mm (all being ≥5mm) while individuals with *P2* have >20 serrated polyps of any size distributed throughout the large bowel (with ≥5 being proximal to the rectum), and *P3* individuals fulfil both *P1* and *P2* criteria.

The proband's family history, questionnaire and medical records were assessed for evidence of FDRs with SPS where confirmation of a diagnosis of SPS in FDRs was determined from medical records. The information on colonoscopy history of SPS-unaffected relatives was also recorded where available.

Results

The 557 probands had a mean cumulative number of serrated polyps of 33 ±37 (mean ± standard deviation). The mean age at SPS diagnosis was 42±15 years and 71% (394/557) were female. Of those, 8.8% (49/557) had ≥1 FDR affected with SPS. For probands with affected FDR, the mean cumulative number of serrated polyps was 48±48 with the mean age at SPS diagnosis of 35±11 years and 79% (39/49) were female. No germline pathogenic variants in CRC/polyposis genes were identified in the familial cases.

The predominant familial relationship was sibling affected relatives with unaffected parents in 32 cases (65%), while 17 (35%) showed a parent-child relationship. For the sibling affected FDR, the most common SPS phenotypes were *P1* (33%) and *P3* (53%). In comparison, the parent-child FDR phenotypes were more evenly distributed *P1* (38%), *P2* (24%) and *P3* (38%).

Conclusions

8.8% of probands with SPS have at least one FDR with SPS, predominantly presenting in their siblings with SPS-unaffected parents. The SPS phenotype for affected siblings was bimodal representing either *P1* (low serrated polyp numbers) or *P3* (high serrated polyp numbers) with the commonality of the presence of large, serrated polyps. The aetiology of familial SPS requires further investigation.

Keywords

Serrated Polyposis Syndrome, Familial, phenotype.

O31 - CAN BUTYRATE PREVENT COLON CANCER? THE AUSFAP STUDY: A RANDOMISED, CROSSOVER CLINICAL TRIAL

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Background and aims

Butyrate produced by fermentation of dietary fiber may reduce the risk of colon cancer. Dietary butyrylated high amylose maize starch (HAMSB) delivers butyrate to the colon. This clinical trial evaluated the effects of HAMSB on polyp burden in participants with Familial Adenomatous Polyposis (FAP).

Method

The study was a double-blind, randomised, placebo-controlled, cross-over trial. Participants

were assigned to 2 groups that ingested 40g HAMSB (Ingredion Inc, USA) or low amylose starch (LAMS) for 6 months, followed by the alternative product for 6 months, and then 6 months with no treatment. Participants underwent video-recorded colonoscopies at baseline, 6, 12 and 18 months to assess polyp number and size, and for collection of polyp and mucosal biopsies. At baseline two colonic tattoos were placed: tattoo 1 where polyps were cleared at each scope to assess polyp initiation; and tattoo 2 where polyps were left in situ the entire study to assess polyp growth or recession. Global polyp burden was assessed by the CIA, with polyp burden in tattoos reviewed independently by 2 gastroenterologists. The primary endpoint was number of colonic polyps. A subset of 14 participants collected faecal samples for analysis.

Results

72 participants were randomised (39 male, 33 female) with 49 completing the study. Mean age at baseline was 37.6 years. Generalised linear mixed models were used to estimate the ratio of mean polyp counts in the intervention compared to placebo period. HAMSB did not affect number (0.9-fold reduction, 95% confidence interval, CI: 0.77–1.06), nor size (0.88-fold reduction, 95% CI: 0.71-1.1 for polyps < 2.4mm) of colonic polyps. HAMSB tended to reduce mean total polyp count in tattoo 1 (0.78-fold reduction, 95% CI: 0.58-1.05, P=0.106) with a 0.72-fold reduction in number of small polyps < 2.4mm in Tattoo 1 (95% CI: 0.5-1.03, P=0.074). Polyp burden in tattoo 2 was not affected by dietary intervention. HAMSB increased mean faecal butyrate concentration compared to LAMS.

Conclusion

This is the first clinical evaluation of a cost-effective food supplement in FAP that delivers significant quantities of butyrate to the colon. Ingestion of HAMSB tended to reduce the initiation of polyp growth without causing regression or growth of existing polyps. Biopsy analysis may determine if this was due to cellular apoptotic or proliferative responses to colonic butyrate. HAMSB may lower the risk of sporadic colonic cancer in the community.

Keywords

Colorectal cancer, butyrate, Familial Adenomatous Polyposis, chemopreventation.

O32 - THE EXOME-WIDE GENOMIC PROFILE OF LYNCH SYNDROME-RELATED COLORECTAL CANCERS DIFFERS BY AFFECTED GENE: IMPLICATIONS FOR DETERMINING PATHWAYS OF TUMOURIGENESIS

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Background and aims

The emergence of gene-dependent cancer risks and the potential for alternate pathways of tumourigenesis has implications for cancer prevention in Lynch syndrome. We aimed to

identify gene-specific genomic differences and genomic evidence for differing pathways of tumourigenesis in colorectal cancers (CRCs) from Lynch syndrome carriers.

Methods

From whole-exome sequenced CRCs, including 21 *MLH1,* 12 *MSH2*, 14 *MSH6*, and 13 *PMS2* carriers and 178 non-hereditary mismatch repair proficient CRCs, we calculated tumour mutational burden (TMB), neoantigen load, tumour mutational signatures, microsatellite instability (MSI), and somatic mutations in *APC*, *CTNNB1*, *KRAS*, *TP53*, and *RNF43*.

The relative timing of mismatch repair deficiency (MMRd) acquisition was estimated by considering if somatic mutations in *APC* matched MMRd 3-bp mutational signature contexts, with absence of *APC* mutations, or *APC* mutations matching MMRd contexts, indicating "early MMRd" and thus potential MMRd-crypt pathway, and non-matching contexts ("late MMRd") suggesting the adenoma pathway.

Results

MSI levels were significantly lower in *MSH6* carrier CRCs compared with CRCs from the other MMR gene carriers (MANTIS $p=3x10^{-4}$, MSIsensor $1x10^{-7}$, MSIseq $1x10^{-4}$). CRCs from *PMS2* carriers showed higher levels of mutational signatures ID1 ($p=2x10^{-12}$) and lower levels of ID2 (p=0.001) and ID7 (p=0.003) compared with CRCs from *MLH1*, *MSH2* and *MSH6* carriers. TMB and neoantigen load were not significantly different between CRCs from the four MMR genes.

The frequency of somatic mutations in CRC driver genes differed significantly across the MMR genes: *APC* was mutated in 9/21 (43%) *MLH1*, 4/12 (33%) *MSH2*, 12/14 (86%) *MSH6* and 10/13 (77%) *PMS2* carrier CRCs while *RNF43* mutations were significantly enriched in *MLH1* and *MSH2* (57% and 42%) compared to *MSH6* and *PMS2* (21% and 15%). *CTNNB1* mutations occurred in 6 (29%) *MLH1*, 3 (25%) *MSH2*, 1 (7%) *MSH6* and 2 (15%) *PMS2* carrier CRCs.

"Early MMRd" was the dominant pathway compared with "late MMRd" for each of the four MMR genes. "Early MMRd" pathway cancers were most common in *MLH1* (76%), followed by *MSH2* (66%), *PMS2* (53%) and *MSH6* (42%) (37% in MMR-proficient CRCs as a reference).

Conclusions

CRCs from Lynch syndrome carriers demonstrated distinct genomic differences based on the defective MMR gene. Genomic evidence showed a high proportion of CRCs in carriers develop MMRd early in tumourigenesis with implications for CRC prevention.

Keywords

Lynch syndrome, tumour mutational signatures, *APC*, pathways of tumourigenesis.

O33 - IL-17A-PRODUCING NKP44(-)ILC3S MAY PROMOTE DUODENAL ADENOMA FORMATION IN FAMILIAL ADENOMATOUS POLYPOSIS

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Objective

Familial adenomatous polyposis (FAP), an inherited gastrointestinal tumor syndrome, is characterized by colonic polyposis and a high incidence of duodenal adenomas, significantly increasing the risk of duodenal cancer compared to the general population. Notably, the variability in duodenal phenotypes among individuals with identical genetic mutations suggests the influence of additional factors, including the local immune response.

Design

This study involved the isolation and analysis of intestinal innate lymphoid cells (ILCs) from normal and adenomatous duodenal tissue obtained during routine endoscopic procedures from 82 FAP patients and 26 non-FAP individuals. We used flow cytometry for phenotypic and functional assessment, complemented by quantitative reverse transcription PCR (qRT-PCR) and bulk RNA sequencing for mucosal mRNA evaluation. In addition, duodenal organoid models were used to investigate the role of ILCs in adenoma development.

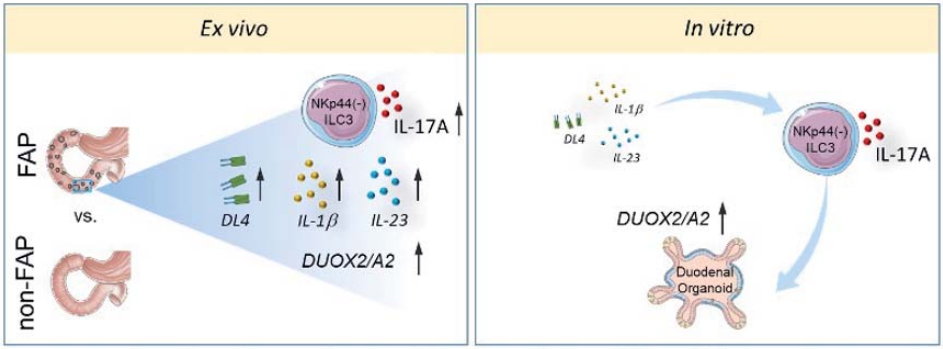
Results

We observed a significant increase in the frequency of ILC3s in both normal and adenomatous duodenal mucosa of FAP patients compared to controls. Furthermore, an association between FAP and increased IL-17A production by NKp44(-)ILC3s in the duodenum was identified. Increased mRNA expression levels of IL1B, IL23A and DLL4 in FAP duodenal adenoma tissues positively correlated with the frequency of IL-17A(+)NKp44(-)ILC3s. In vitro studies showed that culturing NKp44(-)ILC3s with DLL4-expressing OP9 feeder cells and IL1sy/IL-23 significantly enhanced IL-17A production in ILC3s. In addition, duodenal adenomas from FAP patients showed increased expression of dual oxidase 2 (DUOX2) and its maturation factor DUOXA2, both of which are implicated in cancer progression. Functional experiments showed that IL-17A and NKp44(-)ILC3s stimulated DUOX2/DUOXA2 expression in duodenal organoids.

Conclusions

Our findings implicate IL-17A-producing NKp44(-)ILC3s in the pathogenesis of duodenal adenoma formation in FAP, suggesting a novel immunological component in this disease.

Summary



Duodenal accumulation of IL-17A-producing NKp44- ILC3 might promote adenoma formation in FAP

O34 - THE IMMUNE PROFILE OF LYNCH SYNDROME-ASSOCIATED COLORECTAL ADENOMAS PINPOINTS THE MAIN DETERMINANTS OF IMMUNE ACTIVATION

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Background and aims

Colorectal cancers in Lynch syndrome (LS) carriers, characterized by microsatellite instability (MSI) and pronounced immune infiltration, can develop via three pathways: progression from mismatch repair-proficient (MMRp) or MMR-deficient (MMRd) adenomas and MMRd crypt foci. We recently reported changes in the immune environment of normal colonic mucosa and carcinomas from LS carriers. Here, we analyzed the LS adenoma immune phenotype and its relation to clinicopathological features with particular focus on its dependence on MMRd/MSI.

Method

Gene expression profiling was performed using the Nanostring nCounter technology covering 770 immune-relevant genes. In addition, T cell subtype infiltration was quantified by immunohistochemistry in 140 adenomas from 66 LS carriers. Correlation with clinicopathological parameters such as adenoma size, localization, histopathology, and MMR deficiency status was analyzed.

Results

LS adenomas displayed MMRd/MSI in 71% of lesions, with the lowest proportion in *MSH6* carriers. CD3-positive and CD8-positive T cell densities were significantly lower in MMRp

lesions compared to MMRd/MSI counterparts, and in rectal compared to colonic adenomas. Adenomas showed significantly lower CD8-positive and significantly higher FOXP3-positive T cell densities than normal mucosa. Gene expression profiling not only confirmed significant differences in the immune cell composition between adenomas, normal mucosa and carcinomas, it further demonstrated a distinct expression pattern in LS adenomas compared to normal mucosa. Adenomas presented with a significant upregulation of known oncogenes (e.g. *MYC*) and genes involved in angiogenesis and metastasis as well as a downregulation of tumor suppressors such as *CDKN2B*.

Conclusions

The analysis of one of the largest cohorts of adenomas with immune profiling data demonstrates that evidence for an immunosuppressive microenvironment is detectable in most LS adenomas. Significantly different immune profiles between MMRd/MSI and MMRp adenomas indicate that MMRd plays an important role in shaping the local immune environment of emerging adenomas, likely as a result of the MSI-induced neoantigen load.

Keywords

Lynch syndrome, carcinogenesis, adenomas, immune profiling.

O35 - HLA TYPE AS A POSSIBLE MODULATOR OF CANCER RISK IN LYNCH SYNDROME: FIRST DATA FROM THE INDICATE NETWORK

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Background and aims

Lynch syndrome (LS) carriers have a broad lifetime cancer risk range (30-80%). More precise, individual risk estimations for LS carriers would be of high clinical value, allowing tailored cancer prevention and surveillance. Due to the high immunogenicity of LS cancers and a crucial role of the human leukocyte antigen (HLA) repertoire of an individual in determining antigen presentation to the immune system, we hypothesized that a LS carrier's HLA genotype may influence the likelihood of progression from pre-cancerous lesions into cancer.

Method

We established a network, INDICATE, to explore the role of HLA type as a possible cancer risk modifier in LS. Clinical information, genomic and tumor DNA has been collected from Germany, Finland, the Netherlands, UK, Hungary. HLA type has been determined using next-generation sequencing including HLA-A, HLA-B and HLA-C gene loci for HLA class I as well as HLA-DRB1, HLA-DQB1 and HLA-DPB1 for HLA class II.

Results

To date, genomic DNA has been collected from 758 LS carriers. Among 635 individuals with cancer history available, 45% of patients have no cancer history, 55% have been previously diagnosed with cancer. The HLA type of the first 619 LS patients has been successfully determined. The most common alleles were HLA-A*02:01 (allele frequency 28.8%) and HLA-B*07:02 (14.1%) for HLA-A and HLA-B, respectively. In a first analysis, a similar distribution of the supertypes HLA-A02, HLA-A03, HLA-B15 and HLA-B07 was observed among patients with and without cancer history. However, in this interim sample set, a potential association between tumor risk or the number of previous tumors and HLA supertype was observed for HLA-A02, HLA-C01 and HLA-B58.

Conclusions

A person's HLA type could be a cancer risk modifier in LS. If validated with continuous INDICATE enrollment, HLA type may have implications for risk-adapted surveillance and the design of next-generation personalized cancer-preventive vaccines.

Keywords

Cancer risk modifiers, immunology, HLA type.

O36 - PANCREATIC CANCER IS MORE COMMON THAN MELANOMA IN NON-WHITE CDKN2A POSITIVE INDIVIDUALS

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Background and aims

Germline pathogenic and likely pathogenic variants (P/LPV) in CDKN2A predispose carriers to early on-set melanoma and pancreatic cancers, however, cancer incidence and penetrance estimates have been derived primarily from White cohorts. We describe the incidence of melanoma and pancreatic cancers (PC) for individuals with CDKN2A P/LPV by ethnicity.

Methods

Individuals with CDKN2A P/LPV identified at a single diagnostic laboratory from Jan 2012-Nov 2023 were characterized by age, self-reported ethnicity, and melanoma/PC diagnosis. This was performed for any CDKN2A P/LPV and specifically for the high-frequency Hispanic LPV, p.I49T. The rate of cancer diagnosis was compared to consecutively tested BRCA2 positive individuals (positive controls) and pan-cancer panel (28-32 genes) negative individuals (wild type controls) identified from an overlapping time period (2012-2016).

Results

A total of 1,286 individuals with P/LPV in CDKN2A were identified. Melanoma diagnoses were significantly decreased for non-White individuals (4.3%) when compared to White individuals (31%; $p<0.001$). There were no significant differences in PC between ethnicities, and on average PC was more common (6.7%) than melanoma in non-White individuals. A comparison of the Hispanic allele, p.I49T (n=479), revealed a 4.3-fold increase in PC (7.3%) compared to the wild type controls (1.8%; n=31,599)(95% CI 3.0-6.2; $p<0.001$) and a 1.8-fold increase compared to BRCA2 positive controls (4.1%; n=826)(95% CI 1.1-3.0; $p=0.01$).

Conclusions

This work demonstrates that while the frequency of PC was consistent across all ethnic groups, melanoma was significantly less common for non-White individuals with a CDKN2A P/LPV compared to White individuals. PC may be the predominant tumor observed in non-White CDKN2A populations. This result is significant, as melanoma is considered major criteria for CDKN2A testing and CDKN2A variant interpretation. This indicates that non-White individuals may be undertested for CDKN2A and that causal pathogenic CDKN2A alterations may be under-identified. CDKN2A c.146T>C, p.I49T was shown to have a 4x increased risk in the development of PC and should be considered a high-risk allele.

O37 - RECRUITING LYNCH SYNDROME PATIENTS FOR CLINICAL RESEARCH VIA SOCIAL MEDIA IMPROVES PARTICIPANT DIVERSITY

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Background

Recruiting sociodemographically diverse patients with Lynch Syndrome (LS) to clinical research studies can be challenging, evidenced by majority of LS study participants with European ancestry. Leveraging social media for research recruitment may hold promise to reach a more diverse patient population.

Aim

We aimed to compare the reach, yield, and demographics of traditional institution-based recruitment versus social media recruitment of LS patients to a nationwide clinical research study (no in-person visits) about the use of digital and mobile health technology in cancer prevention care.

Methods

Institution-based recruitment included providing study information to patients during clinical care (clinic, endoscopy) and to those attending an in-person Lynch Syndrome Patient Symposium hosted at the institution. Social media recruitment included a single post from the principal investigator's X (formerly Twitter) account. Both recruitment strategies provided the same information and required individuals to reach out to the study team to express interest. For those who expressed interest, the study team requested completion of a brief demographic survey and requested individuals provide verification of LS diagnosis.

Demographics between the two recruitment strategies were compared using Chi-Square for categorical variables and unpaired T test for continuous variables.

Results

94 patients with Lynch Syndrome were provided study information through institution-based recruitment. Of these patients, five (5.3%) expressed interest and four completed all study pre-requirements. The social media posting had 6,500 views, 79 likes, 9 comments, 27 re-postings within X (7 by patient advocacy or health organizations, 16 by healthcare professionals, 4 by patients), and 2 re-postings in other online forums. 105 individuals expressed interest by contacting the research team, 64 completed the required survey, and 12 provided verification of LS diagnosis (**Figure**). Compared to the patients who expressed interest via institution-based recruitment, social media recruitment resulted in younger participants (mean age 27 vs 53, p=0.022), more evenly distributed by sex (48% female vs 100% female, p=0.045) and more diverse participants based on race (p=0.013), and geography (p=0.0207) (**Table**). Social media recruitment did result in individuals *without* LS expressing interest in the study, however this was addressed by asking for verification of LS diagnosis.

Conclusion

Social media recruitment holds promise to improve diversity in clinical research recruitment, though requires verification of eligibility. Future directions include assessing the effectiveness of this recruitment strategy for clinical research that requires in-person visits in addition to telephone/virtual activities.

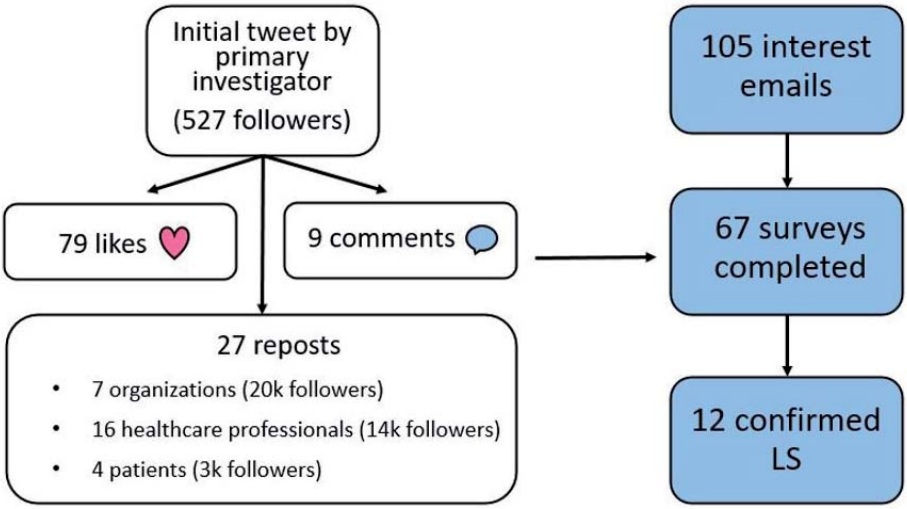

Figure. Social media recruitment reach and yield

Table. Institution vs social media recruitment. Demographic comparison of those who expressed interest in the study and completed the pre-study demographic questionnaire.

Demographics	Institution-based (n=4)	Social media* (n=64)	P valve
Age (mean years)	53	27	**0.0222**
Sex			**0.0453**
Female	4 (100%)	31 (48%)	
Male	0 (0%)	33 (52%)	
Race			**0.0169**
White	4 (100%)	12 (19%)	
Black	0 (0%)	43 (67%)	
Asian	0 (0%)	2 (3%)	
American Indian/ Alaska Native	0 (0%)	3 (5%)	
Multi/ Mixed Race	0 (0%)	4 (6.3%)	
Ethnicity			0.3652
Hispanic	0 (0%)	11 (17%)	
Geography			**0.0207**
Western US	0 (0%)	8 (13%)	
Mountain US	4 (100%)	14 (22%)	
Midwestern US	0 (0%)	11 (17%)	
Southeastern US	0 (0%)	16 (25%)	
Northeastern US	0 (0%)	14 (22%)	
Missing	0 (0%)	5 (8%)	

*Prior to verifying LS diagnosis

O38 - MACHINE LEARNING-BASED ENDOSCOPIC CLASSIFICATION FOR SUPERFICIAL MUCOSAL LESIONS IN HEREDITARY DIFFUSE GASTRIC CANCER

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Introduction

Hereditary diffuse gastric cancer (HDGC) is typically due to pathogenetic variants in *CDH1* and carries 40-60% life-time risk of signet ring cell carcinoma (SRCC). Endoscopic surveillance can inform gastrectomy time by detecting early SRCC. The pale area is the most common endoscopic lesion harbouring SRCC but difficult to diagnose. We previously described the Cambridge criteria to aid endoscopic detection of early SRCC in pale areas. Here, we aimed to validate the performance and interobserver agreement (IOA) of the criteria and to develop machine-learning (ML) tool based on the criteria to improve endoscopic diagnosis.

Methods

CDH1+ patients undergoing endoscopic surveillance were recruited at single institution between Jan 2020 and Aug 2023. Endoscopies were performed with white light and magnifying narrow band imaging. Pale areas were labelled with Cambridge criteria by three HDGC experts during live examination or post-hoc video using endoscopic features **(Table 1)** and blinded to histology. Then ML model was trained and tested based on experts' labels for 100 times on randomly split datasets. By analysing the ML's logic and combining experts' experience, we generated a clinically applicable DT rule. Four gastroenterologists, not experienced in HDGC, first examined and diagnosed 30 pale areas. After training on Cambridge criteria, they then labelled an independent dataset with the features and submitted their diagnosis. The performance of the criteria was analysed based on the sum of positive features, individually or as a panel.

Results

Overall, we enrolled 79 CDH1+ individuals (60.8% females; average age 41.5yr). 215 pale areas were included from 132 endoscopies (25.1% SRCC). HDGC expert endoscopists applying the Cambridge Criteria achieved an accuracy of 81.4%, sensitivity of 85.2% and specificity of 80.1%, with a threshold of ≥3 positive features. The ML model achieved significantly higher accuracy than the simple score (84.4% vs. 81.7%, $p<0.001$). An easy-to-use DT derived from the diagnostic logic of ML was shown in **Figure 1**. The IOA of the criteria varied from low (0.205) to moderate (0.444). Following the Cambridge Criteria training, the average sensitivity and specificity among non-experts increased from 56% to 66% and from 51% to 57%, respectively.

Conclusion

Specific features of pale areas can be combined to give high accuracy and aid less experienced endoscopists in predicting SRCC in HDGC. A user-friendly DT based diagnostic rule potentially improves performance of the criteria.

Table 1 The performance of Cambridge endoscopic criteria applied by HDGC expert endoscopists for diagnosing signet ring cell carcinoma foci in hereditary diffuse gastric cancer.

		Interpretation		Performance				
		Positive (+)	Negative (-)	Accuracy %	Sensitivity %	Specificity %	PPV %	NPV %
A	Shape	Round	Linear	54.4	94.4	41.0	34.9	95.7
B	Demarcation line	Sharp	Faded	80.0	35.2	95.0	70.4	81.4
C	Vessels	Focally irregular	Regular	77.7	74.1	78.9	54.1	90.1
C++	Vessels	Diffusely irregular	Not diffuse irregular	80.0	20.4	100	100	78.9
D	Pit pattern	Irregular	Regular	70.2	25.9	85.1	36.8	77.4
E	Reproducible on dynamic view	Yes	No	27.9	100	3.7	25.8	100
Score ⩾ 3	At least 3 positive features			81.4	85.2	80.1	60.0	94.2

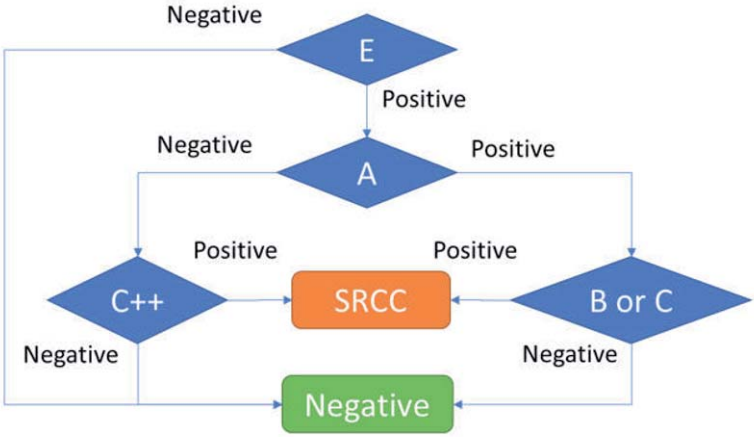

Figure 1. The decision tree-based strategy in diagnosing signet ring cell carcinoma foci on pale areas in hereditary diffuse gastric cancer patients.

O39 - EVALUATING DIFFERENT TESTING APPROACHES FOR IDENTIFYING APC MOSAICISM IN UNEXPLAINED ADENOMATOUS POLYPOSIS: PAVING THE WAY FOR THE NEW SUSCEPTIBILITY GENE DISCOVERY

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Background and aims

Unexplained adenomatous polyposis (UAP) represents a significant clinical burden. This study aimed to: 1) evaluate different testing approaches for identifying somatic *APC* mosaicism to determine its prevalence, and 2) identify new risk genes in UAP cases where *APC* mosaicism has been excluded.

Methods

Eighteen UAP cases were recruited to the Genetics of Colonic Polyposis Study following clinical colorectal cancer/polyposis gene panel testing with no actionable germline variants

identified. Two independent approaches for detecting *APC* mosaicism were evaluated: 1) "Adenoma-first" to identify concordant *APC* somatic mutation in multiple polyps using targeted multi-gene panel sequencing, and 2) "Blood-first" to detect low-level *APC* Pathogenic Variants (PVs) in blood DNA by performing deep targeted sequencing. *APC* mosaicism was confirmed by performing droplet digital PCR (ddPCR) in DNA derived from saliva, blood and normal colonic mucosa. UAP cases where APC mosaicism had been excluded (n=6) underwent germline whole genome sequencing (WGS) to identify predicted loss of function and pathogenic structural variants.

Results

Of the 18 UAP tested (mean±s.d. age dx 42.6±11, adenoma count 84.2±21.9), *APC* mosaicism was identified in 7/18 (39%) UAP cases using the adenoma-first approach where a somatic APC mutation was observed in common in all adenomas and/or tumours sequenced from each of the 7 cases (number of tested lesions = 3.2±1.7). The 7 *APC* mosaic cases had mean±sd dx age of 47.6±7 and 95±11 adenomas. In 4 (22%) cases, ddPCR confirmed the APC variant was present at low levels in the blood (mean±s.d.=1.7±1%), saliva (2.6±2.8%) and normal mucosa DNA (2.2±1.7%). In the remaining 3 (17%) cases, the *APC* mosaic variant was only present in normal colonic mucosa DNA (1.6±2%), suggesting localised APC mosaicism confined to the colon. The blood-first approach detected only a single *APC* mosaic case. Analysis of WGS data from *APC* mosaicism negative cases is underway (n=6).

Conclusions

The adenoma-first approach, coupled with ddPCR of non-neoplastic tissue, provides accurate detection of *APC* mosaicism, including localised *APC* mosaicism, in UAP cases where no germline PV is identified by clinical genetic testing. In this study, *APC* mosaicism constitutes ~39% of UAP, necessitating its investigation prior to new risk gene discovery studies. Unexplained adenomatous polyposis cases without *APC* mosaicism warrants further investigation for new risk genes through WGS.

O40 - MULTICENTRE APPROACH TO IMPROVE THE IDENTIFICATION AND MANAGEMENT OF CMMRD PATIENTS IN SPAIN

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Background and aims

Constitutional mismatch repair deficiency (CMMRD) is a rare and devastating childhood cancer predisposition syndrome caused by biallelic germline pathogenic variants in mismatch repair (MMR) genes (*PMS2*, *MSH6*, *MLH1*, *MSH2*). An accurate and prompt diagnosis is essential for implementing genetic counseling, cancer surveillance and effective treatments. However, clinical diagnosis of CMMRD is challenging due to its extremely low prevalence,

the broad spectrum of associated tumors, and the overlapping phenotype with other cancer syndromes. Moreover, CMMRD genetic diagnosis is hampered by difficulties in *PMS2* analysis and the identification of MMR variants of unknown significance. Our aim is to improve the identification of CMMRD in Spain.

Method

An interdisciplinary group of experts in CMMRD from 22 Spanish institutions has been created aiming at improving identification of CMMRD-suspected patients. Highly sensitive assessment of microsatellite instability (MSI) in blood samples using hs-MSI assay was used to confirm CMMRD diagnosis. Clinical data and 208 biological samples (including 36 blood, 22 urine, 14 oral mucosa, 58 normal tissue, and 39 tumors) from confirmed CMMRD individuals were collected at diagnosis and follow-up. Newly diagnosed tumours were analyzed using the TruSight Oncology 500 assay.

Results

CMMRD diagnosis has been confirmed in five of the thirteen CMMRD suspected patients (1 *PMS2*, 2 *MSH6*, 1 *MSH2*, 1 *MLH1*) registered so far, all presenting positive hs-MSI scores in blood. Our findings increase to 15 the number of CMMRD patients identified in Spain. The twelve tumors analyzed (Burkitt lymphomas, lymphoblastic T lymphomas, a Wilms tumor, high grade gliomas (HGG) and a hepatoblastoma) presented high levels of hs-MSI scores (12.9-51.41). Mutational profiling in four HGG identified drivers and/or actionable mutations in all of them. These 4 HGG showed high tumor mutational burden (>10mutations/MB), being two of them ultrahypermutated (>100 mutations/Mb), supporting their treatment with immune checkpoint inhibitors.

Conclusions

The multi-center network of CMMRD multidisciplinary experts contributes to the improved identification and management of CMMRD patients, prompting the initiation of a national-wide screening of CMMRD in pediatric cancer patients.

O41 - DIGESTIVE BURDEN OF PATIENTS WITH CONSTITUTIONAL MISMATCH REPAIR DEFICIENCY SYNDROME, DIAGNOSTIC PITFALLS, AND EXPERIENCE OF IMMUNOTHERAPY: A REPORT FROM THE C4CMMRD DATABASE

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Background and aims

Constitutional mismatch repair deficiency (CMMRD) is a severe cancer predisposition syndrome caused by bi-allelic germline pathogenic variants affecting a mismatch repair gene. CMMRD is associated with childhood brain tumors, haematological malignancies, and LS-spectrum cancers. We present data on digestive lesions in CMMRD patients from the European C4CMMRD (Care for CMMRD) consortium database.

Method

Data concerning the digestive phenotype of 105 CMMRD patients with at least one digestive lesion over the last ten years were extracted from the European database. Clinical, biological, histological and biomolecular data for each tumor including response to treatment, germline genetic results and endoscopic surveillance outcome were collected.

Results

In the C4CMMRD database 54 patients had at least one digestive lesions for a total of 71 cancers : 59 colorectal, 7 small bowel, 4 gastric and 1 oesophagus tumours. All of them developed gastrointestinal adenomas (1-102). Two patients had digestive polyposis without malignant neoplasia.

Immunochemistry of the MMR protein was available for 28 patients and showed loss of expression of the protein corresponding to the mutated gene in all except in 8 cases with normal staining in tumor and normal tissue. All other digestives tumors (28) tested for microsatellite instability were found to be MSI.

Evaluation of the tumor mutational burden (TMB) was available for 4 patients and showed a very high load, higher to MSI tumors (>200 variants/Megabase). A molecular signature profile was obtained for three tumours with somatic variant of *POLE* in all of them and a combined signature of MSI and *POLE* in one.

Six patients received immunotherapy, initially or after failure of conventional treatment or disease relapse. Three of them are still alive and in remission.

Conclusions

Our results confirm the high risk of digestive cancers in CMMRD patients associated with a phenotype of multiple adenomatous polyps in the digestive tract. These tumors most often present with a loss of expression on IHC and an MSI phenotype as in Lynch syndrome. We also described a high TMB with a good response to immunotherapy as already described in MSI tumors of Lynch patients, suggesting for digestive tumors arising in the context of CMMRD the treatment recommendations of Lynch syndrome should be followed and immunotherapy should be recommended as first-line treatment of large, unresectable, or metastatic colorectal tumors.

Keywords

Constitutional MisMatch Repair Deficiency, digestive cancers, immunotherapy.

O42 - INTERROGATING THE LANDSCAPE OF IMMUNOGENIC FRAMESHIFT MUTATIONS IN PATIENTS WITH LYNCH SYNDROME AND IMMUNE SUPPRESSION PROGRAMS SUPPORTING COLORECTAL CANCER DEVELOPMENT

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Background and aims

Patients with pathogenic germline variants in the mismatch repair (MMR) genes, or Lynch syndrome (LS), are at increased risk of developing colorectal (CRC) and endometrial cancers. These LS tumors are characterized by a high microsatellite instability (MSI-H) phenotype. 1 We and others have identified shared immunogenic frameshift (fs)-neoantigen peptides in MSI-H tumors and fs-specific T cell receptors (TCRs) in primary and metastatic MSI-H tumors. Additionally, strong mucosal T cell infiltrates are associated with later CRC onset.2 Therefore, we hypothesized that fs-neoantigen expression may be seen early in MMR deficient (MMRd) tumor development, but fs-specific T cell activity may be modulated by the immune response in the precancerous microenvironment (PCME) and tumor microenvironments (TMEs).

Methods

Peripheral blood mononuclear cells (PBMCs) and LS normal, precancer (e.g. adenoma) and malignant colon and endometrial tissues were analyzed. Loss of MMR protein expression was assessed with multiplexed immunohistochemistry and immune landscape of PCME and TME with single-cell and spatial transcriptomics. Whole-exome and bulk RNA sequencing were performed to assess fs-neoantigen expression and quality. Functional T cell phenotypes and specificity were analyzed with *in vitro* neoantigen-specific T cell expansion and stimulation

assays4, *ex vivo* flow cytometry and single-cell RNA/TCR sequencing of PBMCs/MMRd lesions.

Results

Shared frameshift mutations encoding potentially highly immunogenic peptides are expressed in MMRd normal mucosa, precancerous adenomas, and tumor lesions of LS patients. In peripheral blood, precancerous tissue, and the TME of LS patients, there are T cells capable of recognizing these peptides *ex vivo*. T cells in tumors relative to normal/precancerous tissue show reduced functional capacity associated with a polarization toward and infiltration of immunosuppressive myeloid cell subsets.

Conclusions

We present a preliminary map of the fs-neoantigen landscape in MMRd tumor development in patients with LS and potential evidence of immune dysregulation/suppression programs which may support neoplastic progression. These results highlight the importance of understanding fs-neoantigen quality and evolution, as well as immune cell differentiation over the course of tumor development, in cancer vaccine design and biomarker selection for immunoprevention efforts.

Acknowledgements

We would like to thank the patients and their families for participating in our study. We also would like to acknowledge the Mount Sinai Biorepository, Genomics, and Human Immune Monitoring Cores for their assistance in tissue processing and next-generation sequencing assays.

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Ethics Approval

The study was approved by the Icahn School of Medicine at Mount Sinai Institutional Review Board, approval numbers: IRB-19–02392, IRB- 21–01317, and IRB-20–00888.

Fig 1. Precancerous immunogenomics profiling identifies potential fs-neoantigen-encoding mutations at early stages of MMRd tumor development in Lynch Syndrome Patients

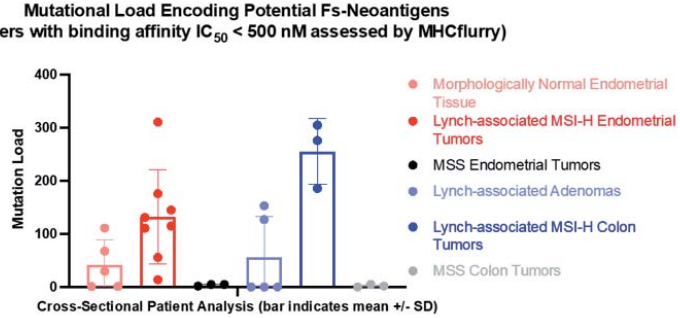
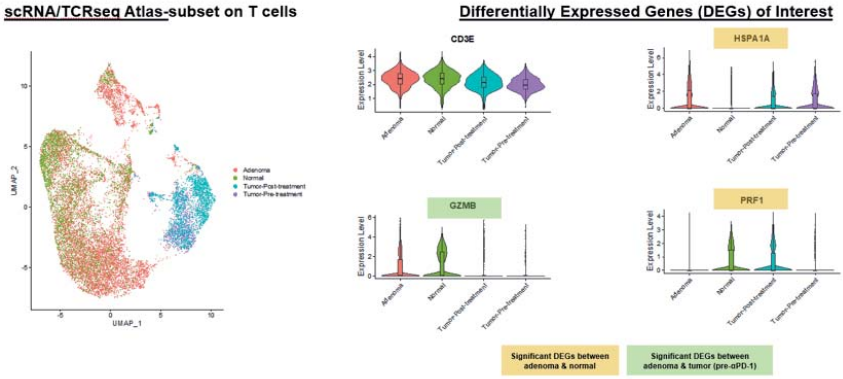


Fig 2. CD8 T cells have increased stress and reduced expression of cytotoxic programs in advanced tumors (pre-αPD-1 treatment) relative to precancerous Lynch Syndrome colon tissue



O43 - OUTCOMES OF LYNCH SYNDROME ENDOMETRIAL CANCER SURVEILLANCE IN A NATION-WIDE COHORT

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Background and aims

Female Lynch syndrome (LS) carriers have an increased risk of developing endometrial cancer (EC). Regardless, research on EC tumorigenesis is scarce and no uniform, evidence-based gynecological surveillance guidelines exist. We therefore described gynecological surveillance outcomes in a nation-wide LS cohort.

Method

For this retrospective cohort study, female LS carriers prospectively registered in the Dutch LS database were included. Carriers were linked to the Dutch national pathology (PALGA) database. The number of carriers with/without gynecological surveillance, ECs (including their characteristics) were assessed, as well as the requisite for adjuvant therapy in endometrioid FIGO IA/IB ECs (EECs) according to current guidelines, and the extent of risk-reducing surgery performed. Overall survival from EC diagnosis was analyzed using Kaplan Meier time to event analyses.

Results

Of 1255 female LS carriers registered, 1050 were at risk for EC and thus included. Of those, 317 of eligible carriers (30.2%) did not have surveillance. In carriers with versus without surveillance, 37 (7.3%) versus 18 (3.3%) ECs were diagnosed; carriers with surveillance more often had *MLH1/MSH2/EpCAM* pathogenic variants (62.8% versus 55.5%, respectively) and were generally younger at database assembly than those without (56.0 years [IQR 48.0-65.0] versus 65 years [IQR 49.0-75.0], respectively). ECs were predominantly of endometrioid

type (93.9% versus 88.9%, respectively) and found in FIGO stage IA (61.3% versus 50.0%, respectively). Adjuvant radiotherapy was required in one patient in both groups. In total, 23.8% of all 1050 carriers opted for prophylactic hysterectomy. Overall survival after EC diagnosis did not differ between carriers with or without surveillance or carriers with EC before LS diagnosis (p=0.197).

Conclusions

In a nation-wide cohort of LS carriers, nearly one-third of eligible carriers did not have gynecological surveillance. Surveillance diagnosed ECs slightly more often in FIGO stage IA, but did not seem to substantially decrease the requisite for adjuvant therapy or affect overall survival. Prospective research should be performed to assess if and to what extent current gynecological surveillance contributes to earlier detection of EC in LS carriers.

O44 - PREDICTORS OF INVASIVE SIGNET RING CELL CARCINOMA IN CDH1 CARRIERS – RESULTS FROM THE MULTICENTER STUDY OF CDH1 OUTCOMES & SURVEILLANCE

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Background

Surveillance of *CDH1* pathogenic variant carriers is challenging, as signet ring cell carcinoma (SRCC) can be indolent for years or have a more invasive phenotype. There are no known factors associated with invasive disease, and, therefore, guidelines recommend prophylactic total gastrectomy for most patients. Previous studies are limited to single centers; therefore, a multicenter consortium was established in 2023 to study outcomes in *CDH1* patients and families. The aims of this ongoing study are to determine clinical, endoscopic and histologic features associated with identification of SRCC foci on upper endoscopy and/or with invasive disease in *CDH1* carriers.

Methods

CDH1 pathogenic/likely pathogenic variant carriers were identified from 8 academic centers in the United States. Clinical, endoscopic, and histological data were collected and compared between individuals with and without SRCC found on endoscopy, and between individuals with localized disease (stage T1a or no cancer) and those with invasive disease (>T1a) found on gastrectomy specimens.

Results

To date, 96 patients have been included. Mean age at diagnosis was 45.6 years and 36.4% were male. Details the clinical management of the study cohort. Eighty-four patients underwent 157 endoscopies (mean 1.8 per patient, range 1-7), with a mean of 34.7 biopsies (+/-24.8) taken in each endoscopy. Twenty-two patients (22.9%) were found to have SRCC foci on endoscopy, the majority (68.2%) found on initial endoscopy and in the proximal stomach. Erythema, nodularity and intestinal metaplasia (IM) were positively associated with SRCC foci on biopsy (57.1% vs. 27.1% [p=0.01], 9.5% vs. 0 [p=0.01] and 18.2% vs. 3.1% [p=0.01], respectively). Of the 37 patients with surgical data, 3 had invasive disease (1 with stage T1b; 2 with stage T4). **Table 1** compares clinical, endoscopic and histological features between patients with and without invasive disease. SRCC foci found on first endoscopy and IM were significantly associated with invasive disease.

Conclusions

These are the initial results of a multicenter study of surveillance outcomes in *CDH1* carriers. Erythema, nodularity and IM were significantly associated with identification of SRCC, while IM and SRCC on first endoscopy were associated with invasive disease. As more patient data are included (anticipated total number of 350), new markers of SRCC invasiveness may be identified to inform endoscopic surveillance in *CDH1* carriers.

Table 1. Comparison of clinical, endoscopic, and histologic features between invasive and non-invasive phenotype.

Parameter	Patients with invasive disease (>T1a)	Patients with non-invasive disease	p-value
Clinical data (n=37)	N=3	N=34	
Age at genetic diagnosis (mean +/- SD)	48.6 (+/- 21.9)	43.1 (+/- 12.7)	0.47
Male (%)	2 (66.7)	10 (29.4)	0.24
Smoking (%)	2 (66.7)	6 (17.6)	0.11
Alcohol use (%)	2 (66.7)	24 (70.5)	1
Proton pump inhibitor use (%)	2 (66.7)	15 (44.1)	0.54
Number of SRCC foci on PTG (mean +/- SD)	16.4 (+/- 12.8)	21.1 (+/- 38.7)	0.43
Endoscopic and histologic data (n=35)	N=3	N=32	
Endoscopic findings (%)			
Thickened mucosal folds	1 (33.3)	1 (3.1)	0.16
Pale spots	1 (33.3)	3 (9.4)	0.31
Erythema	2 (66.7)	14 (43.8)	0.58
Friability	1 (33.3)	1 (3.1)	0.16
Nodularity	1 (33.3)	6 (18.8)	0.49
Polyp	0	13 (40.6)	0.27
Erosions	1 (33.3)	5 (15.6)	0.44
Ulceration	1 (33.3)	1 (3.1)	0.16
Mass	1 (33.3)	0	0.08
Histologic findings (%)			
Intestinal Metaplasia	2 (66.7)	1 (3.1)	0.01
Gastritis	2 (66.7)	14 (43.8)	0.58
Fundic gland polyps	1 (33.3)	9 (28.1)	1
H. pylori	0	3 (9.4)	1
SRCC			
Found on 1st endoscopy	**3 (100)**	**8 (2)**	**0.02**
Number of foci identified on endoscopy (mean +/- SD)	3 (+/- 1.4)	1.5 (+/- 0.5)	0.12

PTG – prophylactic total gastrectomy; SD – standard deviation; SRCC – signet ring cell carcinoma.

O45 - SPECIFICATIONS TO THE ACMG/AMP CRITERIA ENHANCE THE CLASSIFICATION OF MMR VARIANTS

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Background and aims

Gene- and disease-specific expertise can enhance the generic ACMG/AMP guidelines for determining the pathogenicity of variants, but this was unknown for MMR genes. The long-running InSiGHT MMR/Lynch Syndrome Variant Interpretation Committee (VIC) was recently updated to become a ClinGen Variant Curation Expert Panel (VCEP). This VCEP was tasked to develop specifications to the ACMG/AMP criteria starting from the previous InSiGHT recommendations for MMR variant classification.

Method

With our previous classification criteria as a starting point, we systematically assigned new specifications to the ACMG/AMP criteria, based on the experiences of the VCEP addressing discordant interpretations. Specifications for *in silico* predictions, tumour characteristics, and functional assays using Bayesian likelihood ratios that align with the ACMG-AMP framework were developed and agreed upon. ClinGen-trained biocurators applied the updated criteria to 48 variants using the ClinGen Variant Curation Interface. Comparisons were performed with existing ClinVar and InSiGHT criteria base classifications.

Results

The VCEP made 19 specifications to the ACMG/AMP criteria. Four existing VUS variants were reclassified to benign (B) or pathogenic (P) using the updated specifications. *MLH1* c.1889T>A, a variant previously classified likely pathogenic (LP) based on segregation data, tumour characteristics, and *in silico* probability, was reclassified to VUS as per the new ACMG/AMP evidence combinations. In total, 43/48 (90%) of the ACMG/AMP classifications were concordant with existing InSiGHT classifications, but overall fewer variants were classified as VUS, thus improving the ability to classify variants.

Conclusions

The InSiGHT - ClinGen Hereditary Colon Cancer / Polyposis VCEP has implemented new specifications to the ACMG/AMP guidelines, and validated these on a pilot set of 48 MMR variants. Our results show that incorporating gene and disease expertise into ACMG/AMP guidelines increases the yield of classified variants. These specifications will facilitate the work of biocurators, and genetic counselors, which ultimately benefits Lynch Syndrome patients and their families. Once approved, classifications are submitted to ClinVar and the specifications available at https://cspec.genome.network/cspec/ui/svi/. InSiGHT continues to engage with ClinGen in this truly international collaborative effort to classify MMR gene variants.

Keywords

Variant classification.

O46 - AN EVALUATION OF USER EXPERIENCES, PERCEPTIONS AND ATTITUDES TOWARDS FAECAL IMMUNOCHEMICAL TESTING (FIT) FOR RISK-STRATIFIED COLONOSCOPY IN LYNCH SYNDROME PATIENTS

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Background and aims

Lynch syndrome (LS) is an inherited condition characterised by pathogenic variants within mismatch repair genes resulting in an increased risk of colorectal cancer (CRC). In England, the faecal immunochemical test (FIT) guides colonoscopy in symptomatic and screening populations, though it is largely underexplored in LS patients. User experience, attitudes, and preferences towards FIT as a modality for CRC surveillance are explored within both an emergency clinical service implemented during the COVID-19 pandemic, and a longitudinal study "FIT for Lynch" (Project 1 and 2, respectively).

Methods

Paper surveys were posted to eligible LS patients in the UK between June 2020 - March 2021 and September 2021 - July 2023 under Projects 1 and 2. The surveys included items with Likert-scale responses assessing user experience, and FIT attitudes and perceptions. Project 1 also had open-ended questions. Likert responses were numerically coded, and Pearson chi-square tested associations with age and sex at a 5% significance level. Free-text responses were coded thematically.

Results

Of 756 participants across both projects, 89.2% completed surveys (63.2% female). Both projects showed favourable FIT experiences (89.9% in Project 1, 95.5% in Project 2), and positive attitudes and perceptions (see **Figures 1** and **2**). Thematic analysis demonstrated a near equal distribution of negative (n=26) and positive (n=24) feedback from Project 1 respondents, though there were instances where a single response was coded twice (negative and positive). 'Negatives' were largely comprised of misinterpretations in the intended use of FIT (i.e., "…I don't feel it should replace it") or misinterpretation of FIT capability (i.e., "The kit does not check if you have polyps").. Women reported more anxiety using FIT in

Project 1 (p=0.045), whereas older respondents in Project 2 showed higher confidence in FIT's accuracy (p=0.046) and its role in colonoscopic triage (p=0.033).

Conclusions

Overall, FIT was well-received across these two LS cohorts. User experiences and attitudes varied across demographics, especially between age groups. Enhanced support and education may address misconceptions and concerns about FIT and aid in its potential integration into colonoscopic surveillance, though additional research is needed in this space.

Figure 1

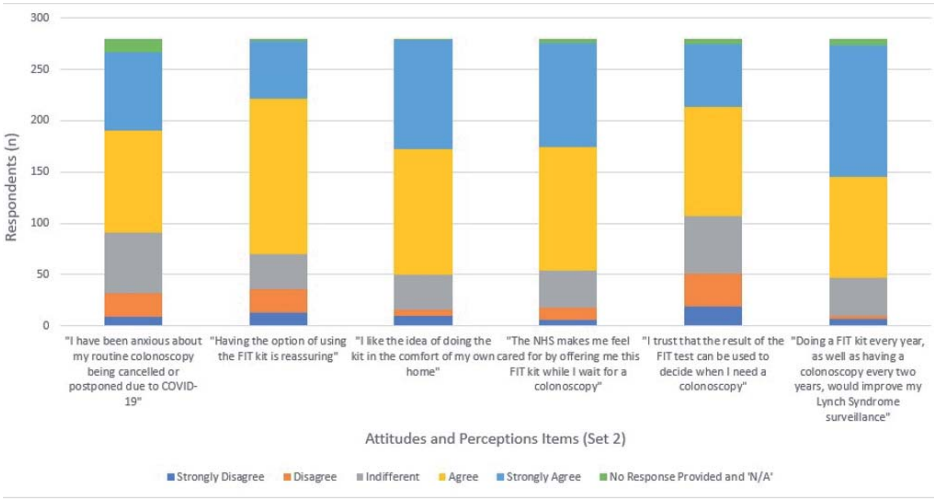
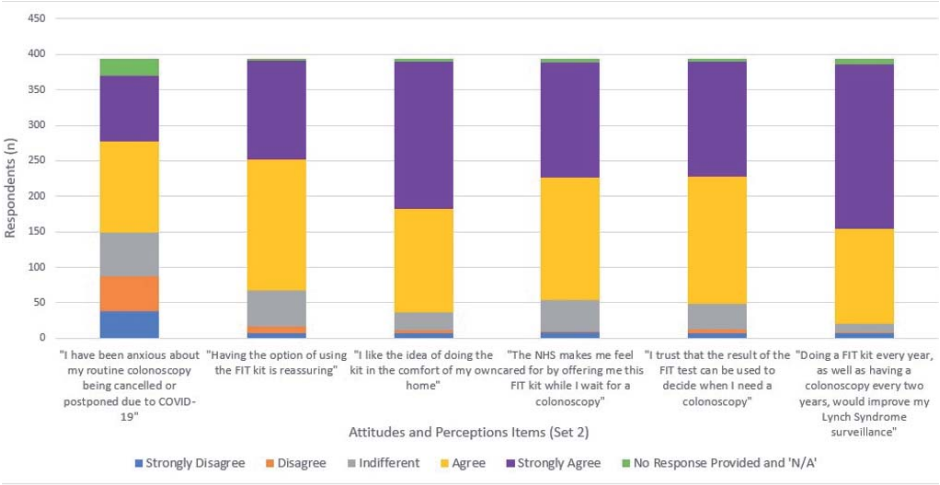


Figure 2


450
400
350
300
250
200
150
100
50
0
Respondents (n)
"I have been anxious about my routine colonoscopy being cancelled or postponed due to COVID-19"
"Having the option of using the FIT kit is reassuring"
"I like the idea of doing the kit in the comfort of my own home"
"The NHS makes me feel cared for by offering me this FIT kit while I wait for a colonoscopy"
"I trust that the result of the FIT test can be used to decide when I need a colonoscopy"
"Doing a FIT kit every year, as well as having a colonoscopy every two years, would improve my Lynch Syndrome surveillance"
Attitudes and Perceptions Items (Set 2)
Strongly Disagree
Disagree
Indifferent
Agree
Strongly Agree
No Response Provided and 'N/A'

O47 - VARIABLE CANCER PENETRANCE AMONG CTNNA1 LOSS-OF-FUNCTION CARRIERS: INITIAL RESULTS FROM THE CTNNA1 FAMILIAL EXPANSION (CAFÉ) STUDY

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Background and aims

Hereditary diffuse gastric cancer syndrome (HDGC) has classically been associated with the CDH1 gene. More recently, loss-of-function (LOF) variants in CTNNA1 have also been implicated as a cause of HDGC, with initial risk estimates showing nearly a 50% cumulative risk of diffuse gastric cancer by age 80. However, as the number of CTNNA1 LOF variant families published in the literature remains limited, the true cancer risk spectrum and penetrance associated with CTNNA1 remains uncertain. The CTNNA1 Familial Expansion (CAFÉ) Study was initiated in 2021 with a goal of better defining CTNNA1 cancer penetrance and risk phenotypes. Herein we present the initial results from the first 34 CTNNA1 LOF variant carriers in the CAFÉ Study.

Methods

Enrollment for the CAFÉ Study opened in May 2021 to any individual who carriers a CTNNA1 loss-of-function variant, regardless of how this variant has been classified by the testing laboratory. All participants signed informed consent for this IRB approved study, and data collection occurred through 12/31/2023. Once consented, participants completed RedCap-based questionnaires collecting information on demographics, and personal and family history of genetic testing and cancer.

Results:

To date, 34 individuals with CTNNA1 LOF variants have been enrolled in the CAFÉ Study, including 24 unique CTNNA1 LOF variants. Of this cohort the median age was 50.5 [IQR 41-59.5], 76% were female, 88% were White, 94% were from the United States, and 59% had a diagnosis of cancer. Gastric cancer was diagnosed in 15% of the cohort with an age range of 20-65, and 29% of the cohort had a first or second degree relative with gastric cancer. Breast cancer was diagnosed in 24% of the cohort with an age range of 45-65, and 56% of the cohort had a first or second degree relative with breast cancer. Sixteen other cancers were diagnosed with CRC and melanoma being most common (N = 2 for both). Phenotypes amongst the families varied substantially including between families with identical CTNNA1 LOF variants and especially with respect to gastric cancer.

Conclusions

The first results of the CAFÉ Study show that breast and gastric cancer are the most common cancers amongst CTNNA1 LOF carriers, and that cancer penetrance is highly variable amongst carriers. Together this data is important for more clearly defining the cancer risk spectrum of CTNNA1 LOF variant carriers and for informing their risk management strategies.

O48 - EMPLOYING INNOVATION TO ENHANCE THE SAFETY AND RELIABILITY OF RESTORATIVE PROPHYLACTIC SURGICAL TECHNIQUES FOR PATIENTS WITH FAMILIAL ADENOMATOUS POLYPOSIS AT A NATIONAL REFERRAL CENTRE

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Background and aims

Total colectomy with ileorectal anastomosis (TC-IRA) and proctocolectomy with ileoanal pouch (RPC) are the traditional restorative prophylactic surgical procedures employed for individuals with familial adenomatous polyposis (FAP). Re-appraisal and innovation have led to modification of these techniques in recent years. Laparoscopic near-total colectomy with ileo-distal sigmoid anastomosis (Lap NTC-IDSA) and robotic restorative proctocolectomy with intracorporeal single-stapled anastomosis (RPC-RISSA) were introduced as modifications to conventional techniques (Lap TC-IRA was modified to NTC-IDSA in 2014. In 2019 robotic RPC-RISSA was introduced as a development of laparoscopic RPC). This study aims to evaluate the temporal trends in operative techniques and postoperative outcomes for prophylactic restorative surgery in FAP patients at a national referral centre.

Methods

A retrospective analysis was conducted using data from patients with FAP who underwent prophylactic restorative surgery between January 2008 and December 2022 at the institution.

Results

253 individuals with FAP underwent major elective prophylactic restorative surgery over the 15-year period; 102 patients underwent TC-IRA, 84 NTC-IDSA, 51 underwent laparoscopic RPC and 16 robotic RPC-RISSA. Temporal trend analysis of anastomotic leakage in rectal-sparing procedures demonstrated 0/84 (0.0%) anastomotic leakage following the Lap NTC-IDSA technique adoption [2014-2022] compared with 8/102 (7.8%) in those undergoing the traditional TC-IRA between 2008 and 2013. No anastomotic leakage was observed

amongst 0/16 (0.0%) patients following the introduction of robotic RPC-RISSA [2019-2022] compared with 5/51 (9.8%) in those undergoing conventional laparoscopic RPC between 2008 and 2018.

Conclusion

This study demonstrates the role of innovation in enhancing the safety and reliability of postoperative outcome in patients undergoing prophylactic minimally invasive restorative surgery for FAP at a British national referral centre.

Keywords (MeSH terms)

familial adenomatous polyposis, colorectal surgery, minimally invasive surgical procedures, robotic surgical procedures, restorative proctocolectomy.

O49 - A FAECAL MICROBIAL SIGNATURE TO OPTIMISE COLORECTAL CANCER SURVEILLANCE IN LYNCH SYNDROME

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Background and aims

Colonoscopic surveillance reduces colorectal cancer (CRC) incidence and mortality in Lynch syndrome (LS) carriers. Still, its invasiveness and high costs highlight the need for a non-invasive approach to predict the presence of neoplastic lesions. This study aimed to identify faecal bacterial biomarkers to discern between carriers with colorectal lesions and those with normal colon.

Method

Two independent Spanish cohorts, training and validation, were recruited through two multicentric studies **(Table 1)**. All participants provided a faecal sample before surveillance colonoscopy for the analysis of the bacterial markers abundances, including Eubacteria (EUB), *F.prausnitzii* (FPRA) and its phylogroups I and II (PHGI and PHGII), *B.fragilis* (BCTF), *B.thetaiotaomicron* (BCTT), B46 (best match BLAST *S.variabile*), *G.morbillorum* (GMLL), and *P.stomatis* (PTST). Participants were classified based on colonoscopy findings as normal colonoscopy (NC), non-advanced adenoma (NAA), and advanced neoplasia (AN, including advanced adenoma and CRC). Statistical analysis included multivariate and univariate tests. A machine learning-based bacterial signature was defined in the training cohort and validated in the validation cohort to distinguish patients with neoplasia (NAA+AN) from those with NC.

Results

The bacterial abundances were analysed according to age, sex, BMI, and germline mutation in the training cohort. BCTF abundance was higher in carriers aged >60 versus 40-60 (p=0.047), and BCTT abundance was higher in carriers between 40-60 years compared to those aged < 40 (p=0.034) or >60 (p=0.038). A lower abundance of PTST was found in AN versus NAA lesions (p=0.045) and patients with neoplasia showed higher abundances of B46 (p=0.027), FPRA (p=0.031), and PHGI (p=0.007).

A bacterial signature was defined, including BCTF, B46, GMLL, FPRA, and PHGII combined with age, to predict the absence of neoplasia. The signature showed 100% sensitivity (SS), 55.6% specificity (SP), 33.3% positive predictive value (PPV) and 100% negative predictive value (NPV). This signature was subsequently validated in the validation cohort, demonstrating 100% SS, 42.5% SP, 49.4% PPV, and 100% NPV.

Conclusions

A new faecal microbial signature to predict the presence of colorectal neoplasia in LS carriers has been identified, showcasing its potential usefulness to improve and personalise LS surveillance, optimising endoscopic resources, and providing a less invasive and cost-effective approach.

Keywords

Lynch syndrome surveillance, gut microbiota, colorectal cancer.

Table 1. Main characteristics of the study participants. NC, normal colonoscopy; NAA, non-advanced adenoma; AA, advanced adenoma; SA, serrated adenoma; CRC, colorectal cancer.

		Training cohort	Validation cohort
Age	mean (range)	50 (25-83)	47 (20-75)
Sex (female)	n (%)	44 (66.7)	75 (65.8)
Diagnosis	NC (%)	54 (81.8)	73 (64.0)
	NAA (%)	7 (10.6)	35 (30.7)
	AA (%)	3 (4.5)	3 (2.6)
	SA (%)	1 (1.5)	2 (1.8)
	CRC (%)	1 (1.5)	1 (0.9)
Mutated gene*	MLH1 (%)	28 (42.4)	21 (18.4)
	MSH2 (%)	14 (21.2)	18 (15.8)
	MSH6 (%)	16 (24.2)	30 (26.3)
	PMS2 (%)	8 (12.2)	9 (7.9)
	EPCAM (%)	0 (0.0)	1 (0.9)
Total	n	66	114

* Currently, mutated gene information is available for 79 out of 114 participants within the validation cohort. Comprehensive data will be procurable by June 2024.

O50 - PROFILING GERMLINE AND SOMATIC MUTATIONS IN CONSECUTIVE EARLY ONSET COLORECTAL CANCER CASES IN A EUROPEAN POPULATION

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Introduction

Early-age onset colorectal cancer (EOCRC) diagnosed <50 years has increased in incidence worldwide. Whilst high risk surveillance exists for selected patients, only ~16% of EoCRC may be attributed to a germline pathogenic variant (PV). This study aims to characterise real-world somatic and germline molecular profiles in consecutive patients with EoCRC across several European centres.

Methods

Consecutive patients at St Marks NHS Trust, London (SMH), Royal Marsden NHS Trust, London (RMH), Chelsea and Westminster NHS Trust, London (CWH) and the Spanish Early Onset Colorectal Cancer Consortium (SECOC) were identified using electronic patient records; clinicopathological, somatic and germline testing data were collected. Patients were included if they had been diagnosed with and histologically confirmed CRC <50, MMR testing for possible Lynch syndrome (LS) and, ideally, subsequent clinically validated NGS somatic testing or germline testing using multi-gene panel tests.

Results

A total of 651 EOCRC patients were identified from 20 centres. SMH cohort (n=175), RMH cohort (n=55), CWH cohort (n=97) and SECOC cohort (n=324, 17 centres, see **Table 1**). The median age was 43 with a range from 14-49. 45% were female. 40/207 (19.3%) patients had a family history of Lynch related cancers in a first degree relative and 207/244 (84.8%) had any reported family history of cancer. Regarding anatomical location 113/456 (24.8%) patients had right sided tumours, 170/456 (37.3%) patients had left sided tumours and 173/456 (37.9%) were rectal.

Across all the cohorts 549/642 (85.5%) of patients were MMR proficient. On somatic next generation sequencing (NGS) analysis, variants were detected in 89/276 (32.2%) EOCRCs with 71 in pMMR and 16 dMMR (2 N/A) of which 66.3% were clinically valuable to personalise treatment and consider eligibility for clinical trials. Germline variants were detected in 86/141 patients (61.0%) with 27 in pMMR and 66 in dMMR CRC (1 N/A). Within 93 patients with dMMR 66 had diagnosed PVs (74%); 29 *MLH1*, 17 *MSH2*, 4 *MSH6*, 11 *PMS2*, 1 APC, 1 *MUTYH*, 1 *BRCA1*, 1 *ATM*, 1 *FANCA*. Of 549 patients with pMMR 27 had diagnosed PVs (4.9%) 5 *MLH1,* 3 *MSH6*, 3 *PMS2,* 7 *APC,* 6 *MUTYH* (1 with coexistent *PMS2* variant), 1 *CHEK2* and 1 *EPCAM*. Lynch syndrome was diagnosed in 80/651 (12.3%) of the cohort. Of that group 14/80 (17.5%) patients presented with pMMR tumours.

Conclusion

Our results support joint somatic and germline multi-gene panel testing for all EOCRC patients, regardless of MMR status or family history. Larger unselected cohort studies would support the validation of testing prediction models and estimates of clinically relevant variant actionability.

Table 1.

Centre	Patients identified
St Marks NHS Trust, London, UK	175
Royal Marsden NHS Trust, London	65
Chelsea and Westminster NHS Trust, London, UK	97
Hospital Fundación Jiménez Díaz, Madrid, Spain	42
Hospital del Mar, Barcelona, Spain	19
Hospital Galdakao, Bizkaia, Spain	19
Hospital de Villalba, Madrid, Spain	6
Hospital Ramón y Cajal, Madrid, Spain	19
Hospital Clínico, Madrid, Spain	28
Hospital Infanta Leonor, Madrid, Spain	30
Hospital Vall d'Hebron, Barcelona	32
Salamanca University Hospital, Salamanca, Spain	43
Gregorio Maranon University Hospital, Madrid, Spain	21
MD Anderson, Madrid, Spain	28
University Clinic of Navarra, Madrid, Spain	5
León University Hospital, León, Spain	2
Vithas Arturo Soria University Hospital, Madrid	2
Hospital Universitario 12 de Octubre, Madrid, Spain	13
Hospital Cliníc de Barcelona, Barcelona, Spain	4
Alcorcón Foundation University Hospital, Madrid, Spain	11

O51 - ILEOCECAL INTUBATION IN LYNCH SYNDROME PATIENTS – GO ALL THE WAY!

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Background and aims

Lynch syndrome (LS) is also associated with extracolonic malignancies. However, there is a paucity of data on ileal cancer associated with LS and consequently no established screening guidelines. Given the ease of ileal intubation during routine colonoscopy, our study aims to analyze the prevalence of ileal cancer in a large cohort of patients with LS and to evaluate the efficacy of ileal intubation as a potential screening method.

Method

This retrospective study analyzed data from 602 colonoscopies performed between 2018 and 2023 in 238 patients diagnosed with LS at the National Center for Hereditary Tumor Syndromes. The study focused on the detection of ileal lesions and evaluated the distribution of pathogenic variants, patient demographics, and personal cancer history. We have additionally extracted patient data from the German Consortium for Familial Intestinal cancer (GCFIC) with both LS and ileal cancer. The incidence of ileal cancer in the patient cohort was compared with the national data. Pathogenic variants (PV) in MLH1, MSH2, and EPCAM were classified as high-risk and PV carriers in MSH6 and PMS2 as low-risk.

Results

The GCFIC has 2859 patients enrolled with pathogenic variants in MLH1, MSH2, MSH6, PMS2, or EPCAM.

Nine ileal carcinomas were detected during the database query, predominantly in male patients (78%) with exclusively high-risk PVs **(Table 1)**. MLH1 accounted for 67% and MSH2 accounted for 33% of these cases. The age of onset ranged from 35 to 76 years (median age 55 y). Among patients with ileal cancer, 89% had a positive history of prior cancer, 67% with at least one colorectal cancer in their medical history. Clinical symptoms were responsible for detecting 72% of cancers, while 14% were detected through screening (colonoscopy). The information for the remaining 14% was not available.

We examined 238 LS-patients (80% of high-risk variants, 20% of low-risk variants) at our center **(Table 2)**. There was a slight female predominance (57.6 %). Ileal intubation was

achieved in 95.7% of all examinations. We detected three advanced ileal adenomas, in addition to the two ileal cancers previously described in the GCFIC data.

Conclusion:

Ileum intubation is an effective and valuable extension of standard colonoscopy, particularly for high-risk groups, such as male patients with MLH1 or MSH2 variants and a history of cancer.

Table 1: Ileal cancers documented in the German Consortium for Familial Intestinal Cancer.

	German Consortium for Familial Intestinal Cancer
Patients with Ileal cancer, n (%)	9 (0.31%)
Onset Age (years), mean ± SD; [range]	55.2±14.59 [35,76]
Female sex, n (%)	2 (22%)
Personal Cancer history (n), mean ± SD; [range]	2.44±2.13 [0,7]
High-risk (likely-) pathogenic variant, n (%)	9 (100%)

Table 2: Baseline characteristics of 602 colonoscopies in 238 carriers of a pathogenic variant in the last 6 years at the National Center for Hereditary Tumor Syndromes.

Base line characteristics	
Total Colonoscopies (n)	602
Patients analyzed (n)	238
Meanage (years), mean ± SD; [range]	47.8 ± 13.4; [17,84]
Female sex, n (%)	137 (57.6%)
Distribution of (likely-) pathogenic variant carriers:	
·MLH1, n(%)	82 (34.5%)
·MSH2, n(%)	100 (42%)
·MSH6, n(%)	37 (15.5%)
·PMS2, n(%)	11 (4.6%)
·MLH1/MSH2, n(%)	3 (1.3%)
·EPCAM, n(%)	5 (2.1%)
Personal history of colorectal cancer, n (%)	102 (42.9%)
Patient's with ≥ 2 colon surgeries, n(%)	25 (10.6%)
Types of colon surgeries:	
·Right hemicolectomy, n (%)	50 (24.4%)
·Transverse colon resection, n (%)	6 (4.6%)
·Left hemicolectomy, n (%)	8 (4.2%)
·Sigmoidectomy, n (%)	10 (4.6%)
·Rectal resection, n (%)	231 (9.7%)
·Colectomy n (%)	8 (6.7)
·Small bowel-resection (jejunal or ileal) n (%)	1 (2.5%)
Mean examinations per patient (n), mean ± SD; [range]	2.55±1.6 [1,9]
Ileal intubation rate, n (%)	576 (95.7%)
Ileal findings (advanced adenoma/cancer) per colonoscopy, n (%)	5 (0.9%)
Ileal findings per patients, n (%)	5 (2.1%)

O52 - PSYCHOSOCIAL DISTRESS AND QUALITY OF LIFE IN FAMILIES WITH A GERMLINE CDKN2A PATHOGENIC VARIANT

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Background and aims

Germline pathogenic variants (PV) in the *CDKN2A* gene play a prominent role in familial melanoma, comprising at least one-third of all cases. Individuals with the Dutch founder *CDKN2A*/p16-*Leiden* PV have an increased lifetime risk of melanoma (70%) and pancreatic cancer (20%). Consequently, skin surveillance is offered at age 12 to those with a confirmed or suspected PV, while pancreatic cancer surveillance is exclusively offered to confirmed carriers at age 40. It is unknown whether the *CDKN2A* PV impacts quality of life (QoL). Therefore, we aimed to assess QoL and psychological distress in individuals from affected families.

Methods

This cross-sectional study included confirmed carriers and those with a 50% risk of the *CDKN2A* PV (at-risk carriers) undergoing cancer surveillance. Individuals ≥18 years were identified from the skin and pancreatic cancer surveillance programs at the Leiden University Medical Center and invited for a one-time, validated self-report questionnaire, including the 12-Item Short Form Health Survey (SF-12), Hospital Anxiety and Depression Scale (HADS), and Cancer Worry Scale (CWS).

Results

A total of 537 individuals were screened for eligibility, of whom 61/249 (25%) individuals under skin surveillance responded (skin surveillance group) and 186/288 (65%) individuals

under combined pancreatic cancer and skin surveillance responded (pancreatic cancer surveillance group). Mean scores for the SF-12 and HADS in both groups were similar to the general population **(Table 1)**. However, cancer-related distress was substantial, with over 40% of all study participants having cancer worries for melanoma. In addition, 44% of participants in the pancreatic surveillance group reported pancreatic cancer worries. Determinants for cancer worries were having a first-degree relative with pancreatic cancer and female sex. More than 80% of the participants felt that benefits of cancer surveillance outweigh the disadvantages.

Conclusions

In conclusion, health-related QoL and general psychological distress in confirmed and at-risk *CDKN2A*PV carriers did not differ from the general population. However, cancer worries played an important role in over 40% of the participants. Cancer surveillance provided reassurance and benefits outweighed disadvantages for the majority of participants. These findings can help clinicians identify individuals who might benefit from psychological support.

Table 1. Descriptive statistics and summary of group comparisons for psychological variables.

Scale	Scale range / threshold	Skin surveillance group		Pancreatic surveillance group		General population	
		N	Mean (±SD) % > threshold	*N*	Mean (±SD) % > threshold	*N*	Mean (±SD) % > threshold
SF-12 PCS	Range 0-100	61	54.3 (6.6)	185	50.5 (8.2)	-	51.7a / 48.1b
SF-12 MCS	Range 0-100	61	47.4 (9.9)	185	51.3 (8.9)	-	48.0a / 51.0b
Total anxiety HADS	Range 0-21 Abnormal threshold ≥ 11	61	4.6 (3.7) 6 (10%)	183	4.0 (3.6) 11 (6%)	199	5.1 (3.6) -
Total depression HADS	Range 0-21 Abnormal threshold ≥ 11	61	2.7 (3.0) 2 (3%)	183	2.7 (2.8) 3 (2%)	199 -	3.4 (3.3) -
Cancer worries for melanoma	Range 8-32 Abnormal threshold ≥ 14	61	12.9 (3.4) 28 (46%)	186	13.3 (3.9) 74 (40%)	-	-
Cancer worries for pancreatic cancer	Range 8-32 Abnormal threshold ≥ 14	61	11.9 (3.6) 13 (21%)	185	13.5 (3.8) 81 (44%)	-	-

[a] age-matched QoL from Dutch general population for skin surveillance.
[b] age-matched QoL from Dutch general population for pancreatic surveillance.
SF-12, 12-item Short Form Health Survey; PCS, Physical Component Scale; MCS, Mental Component Scale; HADS, Hospital Anxiety and Depression Scale; cancer worries measured by the adapted Cancer Worry Scale (CWS).

Table 2. Attitudes toward skin and pancreatic cancer surveillance

Participants from both surveillance groups expressed their attitude toward skin surveillance, while only the pancreatic cancer surveillance group could express their attitude toward pancreatic cancer surveillance.

	Total study population (*N* = 246)	**Pancreatic surveillance group (*N* = 184)**
	Skin surveillance	**Pancreatic surveillance**
	Rather / very much	
Do the follow-up visits convey you a sense of security?	189 (77%)	145 (79%)
Do you think the investigations at follow-up burdensome?	5 (2%)	30 (16%)
Would you worry more about your cancer risk if there were no follow-up?	183 (74%)	139 (76%)
Does the follow-up remind you each time of your cancer risk while you'd rather think less often about it?	46 (17%)	57/183 (31%)
Do the advantages of follow-up outweigh the disadvantages?	204 (83%)	156 (85%)

O53 - DIAGNOSTIC CHALLENGES OF MLH1 CONSTITUTIONAL EPIMUTATIONS INVOLVED IN LYNCH SYNDROME

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Background and aims

Constitutional epimutations are an alternative mechanism to genetic mutations in the etiology of Lynch syndrome. For some patients, the underlying constitutional cause of cancer predisposition is epigenetic, specifically involving promoter hypermethylation of *MLH1* or *MSH2* genes. Our work focuses on *MLH1* epimutations, which can be of 2 different types: primary or secondary. Primary epimutations are pure epigenetic events, labile in the germline, and typically not transmitted to the offspring. Secondary epimutations are associated with a cis-acting genetic alteration and therefore transmitted to the offspring following a Mendelian inheritance pattern. Identifying patients with constitutional epimutations is challenging due to the presence of tumor hypermethylation—a characteristic of sporadic tumors—and the absence of a family history for primary epimutations.

Method

MLH1 epimutation screening was implemented in routine diagnosis of Lynch syndrome in the Molecular Oncogenetics clinical lab of Lille University Hospital (France) in 2009. For patients diagnosed with an epimutation, comprehensive analysis of the *MLH1* gene was conducted to identify variants associated with secondary epimutations. Additionally, presymptomatic diagnosis was offered to relatives.

Results

Seventy-three French patients with a constitutional *MLH1* epimutation were identified in our lab. Among the 55 probands, 84% presented with colorectal cancer as their first tumor, while 15% had endometrial cancer. The median age of onset was 46 y.o. [29-58]. Sixteen patients had multiple tumors. Methylation levels in DNA extracted from peripheral blood mononuclear cells ranged from very low (below 1%) to 50%. Dominant transmission of the epimutation within the family was demonstrated for some of these patients, and a broad spectrum of previously unreported cis-acting genetic alterations associated with *MLH1* promoter hypermethylation were characterized, including single nucleotide variations, a tandem duplication, and the exonic insertion of an Alu element. This information proved valuable for genetic counseling in affected families.

Conclusions

MLH1 constitutional epimutations, though rare, may be underdiagnosed. Diagnosis should be improved. Our findings also provide additional insights into the complexity of molecular mechanisms leading to *MLH1* secondary epimutations and highlights the need for extensive *MLH1* gene analysis in Lynch syndrome patients with constitutional epimutations.

O54 - CANCER RISKS ASSOCIATED WITH GERMLINE PATHOGENIC VARIANTS IN *MLH1, MSH2, MSH6, PMS2,* AND *EPCAM* GENES

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Background and aims

Lynch syndrome (LS) is caused by germline pathogenic variants (PVs) in the mismatch repair genes *(MLH1, MSH2, MSH6, PMS2)* or the *EPCAM* gene. LS is associated with high risks of various cancers, notably cancers of the colorectum and uterus, but gene-specific estimates of risk have been limited, especially for the lower penetrance genes *MSH6* and *PMS2*.

Methods

We examined clinical and genetic records from a consecutive cohort of 1,064,399 individuals referred for hereditary cancer testing at a commercial laboratory between September 2013 and September 2023. Cancer associations of each LS gene were estimated as odds ratios (ORs), with 95% confidence intervals (CIs), from multivariable logistic regression models adjusted for personal and family cancer history, age, ancestry, and sex (where applicable). P-values are based on Wald statistics and are reported as two-sided.

Results

LS PVs were detected in 0.9% (9,651/1,064,399) of the study population. The highest number of PVs were observed in *PMS2* (N=3,587), followed by *MSH6* (N=2,770), *MSH2* (N=1,910), *MLH1* (N=1,376) and *EPCAM* (N=34) **(Table 1)**. PVs in all genes were significantly associated with colorectal cancer, with ORs ranging from 14-fold for MLH1 to 2-fold for PMS2 (Figure 1). PVs in *EPCAM* were too rare for evaluation of cancers other than colorectal cancer. PVs in *MLH1, MSH2, MSH6,* and *PMS2* were significantly associated with uterine cancer, with ORs ranging from 6-fold for *MSH2* to 2-fold for *PMS2*. *MLH1* and *MSH2* PV carriers had 4- and 2-fold increased risks of gastric cancer, respectively. PVs in *MLH1, MSH2* and *MSH6* showed statistically significant but modest (<4-fold) associations with ovarian cancer.

Conclusions

We confirmed a higher prevalence of *PMS2* and *MSH6* compared to *MSH2* and *MLH1* among LS PV carriers. Our data provide gene-specific cancer risks for *PMS2* and *MSH6* where current literature is limited. These results may inform gene-specific cancer risk counseling for LS PV carriers.

Keywords

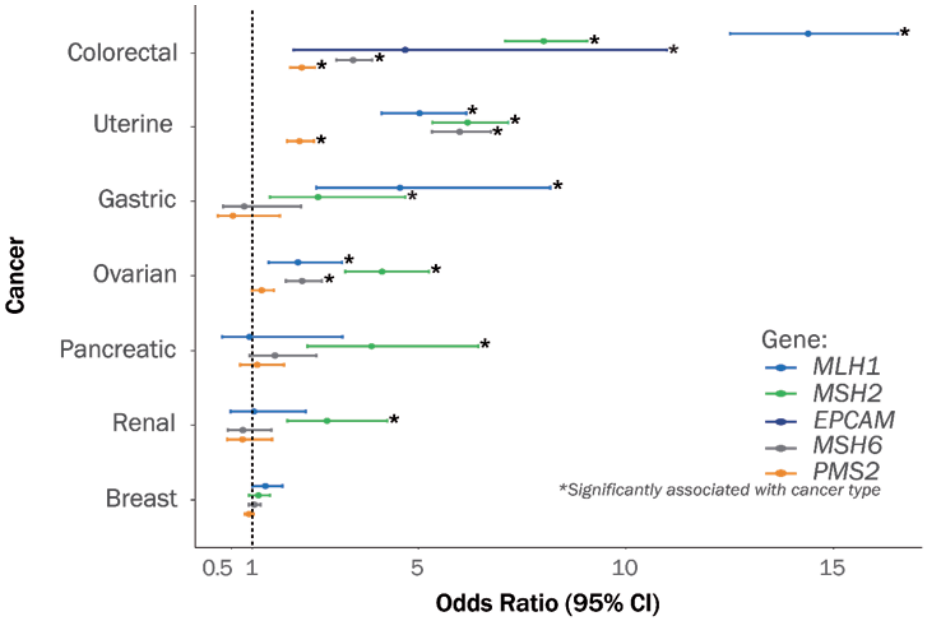
Lynch syndrome, pathogenic variant, prevalence, cancer risk.

Table 1. Prevalence of Lynch Syndrome pathogenic variants (PV) among individuals diagnosed with different cancers.

	Total Patients	Number (%) of Patients with a PV			
		MLH1	*MSH2*	*MSH6*	*PMS2*
Colorectal*	25,408	590 (2.3%)	593 (2.3%)	407 (1.6%)	340 (1.3%)
Uterine	19,328	143 (0.7%)	301 (1.6%)	484 (2.5%)	225 (1.2%)
Gastric	1,059	17 (1.6%)	15 (1.4%)	4 (0.4%)	3 (0.3%)
Ovarian	25,840	29 (0.1%)	88 (0.3%)	121 (0.5%)	96 (0.4%)
Pancreatic	5,747	3 (0.1%)	17 (0.3%)	16 (0.3%)	19 (0.3%)
Renal	2,383	8 (0.3%)	30 (1.3%)	10 (0.4%)	9 (0.4%)
Breast	173,945	69 (<0.1%)	104 (0.1%)	275 (0.2%)	443 (0.3%)
Unaffected	720,506	557 (0.1%)	786 (0.1%)	1,440 (0.2%)	2,227 (0.3%)

*There were 13 individuals with colorectal cancer who had a PV in *EPCAM*.

Figure 1. Gene-specific cancer risks associated with Lynch syndrome genes.



O55 - GENOMIC FEATURES OF POST-COLONOSCOPY COLORECTAL CANCERS IN PEOPLE WITH LYNCH SYNDROME

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Background and aims

In people with Lynch syndrome, frequent colonoscopy with polypectomy does not prevent many colorectal cancers (CRCs). Alternate pathways of non-polypoid tumourigenesis or rapid tumour growth are proposed to underlie these post-colonoscopy (incident) CRCs. We aimed to genomically characterise incident CRCs.

Methods

Incident CRCs from the Australasian Colorectal Cancer Family Registry comprising 17 *MLH1* and 10 *MSH2* carriers were compared with prevalent CRCs (no prior surveillance colonoscopy) from 21 *MLH1* and 11 *MSH2* carriers. Tumour mutational burden (TMB), neoantigen load, tumour mutational signatures, microsatellite instability (MSI), loss of heterozygosity (LOH) and somatic mutations in *APC*, *CTNNB1*, *KRAS*, *TP53*, and *RNF43* were calculated from whole exome sequencing. *APC* mutations that matched mismatch repair deficiency (MMRd) 3-bp mutational signature contexts were indicative that MMRd occurred prior to the *APC* mutation ("early MMRd") and CRC tumourigenesis occurred via a potential MMRd-crypt pathway.

Results

The median time since last colonoscopy was 12 months (range=7-60 months) where most incident CRCs; 1) had reported an adequate bowel preparation and visualisation of the remaining bowel (21/27, 77.8%) and 2) developed after a colonoscopy where no polyps were detected (21/27, 77.8%). The mean age at CRC diagnosis was not significantly different between incident and prevalent CRCs (52.3±10.9 v 46.8±12.2 years).

For the exome-wide genomic features, MSI levels, MMRd related tumour mutational signatures, TMB, and neoantigen load were not significantly different between incident and prevalent CRCs.

APC (48% v 38%), and *CTNNB1* (33% v 25%) mutations were enriched in incident CRCs compared with prevalent CRCs, though not significantly. Somatic *TP53* mutations were not observed in any incident CRC from *MLH1* carriers, in contrast to prevalent CRCs from *MLH1* carriers (33%, p=0.01). In *MLH1* carriers, incident CRCs were significantly enriched for LOH that encompassed both *MLH1* and *CTNNB1*, compared with prevalent CRCs (24% v 0%, p=0.03). No differences were observed for *MSH2* carriers. The proportion of "Early MMRd" related *APC* mutations was not significantly different between incident and prevalent CRCs.

Conclusions

Incident CRCs in *MLH1* carriers demonstrated an enrichment of mutations and LOH events in *CTNNB1* and absence of *TP53* mutations, highlighting a potential alternate pathway of tumourigenesis underlying incident CRC development.

Keywords

Lynch syndrome, post-colonoscopy colorectal cancer, *APC*, *CTNNB1*, pathways of tumourigenesis.

O56 - GERMLINE RESULTS FOLLOWING COMPREHENSIVE GENOMIC TUMOUR SEQUENCING OF 1070 GYNAECOLOGICAL CANCERS

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Tumor-only genomic profiling is an important tool in the clinical care of cancer patients, through the identification of actionable somatic mutations. Genomic tumour sequencing may additionally reveal germline variants of clinical relevance for patients and their at-risk family members.

In January 2022, Fondazione Policlinico Universitario Agostino Gemelli IRCCS started a prospective study (ID: FPG500) that includes patients with 11 different cancer types. DNA and RNA from patient tumor specimens are profiled using TruSight Oncology 500 assay (Illumina).

Between 01/01/2022 – 30/06/2023, 1070 patients with gynaecological cancer (ovarian – OC -and endometrial - EC) were enrolled in the study.

FPG500 customized pipeline for germline-focused analysis included PVs in ESMO/ACMG genes and in other ClinGen actionable cancer predisposing genes (CPGs). Other OMIM genes involved in Mendelian conditions, among which genes associated with autosomal recessive disorders, were interrogated based on clinical phenotype and actionability.

36% of OC and 21% of EC patients were referred to genetic counseling. A total of 201 OC cases underwent genetic evaluation. 178 patients underwent germline analysis: 68% tested positive for at least one PV previously identified through somatic testing.

A total of 78 EC patients underwent genetic evaluation. 58 patients underwent germline analysis: 54% tested positive for at least one PV identified through somatic testing.

Of all PVs founded in CPGs, 9 (ovary) and 11 (endometrial) were off tumour, apparently not related to the clinical presentation. In addition, a diagnosis of a genetic non cancer condition was made in one case of a rare autoimmune disease.

Detailed informations about CPGs genetic evaluation results are available in **Table 1** and **Table 2**.

Patients diagnosed with a hereditary cancer predisposition syndrome include a patient with a MINAS (ATM and BRCA2 PVs) and a patient with biallelic MUTYH PVs.

Cancer genome profiling can improve the identification of mild CPGs and high actionability CPGs in off-tumour context. Acquisition of a blood sample for germline testing at the time of enrolment for testing could reduce the turnaround time and the need for genetic counselling. It is also worth noting that we found 3 additional PVs in patients through genetic counselling and indication for germline panel testing. Therefore, limitations in sensitivity of somatic testing should be always considered by clinicians.

Table 1.

Endometrium CPGs Pathogenic variants								
GENE	Indication to genetic counseling	Confirmed PV	No confirmed PV	Unknown	Patient died	Consent denied	No clinical indication	Germline conversion rate
MSH2	19	9	6	2	0	2	0	60
PTEN	14	0	2	3	1	4	4	0
ATM	12	1	1	7	1	2	0	50
MSH6	11	6	1	3	0	1	0	85,71428571
MLH1	9	2	6	1	0	0	0	25
BRCA2	6	1	2	2	0	1	0	33,33333333
MUTYH	5	3	0	1	0	1	0	100
POLE	4	0	2	2	0	0	0	0
PALB2	4	0	2	1	0	1	0	0
CHEK2	4	0	2	2	0	0	0	0
BRCA1	4	0	1	3	0	0	0	0
RNF43	3	0	0	0	0	1	2	.
PMS2	3	1	2	0	0	0	0	33,33333333
POLD1	2	0	1	1	0	0	0	0
BRIP1	2	1	1	0	0	0	0	50
CDKN2A	2	0	2	0	0	0	0	0
DICER1	2	0	1	1	0	0	0	0
LZTR1	1	1	0	0	0	0	0	100
CDH1	1	0	1	0	0	0	0	0
RAD51D	1	1	0	0	0	0	0	100
CDKN1B	1	1	0	0	0	0	0	100
SDHA	1	1	0	0	0	0	0	100
PTCH1	1	0	0	0	0	1	0	.
TSC1	1	0	0	1	0	0	0	.
RET	1	0	0	1	0	0	0	.
NF1	1	0	0	0	0	0	1	.

Table 2.

Ovary CPGs Pathogenic variants								
GENE	Indication to genetic counseling	Confirmed PV	No confirmed PV	Unknown	Patient died	Consent denied	No clinical indication	Germline conversion rate
BRCA1	102	55	30	16	1	0	0	64,70588235
BRCA2	43	27	10	4	2	0	0	72,97297297
MUTYH	11	5	0	6	0	0	0	100
RAD51C	9	6	0	3	0	0	0	100
ATM	9	4	1	3	0	1	0	80
MSH6	8	2	3	1	1	1	0	40
PALB2	7	6	0	1	0	0	0	100
NF1	7	0	0	1	0	0	6	.
BRIP1	5	3	1	1	0	0	0	75
PTEN	5	0	4	1	0	0	0	0
MSH2	4	1	3	0	0	0	0	25
MLH1	4	3	1	0	0	0	0	75
TP53	4	0	3	1	0	0	0	0
RAD51D	3	1	0	1	1	0	0	100
CHEK2	3	2	1	0	0	0	0	66,66666667
DICER1	2	0	1	0	0	0	1	0
CDKN2A	2	0	1	0	1	0	0	0
PTCH1	2	0	0	0	0	1	1	.
NF2	2	0	0	0	1	0	1	.
SDHA	2	1	0	1	0	0	0	100
SMAD4	1	0	0	0	1	0	0	.
POLE	1	1	0	0	0	0	0	100
SMARCB1	1	0	0	0	1	0	0	.
SMARCA4	1	0	0	0	1	0	0	.
CDH1	1	0	0	0	1	0	0	.
PMS2	1	1	0	0	0	0	0	100
LZTR1	1	0	0	1	0	0	0	.
RET	1	0	1	0	0	0	0	0
EPCAM	1	0	0	1	0	0	0	.
POLD1	1	0	1	0	0	0	0	0

O57 - GERMLINE VARIANTS IN DNA INTERSTRAND-CROSS LINK REPAIR GENES MAY CONTRIBUTE TO INCREASED SUSCEPTIBILITY FOR SERRATED POLYPOSIS IN PARTICULAR WITH PROXIMAL/WHOLE COLON LOCALIZATION OF POLYPS, WITH IMPLICATIONS FOR CLINICAL MANAGEMENT AND THERAPEUTICS

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Background and aims

Serrated polyposis (SP) is characterized by the development of multiple colorectal serrated polyps and increased predisposition to colorectal cancer (CRC). However, the molecular basis of SP, especially in cases presenting family history of SP and/or polyps and/or CRC in first degree relatives (SP-FHP/CRC), is still largely unknown. In a previous study, we proposed the existence of two molecular entities amongst SP-FHP/CRC families, proximal/whole-colon and distal SP-FHP/CRC, according to the preferential location of lesions and somatic events involved in tumour initiation. In the present study, we aimed to further investigate these distinct subgroups of SP patients, in a larger cohort, at the germline level and to identify the genetic defects that may underlie an inherited susceptibility for these two entities.

Methods

Next generation sequencing was performed using multigene analysis with a custom designed panel in a MiSeq platform, in 56 SP patients (with or without FHP/CRC).

Results

We found germline pathogenic mutations in 4/56 patients (*ATM*, *RAD50*, *RAD51C*, and *RNF43* genes), all in the proximal/whole-colon group. We also found variants of unknown significance (VUS) with prediction of probable damaging effect in 22/56 patients (*BLM* (2), *BRCA1, FAN1, FANCA* (2), *FANCL, MSH2, MSH6, NTHL1, PALB2* (2), *PDGFRA, PMS2, PTCH1, RAD51C, RAD51D* (2), *RECQL4, WRN* and *XRCC5* genes. Most of the mutations are in genes involved in DNA repair particularly in genes coding for proteins involved in the Fanconi Anemia (FA) that act downstream of FA complex to facilitate DNA Interstrand-Cross Link repair (ICLR). Notably, mutations in ICLR genes appear to be more frequent in the proximal/whole-colon than in the distal subgroup [14/43(33%) vs 1/13(8%), p=0,063] as opposed to

the non-ICLR genes that were significantly more frequent in the distal group [5/43(12%) vs 5/13(38%), p=0,035].

Conclusions

Germline defects in DNA-ICLR genes may contribute to increase serrated colorectal polyps/carcinoma risk in SP patients, particularly in proximal/whole-colon SP. The inclusion of DNA-ICLR genes in the genetic diagnosis of SP patients, mainly in those with proximal/whole-colon lesions, should be considered and validated by other studies. In addition, patients with germline defects in the ICLR-DNA repair genes may be more sensitive to treatment with platinum-based therapeutics, which can have implications in the clinical management of these patients.

O58 - OPEN-SOURCE BIOINFORMATIC PIPELINE TO IMPROVE PMS2 GENETIC TESTING USING SHORT-READ NGS DATA

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Introduction

The molecular diagnosis of MMR-deficient cancer syndromes is hampered by difficulties in sequencing the PMS2 gene mainly due to the PMS2CL pseudogene(s). Next-generation sequencing (NGS) short reads cannot be unambiguously mapped by standard pipelines, causing a decrease in variant calling accuracy. We aimed to provide a refined bioinformatic pipeline for PMS2 mutational analysis and to explore the prevalence of PMS2 germline pathogenic variants in an unselected hereditary cancer (HC) cohort.

Methodology

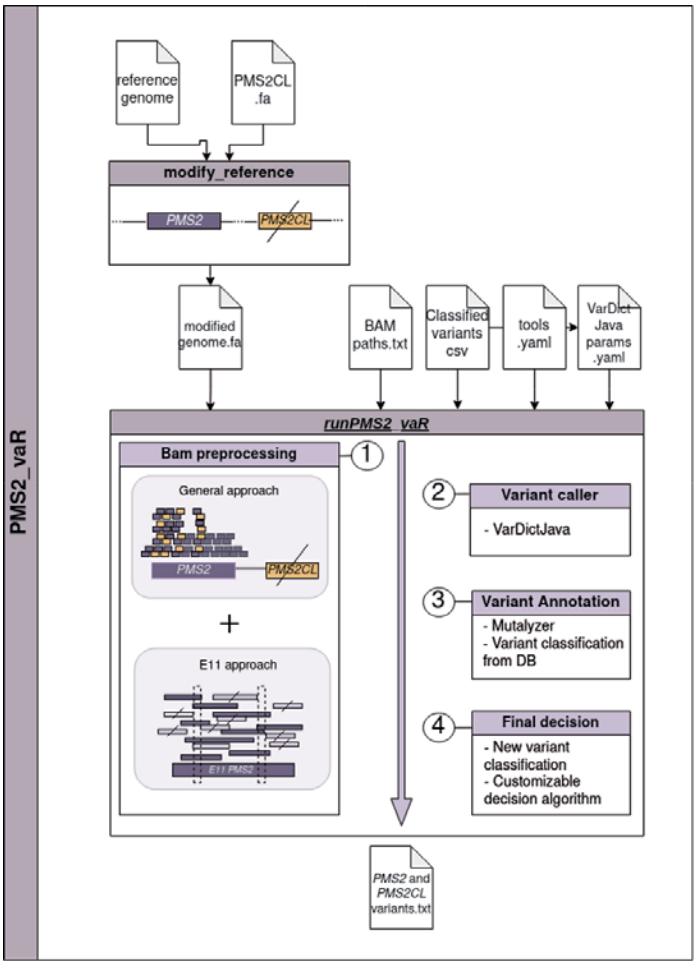
PMS2 mutational analysis was optimized using two cohorts: a group of 192 unselected HC patients for assessing the allelic ratio of paralogous sequence variants, and another cohort of 13 samples enriched in PMS2 (likely) pathogenic variants previously screened by long-range gDNA PCR amplification (LR-PCR). Reads were forced to align with the PMS2 reference sequence; except those corresponding to exon 11, where we only considered those intersecting gene-specific invariant positions (https://github.com/emunte/PMS2_vaR). The accuracy of the refined pipeline was validated in a cohort of 40 patients. The pipeline was subsequently used to screen for PMS2 variants in an HC cohort of 5619 patients.

Results

The PMS2_vaR pipeline showed increased technical sensitivity in the analysis of the validation cohort, from 0.853 to 0.956, identifying all PMS2 pathogenic variants previously identified by Long range-PCR. Fifteen HC cohort samples carried a pathogenic PMS2 variant (15/5619, 0.285%) doubling the estimated prevalence in the general population.

Conclusion

The refined open-source approach (https://github.com/emunte/PMS2_vaR) improved the accuracy of PMS2 mutational analysis, allowing its inclusion in the routine NGS pipeline that could be applied to PMS2 screening.



O59 - TESTING COLONIC GANGLIONEUROMAS AS A CLUE TO THE DIAGNOSIS OF PTEN HAMARTOMA TUMOR SYNDROME

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Background and aims

Gastrointestinal hamartomas, including ganglioneuromas are part of the PTEN Hamartoma Tumor Syndrome (PHTS) and represent one of the major diagnostic criteria. Ganglioneuromas are (sub-)mucosal polyps characterized by ganglion cells with neural proliferation. The incidence of colonic ganglioneuromas in PHTS patients is estimated at 35-53%, but the prevalence of PHTS in persons with ganglioneuromas is unknown. Since ganglioneuromas are rare in the general population, we aimed to investigate the occurrence of PTEN (likely) pathogenic variants (PV) in a nation-wide cohort of ganglioneuromas.

Method

Via the Dutch Nationwide Pathology Databank 229 cases with colonic ganglioneuroma(s) and clinicopathological data, including medical history, were collected. From 206 cases DNA of sufficient quality was isolated from ganglioneuroma tissue and used in single molecule molecular inversion probes (smMIP)-based targeted sequencing of all exons and adjacent consensus splice sites of *PTEN*.

Results

In 15% of ganglioneuroma tissues (31/206) a PV in *PTEN* was found with a variant allele frequency (VAF) between 35 and 65%. In 24 of these 31 cases, PHTS could be confirmed. In 8 other cases reported to have PHTS, a PV PTEN was not detected in the ganglioneuroma tissue. Two of these were reported to have exon deletions, which were not yet evaluated with the current method. Of the 7 newly identified cases three fulfilled PHTS diagnostic criteria (43%) and germline analysis will be performed. In total, at the time of the ganglioneuroma diagnosis, 22/31 cases were already known with PHTS. In these cases, the diagnosis of PHTS was presumably the indication of the colonoscopy and therefore the detection of the ganglioneuroma was not an incidental finding. Finding ganglioneuromas as the first clue to PHTS was seen in 9/184 (5%) of cases.

Conclusions

Due to the wide range of clinical manifestations in PHTS, the diagnosis is difficult and may be easily delayed or missed entirely. Based on the observed frequency of putative PTEN PVs among persons with colonic ganglioneuromas, the presence of ganglioneuroma is sufficiently pathognomonic for PHTS to consider evaluation of PHTS in all persons developing ganglioneuroma.

Keywords

PTEN, PHTS, ganglioneuromas, colon polyps, Cowden syndrome.

O60 - RACIAL AND ETHNIC PATTERNS OF VARIANTS OF UNCERTAIN SIGNIFICANCE (VUS) AMONG PATIENTS WITH EARLY-ONSET COLORECTAL CANCER

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Background and aims

Alongside the rising incidence of colorectal cancer among adults age younger than 50 (early-onset CRC; EOCRC), EOCRC disparities have grown more pronounced. We recently discovered racial/ethnic differences in germline pathogenic variants among EOCRC patients. However, patterns for variants of uncertain significance (VUSs) in a diverse EOCRC population remain uncharacterized. Consequently, we aimed to define the prevalence and spectrum of VUSs among patients with EOCRC by race and ethnicity.

Methods

We included individuals who identified as Ashkenazim, Asian, Black, Hispanic, or White, were diagnosed with a first primary CRC between ages 15-49, and underwent germline genetic testing of 14 CRC susceptibility genes—*APC, BMPR1A, CDH1, CHEK2, EPCAM, MLH1, MSH2,*

MSH6, MUTYH, PMS2, PTEN, SMAD4, STK11, and *TP53*—performed by a nation-wide clinical testing laboratory. A five-tier classification system was applied to all genetic variations. We compared VUSs by race/ethnicity using chi-square tests and multivariable logistic regression adjusted for patient sex, age, CRC site, and number of primary CRCs.

Results

Among 3,980 patients with EOCRC (including 1,001 who identified as non-White), a total of 720 VUSs were identified in 634 patients (15.9%). By race/ethnicity, 8.7% of Ashkenazim, 26.8% of Asian, 22.5% of Black, 17.7% of Hispanic, and 14.5% of White patients carried at least one VUS ($P<0.0001$). The proportion of patients with >1 VUS was also higher among Asian, Black and Hispanic (4.55%, 4.82%, and 3.20%; respectively) versus Ashkenazim and White patients (0% and 1.3%; respectively) ($P<0.0001$). The prevalence of VUSs in *APC, CDH1, CHEK2, MSH2, MUTYH, PMS2*, and *SMAD4* varied by race and ethnicity (all $P<0.05$). Overall, Asian (OR, 2.0; 95%CI 1.35-2.97) and Black (OR, 1.70; 95%CI 1.19-2.44) patients with EOCRC had significantly higher odds of presenting with any VUS versus White patients in adjusted models. Across individual genes, significantly higher odds were observed in adjusted models for VUSs in *MSH2* and *MUTYH* among Asians and Blacks, in *CDH1* for Asians, in *PMS2* and *SMAD4* for Blacks, and in *CHEK2* for Hispanics versus White patients with EOCRC.

Conclusions

Patterns of VUSs varied by race/ethnicity among young patients with CRC in this study. A comprehensive assessment of VUSs across CRC susceptibility genes in diverse EOCRC patients is warranted to guide efforts that will reduce uncertainty and improve clinical care in this population.

BEST ORAL COMMUNICATIONS

CANCER PATHWAY-SOMATIC MUTATIONS, PATHOLOGY

CHEMOPREVENTION

EARLY ONSET COLORRECTAL CANCER

GENETICS

BO1 - ESTIMATING CANCER RISK IN CARRIERS OF LYNCH SYNDROME VARIANTS IN UK BIOBANK

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[2] *MRC Human Genetics Unit, Institute of Genetics and Cancer, University of Edinburgh, Edinburgh, United Kingdom & The Roslin Institute, University of Edinburgh, Edinburgh, United Kingdom*

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[4] *The Centre for Familial Intestinal Cancer, St Mark's: National Bowel Hospital, London, United Kingdom & Inherited Tumour Syndromes Research Group, Division of Cancer & Genetics, Cardiff University, United Kingdom*

[5] *Institute of Ecology and Evolution, University of Edinburgh, Edinburgh, United Kingdom*

Background

Lynch syndrome (LS) is an inherited cancer predisposition syndrome caused by genetic variants affecting DNA mismatch repair (MMR) genes *MLH1*, *MSH2*, *MSH6*, and *PMS2*. Cancer risk in LS is estimated from cohorts of individuals ascertained by family history of cancer, which is known to upwardly bias estimates.

Methods

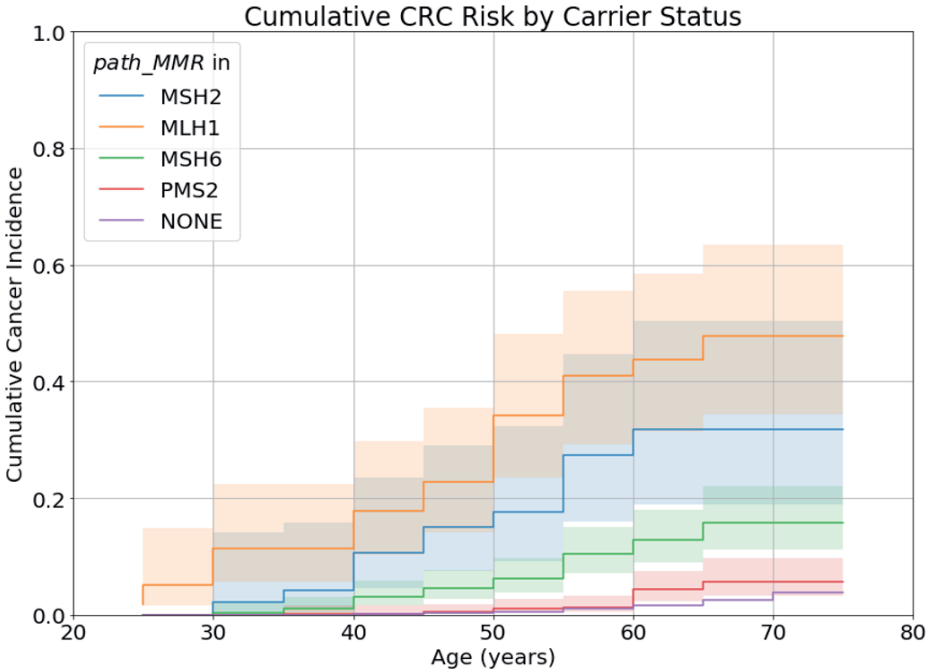
The InSiGHT Database classifies MMR gene variants by pathogenicity through expert panel review of published evidence. 830 carriers of pathogenic or likely pathogenic (*path_MMR*) MMR gene variants from InSiGHT were identified in 454,756 UK Biobank participants using whole exome sequence. Nelson-Aalen survival analysis was used to estimate cumulative incidence of colorectal, endometrial, and breast cancer.

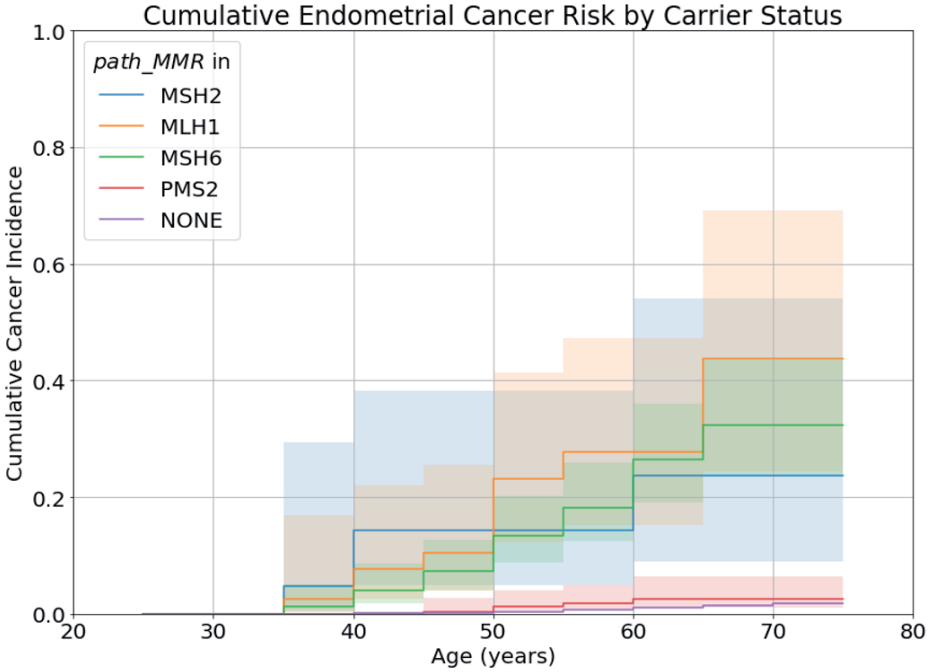
Results

Cumulative incidence of colorectal and endometrial cancer by age 70 was elevated in *path_MMR* carriers compared to non-carriers (colorectal: 11.8% (95% CI: 9.5 - 14.6) vs. 1.7% (1.6 - 1.7), endometrial: 13.4% (10.2 - 17.6) vs. 1.0% (0.9 - 1.0)), but the magnitude of this increase differed between genes. Cumulative breast cancer incidence by age 70 was not elevated in *path_MMR* carriers compared to non-carriers (8.9% (6.3 - 12.4) vs. 7.5% (7.4 - 7.6)). Cumulative cancer incidence estimates in UK Biobank were similar to estimates from the Prospective Lynch Syndrome Database for all genes and cancers, except there was no evidence for elevated endometrial cancer risk in carriers of pathogenic *PMS2* variants in UK Biobank.

Conclusion

These results can be used to inform the management of incidentally identified cases of LS. For example, they support the application of existing colorectal cancer surveillance strategies for LS in incidentally identified cases.




Cumulative Endometrial Cancer Risk by Carrier Status
path_MMR in
MSH2
MLH1
MSH6
PMS2
NONE
Cumulative Cancer Incidence
1.0
0.8
0.6
0.4
0.2
0.0
20
30
40
50
60
70
80
Age (years)

BO2 - PHASE IIA TRIAL OF ENCAPSULATED RAPAMYCIN (ERAPA) IN PATIENTS WITH FAMILIAL ADENOMATOUS POLYPOSIS TO REDUCE INTESTINAL POLYP BURDEN: 6 MONTH INTERIM RESULTS

Carol Burke, MD

Disclaimer: This research was partially funded by the Cancer Prevention Research Institute of Texas under grant DP[19]00[69]

Preclinical data demonstrates mTOR pathway upregulation in Familial Adenomatous Polyposis (FAP). While mTOR inhibition by rapalogues such as rapamycin has demonstrated promise, poor drug bioavailability and toxicity have limited use of these drugs. Encapsulated Rapamycin (eRapa) offers sustained and controlled absorption, thus, we conducted a trial to determine the safety of eRapa and its impact on gastrointestinal (GI) polyp burden in patients with FAP.

This 12-month open label trial included adults with genetic or clinical FAP, intact colon or ileo-rectal anastomosis, and at least 10 adenomas in the remaining colon/rectum. Patients were sequentially enrolled into 3 cohorts: 0.5 mg every other day (Cohort 1), 0.5 mg daily every other week (Cohort 2), and 0.5 mg daily (Cohort 3). Upper and lower endoscopy occurred at baseline and every 6 months. Primary endpoints were safety and tolerability of eRapa and percent change from baseline in polyp burden (sum of all polyp diameters). Polyp outcomes were classified into progressive disease (PD: >20% increase), stable disease (SD: ± 20% change) or partial response (PR: >20% reduction in polyp burden). A combination endpoint of non-progressors included SD and PR.

30 patients were enrolled across the dosing cohorts. 6 patients had an intact colon and 24 had an ileo-rectal anastomosis. Baseline clinical characteristics were comparable and the median age was 43 years (37-62) and 50% of participants were female. 18/30 (60%) patients had evaluable duodenal polyposis. Two related, Grade 3 Serious Adverse Events occurred with no Grade 4/5 toxicities **(Table 1)**. A significant decrease in overall mean polyp burden occurred at 6 months ($p = 0.04$) **(Table 1)**. In the duodenum, 14/18 (78%) patients were non-progressors with 11/18 (61%) of these patients with PR. In the colorectum, 26/30 (87%) patients were non progressors including all with an intact colon, including 15/30 (50%) patients with PR including 4 with an intact colon and **(Figure 1)**.

Treatment with eRapa is safe and well-tolerated with patients receiving 0.5 mg of eRapa every other day demonstrating the greatest reduction in polyp burden with fewest related AEs. Partial response was noted in the duodenum and lower GI tract and all patients with an intact colon were non-progressors. This trial shows the potential benefit of low dose eRapa to prevent progression of duodenal and lower tract polyp burden over the first 6 months of treatment.

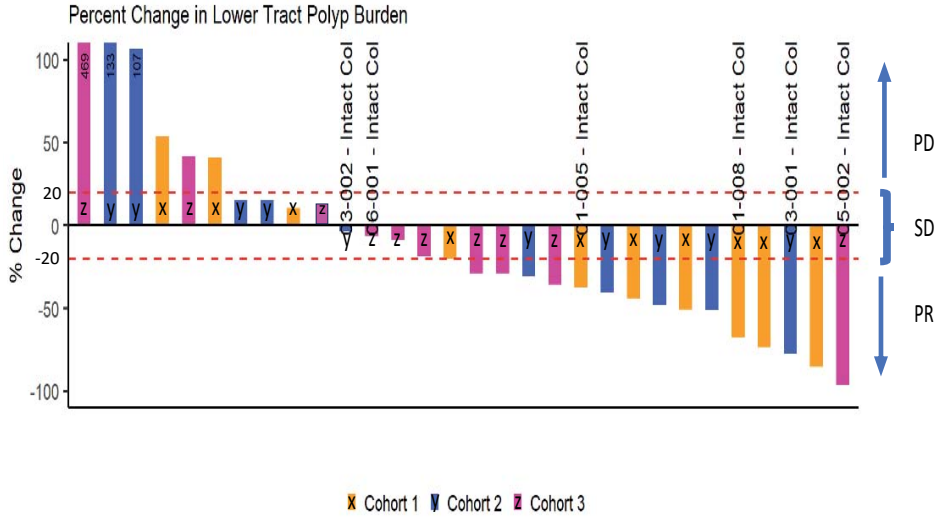
Keyword

FAP, chemoprevention, rapamycin.

Table 1. Adverse Events (AEs) and Change in Polyp Burden.

Adverse Event	Cohort 1, n=10	Cohort 2, n=10	Cohort 3, n=10	Total, n=30	p (χ2)
Any AE, n (%)	7 (70.0)	9 (90.0)	9 (90.0)	25 (83.3)	0.38
Median time to first AE, in days (95% CI)	98.0 [15.0, NA]	35.5 [2.0, 57.0]	6.0 [1.0, 151.0]	29.0 [9.0, 85.0]	
DLTs, n (%)	0	0	1 (10.0)	1 (3.3)	0.36
Serious AEs, n (%)	2 (20.0)	1 (10.0)	2 (20.0)	5 (16.7)	0.79
≥ Grade 3 AEs, n (%)	2 (20.0)	1 (10.0)	4 (40.0)	7 (23.3)	0.27
Related AEs ≥ Grade 3, n (%)	0	1 (10.0)	1 (10.0)	2 (6.7)	0.59
All Related AEs, n (%)	2 (20.0)	4 (40.0)	8 (80.0)	14 (46.7)	0.02
Mean polyp burden (mm)	Overall burden in Upper and Lower GI Locations				Lower Tract Only
Baseline	409.30	288.50	169.90	290.97	265
6 months	280.00	272.00	111.40	224.57	202.37
% reduction	32%	6%	34%	23%	24%
p (paired t-test)	0.1	0.62	0.33	0.04	0.04

Figure 1. Percent Change in Lower Tract Polyp Burden.



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BO3 - COLORECTAL CARCINOMAS FROM PATH_MSH6 CARRIERS DISPLAY A LOWER DEGREE AND/OR LATER ONSET OF MICROSATELLITE INSTABILITY AND EVOKE WEAKER IMMUNE RESPONSES

Noah C. Helderman[1], Marieke E. IJsselsteijn[2], Fabian Strobel[3], Lena Bohaumilitzky[3], Manon van der Ploeg[2], Hans Morreau[2], Magnus von Knebel Doeberitz[3], Aysel Ahadova[3], Noel F.C.C. de Miranda[2], Matthias Kloor[3], Maartje Nielsen[1]

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[2] *Department of Pathology, Leiden University Medical Center, Leiden, the Netherlands*

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Background and aims

Numerous observational and molecular studies focusing on Lynch syndrome (LS) have revealed significant variation in the phenotype and molecular characteristics among carriers of pathogenic variants in mismatch repair genes (*path_MMR*). Recently, we demonstrated that colorectal carcinomas (CRCs) from *path_MSH6* carriers exhibit fewer insertion/deletion mutations compared to CRCs from other MMR mutation groups, raising the question of whether *MSH6* CRCs might display a lower degree of microsatellite instability (MSI) and evoke weaker immune responses.

Methods

To evaluate the degree of MSI, we analyzed mutations at twenty coding microsatellites (cMS) in a cohort of 95 LS CRCs (39 *MSH6*, 18 *MLH1*, 16 *MSH2* and 22 *PMS2* CRCs) and 35 sporadic MSI CRCs, and compared mutation frequencies and mutant allele ratios among the different MMR groups. We further investigated the identified mutation profiles for a possible association with *HLA-A**02:01 status and the predicted immunogenicity of cMS mutation-derived frameshift peptides. To analyze the frequencies and spatial context of immune cells in LS CRCs, we applied a 40-marker imaging mass cytometry panel on a selected set of 40 CRCs (eight CRCs per MMR group and eight sporadic MSI CRCs).

Results

As compared to the other MMR groups, *MSH6* CRCs exhibited significantly lower mutation frequencies and mutant allele ratios across most cMS. In line with these findings, *MSH6* CRCs displayed lowest T cell infiltration levels of all MMR mutation groups, with the infiltrating T cells in *MSH6* CRCs generally showing decreased expression levels of granzyme B and PD-1. The mutant allele ratios of the cMS mutations in *MSH6* CRCs exhibited a negative correlation (*P*=.072; Pearson's r -0.536, 95% CI -0.849 – 0.055) with the predicted immunogenicity of

the resulting frameshift peptides, which may suggest a potential counterselection of cell clones bearing highly immunogenic frameshift peptides.

Conclusions

Our findings suggest that *MSH6* CRCs display a lower degree and/or later onset of MSI. As a consequence, *MSH6* CRCs appear to evoke weaker immune responses as compared to CRCs from other MMR groups. These findings may have important implications for the management of these patients, for instance with regards to immunotherapy and preventive cancer vaccine management, and if confirmed, would reinforce the notion of classifying LS as distinct syndromes associated with specific MMR genes.

Keywords

Lynch syndrome, colorectal cancer, pathogenesis, mutation profile, immune profile.

BO4 - PREVENTING CANCER IN LYNCH SYNDROME: VACCINATION WITH MUTATION-DERIVED NEOANTIGEN-LOADED DENDRITIC CELLS ELIMINATES PRECANCEROUS CELLS

Asima Abidi[1], Harm Westdorp[2], Mark A.J. Gorris[3], Steve Boudewijns[2], Roland P. Kuiper[4], Reno S. Bladergroen[4], Tanya M. Bisseling[5], Maria W.J. van Vugt-van Pinxteren[5], Fokko M. Nagengast[5], Anna L. de Goede[6], Michelle M. van Rossum[7], Alexandra J. Croockewit[8], Marjolijn J.L. Ligtenberg[9], Iris D. Nagtegaal[10], Carl G. Figdor[3], Winald R. Gerritsen[2], Gerty Schreibelt[1], Nicoline Hoogerbrugge[11], I. Jolanda M. de Vries[1]

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[4] *Princess Máxima Center for Pediatric Oncology, Utrecht, the Netherlands*

[5] *Department of Gastroenterology, Radboud university medical center, Nijmegen, The Netherlands*

[6] *Department of Pharmacy, Radboud university medical center, Nijmegen, the Netherlands*

[7] *Department of Dermatology, Radboud university medical center, Nijmegen, The Netherlands*

[8] *Department of Hematology, Radboud university medical center, Nijmegen, The Netherlands*

[9] *Department of Human Genetics, Radboud university medical center, Nijmegen, The Netherlands, Department of Pathology, Radboud university medical center, Nijmegen, the Netherlands*

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Background and aims

Lynch syndrome (LS) is an autosomal dominantly inherited cancer syndrome. Due to pathogenic variants in DNA mismatch repair genes, frameshift mutations occur that lead to proteins with an aberrant C-terminus. These are a source of neoantigens which may serve as excellent targets for immunotherapy since they are expressed by (pre)cancerous cells and not by normal tissues.

Dendritic cells (DC) are the antigen-presenting cells of the immune system. Their decisive role in inducing immunity formed the rationale for DC immunotherapy: DC loaded with tumour antigens are injected into cancer patients to stimulate T cells to eradicate tumours.

Here, LS patients were vaccinated with DC loaded with neoantigens and a tumour associated antigen **(Figure 1)**.

Methods

From 2012 to 2016, in a phase I/II clinical trial, individuals with LS who were either previously affected by colorectal cancer (CRC) or not, received DC loaded with predicted neoantigen peptides from TGF-βRII and caspase-5, and the tumor-related antigen carcinoembryonic antigen (CEA). Patients received up to 3 vaccination cycles, each consisting of 3 weekly injections.

Results

DC vaccinations were well tolerated. All vaccinated patients experienced flu-like symptoms during 2-3 days after vaccination. There were no signs of autoimmunity.

Neoantigen- and CEA-specific T cells were detected in post-treatment skin biopsies in 87% of the patients. In-vitro expanded antigen specific T cells were capable of specifically lysing tumour cells presenting exogenously loaded peptides of all three (neo)antigens included in the trial and against endogenously processed and presented TGF-βRII and CEA.

Higher probability of disease-free 10-year survival post vaccination was found to correlate with the presence of TGF-βRII specific T cells.

Conclusions

In conclusion, preventive DC vaccination is feasible and safe. LS patients who mounted a T cell response against the neoantigen TGF-βRII did not develop cancer in almost 10 years **(Figure 2)**. Moreover, within these 10 years, the tested precancerous lesions did not exhibit detectable levels of the mutated form of TGF-βRII, which is recognized by induced neoantigen-specific T cells. This confirms that T cells prevent the outgrowth of (pre)cancerous cells expressing the mutation, hence prevent the progression from adenoma to carcinoma. So, prophylactic DC vaccination offers a new non-toxic preventive treatment modality for patients with LS.

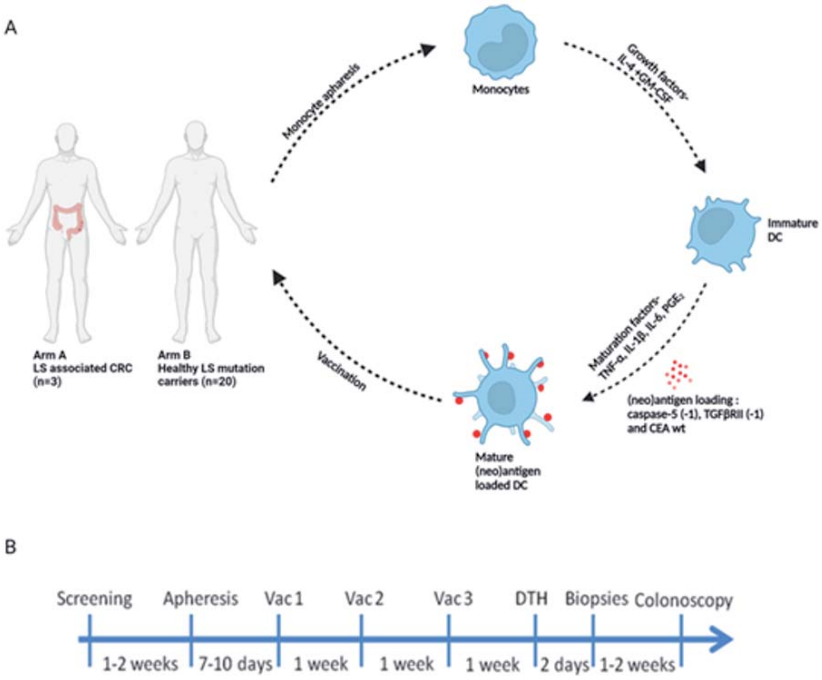

Figure 1- Schematic representation of **A**- the DC maturation and antigen-loading and **B**- the treatment schedule. CEAwt: carcinoembryonic antigen wild type; DC: dendritic cell; GM-CSF: granulocyte-macrophage colony-stimulating factor; IL: interleukin; KLH: keyhole limpet hemocyanin; PGE2: prostaglandin E2; TGFβRII: transfoming growth factor beta receptor II; TNF-α: tumor necrosis factor-alpha.

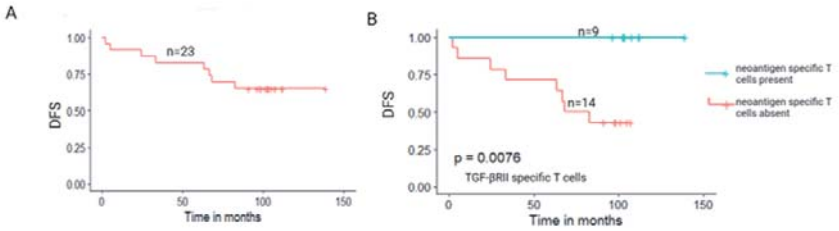

Figure 2: Kaplan Meier Curves showing **A**- disease free survival (DFS) probabilities for all patients included in the trial. **B**- DFS probability for patients with or without neoantigen specific T cells. Presence of TGF-βRII specific T cells have a longer DFS as compared to patients that do not.

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BO5 - DEVELOPMENT AND VALIDATION OF A MIRNA-BASED SIGNATURE, POWERED BY MACHINE LEARNING, FOR PREDICTING 10-YEAR RECURRENCE-FREE AND OVERALL SURVIVAL AFTER CURATIVE-INTENT TREATMENT IN EARLY-ONSET COLORECTAL CANCER

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[3] *Gastroenterology Department, Hospital Clinic of Barcelona, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Universitat de Barcelona, Institut d' Investigacions Biomediques August Pi I Sunyer (IDIBAPS); Barcelona*

[4] *Institute of Biomedical Research of Salamanca (IBSAL-SACYL), University of Salamanca-CSIC, Paseo de San Vicente; Salamanca, Spain*

[5] *Division of Gastroenterology, School of Medicine, University of California San Diego; La Jolla, CA, USA*

[6] *Department of Molecular Diagnostics and Experimental Therapeutics, Beckman Research Institute of City of Hope; Monrovia, CA, USA and City of Hope Comprehensive Cancer Center; Duarte, CA, USA*

Introduction

Patients with early-onset colorectal cancer (EOCRC, diagnosis before age 50 with no hereditary CRC syndrome) have a significant risk of metachronous CRC. Although often used, there is no consensus on intensive endoscopic surveillance after curative treatment. To overcome the lack of prognostic biomarkers that predict recurrence-free survival (RFS), we developed and validated a miRNA signature to identify patients at high risk of recurrence.

Methods

We identified a panel of differentially expressed miRNAs (RFS> vs. <5 years) through small RNA sequencing, followed by Cox-LASSO regression and AUC analysis, in a discovery cohort of stage II-III EOCRC. We then trained a machine learning algorithm (eXtreme Gradient Boosting, XGB) on RT-qPCR miRNA results from a European cohort of stage I-III EOCRC (n=88). This assay was then independently validated in an Asian cohort (n=69).

Results

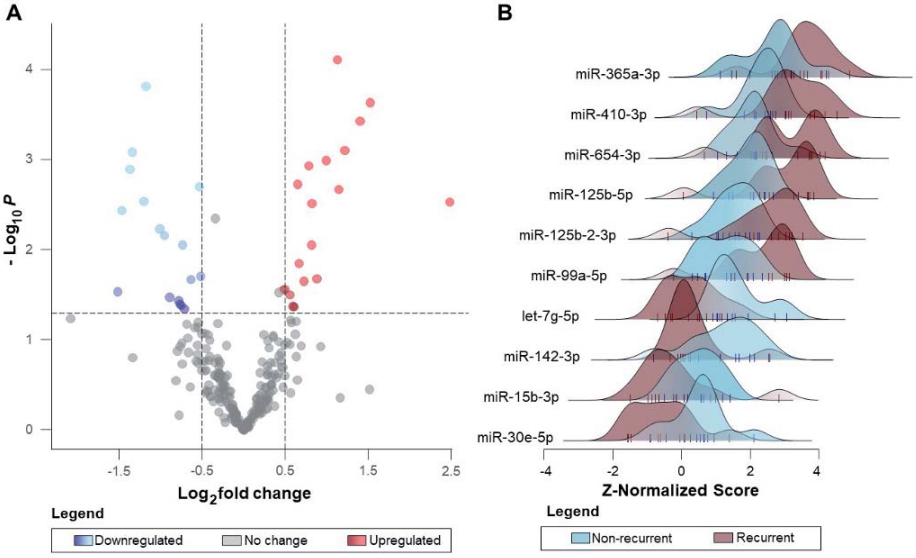
Small RNA sequencing identified 35 differentially expressed miRNAs (**Figure 1A**), and Cox regression prioritized a panel of 10 markers (**Figure 1B**). Six candidates were independently associated with a higher risk of recurrence, while four were associated with a lower risk (**Figure 1C-D**).

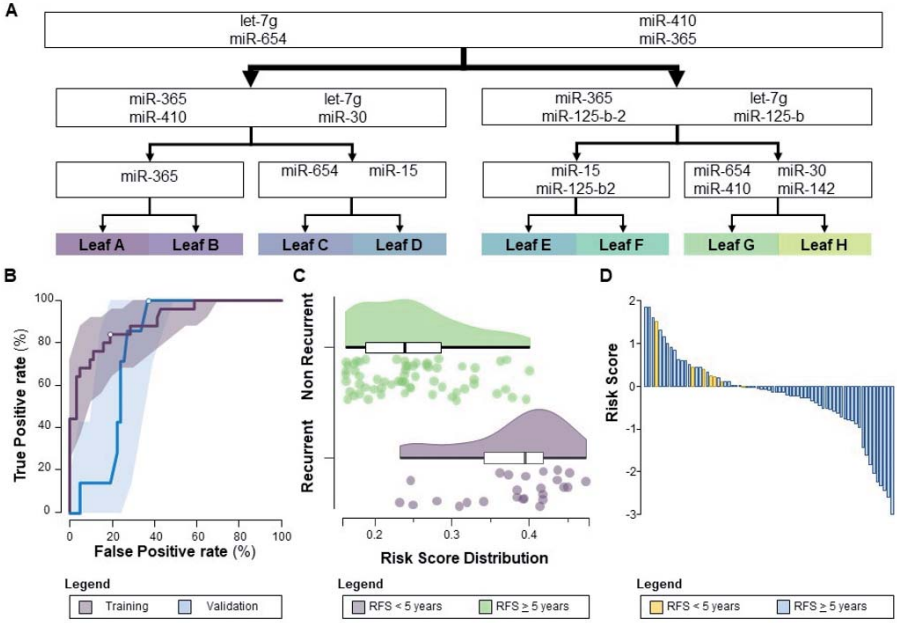
Transitioning from sequencing to RT-qPCR, XGB leveraged all candidates (**Figure 2A**) to train a highly accurate prognostic model (AUC=90.1%, $CI_{95\%}$=83-97%), which was successfully validated (AUC=77.3%, $CI_{95\%}$=66-89%, **Figure 2B**). In the training cohort, EOCRC patients with RFS<5 years were consistently assigned to lower risk scores (**Figure 2C**), a finding that was replicated in the validation cohort with 100% sensitivity (**Figure 2D**).

Patients labeled as high-risk had a statistically higher risk of both 5-year RFS and 10-year RFS in both training (5-year RFS=34.4% vs. 92.4%; 10-year RFS=25.8% vs. 92.4%; log-rank test p<0.0001, **Figure 2E**) and validation cohorts (5-year and 10-year RFS=75% vs. 100% for both; log-rank test p=0.0019, **Figure 2F**). Similarly, RFS event-rates in the training cohort resulted in significant overall survival differences in both the training (10-year OS=52.1% vs. 97.9%; 15-year OS=34.7% vs. 97.9%, log-rank test, p<0.0001) and validation cohorts (10-year OS=77.0% vs. 100%, 15-year OS not reached yet, log-rank test, p=0.013).

Conclusion

A miRNA-based signature, powered by advanced machine learning, can predict up to 10-year RFS and OS after curative-intent treatment of EOCRC. This signature offers an alternative surveillance strategy to overly intensive endoscopic surveillance.


A
- Log10 P
Log2 fold change
Legend
Downregulated
No change
Upregulated
B
miR-365a-3p
miR-410-3p
miR-654-3p
miR-125b-5p
miR-125b-2-3p
miR-99a-5p
let-7g-5p
miR-142-3p
miR-15b-3p
miR-30e-5p
Z-Normalized Score
Legend
Non-recurrent
Recurrent
C
Sex
Location
Stage
Grade
MSI
Recurrent
Color Key
D
Hazard Ratio
of Recurrence [95% CI]
7.2 [1.9 – 26.9]
2.7 [1.3 – 5.7]
2.7 [1.2 – 6.1]
6.0 [1.7 – 20.9]
8.4 [1.8 – 39.1]
6.6 [1.6 – 28.0]
.003 [.00 – .13]
.30 [.10 – .86]
.07 [.01 – .55]
.02 [.00 – .33]
Legend
Protective Factor
Risk Factor


A
let-7g
miR-654
miR-410
miR-365
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miR-30
miR-365
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let-7g
miR-125-b
miR-365
miR-654
miR-15
miR-15
miR-125-b2
miR-654
miR-410
miR-30
miR-142
Leaf A
Leaf B
Leaf C
Leaf D
Leaf E
Leaf F
Leaf G
Leaf H
B
True Positive rate (%)
False Positive rate (%)
Legend
Training
Validation
C
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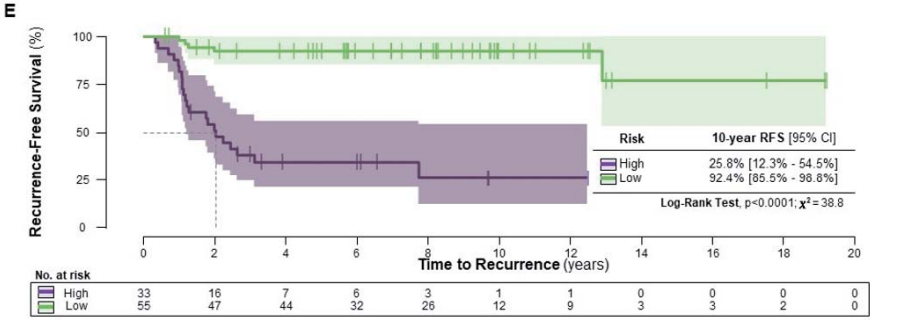
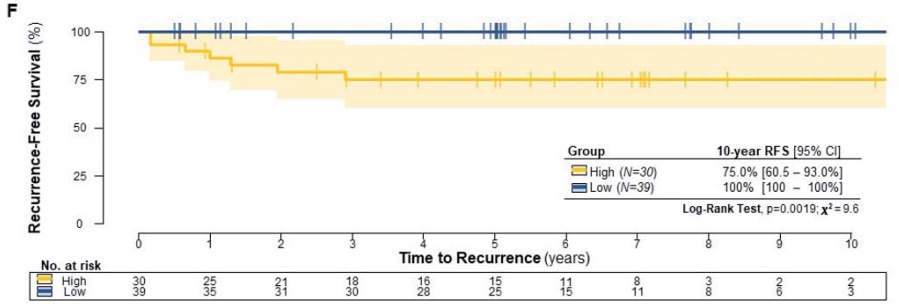
Recurrent
Risk Score Distribution
Legend
RFS < 5 years
RFS ≥ 5 years
D
Risk Score
Legend
RFS < 5 years
RFS ≥ 5 years


E
Recurrence-Free Survival (%)
Risk
10-year RFS [95% CI]
High
25.8% [12.3% - 54.5%]
Low
92.4% [85.5% - 98.6%]
Log-Rank Test, p<0.0001; χ² = 38.8
Time to Recurrence (years)
No. at risk
High 33 16 7 6 3 1 1 0 0 0 0
Low 55 47 44 32 26 12 9 3 3 2 0


F
Recurrence-Free Survival (%)
Group
10-year RFS [95% CI]
High (N=30)
75.0% [60.5 – 93.0%]
Low (N=39)
100% [100 – 100%]
Log-Rank Test, p=0.0019; χ² = 9.6
Time to Recurrence (years)
No. at risk
High 30 25 21 18 16 15 11 8 3 2 2
Low 39 35 31 30 28 25 15 11 8 6 3

BO6 - HOMOPOLYMER SWITCHES MEDIATE ADAPTIVE MUTABILITY IN MISMATCH REPAIR-DEFICIENT COLORECTAL CANCER

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Background and aims

Mismatch repair (MMR) deficient colorectal cancer predictably evolves through the continuous erosion of microsatellite homopolymer sequences in target genes (e.g. *TGFBR2*, *BAX* etc). Curiously, the MMR genes – for example *MSH6* and *MSH3* – also contain coding

homopolymer sequences and these are frequent mutational targets in MMR-deficient cancers. The impact of secondary (i.e. subclonal) MMR homopolymer mutations on MMR-deficient colorectal cancer evolution is unknown.

Method

We leveraged publicly available datasets (including Genomics England and TCGA), whole exome profiling of patient-matched microdissected MSH6-deficient and proficient tumor regions, patient-derived organoid *in vitro* studies, multiplex immunohistochemistry, molecular evolution analyses, and mathematical modelling to profile mutation burden, mutation bias, clonal complexity and immune evasion of MMR-deficient colorectal cancer.

Results

By mapping the clonal topography of MMRd colorectal cancer, we show that subclonal *MSH6* or *MSH3* homopolymer indel mutations in the context of preceding *MLH1/PMS2* (epi) mutation increase mutation rate and shift clonal mutation bias. These results are corroborated by mutation accumulation studies in patient-derived organoids of MMR-deficient colorectal cancer. Lineages carrying secondary MMR mutations show increased neoantigen burden and greater clonal HLA complexity. Spatial immune profiling reveals increased clonal immune cell infiltration. Combined mathematical modelling and molecular evolution studies demonstrate that MMR-deficient cancers balance the immune evasion benefits of secondary MMR mutations against the genotoxic fitness costs of increased genomic mutation rates. Indeed, phylogenetic analyses from multiregion-sampled tumors show that immune-adapted lineages clonally expand following restoration of homopolymer reading frame and MSH6 re-expression.

Conclusions

Overall, our data reveal that MMRd cancers exploit homopolymer microsatellite sequences in *MSH6* and *MSH3* as ON/OFF switches to tune intratumor heterogeneity to immune selection. This reveals layers of mutational complexity and microsatellite biology in MMRd cancer evolution previously hidden in bulk analyses. This evolutionary adaptive process can be traced by examining the mosaic pattern of *MSH6* and *MSH3* labelling in MLH1/PMS2-deficient tumors.

Figure 1

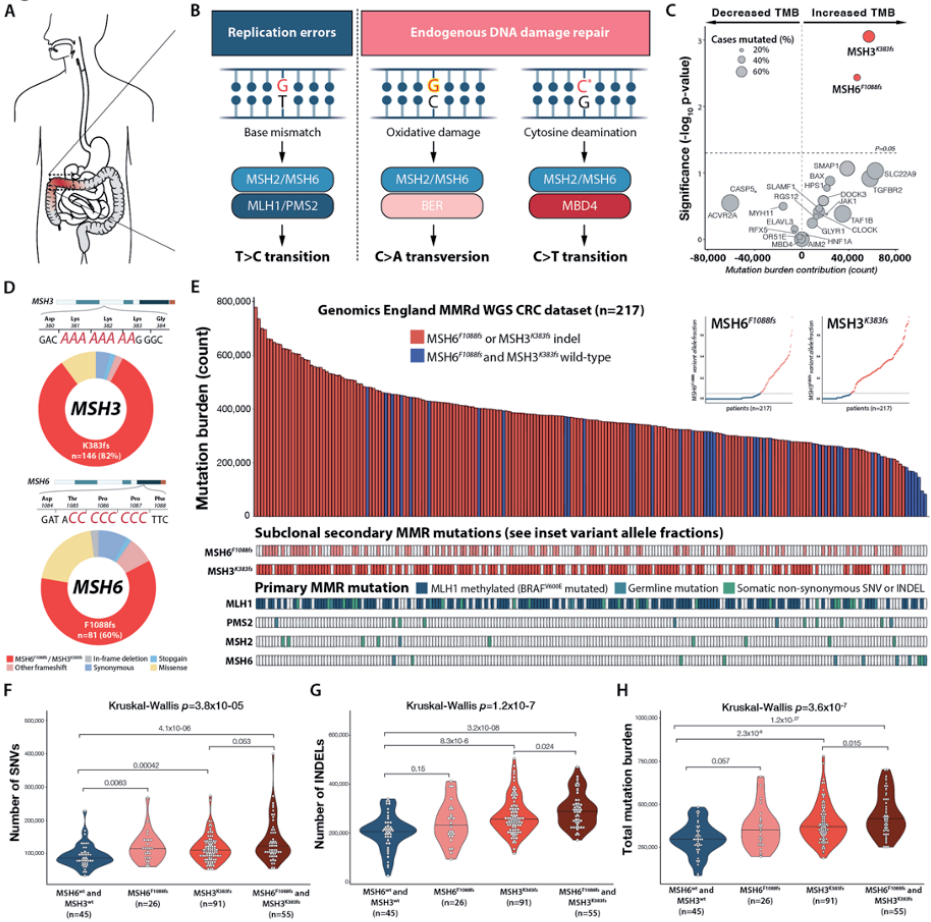

Figure 1. Subclonal MSH6^{F1088fs} and MSH3^{K383fs} homopolymer frameshift mutations drive increased mutation burden in the MMRd CRC Genomics England WGS cohort. | (A) Microsatellite instable colorectal cancer. (B) The MMR system safeguards genomic integrity by detecting and repairing replication-associated mismatches (left, blue) and endogenous DNA damage (right, pink). (C) Volcano plot showing relationship between microsatellite frameshifts in individual genes and total mutation burden in multiple linear regression analysis. (D) Pie charts showing mutation categories for MSH3 (top) and MSH6 (bottom). (E) Cases with MSH6^{F1088fs} and/or MSH3^{K383fs} homopolymer frameshifts (in red) and cases without such mutations (in blue) ranked by mutation burden (n=217). Clonal alterations in MMR genes MLH1, PMS2, MSH2 and MSH6, as well as subclonal MSH6^{F1088fs} and MSH3^{K383fs} frameshift status is indicated in the panels below. Insets show MSH6^{F1088fs} and MSH3^{K383fs} mutation variant allele fraction. Supplementary data shows analysis restricted to BRAF^{V600E} tumours. (F-H) SNV, indel, and total mutation burden according to MSH6^{F1088fs} and MSH3^{K383fs} mutation status. Median values represented by horizontal black lines.

Figure 2

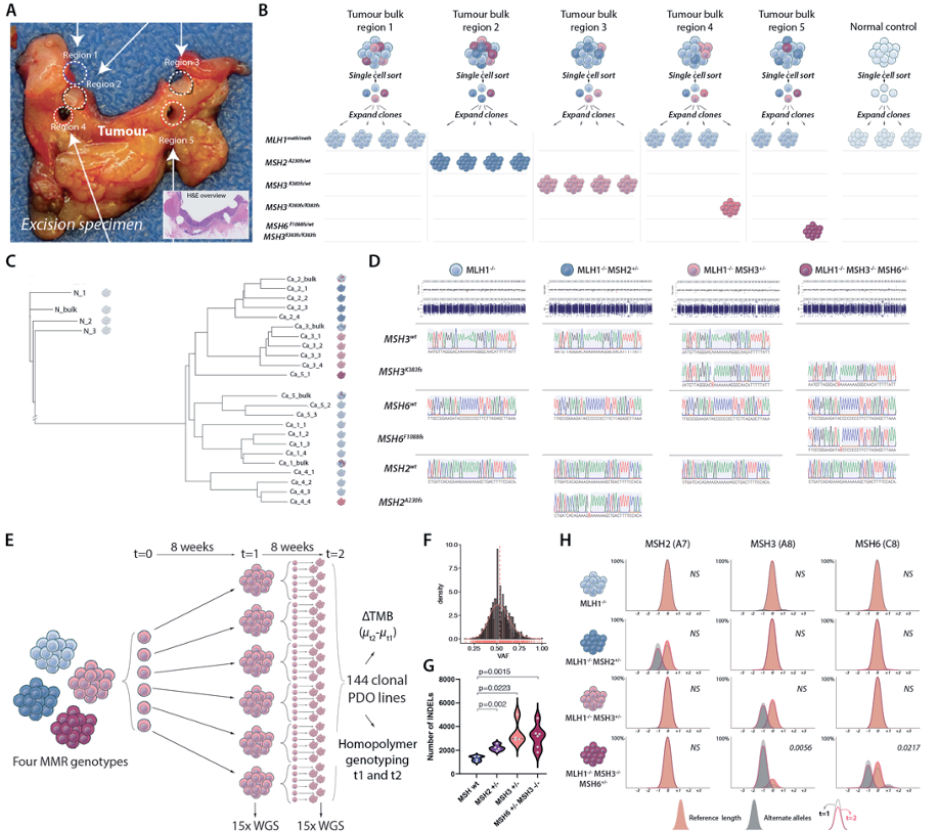

Figure 2. Incremental MMR mutations in patient-derived organoids (PDOs). | (A) Macro picture of sampled excision specimen, tumour regions 1 to 5 indicated. Inset shows corresponding haematoxylin & eosin-stained tumour section. (B) Cartoon showing clonal PDO derivation strategy. Bulk samples were briefly expanded and subcloned at passage 1. Individual clonal organoids were expanded and genotyped at passage 4 (15x WGS). (C) Neighbour joining tree showing lineage relationships of bulk and clonal organoids. (D) Homopolymer genotyping confirms allelic status of MMR homopolymers as shown. (E) Cartoon extended culture mutation accumulation experiment, see text for details. (F) Variant allele fraction density distribution shows symmetric binomial distribution around 0.5 confirming single cell origin. (G) Indel burden across MMR PDO genotypes after 8 weeks of extended culture (Welch's t test). (H) Homopolymer population diversity analysed at t=1 (black) and t=2 (pink). Beige shows reference length and grey shows alternate alleles (Fisher's exact test).

FLASH TALKS

CHEMOPREVENTION

CLINICAL: DIAGNOSIS, ENDOSCOPY, MANAGEMENT, IMPLEMENTATION

EPIDEMIOLOGY AND REGISTRIES

GENETICS

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FT1 - DIAGNOSTIC YIELD AND CHARACTERIZATION OF ENDOSCOPIC SURVEILLANCE IN CDH1 PATHOGENIC VARIANT CARRIERS IN SPAIN

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Background

Approximately 1-3% of gastric cancers (GC) are hereditary, with hereditary diffuse GC being the most significant, primarily caused by a germline pathogenic variant in *CDH1*. Characterized by the multifocal presence of signet ring cell carcinoma (SRCC) in the gastric mucosa, SRCC foci are nearly universal in *CDH1* carriers, described in up to 95% of total gastrectomies. Preventive strategies involve prophylactic total gastrectomy or endoscopic surveillance. However, the effectiveness of endoscopic surveillance yields conflicting results between studies.

Aim

Analyze the diagnostic yield of SRCC under endoscopic surveillance. Additionally, describe endoscopic surveillance characteristics and potential risk factors for GC.

Method

Retrospective multicenter study including *CDH1* carriers under surveillance in 11 Spanish high-risk digestive cancer clinics from 2010 to 2022.

Results

Fifty-nine *CDH1* carriers under surveillance were included, accounting for 175 endoscopies. Of these, 47.5% (n=28) were females, with a median age of 46 years (IQR 31-54). Conventional endoscopy (50.9%) and chromoendoscopy (36.4%) were the most used types. The biopsy strategy primarily employed was the Cambridge protocol (74.5%).

Thirty-seven individuals underwent total gastrectomy; the remaining 22 continued endoscopic surveillance without identifying SRCC. Among gastrectomized individuals, SRCC was identified in 26 (70.3%). Upper gastrointestinal endoscopy identified SRCC in 10 of 22 individuals (4 cases excluded due to incomplete endoscopy information), resulting in a diagnostic yield of 45.5%. SRCC identified by endoscopy primarily depended on random biopsies, consistently presenting as T1 stage **(Figure 1)**.

Regarding factors associated with SRCC detection by endoscopy, chromoendoscopy showed significant superiority (p=0.009). No significant differences were found when comparing different biopsy strategies (Cambridge p=0.457; Sydney p=0.262; targeted biopsies p=0.840) **(Table 1)**. Analyzing potential risk factors for SRCC development revealed no significant differences in sex, family history, tobacco, alcohol, NSAIDs, PPIs, or H. pylori.

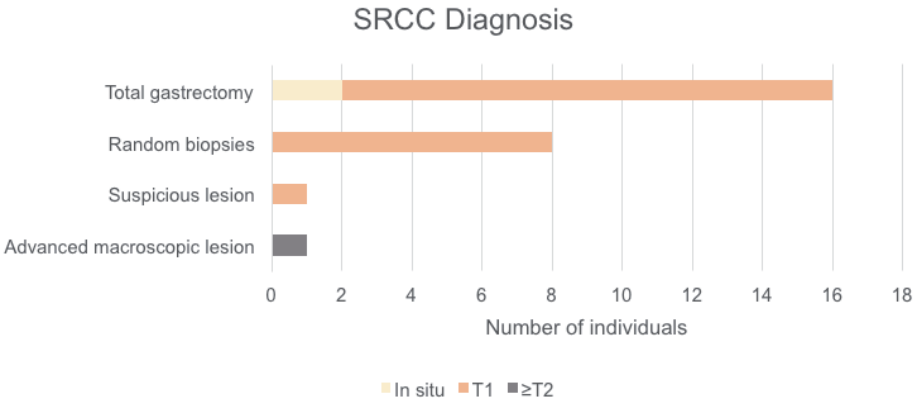
Conclusions

The diagnostic yield of SRCC by upper gastrointestinal endoscopy in *CDH1* carriers is 45.5%. Chromoendoscopy is significantly associated with greater detection. Improving endoscopic techniques and/or utilizing biomarkers is necessary to increase lesion detection.

Table 1. Quality of the endoscopy and SRCC detection.

	Total (N=22)	SRCC detected by endoscopy (N=10)	No SRCC detected by endoscopy (N=12)	P
Endoscopy type				
Chromoendoscopy (N, %)	7 (31,8%)	6 (60%)	1 (8,3%)	0,009
High definition (N, %)	2 (9,1%)	1 (10%)	1 (8,3%)	0,892
Conventional (N, %)	13 (59,1%)	3 (30%)	10 (83,3%)	0,011
Biopsy strategy				
Cambridge (N, %)	17 (77,3%)	7 (70%)	10 (83,3%)	0,457
Sydney (N, %)	1 (4,5%)	1 (10%)	0	0,262
Targeted (N, %)	4 (18,2%)	2 (20%)	2 (16,7%)	0,840

Figure 1. Methods of first SRCC diagnosis.



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FT2 - MESALAMINE FOR COLORECTAL CANCER PREVENTION PROGRAM IN LYNCH SYNDROME (MESACAPP)

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Background

Safe chemopreventive alternatives to aspirin is lacking in Lynch syndrome. In vitro, mesalamine (5-ASA), a well-tolerated drug that had been used for over 30 years in ulcerative colitis, reduces MSI via improvement of replication fidelity. 5-ASA activates a replication checkpoint thereby allowing more time for cells to pass through S-phase leading to less replications errors. Such a chemopreventive effect was not observed upon aspirin/ASA, which has no effect on MSI either. 5-ASA has minimal toxicity as it is delivered to the colon through slow release formulations and immediately inactivated (N-acetylated) within the colonic mucosa. The drug is not systemically active. Thereby it would fulfill all requirements for a designer drug for CRC prevention in LS and an alternative to aspirin. In addition epidemiological data support

its chemopreventive properties in humans as it reduces the risk of CRC in patients with ulcerative colitis.

Aim

The primary aim of the study is to investigate the effect of regular treatment with mesalamine (5-ASA) on the occurrence of any colorectal neoplasia, tumor multiplicity (the number of detected adenomas/carcinomas) and tumor progression in LS patients. The secondary aim is to examine effect on microbiota and gut immunology.

Method

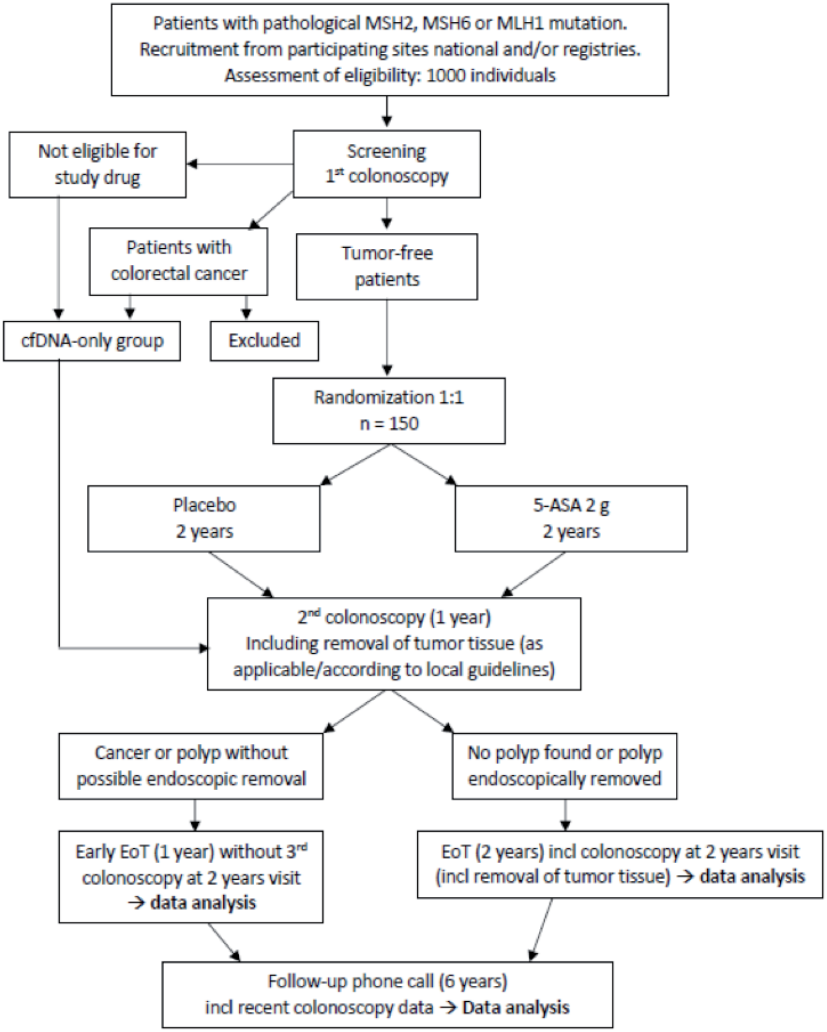
This is an ongoing multicenter, multinational, randomized, 2-arm, double-blind, phase II clinical study with 2000mg mesalamine (5-ASA) or placebo in LS patients for a 2-year treatment. 150 tumor free carriers of a known genetic mutation in a major MMR gene will be randomized 1:1 to receive 2000mg mesalamine or placebo. Tumor free patients, assessed by white light high resolution colonoscopy, will be randomized to the study. Blood and stool samples will be collected for analysis of microbiota, ctDNA and potential biomarkers. Biopsies of the normal tissue of ascending colon and rectum will be taken. Gut immunology will be studied.

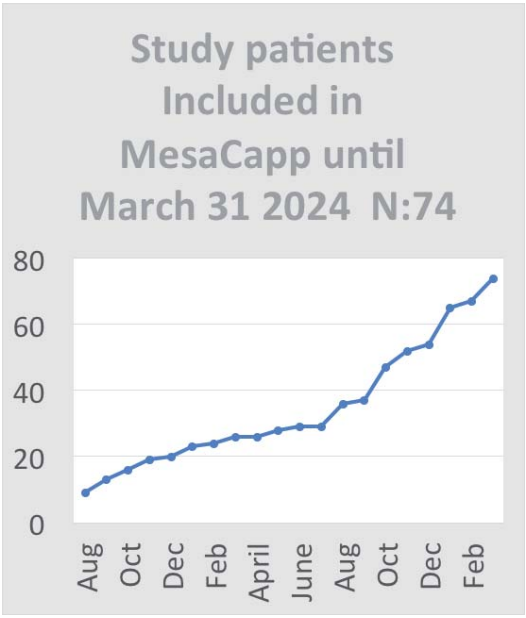
Results

From March 2022-2023 6 sites started to recruit patients in Sweden and Denmark. In Dec 2023 52/150 patients were included **(Table 1)**. The screening log and reasons for non-inclusion in two large Swedish sites is presented in **Table 2**. No adverse effects were observed for the 20 patients that had taken the study-drug over one year.

Conclusion

Chemopreventive alternatives to aspirin needs to be evaluated. Mesalamine is a well-tolerated drug with anti-inflammatory properties in the gut. MesaCapp -study is recruiting until Sept 2025 and interested new sites and patients are welcome.


Patients with pathological MSH2, MSH6 or MLH1 mutation.
Recruitment from participating sites national and/or registries.
Assessment of eligibility: 1000 individuals
Not eligible for study drug
Screening 1st colonoscopy
Patients with colorectal cancer
Tumor-free patients
cfDNA-only group
Excluded
Randomization 1:1
n = 150
Placebo 2 years
5-ASA 2 g 2 years
2nd colonoscopy (1 year)
Including removal of tumor tissue (as applicable/according to local guidelines)
Cancer or polyp without possible endoscopic removal
No polyp found or polyp endoscopically removed
Early EoT (1 year) without 3rd colonoscopy at 2 years visit → data analysis
EoT (2 years) incl colonoscopy at 2 years visit (incl removal of tumor tissue) → data analysis
Follow-up phone call (6 years)
incl recent colonoscopy data → Data analysis


Study patients Included in MesaCapp until March 31 2024 N:74
80
60
40
20
0
Aug
Oct
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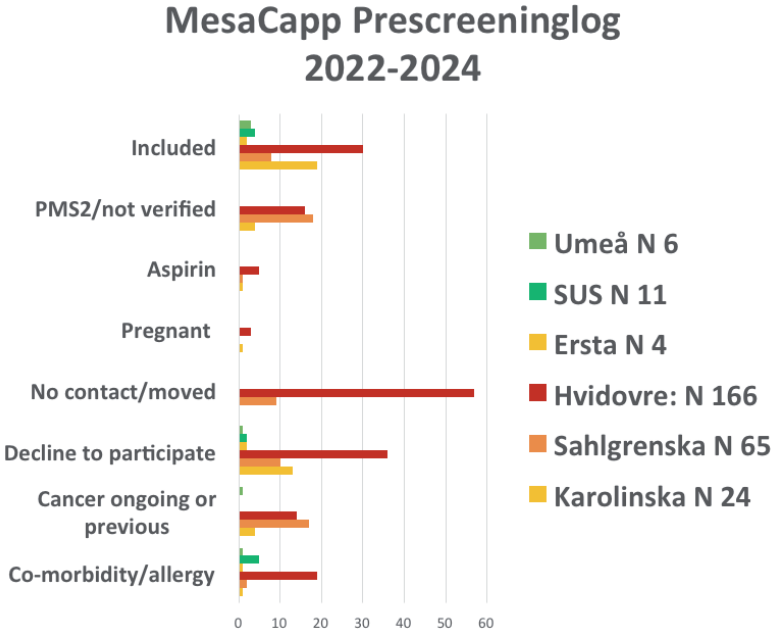
Feb


MesaCapp Prescreeninglog 2022-2024
Included
PMS2/not verified
Aspirin
Pregnant
No contact/moved
Decline to participate
Cancer ongoing or previous
Co-morbidity/allergy
0
10
20
30
40
50
60
Umeå N 6
SUS N 11
Ersta N 4
Hvidovre: N 166
Sahlgrenska N 65
Karolinska N 24

FT3 - A PROSPECTIVE ANALYSIS IDENTIFIED MISMATCH REPAIR GENES AS CANDIDATE PREDISPOSING GENES FOR UVEAL MELANOMA

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Background and aims

Uveal melanoma (UM) is the most common intraocular malignancy in adults. Germline mutations of two genes have been previously identified to be predisposing to UM with a high penetrance: *BAP1*, responsible for the *BAP1* tumor predisposition syndrome (*BAP1*-TPDS), and *MBD4*, whose germline alterations are associated with tumor predisposition, specific tumor mutational signature and good response to immunotherapy. Nevertheless, only a fraction of familial and bilateral/multifocal forms of UM can be explained by *BAP1* or *MBD4* germline alterations, suggesting the existence of other predisposing factors.

Methods

We analyzed a panel of 122 genes by targeted sequencing on germline DNA from a cohort of 381 patients diagnosed with UM at Institut Curie since July 2021.

Results

We identified 30 pathogenic variants (PVs) in 15 hereditary cancer predisposition genes (*ATM* (N=1), *BAP1* (N=3), *BRCA2* (N=3), *ERCC2* (N=1), *FAN1* (N=3), *FANCD2* (N=1), *FANCL* (N=1),

FANCM (N=2), *MBD4* (N=6), *MLH1* (N=1), *MSH2* (N=1), *MSH6* (N=2), *MUTYH* (N=1), *NBN* (N=1), *PMS2* (N=3)). Two patients had PVs in *MSH6*, three in *PMS2*, one in *MLH1* and one in *MSH2*. The frequencies of *MSH2* and *MSH6* PVs were significantly higher than in the gnomAD v2.1.1 database as a control cohort (p=0.047, OR: 22.04 [0.52-143.71]; p=0.03, OR: 7.6 [0.9-28.5], respectively), and higher by pooling the MisMatch Repair (MMR) genes *MLH1*, *MSH2* and *MSH6* (p=0.00073, OR: 10.4 [2.8-27.5]), *PMS2* being excluded from this statistical analysis because its pseudogene prevents the interpretation of public data. One UM tumor was available from these MMR-mutated patients, for the germline *MLH1* PV. This tumor presented a biallelic inactivation of *MLH1*, MSI-H phenotype, and loss of MLH1 and PMS2 expression in immunohistochemistry. Whole Genome Sequencing (WGS) of the tumor revealed a prominent Substitution Mutational Signature (SBS) 6, which is associated with MMR deficiency.

Conclusions

This study confirms MMR genes as potential predisposition factors in UM, as suggested by a previous study identifying a germline *MLH1* PV and biallelic inactivation in a UM. Further analyses will be needed to define the risk to develop UM for patients with a Lynch syndrome.

FT4 - BIRTH COHORT EFFECT ON AGE OF COLORECTAL CANCER ONSET IN LYNCH SYNDROME

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Background and aims

In the general population, the incidence of early onset colorectal cancer (CRC), defined as diagnosis before age 50, is rising, and a birth cohort effect has been observed in that younger generations have higher CRC incidence compared to older generations. Whether hereditary cancer syndromes that predispose to CRC, such as Lynch syndrome, follow similar birth cohort trends as the general population has not been explored previously. The aim of this study was to evaluate birth cohort effects on age of CRC in Lynch syndrome patients.

Methods

Lynch syndrome patients, defined by having a pathogenic or likely pathogenic variant in a mismatch repair gene (*MLH1, MSH2, MSH6*, *PMS2* and *EPCAM*) were identified from an academic registry between 1990-2023. Demographics, genetic data and cancer incidence were collected. Kaplan-Meier curves were used to assess the age of cancer incidence by birth cohorts, and Cox regression was used to adjust for confounders. Analyses were performed separately for each Lynch syndrome-associated gene.

Results

In total, 151 Lynch syndrome patients were identified. Clinical characteristics are shown in **Table 1**. Earlier age onset of CRC was noted by birth cohort (**Figure 1A)**, and between those born before or after 1970 (**Figure 1B**). When the Lynch syndrome genes were analyzed individually, significant differences between those born before or after 1970 were found for *MSH2* and *PMS2* carriers, while results were borderline for *MLH1* carriers and not significant for *MSH6* carriers (**Figure 1C-F**). Using Cox regression to adjust for stage of CRC, birth year and proband status, later birth year was noted to be the only significant predictor for earlier age CRC (HR 1.20, 95% CI 1.14-1.26). Exclusion of probands, to control for ascertainment bias, resulted in similar findings, as non-probands born in later generations were more likely to develop earlier age CRC (HR 1.11 95% CI 1.02-1.21). There were no significant differences between birth cohorts and extra-colonic malignancies (p=0.354).

Conclusions

Younger birth cohorts of Lynch syndrome patients appear to develop CRC at earlier ages compared to older birth cohorts similar to trends observed in the general population.

Interestingly, results were not uniform across all Lynch syndrome genes and warrants further evaluation. Larger studies are needed to validate these results and to further control for ascertainment bias and possibly genetic anticipation.

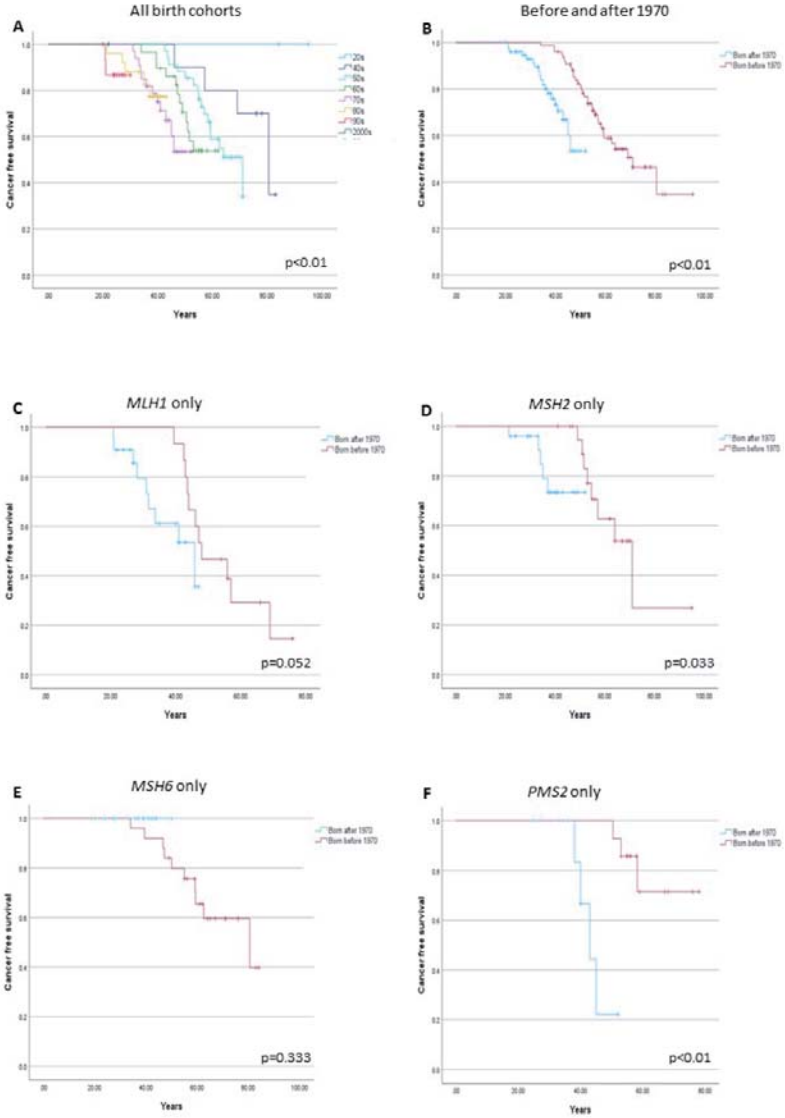

Figure 1. Age of colorectal cancer occurence in Lynch syndrome patients by (A) all birth cohorts; (B) stratified before and after 1970; (C) only in *MLH1*, (D) only in *MSH2*; (E) only in *MSH6*; (F) only in *PMS2*.

FT5 - THE GERMLINE POLD1 C.1420C>A P.(LEU474ILE) VARIANT SEGREGATES WITH ENDOMETRIAL CANCER AND COLONIC ADENOMAS DEMONSTRATING HYPERMUTATION AND DEFECTIVE POLD1 MUTATIONAL SIGNATURES

Khalid Mahmood[1,2,3], Sharelle Joseland[1,2], Mark Clendenning[1,2], Peter Georgeson[1,2], Romy Walker[1,2], Jihoon Joo[1,2], Julia Como[1,2], Susan Preston[1,2], Ryan O'Keeffe[1,2], Mark Jenkins[2,4], Christophe Rosty[1,2,5], Ingrid Winship[7,8], Finlay Macrae[7,9], Emilia IP[10], Rebecca Harris[10], Annabel Goodwin[10], Daniel Buchanan[1,2,7]

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Background and aims

Germline pathogenic variants within the exonuclease domain of the *POLD1* gene predisposes to cancer including endometrial (EC). Tumours from *POLD1* pathogenic variant carriers demonstrate genomic features including hypermutation and tumour mutational signatures (TMS) SBS10c, SBS10d and SBS20. Classification of variants within *POLD1* remains challenging.

Methods

The *POLD1* c.1420C>A p.Leu474Ile variant was identified from clinical multigene panel testing in a woman diagnosed with EC at 62yrs (person 001) and classified as a variant of uncertain significance. Person 001 and two cancer-affected cousins were recruited to the ANGELS study. FFPE tissue from person 001 (EC at 62yrs, 1 adenoma and 2 sessile serrated lesions at 67yrs), person 009 (EC at 56yrs, 3 adenomas at 60yrs), and person 010 (breast

cancer at 72yrs, EC at 73yrs, and colorectal cancer (CRC) at 74yrs and 4 adenomas, 2 sessile serrated lesions and 1 traditional serrated adenoma at 74yrs) were tested using custom multigene panel to determine tumour mutational burden (TMB; SNV+Indel mutations/Mb), microsatellite instability (Mantis) and TMS (COSMICv3.4).

Results

We identified persons 001, 009, and 010 as carriers with a further carrier (018) and three obligate carriers (002, 014, 016) identified but not part of the study (see pedigree). The mean age at first cancer diagnosis of the affected carriers was 62 years. The colonic polyp burden for persons 001, 009, 010 was 14, 18, 21, comprised predominantly of tubular/tubular villous adenomas. The ECs from 001, 009 and 010 each demonstrated hypermutation (41.9, 101.2, 63.3) and the *POLD1*-deficiency related SBS10d (proportions of 0.46, 0.12, 0.84). The CRC from 010 showed a TMB=13.9 and SBS10c (0.15) and SBS20 (0.1), while the breast cancer showed a TMB=16.5 but was absent of *POLD1* or homologous recombination deficiency TMS. Two thirds of the polyps, including adenomatous or serrated polyps, from person 010 showed SBS10c and SBS10d and all were hypermutated.

Conclusion

Tumour and adenoma profiling demonstrated genomic features associated with defective *POLD1*, including hypermutation and SBS10 (c+d) TMS, in three carriers of the *POLD1* c.1420C>A variant supporting a pathogenic classification. This study highlights the utility of somatic profiling for the identification of germline predisposition and further supports incorporating tumour profiling into variant classification approaches for *POLD1*.

FT6 - DIETARY HABITS OF PATIENTS WITH OR WITHOUT HEREDITARY CANCER SYNDROMES- A CROSS SECTIONAL COHORT STUDY

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Background and aims

Lynch syndrome (LS) and familial adenomatous polyposis (FAP), autosomal dominant hereditary colon cancer syndromes are important causes of hereditary colorectal cancer. Modifiable risk factors including diet and obesity are well known contributors to sporadic cancer, but the contribution of modifiable risk factors are less studied in people with increased genetic risk. Few studies of dietary risk in individuals with Lynch syndrome have been contraindicatory and there have been no studies assessing dietary modifiable risk factors in patients diagnosed with family adenomatous polyposis. *Our aim in this study is to understand the difference in the eating habits among individuals with hereditary GI colon cancer syndromes and their unaffected family members.*

Methods

Three hundred fifty four subjects were enrolled in the Family Microbiome Initiative. They completed general questionnaires and Fred Hutch food frequency questionnaires. We investigated the differences in several food groups and Healthy Eating Index (HEI), a score used to approximate food-based diet quality, among FAP patients, Lynch patients, unaffected family members of patients with FAP, unaffected family members of patients with LS, and healthy controls. The HEI score is composed of several food groups including, greens and beans, total vegetables, total wholegrain, total fruit, total dairy, total fatty acids, total added sugars, total sodium and total protein. We used Kruskal-Wallis test for continuous variables and Chi Test for categorial variables to compare subjects by their pathogenic variant status. Predictors of HEI score were derived using a linear regression model.

Results

Of the 354 subjects recruited the median age at enrolment was 35 years (IQR: 1 month-82) and 56% were female. The median age for each group was 36 years, 55 years, 21 years, 46 years, and 15 years respectively. There were significant differences in HEI score among these groups with underlying differences in (list which food groups were different). Unaffected family members of patient with FAP scored the lowest HEI compared to other groups, however there was significant heterogeneity in age and sex among these groups. This finding remained significant even after controlling for age and sex in a multivariable linear regression model.

Conclusion

In this sample of individuals with FAP, LS, their unaffected family members and healthy controls, we observed small differences in dietary quality according to HEI score. There was evidence suggesting a significant difference among fruit, greens, vegetables and dairy. Future directions include larger scale prospective studies in patients with FAP collecting information on diet and following for colorectal neoplasia outcomes over time.

Table 1. Characteristics of subjects with or without genetic pathogenetic variation and their dietary HEI scores.

Characteristic	N/Median with IQR	FAP, N = 45 (12.7%)	Unaffected FAP family members, N = 261 (7.34%)	Lynch, N = 501 (14.12%)	Unaffected Lynch family members, N = 211 (5.9%)	control, N = 2121 (59.88%)	p-value[2]
Age	354 (5, 64)	36 (23, 52)	21 (10, 36)	55 (43, 64)	46 (15, 62)	15 (5, 35)	<0.001
HEI	63.4 (57, 66)	63 (54, 72)	57 (52, 66)	66 (59, 76)	66 (58, 71)	65 (57, 72)	0.050
Sex							<0.001
Female	199 (56%)	31 (69%)	11 (42%)	41 (82%)	7 (33%)	109 (51%)	
Male	155 (44%)	14 (31%)	15 (58%)	9 (18%)	14 (67%)	103 (49%)	
Greens and Beans	3.094 (0.44, 5)	2.76 (1.53, 4.58)	1.76 (0.44, 3.31)	4.40 (2.69, 5.00)	3.92 (1.80, 5.00)	2.63 (1.27, 4.25)	<0.001
Total Vegetables	3.386 (2.67, 5.00)	4.19 (3.13, 5.00)	3.36 (2.33, 4.80)	4.94 (3.44, 5.00)	3.79 (2.67, 5.00)	3.65 (2.59, 5.00)	0.02
Total Wholegrain	3.354 (1.14, 8.95)	3.71 (1.77, 5.54)	2.27 (1.26, 6.30)	4.37 (1.14, 8.95)	3.95 (1.31, 5.07)	4.47 (2.09, 7.74)	0.15
Total Fruit	4.078 (1.07, 5)	3.46 (2.27, 5.00)	1.97 (1.07, 4.69)	4.96 (3.08, 5.00)	5.00 (3.22, 5.00)	5.00 (3.06, 5.00)	<0.001
Total Dairy	5.95 (3.35, 10)	5.73 (4.02, 8.84)	5.14 (3.76, 9.84)	4.87 (3.35, 7.02)	6.85 (4.46, 9.83)	7.14 (4.47, 10)	0.006
Total Fatty Acid	4.678 (2.16, 7.80)	5.05 (3.52, 6.77)	5.70 (3.44, 7.80)	4.31 (3.06, 6.63)	4.27 (2.16, 5.76)	4.06 (2.40, 5.92)	0.041

Characteristic	N/Median with IQR	FAP, N = 45 (12.7%)	Unaffected FAP family members, N = 261 (7.34%)	Lynch, N = 501 (14.12%)	Unaffected Lynch family members, N = 211 (5.9%)	control, N = 2121 (59.88%)	p-value[2]
Total Added Sugars	8.994 (3.16, 10)	8.28 (6.84, 9.73)	9.24 (3.16, 10.00)	9.12 (7.65, 10.00)	9.38 (8.42, 10.00)	8.95 (7.39, 10.00)	0.13
Total Protein	5.12 (3.1, 7.60)	4.9 (3.1, 6.6)	5.1 (3.0, 6.9)	5.6 (4.00, 7.60)	5.00 (4.6, 6.7)	5.00 (3.2, 6.9)	0.3
Total Sodium	4.646 (1.66, 7.82)	4.74 (1.97, 6.16)	4.60 (2.11, 7.82)	4.68 (2.79, 5.97)	4.20 (1.66, 6.17)	5.01 (3.33, 6.65)	0.5
1 Median (IQR); n (%)							
2 Kruskal-Wallis rank sum test; Pearson's Chi-squared test							

Table 2. Associations between demographic factors, pathogenic variant status and HEI score using multivariable linear regression adjusted for age and sex.

Characteristic	Beta (95% CI)[1]	p-value
Status		
Healthy control	Ref	
FAP patients	-3.3 (-6.9, 0.28)	0.07
Unaffected FAP family members	-4.0 (-9.0, -0.36)	0.03
LS Patients	1.(-2.8, 5.1)	0.6
Unaffected LS family members	0.3(-4.6, 5.2)	0.9
Age	0.01 (-0.06, 0.07)	0.8
Male	-2. (-5.1, -0.45)	0.02
[1] CI = Confidence Interval		

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FT7 - ADENOMATOUS POLYPS OF GERMLINE PATHOGENIC PMS2 MUTATION CARRIERS: PRELIMINARY EPIDEMIOLOGICAL AND HISTOPATHOLOGICAL REPORT FROM THE LYNCH-GPS STUDY

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Background and aims

Lynch syndrome (LS) is an inherited condition that increases the risk of cancer occurrence for its carriers, especially colorectal cancer (CRC). LS-associated CRC is caused by germline mutations in one of the mismatch repair (MMR) genes: *MLH1, MSH2, MSH6* or *PMS2*. It is currently accepted that carriers of different germline MMR gene mutations develop LS-CRC in three different carcinogenesis pathways that consequently influence the biological behaviour of the tumour. In the past decade, experts managed to obtain increasing amount of genomic information from LS-CRC samples. However, the phenotypic and genomic information regarding early adenomatous phase of LS-CRC is still relatively sparse. The LYNCH-GPS project (2021-2025) aims to characterise the MMR status of LS-associated adenomas and discover the early mutational events that promote progression into LS-CRC.

Method

Anonymised clinical data from 171 confirmed germline *PMS2* mutation carriers in the Netherlands between 1989-2023 were collected for the analysis of adenoma incidence rate. To determine the MMR status of *PMS2*-associated lesions, a total of 80 *PMS2*-associated adenomas stored in paraffin blocks were requested from PALGA for immunohistochemical (IHC) staining. 39 *PMS2*-associated adenomas were stained with MLH1 and PMS2 antibody heterodimer, while the remaining 41 are currently being processed for IHC. The collection of adenoma specimens for IHC from other MMR gene mutation carriers is ongoing.

Results

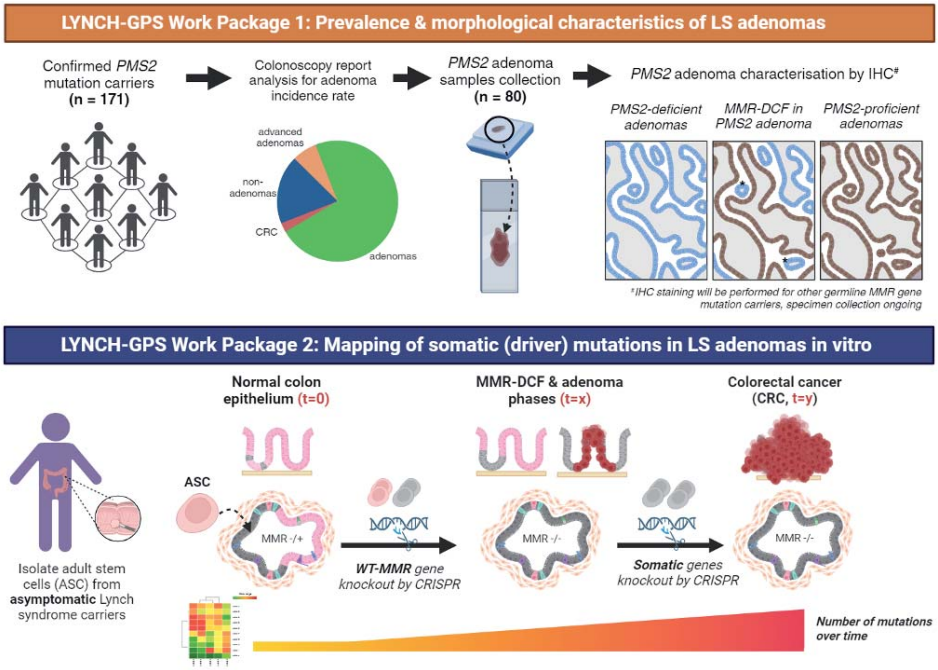
The majority of lesions removed from the colon of germline *PMS2* mutation carriers during surveillance colonoscopy were adenomatous (72.2%). IHC results from 39 *PMS2*-associated adenomas demonstrated that PMS2 protein staining is lost in 5 adenomas: 3 with tubular histology and 2 with tubulovillous histology. Several colonic crypts resembling MMR-deficient crypt foci (MMR-DCF) were discovered in 1 *PMS2*-deficient tubular adenoma.

Conclusion

The adenoma incidence rate among germline *PMS2* mutation carriers were comparable to that of germline *MSH2* and *MSH6* mutation carriers. Our preliminary histopathological findings support the late involvement of *PMS2* deficiency in the progression to CRC (Pathway 1), since the majority of *PMS2*-associated adenomas retained PMS2 protein expression on IHC.

Keywords

Lynch syndrome, PMS2, adenomatous polyp, adenoma incidence rate, immunohistochemistry.



FT8 - OPTIMIZING POLE AND POLD1 VARIANT INTERPRETATION: GENE-SPECIFIC CLASSIFICATION GUIDELINES AND IN VITRO SYSTEM FOR FUNCTIONAL ASSESSMENT

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Germline pathogenic variants within exonuclease domain (ED) of polymerases epsilon and delta (encoded by POLE and POLD1 genes, respectively) cause an autosomal dominant cancer syndrome characterized by increased risk of gastrointestinal polyps, and colorectal, endometrial, ovarian and breast cancers, among other tumors. Proofreading-defective tumors of either constitutional or somatic origin, tend to accumulate thousands of mutations (>10-100 mutations per Mb), which correspond to very specific mutational spectra: mutational signature SBS10a for POLE and SBS10d for POLD1 proofreading deficient tumors. Accurate variant classification of POLE and POLD1 is crucial for a precise clinical management, both in the hereditary cancer field as for precision medicine in cancer. However, interpretation of variants in these genes is challenging, and there is an impending need of reliable functional evidence to improve variant classification.

Our group has developed gene-specific recommendations for POLE and POLD1 variant classification, based on ACMG/AMP guidelines. These recommendations consider population frequencies, computational predictions, co-segregation, phenotypic and tumor data, functional results, and other features. They have been applied to 128 ED variants identified in patients and/or reported in public databases, allowing the classification of 35 variants as (likely) pathogenic or benign.

In parallel, we have developed an in vitro system to assess, in a high throughput manner, the functional impact of POLE and POLD1 ED variants. This method consists of modeling ED variants with CRISPR/Cas9 in the haploid human HAP1 cell line. To assess the pathogenicity/neutrality of ED variants, whole-genome sequencing at coverage 5x is performed at passage 20 in the edited cell lines to evaluate the burden and spectrum

(signatures) of accumulated mutations. To date, a pilot study with two POLE (p.T278K and p.L424V) and two POLD1 (p.L474P and p.S478N) known pathogenic variants has been performed. Additional variants classified as pathogenic and benign are currently being modeled and the system is being adapted for high throughput multiplex variant screening.

FT9 - EVALUATION OF UPPER GASTROINTESTINAL TRACT SURVEILLANCE IN INDIVIDUALS WITH LYNCH SYNDROME (EARLY) - AN INTERNATIONAL REGISTRY

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Background and aims

So far, data on prevalence of gastric adenocarcinoma, small bowel cancer and their respective precursor lesions in patients with lynch syndrome (LS) is scarce, leading to a lack of consensus regarding surveillance of upper gastrointestinal (GI) cancer and screening for risk factors such as Helicobacter pylori. Most studies addressing upper GI cancer in LS were either retrospective, monocentric or lacked key endpoints such as the impact of surveillance on mortality. With a life-time risk of 8 % and 13 % for gastric cancer and small bowel cancer respectively esophagogastroduodenoscopy (EGD) needs to prove similar efficacy as colonoscopy in treating precursor lesions and detecting early cancer in LS carriers.

Methods

Patients with LS are included in a prospective registry and will either be in a surveillance program or without any upper GI surveillance in accordance with the respective local or national guidelines and will be followed up over a course of ten years. All relevant data regarding concomitant medication, occurrence of upper GI cancer, premalignant lesions, Helicobacter pylori infection or cancer related mortality, entered via an electronic case report form, will subsequently be compared between the two groups.

Conclusions

This prospective endoscopic registry will evaluate surveillance of the upper GI tract in a large international registry of LS patients. The primary endpoint is to compare the 10-year cumulative incidence of upper GI tract neoplasms between the different surveillance strategies. Further, we want to identify factors associated with a higher risk to develop upper GI cancer as well as the impact of resecting precursor lesions on cancer prevalence. The database is designed to enable all participating centers to take part with a minimal time investment. So far 16 centers from the German consortium of hereditary colorectal cancer are participating with more than 600 LS carriers.


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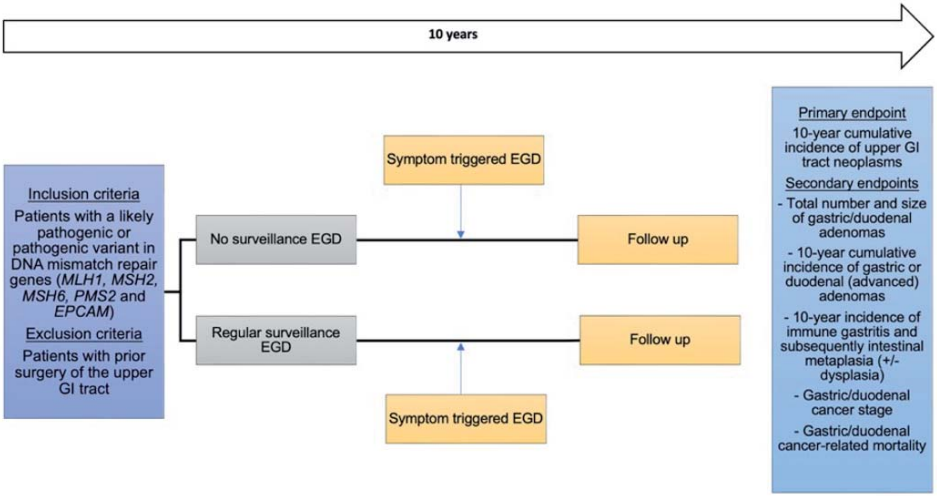
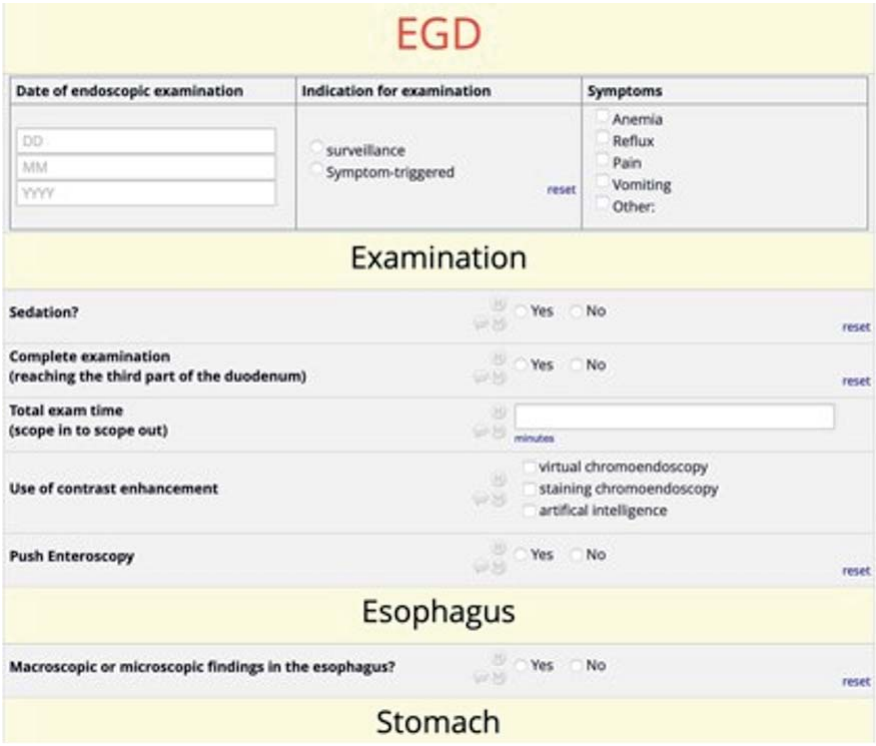

10 years
Inclusion criteria
Patients with a likely pathogenic or pathogenic variant in DNA mismatch repair genes (*MLH1, MSH2, MSH6, PMS2* and *EPCAM*)
Exclusion criteria
Patients with prior surgery of the upper GI tract
No surveillance EGD
Regular surveillance EGD
Symptom triggered EGD
Follow up
Follow up
Symptom triggered EGD
Primary endpoint
10-year cumulative incidence of upper GI tract neoplasms
Secondary endpoints
- Total number and size of gastric/duodenal adenomas
- 10-year cumulative incidence of gastric or duodenal (advanced) adenomas
- 10-year incidence of immune gastritis and subsequently intestinal metaplasia (+/- dysplasia)
- Gastric/duodenal cancer stage
- Gastric/duodenal cancer-related mortality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Reflux
Pain
Vomiting
Other:
Examination
Sedation?
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No
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Complete examination
(reaching the third part of the duodenum)
Yes
No
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Total exam time
(scope in to scope out)
minutes
Use of contrast enhancement
virtual chromoendoscopy
staining chromoendoscopy
artifical intelligence
Push Enteroscopy
Yes
No
reset
Esophagus
Macroscopic or microscopic findings in the esophagus?
Yes
No
reset
Stomach

FT10 - LONG READ SEQUENCING ELUCIDATES COMPLEX GERMLINE VARIANTS IN INDIVIDUALS UNDERGOING HEREDITARY GASTROINTESTINAL CANCER TESTING

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Background and aims

A considerable fraction of patients who undergo clinical genetic testing present with a personal and family history suggestive of hereditary gastrointestinal (GI) cancer syndromes, and often receive negative germline results. Conventional testing, based on short read (SR) sequencing, may fail to detect structural variants (SV) and mobile element insertions (MEI), which could be resolved with long read sequencing (LRS). We sought to evaluate the clinical utility of LRS by exploring cases suspected to have Lynch syndrome (LS) missing heritability, by elucidating DNA variation in cases with abnormal RNA findings and by resolving a conflicting *PMS2* duplication.

Methods

Multiple LRS approaches were leveraged to study cases with negative or dubious germline findings. These included genome sequencing and a multigene hybrid capture panel covering all exons and introns of bona fide hereditary GI cancer genes. Further details about selected cases are included in **Table 1**. Patient samples underwent SR capture DNA and RNA sequencing, as well as MLPA as part of their clinical diagnostic testing.

Results

We identified the genetic cause of LS in 1/17 (5.9%) patients in the LS missing heritability cohort. The proband was a carrier of a novel 90kb *PMS2* exon 1 Inversion and was diagnosed with CRC at age 60, with PMS2 loss in his tumor. The proband has four siblings with LS-related cancers (ages at diagnosis: 51-67). Review of SR RNA data uncovered complete monoallelic expression, supporting the pathogenic nature of the inversion. We also evaluated the use of LRS to resolve cases with abnormal RNA findings. In two cases with >50 colonic polyps where RNA data supported skipping of *APC* exon 14, we uncovered an Alu-mediated complex recombination event in intron 14, in proximity to the splice donor site. Genome sequencing in a proband from a family fulfilling Amsterdam II criteria identified a 3kb SVA (SINE-VNTR-Alu) insertion that caused skipping of *MSH2* exon 2. In a cancer-free case with a suspected *PMS2* exon 11 duplication, we relied on PacBio's Paraphase tool to fully phase *PMS2*/*PMS2CL* haplotypes, which led to the identification of *PMS2CL* variants that drove the false positive result reported by MLPA.

Conclusions

By using DNA LRS, we identified and fully characterized SVs and MEIs, previously missed by conventional approaches. This allowed us to uncover the genetic basis of LS in one patient in the missing heritability cohort, providing evidence to further expand these efforts to larger discovery series. Leveraging available RNA data was crucial to elucidate the effect of the identified DNA variants. Similarly, cases with abnormal RNA findings could be resolved with LRS. Finally, LRS constitutes a new approach to address the uncertainty related to *PMS2* variants located within the region of high sequence similarity to *PMS2CL*.

Table 1. Selected cases for DNA long read sequencing.

Selected cases	Personal cancer history	Ages of diagnosis	Family history[1]	DNA LRS	Findings
LS Missing heritability (n=17)	CRC/Endometrial (n=15) Biliary tract (n=1) Urothelial (n=1)	39-77 (Median 50)	≥ 1 FDR (n=13) ≥ 2 SDRs (n=4)	Hybrid capture	*PMS2* Ex1 Inversion (1/17 cases)
APC Ex14 skipping (n=2)	Proband 1: >50 polyps[2] Proband 2: >50 polyps[3] + Breast cancer	57 71	None 2 FDRs, 1 SDR	Hybrid capture, Amplicon sequencing	*APC* Intron 14 complex rearrangement. Alu-mediated.
MSH2 Ex2 skipping (n=1)	Endometrial	55	2 FDRs	Genome sequencing	*MSH2* c.212-15_212-14 insSVA
PMS2 Ex11 duplication[4] (n=1)	No cancer	NA	1 FDR, 1 SDR, 1 TDR	Hybrid capture	No *PMS2* Ex11 duplication identified

Abbreviations: LS, Lynch Syndrome; CRC, colorectal cancer; FDR, First-degree relative; SDR, Second-degree relative; TDR, Third-degree relative; LRS, long-read sequencing; Ex, exon.

1. LS related cancer types identified: colorectal, endometrial, ovarian, gastric, small bowel, urinary tract, biliary tract, prostate, pancreatic.

2. Adenomatous/hyperplastic.

3. Unspecified type.

4. Detected by multiplex ligation-dependent probe amplification (MLPA).

POSTERS

CANCER PATHWAY-SOMATIC MUTATIONS, PATHOLOGY

CLINICAL: COUNSELLING, PSYCHOSOCIAL

CLINICAL: DIAGNOSIS, ENDOSCOPY, MANAGEMENT, IMPLEMENTATION

EOCRC

EPIDEMIOLOGY AND REGISTRIES

GENETICS

P1 - MULTIPLE COLORECTAL ADENOMAS SYNDROME: THE ROLE OF MUTYH MUTATION AND THE POLYPS' NUMBER

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Background and aims

Multiple colorectal adenomas (MCRAs) can result from APC (AFAP) or biallelic MUTYH (MAP) mutations, but most patients are wild type and known as non-APC/MUTYH polyposis (NAMP). We investigated the management and risk of colorectal cancer (CRC) in MCRAs patients.

Methods

Records of MRCA between 2000 and 2022 were retrospectively analysed. Patients were divided according to the genotype (MAP vs. NAMP) and the number of categorised polyps' burden (group 1: 10-24, group 2: 25-49, and group 3: 50-99 adenomas). Predictors of outcome were CRC-free survival (CRC-FS) and Surgery free-survival (S-FS).

Results

A total of 220 patients were enrolled (NAMP n=178(80.0%)). The number of CRC at diagnosis was higher in group 3 (p=0.01), without significant differences between the genotypes

(p=0.20). At a follow-up of 83(41-164) months, 15(7%) patients developed CRC during surveillance. CRC-FS was not correlated to genotype (p=0.07) or polyps' number (p=0.33), while S-FS was similar in MAP and NAMP (p=0.22) and lower in groups 2 and 3 (p=0.0001). Age, gender, familiarity, prophylactic surgery, polyps' number, time on surveillance, and genotype were not associated with CRC risk.

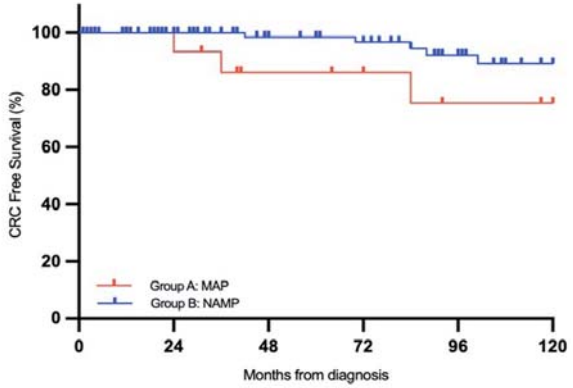
Conclusions

MAP and NAMP have the same CRC risk and no difference in treatment. In patients without cancer at diagnosis, polyps' number is not related to CRC during surveillance, but it influences treatment.

Keywords

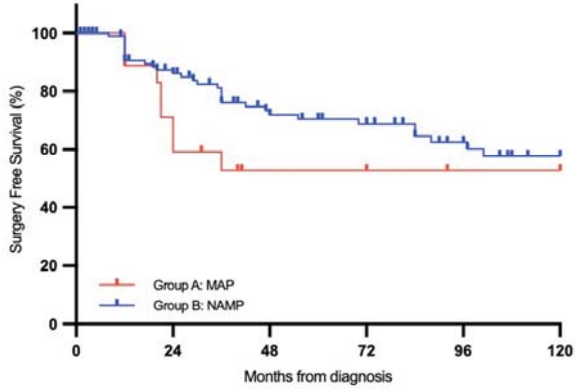
Colorectal cancer; familial adenomatous polyposis; colorectal polyposis; MUTYH associated polyposis.

1a



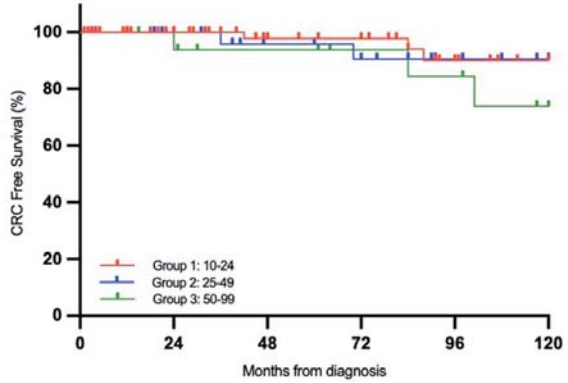
Group A: MAP	17	15	11	9	7	6
Group B: NAMP	106	82	62	55	35	24

1b



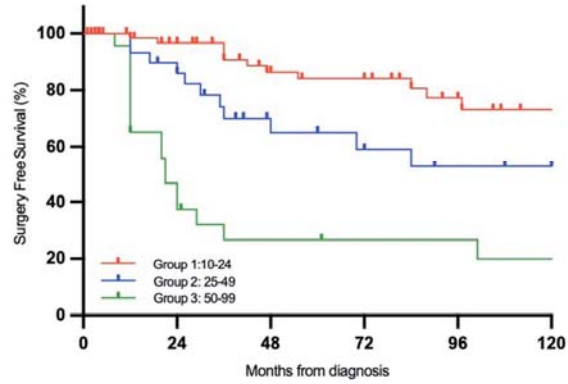
Group A: MAP	19	12	7	6	5	5
Group B: NAMP	108	77	51	43	27	17

2a



Group 1: 10-24	72	54	40	37	20	16
Group 2: 25-49	32	27	21	17	13	9
Group 2: 50-99	19	16	13	11	10	7

2b



Group 1: 10-24	72	54	38	35	20	15
Group 2: 25-49	32	25	14	10	8	5

P2 - DESMOIDS: FINDING FAP IN THE SPORADIC HAYSTACK

Cherryl Caballit, Sue Clark, Ashish Sinha

St Mark's Centre for Familial Intestinal Cancer

Background and aims

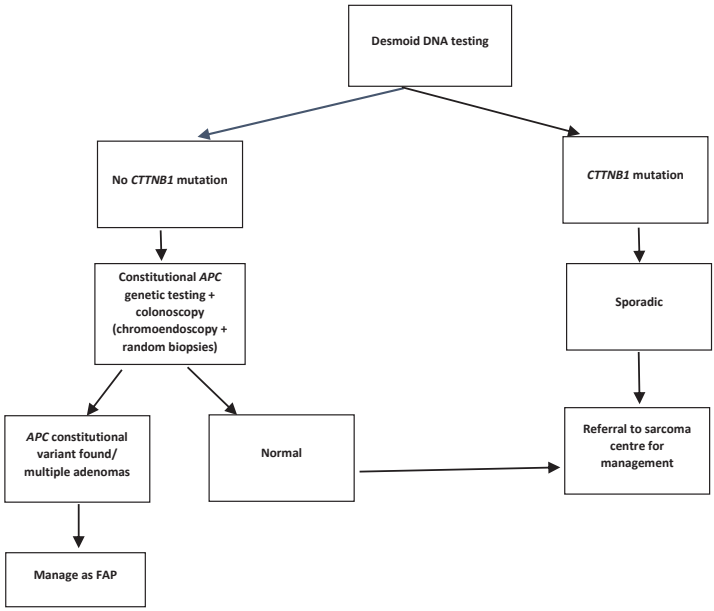
Patients presenting with desmoid are often referred for assessment for possible FAP, although 90% of desmoids are sporadic.

Desmoids seem to result from derangement of the wnt-signalling pathway, due to constitutional *APC* variant and subsequent somatic wild-type derangement in FAP, and to somatic mutation of *CTNNB1* in sporadic cases.

Method

We present our algorithm for approaching this situation. Tumour analysis for somatic *CTNNB1* pathogenic variant is recommended as first line, and if present indicates a sporadic desmoid and no further investigation is required; its absence triggers further investigation for possible FAP.

Figure 1. Algorithm



Results

Recently we have been referred three patients in whom analysis of desmoid DNA has revealed an *APC* pathogenic variant, the assumption being that this is constitutional, and the patient therefore has FAP. However, constitutional DNA testing showed no abnormality in *APC*, there was no evidence of mosaicism; there was no relevant family history and in both cases colonoscopy with chromoendoscopy was normal, as were random biopsies.

Patient	A	B	C
Sex	F	F	F
Age (years)	16	44	62
Desmoid somatic CTNNB1 testing	No mutation detected	No mutation detected	No mutation detected
Desmoid somatic APC testing	c.4510_4513del p.(Ser1504AlafsTer2)	c.4618_*7080del p.(Glu1540-2844del)	c.3183_3187del p.(Gln1062Ter)
Constitutional APC testing	No mutation detected	No mutation detected	No mutation detected
Colonoscopy	No polyps	2 x tubular adenomas with low grade dysplasia (1mm and 3mm)	No polyps

Conclusions

In these cases, therefore, it seems that somatic *APC* mutation has caused sporadic desmoid, something we cannot find reported in the literature.

P3 - ADRENAL TUMOURS IN PATIENTS WITH PATHOGENIC APC MUTATIONS: A RETROSPECTIVE STUDY

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Background

Adrenal tumours are associated with familial adenomatous polyposis (FAP). In the literature, most studies use the clinical definition of FAP (more than 100 adenomatous polyps found in endoscopic studies). However, not all patients that meet clinical criteria for FAP carry pathogenic mutations in the adenomatous polyposis coli (*APC*) gene, as there is genetic heterogeneity responsible for FAP with the polyposis sometimes explained by genetic and environmental factors other than pathogenic *APC* mutations. Reciprocally, not all the patients with pathogenic *APC* variants will fulfil the clinical criteria of FAP. This study aims to investigate the characteristics of adrenal tumours in patients with pathogenic or likely pathogenic *APC* variants.

Method

This is a retrospective cohort study of all patients that carry a constitutional likely pathogenic or pathogenic APC variant as reported in ClinVar or the InSiGHT LOVD database. They were tested by the Molecular Pathology laboratory at the Peter MacCallum Cancer Centre. The study involved collecting patient demographic information, APC variants, indications for scanning, characteristics of adrenal masses, and results from hormonal testing.

Result

90 patients were included with a median age of 27 (IQR: 19.75 – 37.5) at diagnosis. The prevalence of adrenal mass was 26.7% (24/90) among patients with pathogenic or likely pathogenic *APC* variants. 34 adrenal tumours were found among these patients with a median maximal diameter of 17mm (IQR: 12.5 - 23). 25 (73.5%) of the tumours were radiologically assessed as adenomas, 3 (8.8%) were myelolipoma and 6 (17.6%) indeterminate. 14 (58.3%) patients had unilateral tumours, 9 (37.5%) bilateral, and 1 (4.2%) unknown laterality. We observed no genotype-phenotype correlation for adrenal tumours among the pathogenic or likely pathogenic *APC* mutations. No adrenal malignancy was detected and there was limited available data on the hormonal function of these patients with adrenal tumours in this cohort.

Conclusion

The prevalence of adrenal tumours among patients with pathogenic or likely pathogenic *APC* mutations in our cohort is at least two to three times higher than the prevalence reported in the general population based on international population-based studies. As scanning was performed universally for reasons other than suspicion of adrenal tumours (mostly for desmoid disease consideration), this series is not subject to ascertainment bias relating to adrenal glands.

Table 1. Characteristics of adrenal tumours among patients with pathogenic or likely pathogenic mutations.

PARAMETERS	PATIENTS, n = 90	
	No adrenal tumours, n = 66	Adrenal tumours, n =24
Sex		
Male	36 (54.5%)	11 (45.8%)
Female	30 (45.5%)	13 (54.2%)
The median age of FAP diagnosis, years (IQR)	22 (18 - 31)	29 (20 - 43)
The median age when the radiological assessment was conducted, years (IQR)	39 (29 - 50)	49 (35 – 61.75)
Modality		
CT	49 (74.2%)	17 (70.8%)
MRI	17 (25.8%)	7 (29.2%)
Laterality		
Left only		11 (45.8%)
Right only		3 (12.5%)
Bilateral		9 (37.5%)
Unknown		1 (4.2%)
Median maximal diameter, mm (IQR)		17 (12.5 - 23)
Type of adrenal tumours		34 tumours in total
Adenoma		25 (73.5%)
Myelolipoma		3 (8.8%)
Indeterminate		6 (17.6%)

Table 2: Clinical details of the cases.

SEX	AGE	VARIANT		ADRENAL MASS TYPE	MAXIMAL DIAMETER (MM)
Male	47	NM_000038.5: c.1958+1_1958+2dupGT	Likely Pathogenic	Adrenal adenoma	20
Male	34	NM_000038.5:c.531+1G>T	Pathogenic	Bilateral adrenal adenomas	17
Male	52	NM_000038.5:c.3920T>A; NM_000038.5:c.3924dup	Pathogenic	Bilateral adrenal adenomas	L) 26, R) 33
Male	29	NM_000038.5:c.5826_5829del	Pathogenic	Bilateral adrenal adenomas	R) 22, L) unknown
Female	36	NM_000038.5:c.3444_3447del	Pathogenic	Bilateral adrenal adenomas	L) 16, R)32
Female	62	NM_000038.5:c.1885_1886insA	Pathogenic	Bilateral adrenal adenomas (3 in total)	L) inferior 31, L) superior 12, R) 6
Male	24	NM_000038.5:c.2805C>A	Pathogenic	Left adrenal adenoma	20
Female	30	NM_000038.5:c.5952-5955del	Pathogenic	Left adrenal adenoma	12
Female	14	NM_000038.5:c.487C>T	Pathogenic	Bilateral indeterminate adrenal masses	L) 15, R) 10
Female	29	NM_000038.5:c.1259-1269del	Pathogenic	Left adrenal adenoma	18
Male	20	NM_000038.5:c.904C>T	Pathogenic	Bilateral adrenal adenomas	L) 12, R) 12
Female	63	NM_000038.5:c.7016-7064del	Pathogenic	Left adrenal adenoma	15
Female	19	NM_000038.5:c.2805C>A	Pathogenic	Left adrenal myelolipoma	17

Female	43	NM_000038.5:c.3183-3187del	Pathogenic	Left adrenal myelolipoma and right adrenal adenoma	L) unknown, R) 15
Female	40	**	Pathogenic	Left adrenal nodule	
female	26	NM_000038.5:c.423-1G>A	Pathogenic	Left adrenal myelolipoma	41
Female	46	NM_000038.5:c.3183-3187del	Pathogenic	Left adrenal nodule	13
Male	20	NM_000038.5:c.487C>T	Pathogenic	Left adrenal nodule	35
Female	N/A	NM_000038.5:c.487C>T	Pathogenic	Right adrenal adenoma	21
Female	17	NM_000038.5:c.348-352del	Pathogenic	Right adrenal adenoma	11
Male	43	NM_000038.5:c.348-352del	Pathogenic	Right adrenal nodule	15
Male	18	Truncated protein from exon 15*	Pathogenic	Bilateral adrenal adenomas	L) unknown, R) 18
male	23	NM_000038.5:c.2805C>A	Pathogenic	Left adrenal adenoma	12
male	18	NM_000038.5:c.2805C>A	Pathogenic	Left adrenal adenoma	29

* Patient had protein testing only when genetic sequencing was not widely available at the time in clinical practice

**Patients had the genetic testing externally. The exact variant was not available but was labelled as pathogenic on the record.

P4 - TUMORS OTHER THAN COLORECTAL AND ENDOMETRIAL CANCER ARE OFTEN THE FIRST MANIFESTATION OF LYNCH SYNDROME: A CALL TO EXPAND UNIVERSAL SCREENING

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Background

Lynch syndrome (LS) predisposes to a wide spectrum of tumors, the most common being colorectal cancer (CRC) and endometrial cancer (EC). Universal screening for LS by using immunohistochemistry assay for the mismatch repair (MMR) proteins is nowadays proposed only for CRC and EC.

Aims

To define incidence of LS-associated cancers; to define the incidence of Non-CRC/EC tumors as the first manifestation of LS.

Materials and methods

Inclusion criteria: patients with germline pathogenetic variants (GPVs) of *hMLH1, MSH2, MSH6, PMS2 and EPCAM* followed at University of Padova & Veneto Institute of Oncology centers for inherited tumors. Exclusion criteria: GPVs class 3; incomplete clinical data.

Clinical and genetic data were retrospective collected. Duration of follow up was defined from the first tumor and/or the first genetic consultation and last control or death.

Categorical variables were analyzed with the Chi-square Test and continuous variables with the Mann-Whitney U Test, with statistical significance at $p<0.05$.

Results

394 patients were enrolled; 225 (57%) were women. The median (range) age at diagnosis was 43(18-88) years. The median (range) follow-up was 72 (12-336) months. The following GPVs were found: *MLH1*= 148(37.6%); *MSH2*= 205(52%), *MSH6*= 23(5.8%), *PMS2*= 4(1%), *EPCAM*=14(3.6%). 330 patients (79%) had cancer. 139 (35%) patients had a tumor Non-CRC/EC: it was the first tumor in 87(22%) cases: ovary=14(6.2%), breast=12(5.8%), urothelial=19(4.8%), pancreas=10(2.5%), thyroid=9(2.2%), stomach= 8(2%), CNS= 8(2%), small bowel= 4(1%), sarcoma= 2(<1%), prostate= 1 (<1%). These Non-CRC/EC cancers were diagnosed before the genetic test in 50(12,7%) patients.

Genotype was not significantly related to sex (p=0.39), age of onset of colon cancer (p=0.08), endometrial cancer (p=0.12) and other cancer (p=0.22). Urothelial tumors are more often related to *MSH2* than *MLH1*GPVs (p=0.002).

Out of the patients with a first Non-CRC/EC tumor, 20 (23%) developed afterwards CRC (n=15) and EC (n=5), at a median (range) time after the diagnosis of the primary tumor of 3 (1-30) and 6 (1-7) years, respectively.

Conclusions

Non-CRC/EC cancers are the first tumor in a high percentage of LS patients, and they are often found before than genetic diagnosis. These data suggest to expanding universal screening for LS to other malignancies.

P5 - A SCORE TO PREDICT THE LIKELIHOOD OF DETECTING ADVANCED COLORECTAL NEOPLASIA AT COLONOSCOPY IN ADULTS YOUNGER THAN AGE 45

Carole Macaron, Sarah Wehbe, Robert Butler, David Liska, Carol Burke

Cleveland Clinic

Background

Colorectal cancer (CRC) screening is not currently recommended for average- risk adults younger than 45 years. Identifying patients < 45 years of age at heightened risk of advanced colorectal neoplasia (AN) is warranted to help tailor recommendations for colonoscopy.

Aim

To identify risk factors for AN and develop and validate a model to estimate the likelihood of detecting AN in adults, age <45 years.

Method

We performed a cross-sectional analysis of adults ages 18-44 years who underwent a colonoscopy for any indication at Cleveland Clinic between 2011-2021. We used backward elimination multivariable logistic regression methods to construct a reduced model based on significant associations (P<.05) between risk factors [Age, gender, race, ethnicity, body mass index (BMI), tobacco use, diabetes (ICD codes, or Hba1c >6.4%), hyperlipidemia (ICD codes or LDL> 130 mg/dl), family history of CRC, and colonoscopy indication] and the presence of AN [tubular adenoma (TA) >10 mm or with any villous features or high-grade dysplasia (HGD), sessile serrated lesion (SSL) >10 mm or with dysplasia, traditional serrated adenoma (TSA) or invasive adenocarcinoma] in a randomly selected training set and confirmed the associations in a validation set. For the analysis, iron deficiency anemia, rectal bleeding, positive stool screening test, family history of CRC, mass on abdominal imaging and weight loss were classified as high-risk indications for colonoscopy while all other indications were considered low risk. We used the reduced model-adjusted coefficients to develop a risk score for detection of advanced colorectal neoplasia.

Results

AN was detected in 635(4.9%) of the 12987 participants included. The reduced logistic-regression model based on the training set identified independent risk factors for AN: gender (p= 0.0083), BMI (p= 0.0017), presence of first degree relative with CRC (age <60, p <0.0001; age >60 p= 0.0023), tobacco use (current vs. never, p<0.0001, former vs. never, p=.0116) and hyperlipidemia (p= 0.03) **(Table)**. In the validation set (N=6,493), the model exhibited moderate discriminatory power (c-statistic 0.64). We developed a score that estimated the likelihood of detecting advanced neoplasia in the complete dataset, from 2.5% for patient scoring 1, to >15% for patient scoring 10 or more **(Figure)**.

Conclusion

We developed and internally validated a simple score using clinical factors which successfully predicts the likelihood of detecting AN in adults younger than 45 years undergoing colonoscopy. Once externally validated, the proposed risk score may be useful for counselling or for individualized prevention studies.

Figure. The prevalence of advanced colorectal neoplasia in the complete dataset by risk score.

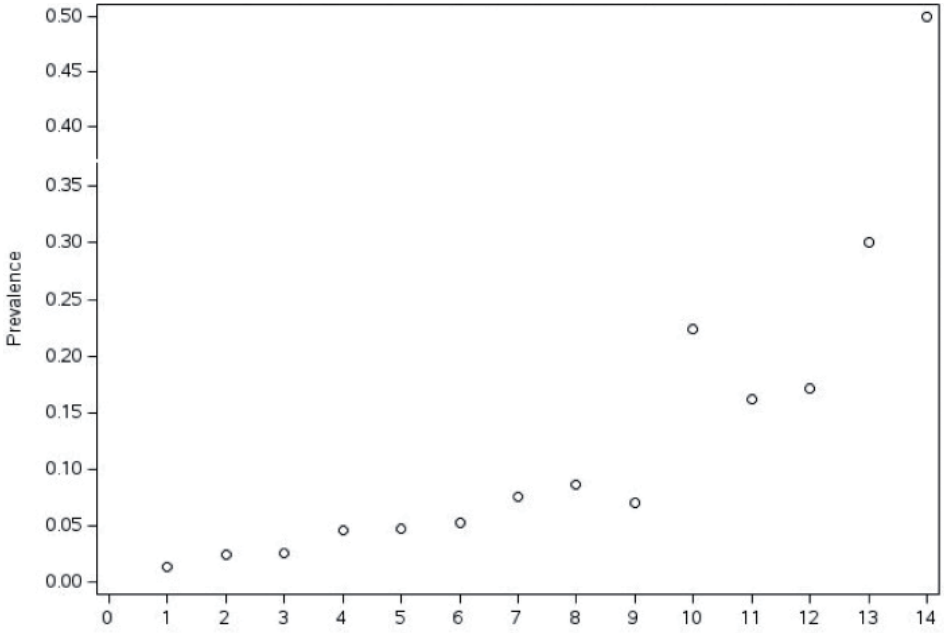

Table. Association between clinical factors and advanced colorectal neoplasia in a multivariable analysis and development of the risk score (N=6,495; test set). Model-adjusted coefficients were divided by the smallest coefficient and then rounded up to the nearest full integer to avoid decimals.

Full Model					
Variable	**Contrast**	**Coefficient**	**p-value**		
Intercept		-4.1292	<0.0001		
Colonoscopy High Risk Indication	Yes vs. No	0.2304	0.0593		
Gender	Male vs. Female	0.3442	0.0045		
BMI kg/m^2 (continuous)		0.2082	0.0045		
Tobacco Use	Former vs. Never	0.3537	0.0112		
	Current vs. Never	0.6556	<0.0001		
Diabetes	Yes vs. No	0.3739	0.2786		
Family History of CRC	1st Degree Relative < 60 vs. None	1.0103	<0.0001		
	1st Degree Relative ≥ 60 vs. None	1.0327	0.0018		
Alcohol consumption	Never vs. Current	0.2269	0.0875		
	Former vs. Current	0.2112	0.3325		
Hyperlipidemia	Yes vs. No	0.3856	0.0291		
Aspirin use	Yes vs. No	0.3149	0.0873		
Reduced Model					
Variable	**Value**	**Coefficient**	**Scaled Coefficient**	**Risk score**	**p-value**
Intercept		-4.0254	-	-	<0.0001
Colonoscopy High Risk Indication	No			0	0.0724
	Yes	0.2192	1	1	
Gender	Female			0	0.0083
	Male	0.318	1.45	1	
BMI kg/m^2 (continuous)	BMI < 25	0.2282	1.04	1	0.0017
	BMI ≥ 25 and BMI < 30			2	
	BMI ≥ 30			3	
Tobacco Use	Never			0	0.0116 <0.0001
	Former	0.3495	1.59	2	
	Current	0.6681	3.05	3	
Family history of CRC	None			0	<0.0001 0.0023
	1st Degree Relative < 60	0.9978	4.55	5	
	1st Degree Relative ≥ 60	1.0061	4.59	5	
Hyperlipidemia	No			0	0.03
	Yes	0.3827	1.74	2	

P6 - ADDRESSING UNCERTAINTY IN HEREDITARY COLORECTAL CANCER RISK: THE ROLE OF A REGIONAL MULTIDISCIPLINARY TEAM MEETING

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Hereditary colorectal cancer (CRC) syndromes associated with excess lifetime cancer risk yet are associated with significant uncertainty within diagnosis and clinical management. We evaluated the role of a novel specialist multidisciplinary meeting for hereditary CRC in how it aided clinicians deal with uncertainty through peer support, responding to diagnostic uncertainty, and clinical management including lifelong surveillance.

Methods

The St Mark's Hospital Centre for Familial Intestinal Cancer leads a collaborative regional virtual hereditary CRC multidisciplinary meeting. High-risk individuals are discussed by a team of expert gastroenterologists, surgeons, specialist nurses, clinical geneticists and regional mainstreaming clinicians. A retrospective analysis of meeting minutes from its inception in June 2020 until March 2023 was performed. Descriptive statistics were employed to ascertain clinicopathological data, clinical queries and whether MDT recommendations were in keeping with current guidelines.

Results

A total of 260 cases were discussed from 13 institutions, representing an average caseload of 3 patients a week, with 84.6% of cases external to St Mark's. A prior personal history of cancer within the past year was present in 79.6% of cases (CRC in 56.9%), and a family history of CRC or non-CRC in 45.4% and 26.8% cases respectively. In thematic analysis uncertainty related to genetic testing was considered in 56.9% of cases, with Lynch-like syndrome a putative diagnosis in 30% of cases. Surveillance related queries represented 21.1% and mainstreaming 11% of cases. The MDT assisted in decision-making for 61 cases of inconclusive or conflicting genetic testing results (not currently covered in guidelines) including advice for review of variants of unknown significance in 17 cases. Management was recommended beyond the scope of existing guidelines in 24.6% of cases.

Conclusions

This hereditary CRC MDT provided clinicians from regional institutions with support in areas of uncertainty in diagnosis and clinical management, particularly surveillance, and supporting

clinical decision-making where guidelines were limited. This model could be expanded to support complexity in clinical care in other geographical regions or other health conditions.

Table 1: Genetic testing strategies and examples of uncertainty, adapted from *BSG guidelines*[20] *and NHS National Genomic Testing Directory*[22]

Risk category	Somatic or constitutive testing	Eligibility	Testing	National Genomic Test Directory	Examples of Uncertainty
Family history of CRC	Somatic Constitutive	Moderate-risk, high-risk families Amsterdam criteria families where MMR testing is not possible	dMMR/pMMR Panel testing of affected individuals or unaffected testing	R210 for dMMR cancers	Unexplained dMMR tumours No germline pathogenic variant identified
CRC	Somatic	Universal testing	dMMR/pMMR and subsequent testing as defined by NICE DG27 guideline	R210	Somatic VUS Possible germline variants detected on somatic testing
Early onset colorectal cancer (EOCRC)	Constitutive	Diagnosis of CRC at 40 years and under	Panel testing determined by MMR status	R211	Can genetic testing alter clinical management?
Lynch-like syndrome	Somatic	dMMR tumours without hypermethylation/*BRAF* pathogenic variant and no constitutional pathogenic variant in MMR genes	Somatic testing panel	R210	There is no agreed definition of Lynch-like syndromes, particularly for non-colorectal cancers
Serrated polyposis syndrome	Constitutive/ somatic	Diagnosis of exclusion	Exclude known predisposition syndromes	R211	Heterogeneity in clinical phenotype and limited yield of genetic testing
Multiple colorectal adenoma (MCRAs)	Constitutive	MCRAs under 60 years of age with ≥10 adenomas, or patients over 60 years of age with ≥20 adenomas, or ≥10 with a family history of multiple adenomas or CRC	Gene panel testing	R211	*MUTYH* heterozygosity *APC* mosaicism. Surveillance for FDRs is not defined

dMMR- mismatch repair deficient, pMMR- mismatch repair proficient, MMR- mismatch repair

P7 - COMBINED COLORECTAL AND ENDOMETRIAL SURVEILLANCE UNDER SEDATION IN WOMEN WITH LYNCH SYNDROME

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Background and aims

According the Dutch guideline, women with Lynch syndrome are under intensive colorectal and endometrial cancer surveillance. They are advised to undergo a biannually colonoscopy starting the age of 25 and an annual gynecological examination with transvaginal ultrasound and endometrial biopsy between 40 and 60 years of age. Endometrial biopsy is often experienced as being a painful procedure, resulting in some women not undergoing it. The aim of this study was to improve quality of patient centered healthcare by combining the colonoscopy and endometrial biopsy under sedation by midazolam, resulting in less outpatient clinic visits and reduction of pain.

Method

In total, 21 patient surveys and forty healthcare professional surveys were sent to professionals after the colonoscopy in combination with gynecological examination.

Results

Eighteen (86 %) of the patients responded to the survey. A significant reduction of pain was shown while given endometrial biopsy under sedation: mean no sedation pain score 5.7 [3 – 10], mean sedation pain score 2.7 [1 – 7], $p = < 0.001$. All patients considered combining the outpatients visits as an improvement. 29 (73%) healthcare professional surveys were send back by a total of fourteen unique healthcare professionals. They unanimously considered the pilot as an improvement in patient care. However, logistics and the efficiency in the healthcare process could be improved.

Conclusions

Combined colonoscopy with endometrial surveillance under sedation in women with Lynch syndrome results to a reduction of pain and improvement of patient centered healthcare.

Focus groups will be scheduled to further optimize the organizational process making it applicable to other centers and accessible for all women with Lynch syndrome.

Keywords

Lynch syndrome, endometrial surveillance, colorectal surveillance.

P8 - UROLOGICAL FOLLOW-UP OF LYNCH SYNDROME: UTUC INCIDENCE AND MUTATIONAL PATTERNS IN A DEDICATED OUTPATIENT CLINIC

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Background and aims

Lynch Syndrome (LS) is an autosomal dominant genetic disorder linked to various cancers, with Upper Tract Urothelial Carcinoma (UTUC) being the third most common manifestation. This study presents findings from our dedicated Lynch Syndrome outpatient urological clinic, aiming to assess UTUC occurrence, pathogenic variants, and follow-up strategies for LS patients.

Method

From 2021 to 2023, we observed 30 LS patients at a specialized tertiary referral center. We collected data on LS pathogenetic variants, medical and family histories, and initiated a rigorous follow-up protocol. Genetic diagnoses were established through immunohistochemistry (IHC) and DNA sequencing. We assessed concordance with Bethesda, EAU guidelines, Amsterdam I, and Amsterdam II criteria. The follow-up involved biennial Ultrasound (US), urinalysis, and urinary cytology. In high-risk cases (age >50 years, MSH2 pathogenetic variants, family UTUC history), we alternated CT-scans with US annually.

Results

Of patients, 53% (n=16) received their LS diagnosis post-cancer, while 47% (n=14) were identified via genetic counseling after degree relative diagnosis. Median time after LS diagnosis was 86 (IQR 32-118) months. Our cohort included 11 colorectal cancer cases (36%), 4 UTUC (13%), and others with endometrial, gastric, skin cancer, and rare LS-related cancers. MSH2 pathogenetic variants were found in 48% (n=14), MLH1 in 31% (n=9). MSH2 was common in colorectal cancer (36%) but had greater incidence in UTUC (50%). Median age at UTUC diagnosis was 60 years, surpassing colorectal cancer (49 years, p=0.007). Bethesda and EAU guidelines showed higher accuracy (86% and 89%) than Amsterdam I/II (60%). During the observational time, no new tumor diagnosis was made.

Conclusions

The establishment of a dedicated Lynch Syndrome clinic over the past two years has yielded valuable insights into common pathogenetic variants. These findings have the potential to play a pivotal role in developing mutation-specific follow-up protocols and to enhance earlier UTUC diagnosis among this particular population.

P9 - IN-SITU CHARACTERISATION OF SIGNET RING CELL FOCI IN HEREDITARY DIFFUSE GASTRIC CANCER PATIENTS

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Background and aims

Hereditary diffuse gastric cancer (HDGC) is predominantly attributed to *CDH1* germline mutations and carries a cumulative lifetime risk of diffuse gastric cancer (DGC) of up to 70%. Definitive treatment remains prophylactic total gastrectomy associated with significant morbidity. There remains a need for alternate treatment strategies where precursor signet ring cell (SRC) foci provide a unique opportunity to understand genetic events that drive disease progression which will facilitate risk stratification and uncover novel therapeutic targets. Hence, this study aims to characterise epithelial cell landscape in HDGC focusing on SRC foci.

Method

A cohort of 20 HDGC patients from 5 families was constructed with a range of pathogenic variants. Within 3 families, there were members with varying prevalence of SRC foci. Using biopsy samples from baseline and surveillance endoscopies, immunohistochemistry staining of E-cadherin, p53, Claudin 18.2, Her2 and PD-L1 was optimised, and scoring performed to compare phenotypically normal gastric mucosa with SRC foci/ DGC lesions.

Results

38 SRC foci and 2 DGC lesions were found across all biopsies. All SRC foci and DGC lesions displayed aberrant E-cadherin where 9/40 (22.5%) and 31/40 (77.5%) displayed absent and decreased expression respectively. In contrast, normal gastric mucosa displayed normal E-cadherin expression in keeping with retained function from wild-type allele. 20/40 (50.0%) SRC foci displayed absent p53 expression while 7/40 (17.5%) SRC foci and DGC lesions showed over-expression and remaining 13/40 (32.5%) SRC foci were wild-type. 13/40 SRC foci and DGC lesions (32.5%) showed positive Claudin 18.2 expression with at least moderate staining in ≥40% of SRC. All cases had negative Her2 and PD-L1 staining. There was no correlation between expression of E-cadherin with p53 and Claudin 18.2.

Conclusions

E-cadherin loss from inactivation of the second allele is likely to be an early initiating event and aberrant p53 may be a useful marker for progression. In addition, Claudin 18.2 has emerged as a potential therapeutic target. Moving forward, a larger cohort will be selected to explore the comprehensive epithelial/stromal/immune cellular landscape in HDGC surrounding the

early SRC lesion using multiplex imaging (Akoya PhenoCycler Fusion platform). We anticipate early SRC is surrounded by a unique tissue microenvironment and its epithelial profiling will provide insights on the status of neoplastic progression.

Keywords

CDH1 mutation; hereditary diffuse gastric cancer; signet ring cell foci.

P10 - ASSESSING THE RISK OF HEREDITARY UPPER TRACT UROTHELIAL CARCINOMA AT A HIGH-VOLUME CENTER USING THE PREMM 5 MODEL FOR LYNCH SYNDROME

Pietro Scilipoti[1], Giulio Zagaria[1], Francesco Cattafi[1], Alessandro Mannucci[2], Marta Puzzono[2], Poliani Laura[2], Giulia Martina Cavestro[2], Francesco Montorsi[1], Alberto Briganti[1], Marco Moschini[1]

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Background and aims

Lynch syndrome (LS) encompasses a broad spectrum of extracolonic tumors, including upper tract urothelial carcinoma (UTUC). UTUC can be misclassified as sporadic disease in up to 10-20% of cases. Despite current guidelines recommend Amsterdam criteria for Lynch syndrome (LS) screening in UTUC patients, the PREMM 5 model shows superior accuracy in this context, though it is not yet integrated into urological practice. The aim of our study is to evaluate the risk of UTUC misclassification by validating the PREMM 5 model and characterize clinicopathological differences between sporadic cases and those at high risk for hereditary UTUC.

Method

We analyzed a prospectively collected database of 246 UTUC patients treated at a tertiary referral center from 2015 to 2022. We utilized the PREMM 5 model to evaluate the risk of hereditary upper tract urothelial carcinoma (UTUC) and compared it to Bethesda and Amsterdam II criteria. Clinicopathological characteristics were analyzed, and Kaplan-Meier plots predicted cancer-specific and all-cause mortality.

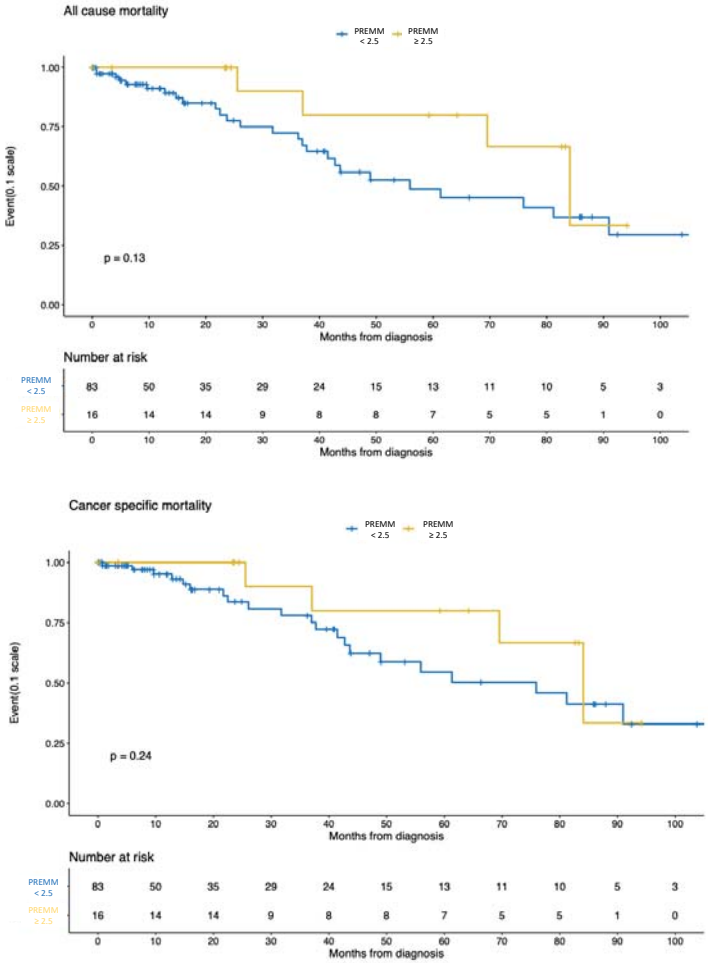
Results

Among 246 UTUC patients, 99 had a complete follow-up and PREMM 5 score. Among them, 16% (N=16) were identified as high risk for hereditary UTUC (PREMM 5 score $\geq$ 2.5%), while 9 (9%) and 6 (6%) patients were categorized at higher risk based on Bethesda and Amsterdam II criteria, respectively. Patients at higher risk of hereditary UTUC were younger then sporadic UTUC patients (57 vs. 72 years, $p<0.006$) and were mostly males (81% vs 66%, $p<0.006$). Bilateral disease was more common in patients with higher risk of hereditary UTUC (13% vs. 7%, $p<0.006$), same as for previous history of bladder cancer (43% vs 28%, $p<0.006$). The most common tumor location was the renal pelvis for patients with higher risk of hereditary UTUC (31% vs. 20%, $p=0.01$), compared to ureteral location that was most

common in the sporadic UTUC group (25% vs 18%, p=0.01). No difference was observed in cancer specific and all-cause mortality (Figure 1, all p >0.05).

Conclusions

The PREMM 5 model identified a 16% rate of patients at a higher risk for hereditary UTUC, surpassing the detection rates of Amsterdam II and Bethesda criteria. Despite distinct clinicopathological features in hereditary UTUC, no significant differences in survival were observed. Early identification through reliable tools like PREMM 5 is crucial for accurate diagnosis and effective management in both patients and their relatives.



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P11 - LYNCH SYNDROME SCREENING IN PATIENTS WITH YOUNG-ONSET EXTRA-COLORECTAL LYNCH SYNDROME-ASSOCIATED CANCERS

Atsushi Yamada[1,2], Yukari Doi[3], Sachiko Minamiguchi[3], Tomohiro Kondo[1], Tomohiko Sunami[1], Takahiro Horimatsu[1], Junzo Hamanishi[4], Masaki Mandai[4], Etsuro Hatano[5], Takashi Kobayashi[6], Kazutaka Obama[7], Hiroshi Seno[8], Hironori Haga[3], Masako Torishima[9], Hiromi Murakami[9], Takeshi Nakajima[9], Takahiro Yamada[9], Shinji Kosugi[9], Kokichi Sugano[10], and Manabu Muto

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Background and aims

Lynch syndrome (LS) is a hereditary cancer syndrome caused primarily by pathogenic germline variants in one of mismatch repair (MMR) genes (*MSH2*, *MLH1*, *MSH6*, *PMS2*). Identification of LS probands are crucial to reduce cancer related deaths in affected families. In addition to colorectal and endometrial cancers to which universal screening is recommended, LS screening covering broader spectrum of cancer types is needed. In the current study, we aimed to elucidate the rate of dMMR tumors and to evaluate the outcome of LS screening in young-onset extra-colorectal LS-associated cancers.

Method

This is a retrospective study conducted in a referral hospital in Japan. We utilized a total of 309 tissue samples of endometrial, ovarian, gastric, urothelial, pancreatic, biliary tract, and

adrenal cancers in patients younger than 50 years of age. Immunohistochemistry (IHC) for MMR proteins was retrospectively performed. Clinicopathological information and the results of genetic testing when available were obtained from medical charts. The study protocol was approved by the Ethics Committee of Kyoto University Graduate School and Faculty of Medicine. (R1978-1)

Results

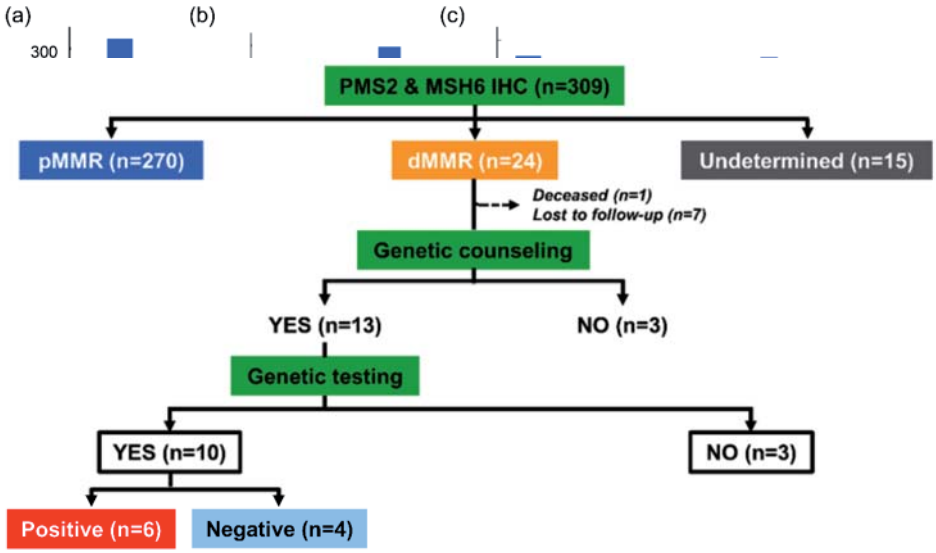
There were 24 dMMR tumors (24/309, 7.8%) including 18 endometrial (18/119, 15.1%), three ovarian (3/65, 4.9%), two urothelial (2/41, 4.9%), and one gastric (1/57, 1.8%) cancers. Meanwhile, 270 tumors were determined to be pMMR (87.4%) whereas MMR status could not be determined in 15 (4.9%) tumors due to insufficient staining. Synchronous and/or metachronous CRCs were presented in four of 24 patients (16.7%) with dMMR tumors in contrast to three of 270 patients (1.1%) with pMMR tumors. Moreover, among 273 patients for whom information on family history was available, familial occurrence of CRC and other LS-associated cancers were significantly enriched in patients with dMMR tumors. At the time of IHC analysis, only 16 of 24 patients with dMMR tumors continued the follow-up at our hospital. Among these 16 patients, five patients with endometrial and one patient with urothelial cancers were diagnosed as LS with positive pathogenic variants in MMR genes.

Conclusions

We reported the outcome of IHC for MMR proteins performed in multiple types of young-onset extra-colorectal LS-associated cancers. Our study demonstrated the feasibility of comprehensive LS screening program incorporating young-onset patients with various types of extra-colorectal LS-associated cancers.

Keywords

Lynch syndrome, Lynch syndrome associated cancer, young-onset, screening, deficient mismatch repair.


(a)
(b)
(c)
300
PMS2 & MSH6 IHC (n=309)
pMMR (n=270)
dMMR (n=24)
Undetermined (n=15)
Deceased (n=1)
Lost to follow-up (n=7)
Genetic counseling
YES (n=13)
NO (n=3)
Genetic testing
YES (n=10)
NO (n=3)
Positive (n=6)
Negative (n=4)

P12 - A REVIEW OF GASTRIC SURVEILLANCE OUTCOMES IN MUTYH ASSOCIATED POLYPOSIS

Victoria Cuthill, James Cockburn, Andrew Latchford, Susan Clark

St Mark's Centre for Familial Intestinal Cancer

Background and aims

Upper gastrointestinal screening for patients with *MUTYH* associated polyposis (MAP) is recommended from the age of 35 years. The duodenal disease in MAP has been characterised (Thomas *et al*, 2021) but there are few data about gastric pathology in MAP. Given the increasing problem of gastric disease in familial adenomatous polyposis (FAP), we reviewed outcomes of gastric surveillance in patients with MAP.

Method

Patients who had homozygote or compound heterozygous *MUTYH* pathogenic variants were identified from a prospectively maintained database. Medical records, pathology and endoscopy reports were reviewed. As this was a service evaluation, ethical approval was not required.

Results

157 patients with MAP were identified. The median length of endoscopic follow-up was five years (range 1-25 years). The median number of oesophago-gastro-duodenoscopies (OGDs) per patient was two (range 1-14). The median age at first OGD was 50 years (range 25-81 years).

Fundic gland polyps (FGPs) were identified in 61 patients (39%), at a median age of 52 (range 35-81 years). Forty-three patients (70%) had <10 FGPs, 14 (23%) had 10-50 FGPs and four patients (7%) had 50-100 FGPs. All FGPs were under 5mm.

Gastric adenomas were detected in one patient only, who developed a 2-3mm gastric adenoma detected at age 69, and a further 20mm adenoma detected two years later in the proximal stomach; both adenomas were removed. No gastric cancer has been identified.

Conclusion

FGPs and gastric adenomas appear to be less common that seen in FAP. Larger cohorts will be required to better understand gastric cancer risk and the natural history of gastric adenomas in MAP. Optimal gastric surveillance is not yet defined. Current surveillance intervals based on duodenal findings seem appropriate.

Reference

Thomas, L., Hurley, J., Sanchez, A. (2021) Duodenal Adenomas and Cancer in *MUTYH* Associated Polyposis: An International Cohort Study. *Journal of Gastroenterology*, **160** (3) pp. 952-954.

P13 - CODESIGN OF THE LYNCH CHOICES PATIENT DECISION SUPPORT WEBSITE WITH PATIENTS AND OTHER EXPERT STAKEHOLDERS: REPORT ON AN INTERVIEW STUDY AND DIGITAL PATIENT EVALUATION SURVEY

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Background and aims

Lynch syndrome ('Lynch') is the most common genetic cancer predisposition, leading to gene-, gender- and age-specific cancer risks and management options. Increased genetic testing is leading to more diagnoses. Families are presented with complex choices. The study presented here was part of a larger project to codesign a patient decision aid website called Lynch Choices. Semi-structured interviews exploring support needs were followed by think-aloud interviews with patients looking at a prototype website, aimed at answering the research questions: 1) How do patients understand the personalised cancer risks presented in Lynch Choices? 2) How can they be supported to make decisions in line with their values and preferences? Subsequently, a digital survey was advertised to a larger sample size of Lynch patients to explore usefulness, meaningfulness and to request their feedback to inform future iterative refinement of the Lynch Choices website.

Methods

Twenty patients with Lynch were interviewed. Patients looked at sections of the prototype website containing decision aids regarding taking aspirin or having a hysterectomy to lower cancer risk. Verbatim transcripts were analysed using the Person-Based Approach. A Table of Changes was used to track positive, neutral and negative comments. MoSCoW prioritisation determined changes to the website. Reflexive thematic analysis was performed on exemplar quotes from interview transcripts to unpack meaning about decision support needs. Following refinement to the Lynch Choices website, a larger, 'real world' sample was invited to review and rate the website via a digital survey.

Results

Patients' lived experience shared in interviews informed iterative refinement to a prototype patient decision support website, including changes to visual presentation of cancer risks. Needs and preferences in line with personal values were identified and incorporated into Lynch Choices.

Conclusions

Patients with Lynch have varied lived experiences informed by their history and beliefs. Gaps in care were experienced, particularly with respect to information and decision support.

This interview study highlighted support needs to empower people with Lynch to obtain personalised, trusted, up-to-date information about cancer risks and choices. Personal decisions about Lynch-related cancer risk management are best navigated in partnership with health professionals through shared decision-making.

P14 - OUTCOMES FROM THE NHS ENGLAND LYNCH SYNDROME TRANSFORMATION PROJECT: FINDING THE MISSING 95%

Kevin J Monahan[1,2], Laura Monje-Garcia[1,2], Paul Fleming[3,] Simone Gelinas[3], Hardy Steven[4], Adam Shaw[3]

[1] *North Thames Genomic Medical Service*

[2] *St Mark's Hospital, London*

[3] *South East Genomic Medical Service*

[4] *National Disease Registration Service, NHS England*

Background and aim

We have previously reported baseline data from the NHS England National Lynch syndrome (LS) Transformation project[(1)]. Briefly, this project aims to deliver on guidelines in the UK, which recommend universal testing of newly diagnosed colorectal (CRC) and endometrial cancer (EC) for Lynch syndrome (LS). There was strong evidence of variation in delivery of these guidelines by clinical services. National baseline data from 2019 demonstrated that only 41% of colorectal cancer patients received any form of index tumour 'mismatch repair '(MMR) testing, and only 28% of eligible patients received constitutional testing for LS. We can now report interim outcomes from this national quality improvement programme.

Method

A national oversight group was formed in May 2021. Regional LS project teams were established in each of 7 regional Genomic Medical Service Alliances (GMSAs), consisting of a LS doctor, nurse and project manager. Each CA was tasked with identifying and supporting a responsible 'Lynch syndrome champion' within each local cancer team, and regional teams use high quality data to develop tailored quality improvement plans for each cancer team, and multiple level training programmes for the national workforce, including workshops, online modules and individual clinician support. Data analysis has been performed by the National Disease Registration Service (NDRS), and includes comprehensive diagnostic and clinicopathological data for all cancer patients diagnosed across England (totalling >44,000 cancers annually), including somatic and constitutional testing outcomes.

Results

In total 176 Lynch syndrome champions have been appointed in CRC and EC teams (>95% coverage nationally). Over 2000 multidisciplinary cancer team members have undergone training. Tumour MMR testing rates increased to colorectal and endometrial tumour mismatch repair testing on the Lynch testing pathway from 43% to 93% nationally for CRC (and from 19% to 93% for endometrial cancer), equally across all geographies in England which all

achieve >90% MMR testing rates **(Figure 1)**. To facilitate constitutional testing, 66 new mainstreaming services have been developed within cancer teams, offering genetic testing locally without referral to clinical genetics services (29% of all cancer teams in England). In subgroup analysis, the time to genetic test following index somatic testing was 21 days in 'mainstreamed' patients, versus 287 days for those referred to regional clinical genetics services.

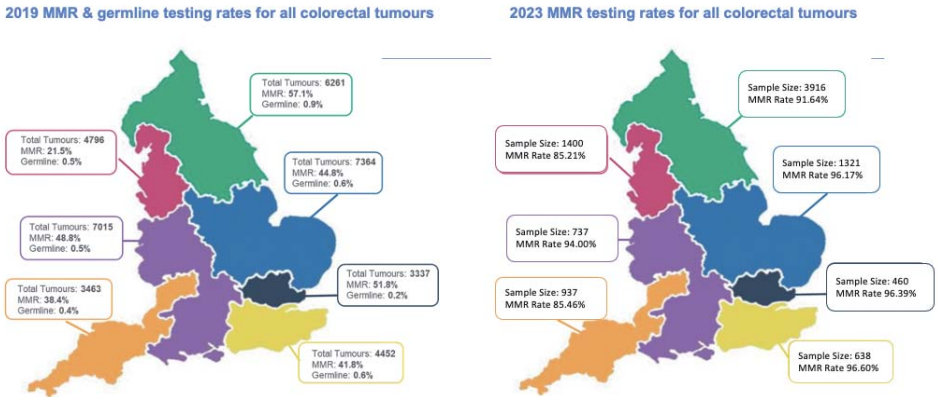
Through this programme we have ascertained 9030 historically diagnosed people with LS for a National Registry of LS, including 8471 eligible for a Nationally coordinated screening programme launched in 2023. Each month an additional 100 new diagnoses are being made across England, achieving the project goal of diagnosing >1000 new cases of LS annually.

Conclusions

Through the NHS England LS Transformation project an improvement in somatic and constitutional MMR testing for LS has been implemented, facilitating systematic delivery of universal testing for LS nationally, with equity of access to testing across all geographies. It is estimated by late 2025 that 57% of cancer teams will offer mainstream constitutional testing for LS.

1. Monahan KJ, Ryan N, Monje-Garcia L, et al. The English National Lynch Syndrome transformation project: an NHS Genomic Medicine Service Alliance (GMSA) programme. BMJ Oncology 2023;2:e000124. doi: 10.1136/bmjonc-2023-000124.

Figure 1.



P15 - THE SPIGELMAN STAGING SYSTEM AND THE RISK OF DUODENAL AND PAPILLARY CANCER IN FAMILIAL ADENOMATOUS POLYPOSIS. A SYSTEMATIC REVIEW AND META-ANALYSIS

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Background and aims

Individuals with familial adenomatous polyposis (FAP) have an almost 20% lifetime risk of duodenal cancer, currently the leading cause of death in FAP. The Spigelman staging system provides guidance on the surveillance intervals and the timing of prophylactic surgery. Still, its accuracy in predicting duodenal and papillary cancer development has not been systematically evaluated. We investigated the sensitivity and cancer risk of the Spigelman stages.

Method

We performed a PRISMA-compliant systematic review on PubMed, MEDLINE, EMBASE, and Cochrane to assess three primary endpoints: the stage-specific risk of cancer, the sensitivity of Spigelman stage IV to cancer, and the endoscopic and histologic risk factors for cancer. Throughout this systematic review, duodenal and papillary cancers were assessed both individually and together. We pooled effect sizes using a random-effects model.

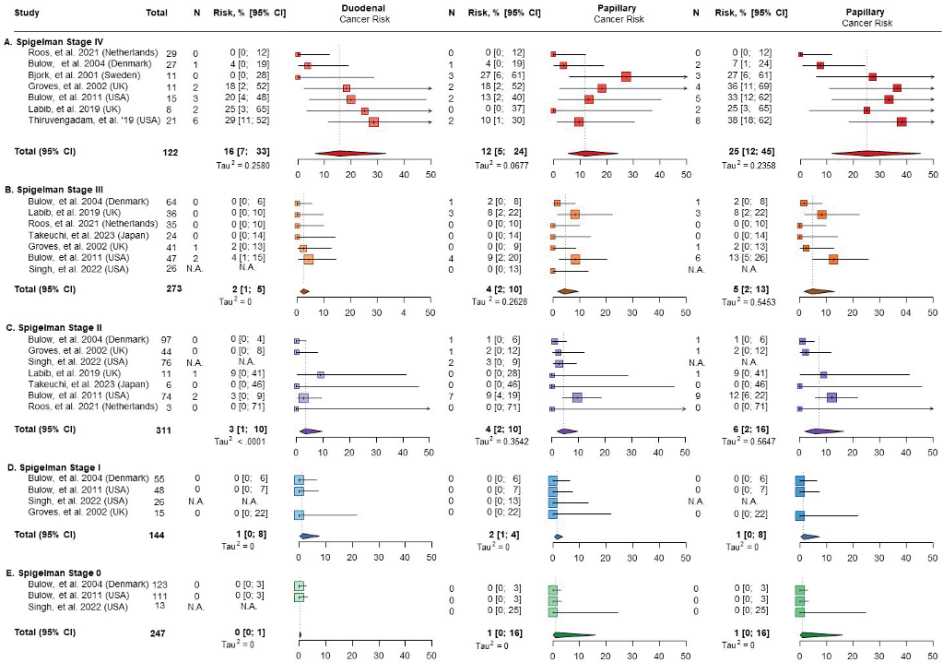
Results

After removing duplicate entries, we screened 1170 records and included 27 studies for quantitative analysis. Pooling results from 1097 FAP patients, we estimated a high risk of duodenal and papillary cancer for Spigelman stage IV (25%, $CI_{95\%}$=12-45%, **Figure 1A)**, an intermediate risk for stages III and II (5% and 6%, respectively, **Figure 1B** and **1C)**, and a very low risk for stages I and 0 (1% for both, **Figure1 D** and **1E**, respectively).

However, the pooled sensitivity of Spigelman stage IV for papillary and duodenal cancers was low (51%, $CI_{95\%}$=42-60%, **Figure 2A**), especially for papillary adenocarcinoma (39%, $CI_{95\%}$=16-68%, **Figure 2B)**. We investigated the reasons behind these low values and observed that the only risk factors significantly associated with duodenal cancer were the presence of polyps >10 mm (pooled RR= 1.9) and polyps with high-grade dysplasia (pooled RR=7.6). Risk factors associated with papillary cancer, instead, included a papilla with high-grade dysplasia (pooled RR=7.1) or >10 mm (pooled RR=3.5). The evidence on other risk factors (tubulovillous histology and >20 polyps) was inconclusive.

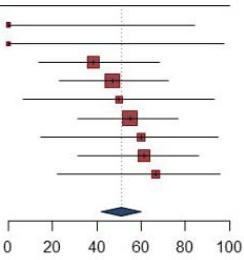
Conclusions

The current Spigelman system had a low sensitivity for duodenal and papillary cancer. Two variables included in the Spigelman classification (duodenal villous histology and polyp count) and the lack of papilla-specific variables likely contributed to the low sensitivity values for duodenal and papillary cancer, respectively. While clinicians may be familiar with the current form of the Spigelman classification, there is an urgent need to update it.



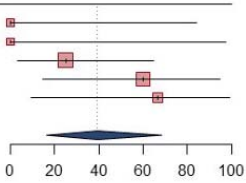
(A) Study	Events	Total	Weight	Sensitivity, % [95% CI]
Singh, et al. 2022 (USA)	0	2	2.1%	0 [0; 84]
Burke, et al. 1999 (USA)	0	1	1.9%	0 [0; 97]
Sourrouille, et al. 2017 (France)	5	13	15.6%	38 [14; 68]
Thiruvengadam, et al. 2019 (USA)	8	17	21.5%	47 [23; 72]
Bulow, et al. 2004 (Denmark)	2	4	5.1%	50 [7; 93]
Bulow, et al. 2011 (USA)	11	20	25.2%	55 [32; 77]
Bjork, et al. 2001 (Sweden)	3	5	6.1%	60 [15; 95]
Augustin, et al. 2019 (USA)	8	13	15.6%	62 [32; 86]
Groves, et al. 2002 (UK)	4	6	6.8%	67 [22; 96]
Total (95% CI)		**81**	***100.0%***	**51 [42; 60]**

Heterogeneity: $Tau^2 = 0$ [0.0 – 0.7]



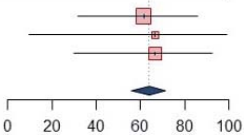
(B) Study	Events	Total	Weight	Sensitivity, % [95% CI]
Singh, et al. 2022 (USA)	0	2	10.0%	0 [0; 84]
Burke, et al. 1999 (USA)	0	1	9.0%	0 [0; 97]
Thiruvengadam, et al. 2019 (USA)	2	8	36.1%	25 [3; 65]
Bjork, et al. 2001 (Sweden)	3	5	28.9%	60 [15; 95]
Groves, et al. 2002 (UK)	2	3	16.0%	67 [9; 99]
Total (95% CI)		**19**	***100%***	**39 [16; 68]**

Heterogeneity: $Tau^2 = 0$ [0.0 – 7.0]



(C) Study	Events	Total	Weight	Sensitivity, % [95% CI]
Augustin, et al. 2019 (USA)	8	13	53.6%	62 [32; 86]
Groves, et al. 2002 (UK)	2	3	11.6%	67 [9; 99]
Thiruvengadam, et al. 2019 (USA)	6	9	34.8%	67 [30; 93]
Total (95% CI)		**25**	***100.0%***	**64 [56; 71]**

Heterogeneity: $Tau^2 = 0$ [0.0 – 0.2]



P16 - EFFECT OF A POLYGENIC RISK SCORE FOR COLORECTAL CANCER INCIDENCE IN PATIENTS WITH EARLY-ONSET, FAMILIAL, OR HEREDITARY COLORECTAL CANCER

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Background and aim

Polygenic risk scores (PRS) based on common CRC-associated SNPs can be used to stratify individuals for CRC risk. The study investigates to which extent a PRS affects CRC risk in individuals of different, clinically or molecularly defined CRC risk groups, using a large cohort from the German Consortium for Familial Intestinal Cancer (GC-FIC).

Methods

A total of 1,839 European-descendant individuals from the GC-FIC were stratified according to low (< 20%), intermediate (20-80%), or high (>80%) PRS (based on 95 CRC-associated SNPs) for the following risk groups: (i) genetically confirmed Lynch syndrome [LS; n=679 with CRC and n=422 CRC-free carriers]; (ii) sporadic early-onset CRC (< 50 years) [SEO-CRC; n=518], (iii) CRC patients with a positive family history for CRC [F-CRC; n=220], and (iv) MSI/dMMR CRC [n=144] vs. pMMR CRC [n=485]. Multivariable logistic regression and Cox proportional hazards models were applied to estimate odds ratios (OR) and to compute lifetime incidences, respectively, in comparison to 3,119 population based controls and sporadic late-onset CRC patients, age at diagnosis ≥60 years [SLO-CRC; n=97]).

Results

In all risk groups, we found a significantly increased PRS in individuals with CRC compared to population controls ($p < 0.02$). The SEO-CRC and F-CRC risk groups both showed a two times increased CRC risk in the high PRS category compared to the medium one (OR=2.01/ OR=2.35, $p < 0.001$, respectively). This translates into a cumulative lifetime risk of CRC before 75 years in the high PRS category of 23% and 13%, respectively. We did not find a significant difference in PRS between SEO-CRC and SLO-CRC cases. In the LS group, CRC patients did not have an increased PRS compared to CRC-free LS carriers ($p=0.13$). Finally, non-LS individuals with MSI/dMMR tumors have a significantly lower PRS compared to individuals with pMMR tumors ($p < 0.001$), but no difference in PRS compared to LS CRC individuals ($p=0.82$).

Conclusions

According to the preliminary analysis, PRS stratifies the CRC risk significantly in some clinically or molecularly defined risk groups. Since the vast majority of CRC-free LS carriers are relatives of the CRC LS carriers, no significant PRS difference might be expected and is in line with previous studies. The lacking PRS effect comparing SEO and SLO CRC cases can be partly explained by the sample size. Thus, larger collaborative studies are needed to finally explore whether PRS can individualize CRC risk stratification in different risk groups.

Keyword

Family History, Lynch syndrome, Polygenic Risk, Risk Stratification, Risk assessment, hereditary tumor syndromes.

P17 - OPTIMIZING SURVEILLANCE STRATEGIES FOR GASTRIC CANCER IN LYNCH SYNDROME: A COST-EFFECTIVENESS ANALYSIS

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Introduction

Lynch syndrome (LS) is one of the most common hereditary colorectal cancer syndromes, caused by germline pathogenic variants (PV) in *MLH1, MSH2/EPCAM, MSH6, PMS2*. Gastric cancer risk in LS is up to 11%, varying by PV, versus 0.9% in the general population, and is the first LS spectrum cancer in 39% patients. Most LS gastric cancer have a precancerous (Correa) cascade amenable to endoscopic intervention. Guidelines recommend upper endoscopy (EGD) every 2-4 years starting age 30-40, performed at time of colonoscopy. We present a comparative effectiveness study of EGD surveillance for gastric cancer in LS by PV.

Methods

We developed a decision-analytic model comparing no surveillance (NSV) vs EGD surveillance at 2, 3, and 4-year intervals (Q2SV, Q3SV, Q4SV, respectively) for gastric cancer in LS, starting at age 35. In the NSV strategy, individuals received EGD only for symptomatic gastric cancer. In the surveillance strategies, individuals had EGD alongside colonoscopy. Dysplasia and early gastric cancer were treated with endoscopic resection. We calibrated the model to lifetime incidence of gastric cancer by grouping PV into high (MLH1/MSH2, 7.4%), intermediate (MSH6, 5.3%), and low/population risk (PMS2, 0.9%). Utilities measuring quality of life, costs and complication rates of intervention were derived from literature. Standard 3% discounting was applied over a 65-year time horizon. Outcomes were reported as incremental cost effectiveness ratios (ICERs). Willingness-to-pay (WTP) threshold was set a priori at an ICER of $100,000 per quality-adjusted life-year (QALY).

Results

NSV, Q4SV was cost-effective for high (MLH1/MSH2) and intermediate-risk (MSH6) individuals, with ICER of $47,171 and $72,536/QALY, respectively **(Table 1)**. Q2SV and Q3SV exceeded the WTP threshold for both high and intermediate-risk individuals. For low-risk individuals (PMS2), no strategy was cost-effective. Surveillance strategies showed 49-63% reduced risk of gastric cancer-related death compared to NSV.

Discussion

Our modeling analysis shows that EGD surveillance every 4 years for carriers of MLH1, MSH2 and MSH6 appears to be cost-effective for gastric cancer prevention. For PMS2 carriers, EGD surveillance was not cost-effective. Future studies are needed to better understand the risk of gastric and small bowel cancer in LS and identify the risk factors (including H*elicobacter pylori*, specific PV, family history) to best inform risk stratification in practice.

Table 1.

	Primary Outcomes					Secondary Outcomes		
Surveillance strategy, in order of cost effectiveness	Cumulative cost, $USD	Incremental cost, $ USD	Effectiveness, QALY	Incremental effectiveness, QALY	ICER, $USD/QALY	Number of EGDs, per patient	Lifetime incident cancer, per 1,000	Cancer deaths, per 1,000
High risk								
NSV	5,940		21.36			0.07	74	69
Q4SV	14,517	8,577	21.54	0.18	47,171	10.5	43	34
Q3SV	16,633	2,116	21.55	0.0079	268,942	13.7	42	33
Q2SV	20,796	4,163	21.56	0.0078	533,870	20.3	40	32
Intermediate risk								
NSV	4,540		21.45			0.05	52	49
Q4SV	13,613	9,073	21.57	0.13	72,536	10.6	33	26
Q3SV	15,754	2,141	21.58	0.005	431,547	13.9	32	25
Q2SV	19,955	4,201	21.58	0.0049	862,306	20.5	31	25
Low risk								
NSV	509		21.81			0.009	9	8
Q4SV	8,931	8,420	21.82	0.017	487,041	11.3	4	3
Q3SV	11,171	2,241	21.83	0.001	2,343,947	14.8	4	3
Q2SV	15,600	4,429	21.83	0.0009	4,779,609	21.9	4	3

$USD, US dollars; QALY, quality adjusted life years * ICER may not add up to due to rounding

P18 - PATHOGENIC VARIANTS IN RNF43 IN PATIENTS WITHOUT SERRATED POLYPOSIS SYNDROME

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Background and aim

Serrated Polyposis Syndrome (SPS) is characterized by multiple and/or large serrated polyps in the colon and an increased risk of colorectal cancer (CRC). The etiology is largely unknown, but in a small subset of patients, monoallelic pathogenic variants (PVs) in *RNF43* are detected. The Gene-Disease relationship between SPS and *RNF43* has been ruled as definite by Clinical Genomics (ClinGen) Hereditary Cancer Gene Curation Expert Panel, based on both genetic and experimental evidence. Thus, RNF43 is often integrated in gene panels when suspecting hereditary GI-cancer. To date, however, the penetrance and phenotypic spectrum of patients carrying PVs in RNF43 are poorly described. We present three patients with PVs in RNF43 that have been incidentally detected as part of genetic testing.

Methods

The patients were non-related and referred to the Dpt. Of Clinical Genetics, University Hospital in Copenhagen, due to a suspicion of hereditary cancer. They underwent genetic counselling and testing as part of the routine work-up.

Results

Patient I was a 61-year-old female, who had recently been diagnosed with gastric adenocarcinoma. A colonoscopy revealed a tubular adenoma but was otherwise normal. **Patient II** was a 32-year-old healthy female, who had a family history of cancer, including breast cancer. The patient had no abdominal symptoms. A colonoscopy is planned for January 2024. **Patient III** was a 54-year-old healthy female, who had a family history of breast cancer. She had previously undergone multiple colonoscopies due to Inflammatory Bowel Disease. The last colonoscopy was performed in 2018 and no polyps were detected.

All patients were genetically tested with a NGS panel comprising 45 genes associated with hereditary cancer including *RNF43*. A nonsense variant in *RNF43* [NM_017763.6], c.1948C>T p.(Arg650Ter) was identified in patient I and II. The variant was classified as likely pathogenic according to ACMG guidelines. Patient III carried another nonsense variant in *RNF43* [NM_017763.6], c.1009C>T, p. (Arg337Ter) also classified as likely pathogenic.

Conclusions

We report three patients with germline PVs in *RNF43* of whom none had a family- or personal history of serrated polyps or CRC. Our findings suggest a low penetrance of *RNF43*–related SPS. The results highlight the complexity of genetic counseling in *RNF43*-positive families – particularly in families without polyposis. Further research is needed to elucidate the role of *RNF43* in the risk of SPS and CRC.

Keywords
Serrated Polyposis Syndrome. Polyposis.

P19 - GASTRIC ADENOCARCINOMA AND PROXIMAL POLYPOSIS OF THE STOMACH (GAPPS) IN DENMARK

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Background and aim

Gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS) is a rare autosomal dominantly inherited gastric cancer syndrome characterized by fundic gland polyposis of the stomach (>100) and an increased risk of gastric cancer. Pathogenic variants are detected in the promotor 1B of the *APC* gene. There is no established surveillance program for managing GAPPS. Here we present the first known Danish family with GAPPS.

Methods

Two brothers at 43 and 39 years old were referred to genetic counseling after the loss of their 46-year-old brother due to gastric cancer (adenocarcinoma). There was a family history of gastric cancer on both his fathers' and mothers' side. Endoscopy of the oldest brother revealed fundic gland polyposis.

Results

An initial restricted gene panel of the deceased brother revealed no pathogenic variants, but later additional analysis revealed a variant in the promotor 1B of *APC*, c.-191T>C. Genetic counseling and testing were initiated, and an additional six family members carried the variant. Endoscopy in these family members revealed fundic gland polyposis, often severe, at an early age. Because of severe polyposis, three family members underwent prophylactic gastrectomy at 28, 28 and 37 years old, respectively. Histopathological examination revealed low-grade dysplasia in two of the patients and both low-grade-and high-grade dysplasia in the remaining patient. Other family members were offered surveillance with regular gastroscopies. One family member had pre-implantation genetic testing that resulted in a viable pregnancy.

Conclusion

GAPPS is a rare, hereditary condition that increases the risk of gastric cancer. However, the penetrance is unknown as is the phenotypic variability. Our case illustrates the difficulty of

surveillance and genetic counseling in these patients and their families. Furthermore, our case demonstrates that sequencing of the promotor region of *APC* should be considered in families with a history of gastric adenocarcinoma and fundic gland polyposis.

Keywords

APC; Polyposis; gastric cancer.

P20 - GASTROINTESTINAL MANIFESTATIONS IN PATIENTS WITH GASTRIC ADENOCARCINOMA AND PROXIMAL POLYPOSIS OF THE STOMACH (GAPPS): A SYSTEMATIC REVIEW WITH ANALYSIS OF INDIVIDUAL PATIENT DATA

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Background and aim

Gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS) is an autosomal dominant syndrome characterized by fundic gland polyps (FGP) as well as an increased risk of gastric cancer. The syndrome has been recognized as a clinical entity for less than a decade. A clinical suspicion may be complex and can vary from incidental findings of FGPs at gastroscopy to obstructive symptoms with dyspepsia and vomiting. The diagnosis is established by genetic detection of a pathogenic variant in the promotor 1B region of the APC gene. As of yet there are no established clinical criteria for the diagnosis. To increase knowledge of the condition and to discuss possible genetic testing and surveillance strategies, we performed a systematic review of all reported patients with GAPPS.

Methods

This review was organized according to PRISMA guidelines. The search, which was conducted on September 7th, 2023, was applied to MEDLINE and restricted to only humans and papers in the English language. Only the studies on patients/families with GAPPS verified by identification of a pathogenic variant in the *APC* promoter 1B were included.

Results

Twelve publications with a total of 113 patients were identified. In all instances the diagnosis was genetically verified with reports of four different variants within the *APC* promotor 1B region. Eighty-eight patients (90.1%) had gastric polyps, of these seven patients had low-grade dysplasia and four patients had high-grade dysplasia. Thirty-seven patients (45.7%) underwent gastrectomy. There were no reports of duodenal polyps (0%). Gastric cancer

was found in 31 patients (30.1%) with a median age of 48 years (range 19-75). Twenty-six patients died (23%) of which 19 had developed gastric cancer (73.1%). One patient was diagnosed with metastatic colorectal cancer (2.2%) and died at 73 years of age. Nineteen patients had colorectal manifestations with <20 polyps (41.3%).

Conclusion

Patients with a pathogenic variant in the *APC* promoter 1B region have an increased risk of gastric polyposis and early-onset gastric cancer. However, there is considerable variation in clinical expression and penetrance, which makes decisions on surveillance and the timing of prophylactic gastrectomy challenging.

Keywords

Systematic review, gastric adenocarcinoma and proximal polyposis of the stomach, gapps, *APC*, promotor region, gastric polyps.

Figure 1. PRISMA flowchart.

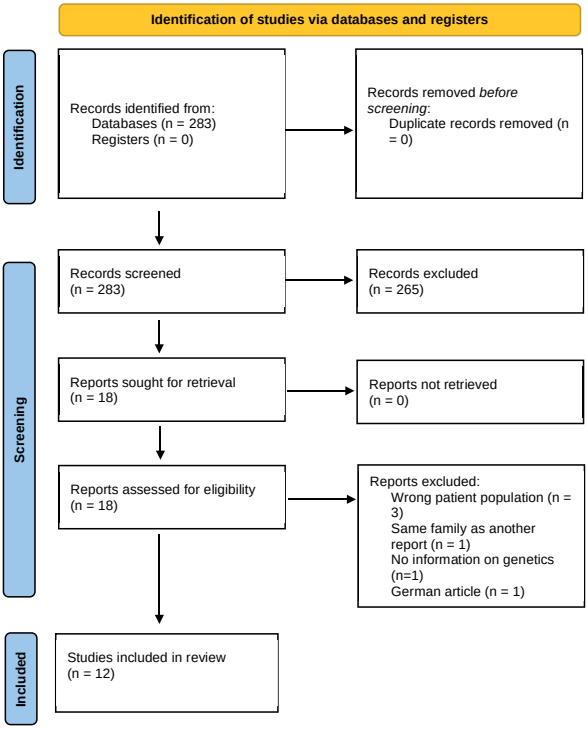

Table 1. Summary of patient characteristics.

Parameter	*n*	%
Number of patients	113	
Number of families	27	
Mean age of diagnosis (years)	43 (n=82)	
Age range (years)	10-92	
Gender M/F	18/36	33%/66%
Obligate carriers	15	13%
Reported pathogenic variant Pathogenic variants	98	77.8%
- *APC* variant: c.-191T>C	52	53.1%
- *APC* variant: c.-195A>C and c.-125delA	33	33.7%
- *APC* variant: c.-192A>G	2	2%
- *APC* variant: c-191T>G	1	1%
- Unspecified	10	10.2%
Gastric polyposis	88 (n=97)	90.1%
Low-grade dysplasia/low- and high-grade dysplasia/ unspecified	7/5/5 (n=24)	29.2%/ 20.8%/ 20.8%
Duodenal polyps	0 (n=83)	0%
Colorectal polyps	19 (n=46)	41.3%
Gastric cancer diagnosis	31 (n=103)	30.1%
- Adenocarcinoma	22	70.9%
- Unknown histopathology	9	16.1%
- Median age (years)	48 (n=25)	
- Age range (years)	19-75	
- Gender M/F	5/17	22.7%/ 77.3%
Colorectal cancer	1 (n=46)	2.2%
Mortality	26 (n=112)	23.2%
- Death from gastric caner	19	73.1%
- Death from colorectal cancer	1	3.8%
- Unspecified	6	23.1%
- Median age (years)	52 (n=12)	
Gastrectomy	37 (n=81)	45.7%
- Median age (years)	37 (n=15)	
- Age range (years)	19-66	

The figures in brackets denote the number of patients where there was a specific information on the event.

P21 - CANCER RISK AND MORTALITY IN PEUTZ-JEGHERS SYNDROME AND JUVENILE POLYPOSIS SYNDROME – A NATIONWIDE COHORT STUDY WITH MATCHED CONTROLS

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Introduction

Juvenile Polyposis Syndrome (JPS) and Peutz-Jeghers Syndrome (PJS) are hereditary syndromes characterized by hamartomatous polyposis in the GI-tract and an increased risk of cancer. Patients are recommended surveillance but long-term estimates of the cancer risk are lacking. We compared the risk of cancer and mortality in JPS and PJS patients with matched controls.

Methods

Patients with PJS and JPS were identified through Danish registries including the Danish National Pathology Register. Cases were matched with 50 controls on year of birth, sex, and postal code at birth. The Danish Cancer Registry provided data on incidences of any cancer. Date of death was extracted from the National Cause of Death Registry. Follow-up began January 1st, 1982, or the day of birth, and ended on the date of emigration, death, loss of follow-up, or end of study on December 31st, 2021. Incidence ratios (IR) for cancer and mortality were calculated per 100,000 person-years, and Hazard Ratios (HR) were estimated by Cox proportional hazards regression with age as the timescale.

Results

Fifty two patients with PJS (58% males) and 2,580 matched controls (58% males) were included. IR for cancer in cases was 1,110 (CI 95% 628-1,800) and 268 per 100,000 years (CI 95% 232-308) in controls, equaling a 6-fold increased risk of cancer in PJS patients (HR 6.2 [CI 95% 5.6-10.7, p<0.001]). The mortality rate was 1,170 (CI 95% 688-1,840), compared with 398 per 100,000 years (CI 95% 353-446) in controls, resulting in a 3-fold increased risk of death in patients with PJS compared with controls (HR 3.4 [CI95% 1.5-7.9, p=0.003]).

Sixty patients with JPS (52% males) and 2,981 matched controls (52% males) were included. IR for cancer was 514 (CI 95% 247-927) in JPS patients, and 257 per 100,000 years (CI 95% 224-292) in controls. Thus, the risk of cancer was doubled for JPS patients (HR 2.3 [CI 95% 1.2-4.5. p=0.0134]). The mortality rate in cases was 370 (CI 95% 159-716), compared with 293 per 100,000 years (CI 95% 259-331) in controls. Thus, patients with JPS had an insignificant 20% increased risk of death compared to controls (HR 1.2 [CI 95% 0.38-3.73, p=0.767]).

Conclusions

Our results showed a significantly increased risk of cancer in both patient with PJS and JPS and an increased mortality in PJS. This implicates a potential for optimized surveillance.

Keywords

Polyposis; peutz; cancer; mortality.

P22 - PATIENT-DERIVED ORGANOID MODEL FOR PREDICTION OF MMR (MISMATCH REPAIR) GENE FUNCTION AND CANCER RISK IN PATIENTS WITH GERMLINE VARIATIONS OF MMR GENES

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Background & aim

Lynch syndrome (LS) patients carry mutations in DNA mismatch repair (MMR) genes, such as MLH1, MSH2, PMS2, MSH6 and EPCAM, and have high risk of multiple cancers, including colorectal cancer. However, individual cancer development risk is various, and clinical significance of some variants, including VOUS (variants of unknown significance), is uncertain. To identify the patients with high risk of tumor development, we aim to develop the individualized functional assay model for variations of MMR genes.

Methods

Patient-derived organoid (PDO) was derived from normal colon of LS patients and healthy person. Organoids were exposed to N'-Mehtyl-N'-Nitrosoguanidine (MNNG), ATR inhibitor and O6-benzylguanine (O6BG), which is MGMT inhibitor. After the organoids were pre-treated with O6-benzylguanine and ATR inhibitor, they were treated with MNNG for 24 hours. All chemicals were dissolved in dimethyl sulfoxide and diluted in deionized water. The DNA damage of organoids was measured by immunofluorescence, WB, and immunofluorescence stain of γH2AX, which is a DNA damage recognition marker for detecting in earlier phase of DNA damage. In addition, to confirm increased mutation accumulation, we performed whole genome sequence analysis in normal and MLH1-mutated PDOs.

Results

The treatment of MNNG induced the apoptosis of organoids, and with additional treatment of ATR inhibitor and O6-benzylguanine showed significant apoptosis in normal organoid group. Then, we found that the cytotoxic effect by combined treatment of MNNG, O^6BG and ATR inhibitor in normal organoids were more sensitive than in MMR gene (MLH1, MSH2, PMS2, MSH6) mutated PDOs. In addition, in DNA damage response by analyzing the expression of γH2AX (immunohistochemical stain, WB, and immunofluorescence study), we found higher expression of γH2AX in normal organoids than MMR gene (MLH1, MSH2, PMS2, MSH6) mutated PDOs after treatment of MNNG and O6BG. Furthermore, in whole genome sequence analysis after treatment of MNNG, MLH1-mutated PDOs showed significant increase of mutational signature of single base substitutions(SBS) signature 14, which is associated with defective DNA mismatch repair, compared to normal organoids.

Conclusion

The MMR gene function of individual LS patients could be evaluated by measurement of DNA damage response using MNNG/O^6BG-treated individual PDO model, suggesting an individualized prediction model of CRC risk.

P24 - IDENTIFICATION AND EVALUATION OF DISCORDANT MUTYH VARIANT CLASSIFICATIONS IN CLINVAR

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Background and aim

MUTYH-associated polyposis (MAP) is linked to pathogenic variants in the MUTYH gene. Carriers of biallelic pathogenic variants have an increased lifetime risk of developing colorectal cancer (CRC), with or without polyposis. Pathogenicity classification of variants in MUTYH is important in ensuring accurate diagnosis in cases of polyposis or CRC, and if not affected, is critical in the assessment of personal risk to ensure appropriate risk management and screening. Discordances in the pathogenicity classifications in public variant databases, such as ClinVar, however present ongoing challenges in the classification of MUTYH variants. This project aims to identify discordant interpretations of variants in the MUTYH gene as listed in ClinVar, explore the reasons behind identified discordances and use this information to inform possible gene-specific modifications of the ACMG criteria.

Methods

Variants in the ClinVar database with recorded discordant classifications (clinical significance of 'conflicting interpretations') were selected for this analysis. Submissions from each ClinVar entry were collated and compared to identify areas of difference in the classification justifications. These differences were categorised into relevant groupings of ACMG criteria for each variant. This data was collated and analysed using descriptive statistics to identify any trends in reasons for classification differences across the set of variants categorised as discordant.

Results

As of January 2023, 148 MUTYH variants were recorded in ClinVar with conflicting interpretations. For an initial subset of these variants (n = 63), the primary ACMG/AMP criteria groups with identified discordances were computational evidence (discordant in 20.8% of variants), functional studies (discordant in 14.6% of variants), and case history (inclusive of both family history and previous cases) (discordant in 12.5%).

Conclusion

Results show that computational evidence criteria may benefit from MUTYH specific modifications as this criteria group was a frequently observed reason for discordance in pathogenicity classifications. Other discordances identified may be resolved with increased information sharing between laboratories or publication of data. Results from this research will be used to support the ongoing efforts of the MUTYH variant curation expert panel in creating gene-specific ACMG/AMP modifications.

P25 - MULTI-OMIC CHARACTERIZATION OF EARLY-ONSET COLORECTAL CANCER

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Background and aim

The identification of novel inherited CRC susceptibility through WES studies has not been as successful as expected, possibly due to the genetic heterogeneity of CRC. Our aim is to integrate the germline and tumor omics from a phenotypically homogeneous cohort of EO-pMMR CRC to identify novel genes of susceptibility to CRC.

Method

WES and RNA-seq were performed on paired normal-tumor tissues from 46 EO-pMMR CRC patients. Calling and variant annotation were performed on WES data (GATK). Additionally,

rare germline variants suspected of high-functional impact were prioritized. RNA-seq data were processed following GTEx protocol. Somatic methylation data were analyzed with ChAMP. Tumor endophenotyping consisted on the analysis of TMB (pyTMB), mutational signature profile (SigProfiler), consensus molecular subtyping (CMSCaller) and GSEA (g:Profiler) on tumor omic-data. To identify novel hereditary candidate genes, differential expression analyses on individual tumor data were compared with the previously prioritized variants found at germline level (all vs one approach).

Results

The CMS classification clustered CRCs into CMS1 (9%), CMS2 (23%), CMS3 (15%) and CMS4 (38%). Pathway analysis on RNA-seq data and methylation patterns agreed with this CMS classification. Tumor mutational signature analysis also showed molecular heterogeneity in our cohort. All tumors presented with at least one of the so called 'flat' signatures. 17/46 tumors exhibited signatures associated with DNA repair pathways, but TMB values or signature percentage did not support the biological involvement of these mechanisms in CRC tumorigenesis. The 'All vs one approach' revealed expression alterations in 11 genes in which previously prioritized germline alterations have been identified. The altered genes were involved in different pathways, like Wnt/beta-catenin pathway (*LEF1* and *TRABD2A*), cellular adhesion (*CDH26* and *CDHR2*), GTPase interaction (*PLEKHG6* and *ARHGAP10*), elongation and transcription factors (EEF2K and TCEA3) and genes involved in metabolic processes (*ZC3H12C* and *ADCY4*).

Conclusions

1. Tumor endophenotyping revealed the existence of molecular heterogeneity in an apparent pMMR CRC homogeneous cohort.
2. The integration of germline and tumor omic data facilitates the prioritization of hereditary risk variants, increasing the probabilities of identifying novel susceptibility genes for CRC.
3. 11 genes are proposed as candidate for hereditary susceptibility to CRC.

P26 - NATIONWIDE SURVEY ON LYNCH SYNDROME IN KOREA: THE STATUS QUO OF DIAGNOSIS, PATHOLOGY, AND TREATMENT

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Background and aim

Lynch syndrome (LS) is a heritable genetic condition caused by mutation in DNA mismatch repair (MMR) genes; *MLH1, MSH2, MSH6,* and *PMS2*. Although the diagnosis methods have evolved from pedigree to genetic tests, the characteristics of LS in the Korean population have not been updated recently, and there are no nationwide studies on surveilling them. This study aims to demonstrate the clinicopathological features with diagnosis and treatment of current Korean LS with a nationwide multicenter cohort.

Method

Colorectal cancer (CRC) patients who underwent surgery from 2004 to 2023 at the eight tertiary hospitals in South Korea were reviewed. The LS cohort included either hereditary nonpolyposis colorectal cancer (HNPCC) by the Amsterdam II criteria, or LS by detecting MMR mutations from the germline genetic tests. The cohort also included highly suspected LS with MMR protein deficient from the tissue immunohistochemistry (IHC) such as deficiency of MSH2 and MSH6, only MSH6, or only PMS2. The other hereditary CRCs such as familial adenomatous polyposis, and loss of MLH1 in the IHC without the genetic tests were excluded.

Results

In the highly suspicious Korean LS cohort (N=218), 52 (23.9%) fulfilled Amsterdam II criteria and 49 (22.5%) had been detected MMR gene mutation, and 24 of them were included in both. The mean diagnosed age for CRC was 54.5 years old with a standard deviation of 15.7, and 58.3% (n=127) were male. The right-sided colon cancer patients were more than half (n=127, 58.3%) of the cohort. Poorly differentiated adenocarcinoma was reported in 24 patients (11.0%), and 71.6% were relatively early stages (pathologic stage I and II, 25.7% and 45.9%, respectively). Extracolonic cancer was reported in 43 patients (19.7%), and the most common site was the stomach (19/43, 44.2%) followed by the endometrium (11/43, 25.6%). While 26 patients (11.9%) underwent extended resection (subtotal, total colectomy, and total proctocolectomy), most of the patients (n=186, 85.3%) were treated by segmental resection; right or left colectomy, (low/ultralow) anterior resection, and abdominoperineal resection. Deficiency in both MSH2 and MSH6 was reported in 106 patients (48.6%), and deficiency in only MSH6 or PMS2 was found in 22 (10.1%) and 19 (8.7%) patients, respectively.

Conclusion

For the past two decades, the Korean LS could be characterized as more male patients who underwent segmental resection with relatively early staged right-sided colon cancer.

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P27 - UPPER GASTROINTESTINAL DISEASE MANAGEMENT AFTER INTESTINAL TRANSPLANTATION FOR FAP-ASSOCIATED DESMOID

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Background and aim

Intestinal transplantation (IT) is a treatment option for patients with life-threatening small bowel mesenteric desmoid. Duodenal polyposis is a well characterised issue in FAP. But gastric adenomas and cancer appear to be increasing, and to be associated with desmoid, so may be a particular issue in transplant patients, who are also exposed to immunosuppression. Our aim was to characterise upper gastrointestinal (UGI) disease in this group.

Method

We identified patients on our prospectively maintained polyposis registry who had undergone IT for desmoid. Electronic patient records were reviewed for pre-transplant demographic data and general disease characteristics, as well as transplantation and pre-/post- transplantation UGI endoscopic data.

Results

From 1994 to 2023, 10 of our patients underwent IT.

Pre-transplant, UGI endoscopy was performed in all 10, and available for eight, patients, a median of 19 (range 1-51) months before the transplant. Three patients (30%) had two or more gastric adenomas (GAs); one underwent endoscopic mucosal resection (EMR) for a 4mm GA. Six patients (60%) had two or more duodenal adenomas (DAs) and four (40%) had twenty or more, two (20%) having modified Spigelman Stage III or more.

All 10 patients also underwent UGI endoscopy post-transplantation, with findings available for seven. Timing of first follow-up UGI endoscopy post-transplant was median 55 (range 17–88) months. Two patients (20%) had GAs. Three patients (30%) had at least four DAs, all equating to modified Spigelman stage II; one individual underwent cold snare polypectomy for 15 DAs.

Of these seven, one died from rejection, three have shown no interval change or significant pathology. One required two successive procedures (at 83 and 99 months post-transplant), demonstrating two DAs on the first (EMR performed) and three on the second (EMR planned); another, had a DA >10mm at 67 months (EMR also planned); the third had both

ampullary and non-ampullary DAs on two procedures within a year of transplant –EMR was performed, but residual non-ampullary DA remained at 34 months, and the patient died from metastatic adenocarcinoma of UGI origin at 45 months post-transplant.

Conclusions

These data have not shown UGI disease peri-transplantation to be a major issue, although one patient died from metastatic UGI cancer.

A careful and consistent approach to surveillance, therapy, and surgical decision-making is needed in this challenging group.

Keywords

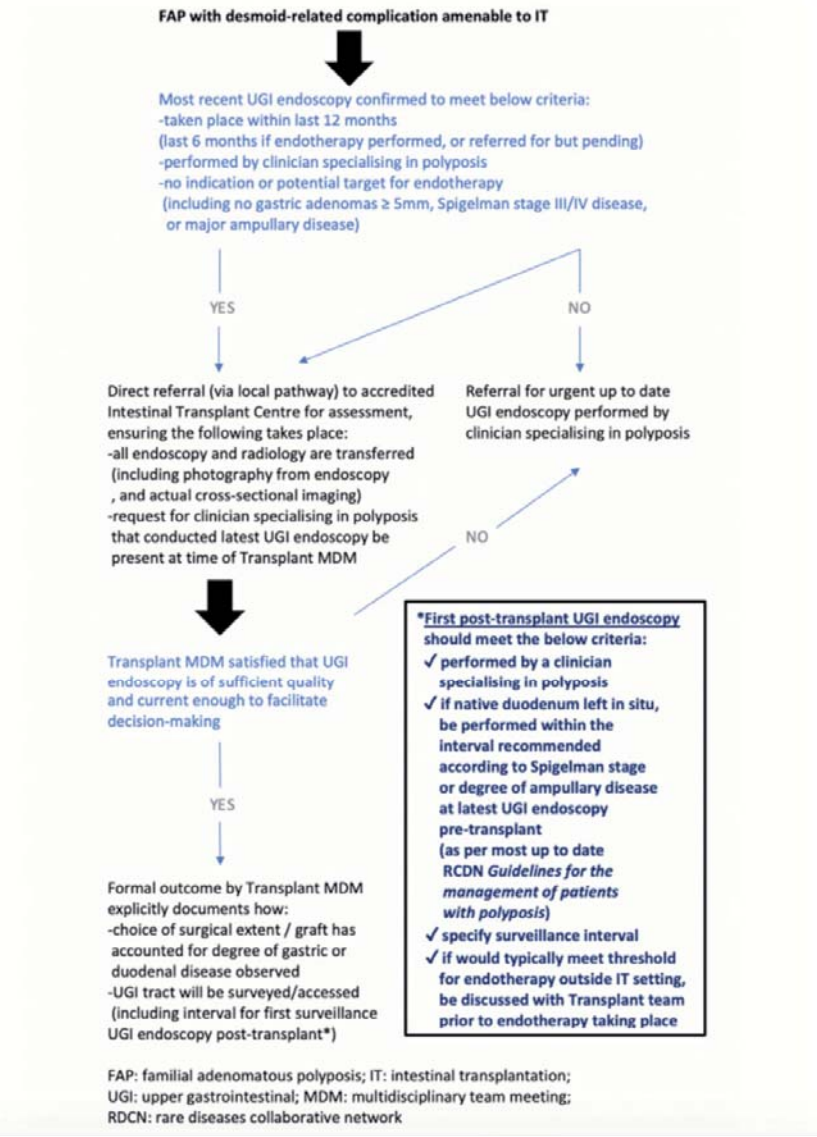
FAP, Desmoid, Intestinal Transplant, Upper Gastrointestinal.

Table 1. Transplantation information of patients.

Patient number	Age (years)	Sex	Desmoid location	Desmoid complication	Year of transplant	Graft type	Graft Constituents	Post-transplant survival (months)
1	39	F	A, M	Infective	2020	Modified MVT†	Small Intestine, Colon, Partial Gastric, Pancreas	36
2	54	F	A, M	Fistulation	2013	IIT	Small Intestine, Colon	121
3	38	F	A	Fistulation	2014	IIT	Small Intestine, Abdominal wall	113
4	39	F	M	Infective	2018	MVT†	Small Intestine, Colon, Partial Gastric, Liver, Pancreas, Autologous Kidney	63
5	51	M	M	Other	2022	MVT†	Small Intestine, Partial Gastric, Liver, Pancreas	11
6	40	F	M	Short bowel	2008	Modified MVT†	Small Intestine, Gastric, Pancreas, Kidney	63*
7	42	M	A, M	Fistulation	2018	Modified MVT†	Small Intestine, Colon, Partial Gastric, Pancreas	60
8	33	F	M, P	Infection	2020	MVT†	Small Intestine, Partial Gastric	38
9	40	M	M	Short bowel	2014	IIT	Small Intestine, Abdominal Wall	96*
10	38	M	M	Obstruction	1994	Modified MVT†	Small Intestine, Gastric, Pancreas, Kidney	73*

MVT: multivisceral transplant; IIT: isolated intestinal transplantation
A: abdominal wall; M: mesenteric; P: pelvic; R: retroperitoneal
†Foregut resection carried out as part of transplantation surgery
*Passed away at time-point described

Figure 1. Proposed algorithm for management of upper gastrointestinal disease in FAP patients with desmoid-related complications referred for intestinal transplantation.



FAP: familial adenomatous polyposis; IT: intestinal transplantation; UGI: upper gastrointestinal; MDM: multidisciplinary team meeting; RDCN: rare diseases collaborative network

P28 - LI FRAUMENI SYNDROME PREDISPOSES TO GASTRO-ESOPHAGEAL JUNCTION TUMOURS

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Background and aim

An association between Li-Fraumeni Syndrome (LFS), caused by germline pathogenic variants in TP53, and gastric adenocarcinoma has been recognised. The risk of esophageal cancer is less well characterised with conflicting reports. Further, gastro-esophageal junction (GEJ) tumours are variably classified as esophageal, gastric or a separate entity, depending on the classification system. In this context, expert consensus guidelines differ, and routine upper endoscopy screening is not universally recommended. There is, however, a biological basis to suspect TP53 as a driver of upper gastrointestinal (GI) cancers. The Cancer Genome Atlas Program (TCGA) classified sporadic esophageal and gastric cancers into distinct molecular phenotypes; pathogenic variants in TP53 were seen in the 'chromosomal instability' subclass which accounted for 49% of adenocarcinomas. Our GI risk management clinic routinely offers upper endoscopy at the same time as colonoscopy in LFS. We sought to assess rates of upper GI cancers and their characteristics in our LFS cohort, with comparison to results from TCGA cohort.

Method

A retrospective review of adult patients seen in our centre between January 2000 to May 2023 with clinical class 4 or 5 germline pathogenic variants in TP53 was performed. Separately, data of each adenocarcinoma case from TCGA were obtained from the cBioportal database for review.

Results

Sixty-five patients with LFS (57% female, mean age 34.5 years) were identified; 53.8% had undergone ≥1 upper endoscopy. Four patients (6.2%) were diagnosed with GEJ adenocarcinoma, with no other upper GI cancers; two cases were asymptomatic **(Figure 1)**. No cases were associated with prior family history of GI malignancy or abdominal radiotherapy. Further, five patients were diagnosed with a premalignant lesion (Barrett's esophagus, gastric intestinal metaplasia and a dysplastic fundic gland polyp). In comparison from TCGA, amongst sporadic esophageal/probable esophageal adenocarcinomas, 76.4% had somatic pathogenic variants in TP53, with decreasing prevalence in indeterminate GEJ (61.1%), gastric/probable gastric GEJ (46.0%) and gastric non-cardia (39.9%) adenocarcinomas **(Figure 2)**.

Conclusion

Our novel observations suggest the GEJ and lower esophagus are particularly vulnerable regions in LFS, in keeping with the molecular phenotyping seen in sporadic GEJ tumours. We advocate for routine endoscopic surveillance in LFS, with particular focus on the GEJ.

Figure 1. Panel of endoscopic, surgical and histologic findings in one case of GEJ adenocarcinoma on screening: Top, left: a subtle GEJ nodule at endoscopy (blue arrow); Top, right: endoscopic biopsy (H&E stain, x40) demonstrating adenocarcinoma adjacent to squamous mucosa; Bottom, left: surgical resection specimen with irregularity at GEJ (yellow arrow); Bottom, right: resection histology (H&E stain, x40) demonstrating adenocarcinoma confined to the mucosa, at the GEJ.

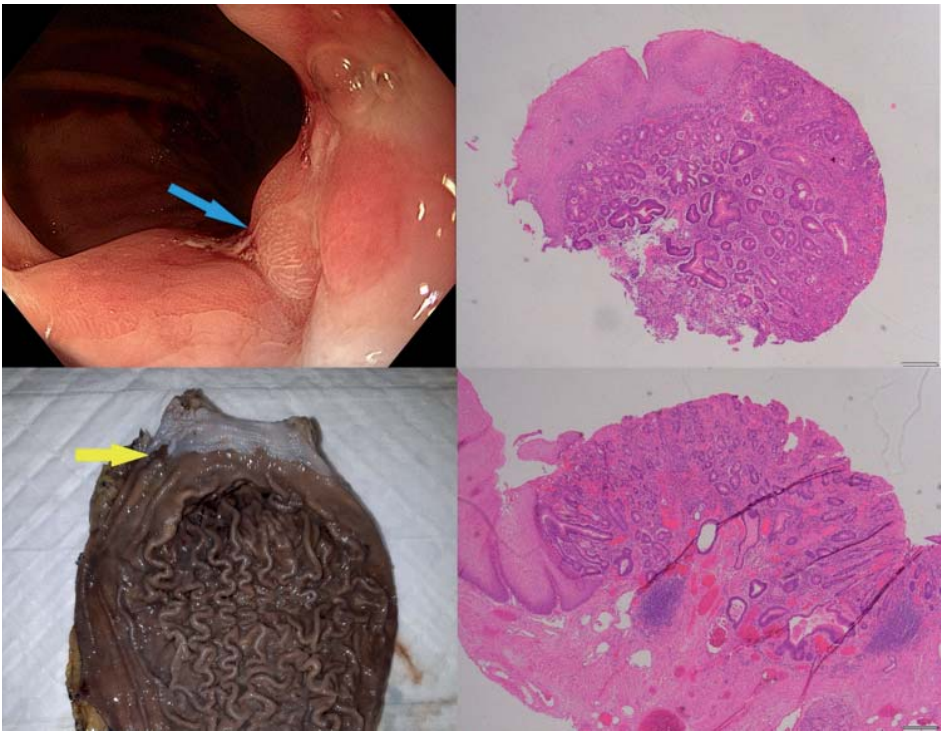
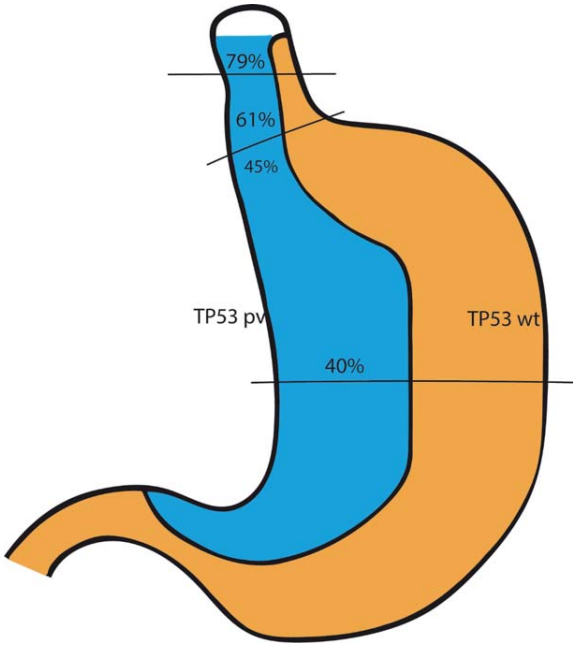


Figure 2. Relative proportions of somatic pathogenic variants (pv) and wild type (wt) in TP53 in each adenocarcinoma subgroup in the TCGA cohort. Esophageal/probable esophageal, indeterminate and gastric/probable gastric groups were GEJ tumours.



P29 - THE KRAS C.34G>T P.G12C SOMATIC MUTATION IS A BIOMARKER OF GERMLINE BIALLELIC MUTYH PATHOGENIC VARIANT CARRIER STATUS

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Background and aim

Carriers of germline biallelic pathogenic variants in the base excision repair gene *MUTYH* develop colonic polyposis and colorectal cancer (CRC). The *KRAS* c.34G>T p.Gly12Cys (*KRAS* p.G12C) somatic mutation has been associated with CRCs from biallelic *MUTYH* carriers but is infrequently observed in CRCs from non-carriers. The aim of this study was to determine the diagnostic utility of the *KRAS* p.G12C somatic mutation as a biomarker of germline biallelic *MUTYH* carrier status.

Method

Non-Lynch, CRC-affected proband participants from the Colon Cancer Family Registry (CCFR), ascertained from both population and clinic-based recruitment, underwent germline testing of the *MUTYH* gene with identified pathogenic variant carriers classified as either biallelic (homozygous or compound heterozygote carriers) or monoallelic (heterozygote carriers). All probands with available FFPE CRC tissue DNA were tested for somatic mutations in codons 12 and 13 of *KRAS* using Sanger sequencing. The diagnostic accuracy of *KRAS* p.G12C was determined overall and stratified by clinicopathological features.

Results

MUTYH and *KRAS* testing was completed on 5,684 participants who developed 5,837 CRCs (50.6% male; mean age at first CRC diagnosis (±SD) 54.7±12 years; 81% white). Biallelic and monoallelic *MUTYH* carriers represented 0.6% (35/5684) and 1.6% (93/5684) of the cohort, respectively. N=35 biallelic *MUTYH* carriers developed 42 CRCs (51.4% male; mean age at first CRC diagnosis 49.9±11.9 years, range 31-81 years). The *KRAS* p.G12C mutation was identified in 2.5% (147/5,837) of all CRCs. A *KRAS* p.G12C mutation positive CRC was identified in 21 biallelic carriers representing a sensitivity of 0.60 (95% CI=0.40-0.80), specificity of 0.98 (0.97-0.98) and AUC 0.79 (0.72-0.86), with positive likelihood ratio (+LR) of 28 (20-38) and negative likelihood ratio (-LR) of 0.41 (0.27-0.61). Limiting to younger-onset CRC improved predictive accuracy, with AUC increasing to 0.80, 0.83 and 0.99 for people diagnosed ≤60, ≤50 and ≤40 years, respectively, but these age restrictions would result in missing 4 (19%), 6 (29%), and 14 (67%) *KRAS* p.G12C positive biallelic *MUTYH* carriers, respectively.

Conclusions

The *KRAS* p.G12C somatic mutation represents a highly specific and diagnostically useful biomarker for identifying germline biallelic *MUTYH* carriers, particularly in people with younger-onset CRC.

Keywords

Colorectal cancer, *MUTYH*, *KRAS* somatic mutation, somatic biomarker, biallelic carrier diagnosis.

Conflicts of interest

None.

P30 - RISK FACTORS, CLINICOPATHOLOGICAL CHARACTERISTICS AND MOLECULAR FEATURES OF COLORECTAL CANCERS FROM PEOPLE WITH SERRATED POLYPOSIS SYNDROME

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Background and aim

People with Serrated Polyposis Syndrome (SPS) have an increased risk of developing colorectal cancer (CRC). This study aimed to characterise the clinicopathological and molecular features associated with CRC development in SPS.

Method

Participants who met the 2010 or 2019 WHO criteria for SPS were recruited to the Genetics of Colonic Polyposis Study. Clinicopathological characteristics were collated from medical records. Molecular testing enabled 74 CRCs to be categorised into three subtypes: 1) MMR-deficient (MMRd)/*BRAF* p.V600E mutation/CIMP-positive; 2) MMR-proficient (MMRp)/*BRAF* p.

V600E mutation/CIMP-positive, and 3) MMRp/*BRAF*-wildtype/CIMP-negative, with subtypes 1 and 2 considered serrated pathway CRCs and subtype 3 considered adenomatous pathway CRCs. Whole exome sequencing (WES) was performed on 26 SPS CRCs and compared with 37 age, sex and molecular subtype matched, non-SPS CRCs (controls) to identify SPS-specific genomic features.

Results

Of 807 SPS cases, 132 (16.4%) developed 177 CRCs. CRCs were predominantly in females (66.7%), located in the proximal colon (54.9%), were MMRp (64.7%) and observed at the time of SPS diagnosis (69.7%). SPS cases with CRC had an older age of SPS diagnosis (54.6±16.8yrs) compared with cases without CRC (41.8±14.9yrs, $P=1.8x10^{-15}$). CRC development was associated with a higher total polyp count (4th v 1st quartile, OR=4.0, 95%CI=2.3-7.2, $P=1.2x10^{-06}$), and the presence of extra-colonic cancers (OR=2.2, 1.1-4.4, $P=2.4\ x10^{-02}$). The presence of at least one traditional serrated adenoma (OR=4.9, 2.6-9.1, *P*=7.85e-07) or conventional adenoma (OR=3.9, 2.3-7.1, P=2.23e-06) was associated with an increased CRC risk.

Adenomatous pathway CRCs (subtype 3) comprised 46% of CRCs followed by subtype 1 (32.4%) and 2 (21.6%). The adenomatous pathway CRCs were associated with male sex ($P=2.49x10^{-02}$), younger age at CRC diagnosis($P=3.13x10^{-04}$), and the distal and rectal location (*P*=7.87e-05), when compared with serrated pathway CRCs (subtypes 1 and 2 combined). WES analysis identified 24 somatic mutations that were enriched in SPS CRCs compared with non-SPS CRCs (*Ps*<0.05).

Conclusions

We found CRC in SPS to be heterogeneous with regards to sex, age at diagnosis and anatomical location and displays a complex aetiology related to multiple risk factors and molecular pathways of tumourigenesis with nearly half of CRCs in SPS developing via an adenomatous pathway, highlighting the importance of adenomas in people with SPS.

Keyword

Colorectal Cancer, Serrated polyposis Syndrome, Tumour heterogeneity.

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P31 - ADDRESSING PATHOGENICITY CLASSIFICATIONS OF MLH1 LEGACY MISSENSE AND SPLICE SITE VARIANTS USING CURRENT MMR GENE SPECIFICATIONS TO THE ACMG/AMP CRITERIA

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Background and aim

DNA variant pathogenicity classifications may change as more experience is assembled in clinical practice, diagnostic laboratories and published literature. Gene specific updates to the ACMG/AMP criteria for pathogenicity may also impact classifications. ClinGen Variant Curation Expert Panels (VCEPs) are charged with maintaining currency of pathogenicity assignments including as published on ClinVar. The aim of this study is check Class 3 *MLH1* variants published in the ClinVar database not reviewed since November 2021 using the latest MMR specific modifications to the ACMG/AMP criteria (v1.0). Data published since the last review and a consensus outcome of any discordant variants informed the study. Reasons for any change in pathogenicity were analysed.

Method

MLH1 VUS variants not reviewed since Nov 2021 recorded on ClinVar as of Nov2023 were selected. Variants were filtered to ensure a high likelihood of classification change by selecting: (A) those with multiple submitters; (B) missense type; (C) documented association with GI cancers; (D) prior probability of ≥0.9 assessed using *in silico* tools (Align-GVGD, MAPP, PolyPhen-2.1, MutPred, SIFT and Mutation Assessor); and (D) gnomAD v4.0.0.0 allele frequency of ≤0.00002. For some variants, the submitting laboratory was contacted to see if further information was available. Mastermind (Chunn et al., 2020) was used to identify any literature related to the variants of interest. Reclassification was done using the MMR specific modifications to the ACMG/AMP criteria (v1.0).

Results

Twenty-three variants meeting the inclusion criteria were found, two were reclassified to LP and the other 21 remained VUS. All reclassified variants had new information only available after the time of initial interpretation dictating the reclassification. From those not reclassified, 2 had no literature, 10 had new literature which did not influence reclassification and 9 had no new literature. To date, submitting laboratories have not added new information.

Conclusions

This study provides a framework for systematically addressing legacy variants recorded on ClinVar to assist the InSiGHT ClinGen VCEP's legacy responsibilities. From this sample ~10% of the variants selected had new information available allowing reclassification to enhance clinical utility. Conversely, lack of updated detailed literature and familial studies often precludes reclassification.

P32 - QUANTIFYING 'JUST-RIGHT' APC INACTIVATION FOR COLORECTAL CANCER INITIATION

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Background and aim

Inactivation of the tumour suppressor gene APC drives around 80% of colorectal cancers (CRCs). Wild type APC acts as a scaffold protein for β-catenin degradation, functioning as a tumour suppressor via regulation of the Wnt pathway *(Hart et al. 1998; Fearnhead, Britton, and Bodmer 2001)*. This activity involves several protein domains, including short repeat sequences known as 20 amino acid repeats (20AARs), which bind to β-catenin. Mutations upstream of the first 20AARs sequence result in complete loss of binding activity and hence results in maximal constitutive Wnt activity *(Kohler et al. 2008; Schneikert, Grohmann, and Behrens 2006)*, but are rarely observed in CRCs. Most mutations occur in the Mutation Cluster Region (MCR), delimited by the first 20AAR and the SAMP repeat (codons 1260-1359), which results in intermediate Wnt activity *(Chandra, Behrens, and Schneikert 2009)*. Thus, even though the naive expectation is that complete loss of a tumour suppressor gene's function should be optimal for tumorigenesis, most lesions have mutations that do not fully inactivate APC. Whilst nearly all CRCs have elevated Wnt signalling, they appear to have far less than maximal.

Although the genetic data supports the 'just-right' model, the associations could be driven by mutational processes, rather than selection. Here, we propose a novel mathematical and computational approach that allows us to quantify the probability of CRC progression of cells with different APC genotypes, controlling for the underlying mutational processes in the healthy colon. If all APC genotypes provided the same selective advantage (uniform CRC risk model), we would expect a uniform distribution upon correcting by mutational biases. Applying our computational model to mutational data from 2,023 CRCs from the Genomics England Project, we obtain quantification of the selective advantage of certain APC genotypes for CRC progression, and relate this to Wnt activity. Furthermore, we investigate tumour heterogeneity in relation to Wnt activity based on the anatomical site of the lesion and the presence of additional mutations on secondary Wnt regulators. Orthogonal evidence is obtained by analysing genetic variability in cancers in FAP patients.

Method

We develop a mathematical framework characterising the initial stages of CRC to test and quantify the 'just-right' hypothesis for APC inactivation. In the model, the genotype of initiated colonic stem cells is defined by the position and class of the mutations on the two

alleles, where we consider all major mutation classes underlying APC inactivation: nonsense mutations, frameshifts and copy number alterations.

As APC is the first step in the microsatellite-stable adenoma-to-CRC pathway, we parametrize the model using sequence data of healthy colonic crypts *(Lee-Six et al. 2019)*. Combining mutational signature data with the genomic context of APC and estimates for copy-number alterations in healthy tissue, we establish the probability that a genotype occurs in a healthy crypt. This is compared to the frequencies in large CRC cohorts, including the Genomics England Project (100KG) and The Cancer Genome Atlas Program (TCGA), allowing us to quantify the CRC progression probabilities of APC genotypes, defined as the probability that a cell with the given genotype transforms into CRC.

We relate APC genotype to the level of Wnt activity by determining the total number of 20AARs repeats retained across both alleles, which inversely correlates with Wnt activation *(Kohler et al. 2008; Schneikert, Grohmann, and Behrens 2006)*, allowing us to test the 'just-right' model for APC-driven Wnt activation.

The model is applied to different subsets of tumours, including lesions with different genetic background or in different anatomical sites. We quantify the difference in mean APC inactivation across pairs of tumour subsets, providing a framework to test if con-founders result in a statistically significant shift in the fitness landscape of APC genotypes.

Results

Using sequence data from 2,023 CRCs from the 100,000 Genomes Project, we find that the relative advantage of APC double mutants depends on the total number of 20-amino acid degradation repeats (20AARs) domains translated across both alleles. In agreement with the 'just-right' model, an intermediate number of 20AARs retained provides the greatest advantage to the growing tumour. Cells retaining a total of two 20AARs have around a 100-fold increase in probability of progressing into CRC compared to crypts with zero 20AARs, in which APC function is most inactivated. The quantities derived are consistent with independent CRC cohorts (TCGA) and data from FAP patients . While our analysis shows a maximal progression risk for specific APC genotypes, a considerable proportion of tumours in the 100KG cohort develop through 'non-optimal' APC mutations. Thus, we find a probabilistic, continuous fitness landscape of APC mutations and corresponding Wnt activity, where factors such as environment or background genetics might modulate the CRC progression probabilities of cells with different APC genotypes. We next studied site-specific mutational processes and selective pressures and genetic background as explanatory factors of variability in APC genotypes.

Upon correction for anatomical site-dependent active mutational biases, we find that the just-right level of APC inactivation is significantly different for distal versus proximal tumours Tumours in the proximal colon retain a significantly higher number of 20AARs, suggesting that they benefit from lower Wnt activation. This could be due to differences in baseline Wnt levels or differences in selective pressures for Wnt activity, including varying immune pressures. In hypermutated tumours (POLE-mutant and MSI cancers in the 100KG cohort), even though the distribution of mutations on APC is considerably different in both cancer

types, upon correction for the adequate mutational signatures, we recover the same pressure for 'just-right' Wnt as in MSS, stressing the importance of accounting for context-specific mutational biases in the cancer. Moreover, this finding points to POLE alterations and MSI status preceding Wnt dysregulation in tumorigenesis.

We find significant differences in APC genotypes in tumours with additional mutations of Wnt regulators, providing evidence of pathway-level selection for optimal Wnt activity at tumour initiation. This analysis provides a framework to measure the quantitative effect on Wnt activity of secondary Wnt regulators. In particular, we find that AMER1 mutations are associated with APC mutations that result in lower Wnt activation, pointing to AMER1 as a secondary Wnt driver that tops up sub-optimal APC genotypes to achieve 'just-right' Wnt activity at early stages of tumorigenesis. We also find that lesions with mutations on TCF7L2 are associated with APC genotypes that result in higher Wnt activity, suggesting that TCF7L2 mutations result in decreased Wnt activity, whilst our analysis suggests that mutations on SOX9, FBXW7 and BCL9L, act as Wnt-upregulators. Analysis of FAP polyp (n=12) and cancer (n=4) novel data shows that mutations on secondary Wnt regulators are present at early stages and precede malignant transformation, providing further evidence for the 'just-right model' of Wnt activity as well as the 'mini driver' modulator model, where the 'just-right' level of Wnt activation required for cancer progression results from co-selection of APC mutations and secondary mutations that top up or down the effect of APC *(van Ginkel et al. 2023)*.

Conclusions

By integrating several independent somatic and cancer datasets with mathematical modelling, our analysis measures the oncogenic effect of APC genotypes in relation to their Wnt activation levels, quantitatively demonstrating that maximal tumour suppressor loss does not imply maximal cancer risk. In sporadic and familial MSS CRC, double allelic inactivation of the tumour suppressor APC is under selection to retain an intermediate level of function, resulting in submaximal Wnt pathway activation. Cells with APC mutations that result in intermediate Wnt activity are at 100x advantage to progress to CRC compared to cells with maximal Wnt. The Wnt activation level that maximises cancer progression potential depends on the anatomical site of the lesion, and is modulated by additional mutations on secondary Wnt drivers, with AMER1 as a main regulator. The fitness landscape of APC genotypes observed in sporadic MSS CRC is consistent across hypermutated CRCs and tumours in FAP patients, suggesting a canonical role of Wnt regulation for CRC initiation.

Inactivation of tumour suppressor genes is a key step in the development of cancer. While a high degree of variability in the fitness conferred by specific mutations of oncogenes is expected, that maximal suppression of APC does not lead to maximal cancer risk is remarkable. The reason for the paucity of colorectal cancers with complete APC inactivation is currently unclear; however, as with hyperactivation of other cancer signalling pathways, e.g. excessive RAS signalling inducing senescence *(Li et al. 2018)*, overstimulation of the Wnt pathway is likely to be toxic. This hypothesis is supported by a recent study of activation-induced

lethalities in cancer, pointing to the conditional activation of signalling pathways as a new source of selective cancer vulnerabilities, with APC-driven Wnt activation in colorectal cancers as a clear candidate *(Chang et al. 2023)*. Whilst exploiting hyperactivation of cancer pathways for therapeutics is a promising paradigm shift, it is imperative to quantify the contribution of specific mutations to different activation levels and elucidate context-dependent advantages, accounting for the biases of active mutational processes in the cancer. Furthermore, as demonstrated by our strategy considering the number of 20AARs to connect APC genotypes with Wnt pathway, it is key to assess genotypes in the context of the associated signalling pathways as opposed to adopting solely a mutation-centric lens. With a growing amount of available data on somatic mutational processes and tumour mutational landscapes, pathway informed computational approaches as proposed in this work could help to quantify how the forces of selection and mutation combine to shape cancer evolution.

P33 - GASTRIC CANCER IN PATIENTS WITH FAMILIAL ADENOMATOUS POLYPOSIS: A CHALLENGING ENDOSCOPIC DIAGNOSIS?

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Background and aim

Familial adenomatous polyposis (FAP) is associated with several extra-colonic manifestations, including duodenal polyposis and desmoid tumors. Recently, gastric cancer has been recognized as an emerging concern in FAP. This case series presents the experience with gastric cancer in a FAP expert center.

Method

FAP patients with gastric cancer were identified by review of electronic medical records. The clinical characteristics of these patients, including comorbidities, type of surgery and survival data were collected.

Results

A total of eight FAP patients were diagnosed with gastric cancer, seven of whom were diagnosed in recent years (2018 - 2023). Mean age at cancer diagnosis was 50 years, ranging from 33 to 64 years, with the majority being female (n = 7). Six out of eight cases were located in the proximal stomach amidst extensive (carpeting) fundic gland polyps, while two were located in the distal stomach. All patients were under surveillance, with a mean endoscopic follow-up of 112 months and a mean number of upper-gastrointestinal surveillance endoscopies of 10 (range 2 - 19) until cancer diagnosis. Five of eight gastric cancers were diagnosed at endoscopy. Those not recognized during surveillance (n = 3) were all located in the proximal stomach in an area of carpeting fundic gland polyposis. Prior to the diagnosis of gastric cancer, all patients were diagnosed with at least one dysplastic lesion in the stomach. The highest Spigelman stage was II in five patients, III in one patient and IV in two patients. Of the eight gastric cancer patients, three survived, four died within 24 months (range 1 – 24) and one is currently receiving palliative therapy.

Conclusion

In recent years, there has been an increase in the diagnosis of gastric cancer in FAP patients. Proximal gastric cancer are always located within (abundant) carpeting fundic gland polyposis which may contribute to challenging endoscopic recognition, even by expert endoscopists. To prevent gastric cancer incidence and mortality, improved endoscopic surveillance of the stomach is urgently needed.

P34 - RISK FACTORS FOR PROXIMAL GASTRIC ADENOMAS IN PATIENTS WITH FAMILIAL ADENOMATOUS POLYPOSIS

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Background and aim

Gastric cancer is a more recently recognized challenge in the management of familial adenomatous polyposis (FAP). Proximal gastric adenomas are believed to be precursor lesions. This study aimed to describe the incidence of these gastric adenomas in patients with FAP and identify potential risk factors for their development.

Methods

Data was retrospectively collected from FAP patients who had undergone esophagogastroduodenoscopy (EGD) between 2015 and 2023 at our academic center. All diagnoses of proximal gastric adenomas were histologically confirmed. A multivariable Cox proportional hazard regression analysis was performed to identify risk factors for proximal adenoma development.

Results

Among the 196 FAP patients who underwent EGD, 33 (17%) were diagnosed with proximal gastric adenomas. The median age at diagnosis was 48 years (range 19-80). A total of 105 proximal adenomas were identified, with 61% detected in female patients. The majority (89%) of the proximal gastric adenomas were found in patients with at least 50 fundic gland polyps. High-grade dysplasia was found in 10 (9.5%) proximal gastric adenomas. In the Cox proportional hazard regression analysis, carpeting fundic gland polyposis ≥100 (HR = 8.94; $p < 0.001$), biliary reflux following duodenectomy (HR = 1.92; $p = 0.017$) and the use of proton pump inhibitors (HR = 1.78 ; $p = 0.014$) were risk factors for proximal gastric adenoma development. An advanced Spigelman stage (III/IV) (HR = 0.37; $p < 0.001$) was associated with a significantly lower risk of developing proximal gastric adenoma compared to a lower Spigelman stage (0-II) .

Conclusions

Proximal gastric adenomas are commonly detected in FAP patients. Carpeting fundic gland polyposis (≥ 100), biliary reflux and use of PPIs were identified as risk factors for their

development. These results emphasize the need for careful assessment of the gastric mucosa in FAP patients, particularly in those with numerous fundic gland polyps where adenomas might be more easily missed. Those with advanced duodenal disease had a significantly lower risk of developing proximal gastric adenomas, indicating that endoscopic surveillance of the upper-gastrointestinal tract should not solely rely on the Spigelman stage.

P35 - FUNCTIONAL OUTCOMES AND QUALITY OF LIFE AFTER ILEORECTAL VERSUS ILEAL POUCH-ANAL ANASTOMOSIS IN FAMILIAL ADENOMATOUS POLYPOSIS

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Background

Patients with familial adenomatous polyposis (FAP) generally undergo total colectomy and ileorectal anastomosis (IRA) or proctocolectomy and ileal pouch-anal anastomosis (IPAA) to prevent colorectal cancer. We aimed to study functional outcomes and quality of life after each procedure.

Methods

This is a cross-sectional study, in which FAP patients from European FAP expert centers who underwent IRA or IPAA were invited to fill out four validated questionnaires on bowel function (bowel frequency and Vaizey score), urinary function (international prostate symptom score (IPSS)), sexual function (Female Sexual Function Index (FSFI) and the International Index of Erectile Function (IIEF)) and quality of life (short form-36 (SF-36)). Multivariable analysis was performed to adjusted for potential confounders.

Results

Questionnaire packages were sent to 283 patients and filled out by 207 (73%): 78/113 IRA patients and 129/170 IPAA patients (median age 52 versus 45, respectively). The mean daily bowel frequency was 5.2 in the IRA group and 7.6 in the IPAA group and antidiarrhoeal medication was used by 43/123 (35%) of the IPAA patients and 12/70 (17%) of the IRA patients ($p = 0.02$ in multivariable analysis). No difference in fecal urgency was observed.

Reported urinary and sexual outcomes were comparable between the groups. No difference was observed for most SF-36 subscales, except for higher scores for mental health and role functioning/emotional in the IPAA group. The mean daily bowel frequency was associated with quality of life.

Conclusion

Patients with IRA and IPAA report comparable functional outcomes and quality of life, except for better outcomes on bowel function in the IRA group. These findings can inform physicians and patients when deciding on the type of (procto)colectomy.

P36 - A GWAS IN SWEDISH COLORECTAL CANCER FAMILIES WITH GASTRIC- AND PROSTATE CANCER

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Aim

To identify candidate loci, -genes and -SNPs that could be associated with an increased risk of CRC, gastric- and prostate cancer.

Background and Introduction

A new syndrome with an increased risk of colorectal, gastric and/or prostate cancer inherited as a complex disease was suggested in our previous studies. The data favoured this syndrome inherited as a complex disease. Therefore, a Genome Wide Association Study (GWAS) was conducted on colorectal cancer patients, with prostate-, and/or gastric cancer in their families. This is a genome wide association study of 685 colorectal cancer cases (with at least one case of gastric and/or prostate cancer in the family and 1642 healthy controls, followed by a search for candidate SNPs on these haplotypes, suggested candidate genes associated with an increased risk of these three tumour types.

Results

A logistic regression model was used, 50 loci with 58 haplotypes were found with p-value of 5E X 10^{-6} and odds ratio between 1.38-6.52. The loci on chromosome 9 (9p24.3) was found to be significant with the p-value of 2.41E-08 and odds ratio of 2.3. Within the suggested haplotype regions, many had protein coding genes. Many of the suggested genes within the loci have been previously reported to be involved in different cancers including colorectal, gastric, and prostate cancer. WGS and WES in a subgroup of these cases identified 33 candidate SNPs in 14 loci, 17 SNPs in 11 loci could be tested in MALDI-TOF SNP analysis in a larger cohort of colorectal cases and healthy controls. For 4 of the tested 11 loci (2q33.1, 4q31.1, 4q31.3 and10q11.21) there was a support for an increased risk of colorectal, gastric-, and/or prostate cancer.

Conclusion

Our study identified candidate loci, - genes and -SNPs that could be associated with increased risk of CRC, gastric- and prostate cancer.

Many of the genes found in our loci have been implicated also in other cancers, besides those selected for the study, this further supports an increased cancer risk of varying degree for different tumours.

Further studies of these loci/genes are warranted to determine the actual risk at the loci. Moreover, we consider the study as a proof of principle; it is possible to use the design in this paper to find SNPs associated with disease in risk haplotype regions.

P37 - THE ROLE OF FERROPTOSIS IN SPORADIC AND FAMILIAL INTESTINAL CANCERS

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Background and aim

Colorectal cancer (CRC) is the third most common cancer worldwide and has a 53% survival rate over 10-years. Sporadic CRC account for around 70% of CRC cases and have no known genetic cause. Familial Adenomatous Polyposis (FAP) and MUTYH-Associated Polyposis (MAP) are hereditary syndromes accounting for around 2% of CRC. FAP and MAP are characterised by the development of tens to thousands of benign intestinal polyps (adenomas), resulting in a substantially increased risk of CRC. FAP and MAP represent accelerated models of sporadic CRC development.

Inactivation of *APC* by somatic mutation is known to be the initiating event in up to 80% of sporadic CRCs, and in FAP and MAP adenomas. Increasing genomic instability, in addition to other mechanisms, is associated with tumour progression. Ferroptosis is a mechanism of programmed cell death, known to play a role in tumour suppression in sporadic CRC, although it has not been studied in adenomas or CRC from FAP or MAP. As highly proliferative cells, it is hypothesised that FAP and MAP adenomas, in addition to sporadic CRC, are vulnerable to ferroptotic inducers. This study aims to understand the role of ferroptosis in sporadic and familial adenomas and CRC.

Methods

2D sporadic colorectal (CR) cell lines, MC38 and Caco2 and 3D patient derived sporadic CRC and matched normal mucosa, from the same patient, organoid lines were treated with ferroptotic inducers Erastin and RSL3. Cell viability was assessed with an MTT assays and flow cytometry. Whole transcriptome analysis (RNASeq) of FAP and MAP CR and duodenal adenomas and normal mucosa was used to determine the expression of genes associated with ferroptosis. Gene expression in FAP and MAP CR adenomas were validated using western blot in an additional series of 11 FAP or MAP CR adenomas and 2 normal mucosa.

Results

Erastin and RSL3 can induce death by ferroptosis in 2D cell lines **(Figure 1)** and in patient derived organoids as seen by reduction in cell viability. FAP and MAP CR, but not duodenal adenomas, are expected to be vulnerable to ferroptosis as seen through increased expression of genes such as *SLC7A11* and *GLS2* **(Figure 2)** and increased protein abundance.

Conclusion

This study provides further knowledge of the role of ferroptosis in sporadic and familial intestinal tumour development. The results suggest that sporadic CRC, and FAP and MAP adenomas are vulnerable to ferroptosis. Inducing ferroptosis in these tumours could be of therapeutic relevance.

Keywords

FAP, MAP, Ferroptosis, polyposis, CRC.

Figure 1.

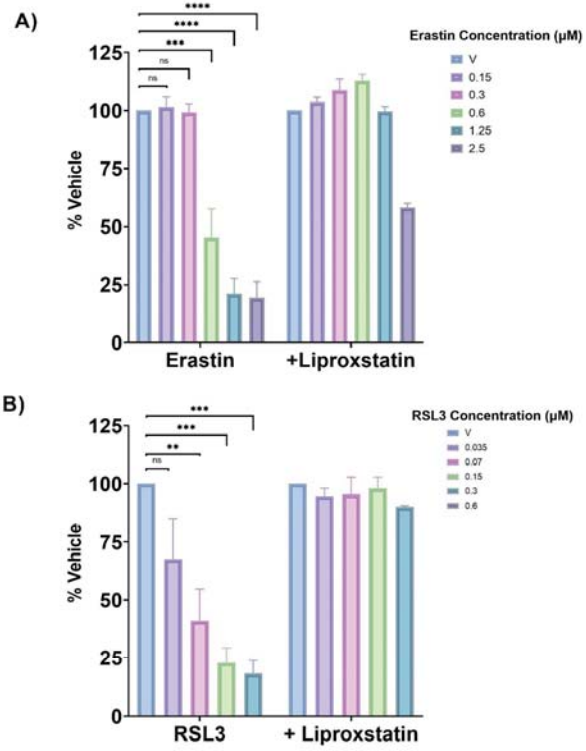
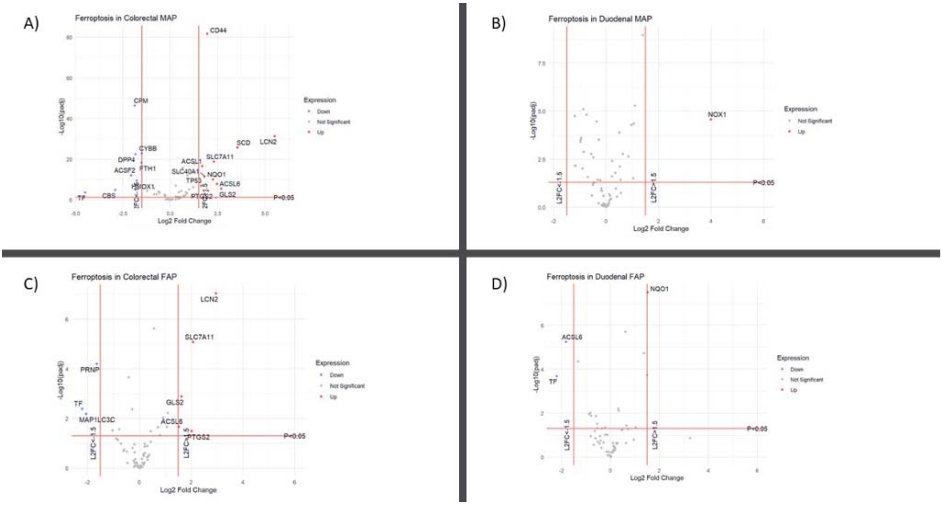


Figure 2.


A)
Ferroptosis in Colorectal MAP
CD44
CRM
CYBB
SCD
LCN2
DPP4
ACSF2
FTH1
ACSL3
SLC7A11
SLC40A1
NQO1
TP53
HMOX1
ACSL6
TF
CBS
PTGS2
GLS2
P=0.05
Expression
Down
Not Significant
Up
-Log10(padj)
Log2 Fold Change
B)
Ferroptosis in Duodenal MAP
NOX1
L2FC<-1.5
L2FC>1.5
P=0.05
Expression
Not Significant
Up
-Log10(padj)
Log2 Fold Change
C)
Ferroptosis in Colorectal FAP
LCN2
SLC7A11
PRNP
TF
GLS2
MAP1LC3C
ACSL6
L2FC<-1.5
L2FC>1.5
PTGS2
P=0.05
Expression
Down
Not Significant
Up
-Log10(padj)
Log2 Fold Change
D)
Ferroptosis in Duodenal FAP
NQO1
ACSL6
TF
L2FC<-1.5
L2FC>1.5
P=0.05
Expression
Down
Not Significant
Up
-Log10(padj)
Log2 Fold Change

P38 - SCREENING FOR ENDOMETRIAL PRECURSOR LESIONS IN FEMALE LYNCH SYNDROME CARRIERS

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**Authors equally contributed to this paper*

Background and aim

Patients with Lynch Syndrome (LS) have a predisposition for developing colorectal cancer (CRC), but females are equally – if not more - at risk for developing endometrial cancer (EC). While the carcinogenesis pathway in LS carriers for CRC has been studied extensively, information on the development of EC remains scarce. The aim of this study was to gain insight into changes in morphology and specific molecular markers of EC in Lynch patients.

Method

In this retrospective cohort study, female LS carriers in the Dutch national LS database were included and linked to the Dutch National Pathology database (PALGA). Endometrial tissue was retrieved of female carriers who had at least two biopsies within ten years prior to their (prophylactic) hysterectomy. These samples were revised for possible anomalies in morphology and ARID1A, PTEN and DNA mismatch repair (MMR) protein expression.

Results

79 endometrial tissue samples of 28 female LS carriers were retrieved and revised. 5 carriers developed EC, 9 carriers developed endometrial hyperplasia, and 14 carriers did not develop

any anomalies. In all 5 carriers with EC, premalignant changes in tissue morphology and aberrant staining of PTEN and DNA MMR proteins was observed in tissue samples prior to EC diagnosis. In 8 carriers with hyperplasia, morphological anomalies such as crowding or irregular architecture were seen in the tissue samples prior to hyperplasia diagnosis. ARID1A expression was conserved in all but 3 carriers, whom had developed endometrial hyperplasia or EC. Loss of expression/abnormal expression of PTEN was seen in 7 LS carriers with hyperplasia (7/9) and in 4 carriers without anomalies (4/14). Focal or diffuse DNA MMR protein loss was found in 8 carriers with hyperplasia (8/9) and 3 controls (3/14). **Conclusions.** Based on this retrospective cohort study, morphological alterations and PTEN/MMR gene expression aberrations seem associated with malignant progression in Lynch syndrome associated endometrial cancer. Further studies are needed to determine the value of these findings in endometrial surveillance and possible timing of surgery in this patient group.

Keywords

Lynch Syndrome, endometrial carcinoma, hyperplasia.

Conflicts of interest

The presenting author declares no conflict of interest.

P39 - YOUR INSIGHT

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Background and aim

The biennial meeting is an opportunity for InSiGHT Council and members to meet, and for members to find out more about and contribute to their society, and its activities. We aim to provide information on the membership and activities of InSiGHT, its Council and secretariat since the last meeting.

Method

Data were obtained from the InSiGHT membership database, minutes of Council meetings and Google Analytics.

Results

During the period 2023-2024 there were 171 fully paid up members of InSiGHT (97 MDs) and 12 honorary life members. The breakdown by country was: Australia 16, Austria 1, Belgium 2, Brazil 1, Canada 4, China 5, Denmark 8, Finland 5, Germany 9, Ireland 2, Israel 3, Italy 7, Japan 6, South Korea 1, Luxembourg 1, New Zealand 9, Norway 2, Rwanda 1, Singapore 1, Spain 11, Sweden 3, Switzerland 2, The Netherlands 13, UK 24, USA 44.

Details of current Council members and their roles will be presented, along with information on how to stand for Council. The voting system for Council has been updated to allow voting by e-mail, allowing members who cannot attend the meeting to remain engaged.

In the period from 1 July 2033- 1 Jan 2024 there were 60000 hits on the website, by 7600 users. The most visited pages were those on the biennial meeting, the variants database and information on Lynch syndrome. Visitors to the site were from countries shown in **Figure 1**, the most being from the USA, followed by UK, Germany, Australia, China and Italy.

Jackie Hawkins has retired, and the secretariat is now comprised of Vicky Cuthill and Sue Clark.

Conclusions

InSiGHT continues to have a broad international audience, membership and Council, and welcomes new members. The website is visited frequently.

Keywords

Society.


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ueg

Figure 1.


Users by Country ID
COUNTRY
USERS
United States 1.8K
United Kingdom 1K
Germany 648
Australia 645
China 372
Italy 342
Spain 314
Last 12 months
View countries

P40 - UK HEREDITARY GASTROINTESTINAL POLYPOSIS SYNDROMES RARE DISEASE COLLABORATIVE NETWORK: THE FIRST TWO YEARS

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Background and aim

There is good evidence that registry-based specialist care results in better outcomes in familial adenomatous polyposis (FAP). We have worked with NHS(England) to create a UK-wide hereditary gastrointestinal polyposis syndromes rare disease collaborative network, covering FAP and rarer syndromes including MutYH associated polyposis, Peutz-Jeghers syndrome (PJS) and juvenile polyposis syndrome (JPS). The aim of the network is to support and develop the centres to allow equality of patient access to specialist care across the country, identify research priorities and undertake collaborative research.

Method

The network comprises seven centres, each with a multidisciplinary team focussed on management of and research in polyposis syndromes.

Results

We hold a monthly virtual and biennial in person meeting. Service specifications, KPI's and guidelines have been agreed. A consensus statement from the group has been published; this recommends all patients with these conditions should be referred to their local centre for discussion of key management decisions (eg timing/type of prophylactic surgery, management of difficult manifestations), and ideally have all of their management at the nearest specialist centre within the network.

The relevant professional associations (UK-CCG, ACPGBI, BSG) have been supportive, and publicised information about the network.

Management of desmoid disease has been identified as a research priority, and funding obtained for a PhD student to undertake this.

In 2022 the network cared for 2200 patients with FAP, 250 with PJS and 219 with JPS; 2500 specialist endoscopies and 131 operations were performed.

Conclusions

Ongoing effort is required to ensure that appropriate patients can access the expertise of the network.

Keywords

Specialist network.

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P41 - COMPREHENSIVE ANALYSIS OF GERMLINE VARIANTS IN A SWISS COHORT OF EARLY-ONSET COLORECTAL CANCER PATIENTS

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Introduction

About 1% of colorectal cancer (CRC) cases are diagnosed before age 40. Even after appropriate clinical and biological investigation, many of these patients do not have an identifiable hereditary or familial risk component. The main objective of this project is to identify genetic variants predisposing to CRC at a very young age using constitutional whole-exome sequencing (WES) and parent-offspring trio analysis.

Methods

Eligible patients were diagnosed with CRC <41 years. In a first step, we performed a custom germline CRC gene-panel testing (n=20 genes). After exclusion of patients carrying germline likely pathogenic/pathogenic (LP/P) variants, WES was performed.

Results

Fifty-six patients took part in this project. Three out of 56 (5.4%) early-onset CRC patients were found to carry heterozygous LP/P variants in *PMS2, MSH6* and homozygous LP/P variants in *MUTYH*, respectively. After exclusion of these carriers of germline LP/P variants and individuals without formal consent for exome testing, WES was performed on 33 index patients plus 2 trios. Four patients have been identified as carriers of germline LP/P variants in *BRCA1* (n=2), *BRCA2* (n=1) and *HOXB13* (n=1). Multiple variants of unknown significance (VUS) have been identified including some very interesting ones. Few of them might have the potential to play a role as CRC susceptibility genes, such as the base excision repair genes *OGG1* and *MBD4*. The 2 trio analyses did not identify *de novo* or recessive LP/P variant potentially involved in colorectal carcinogenesis. No secondary findings in actionable genes (ACMG SF v3.2, 2023) have been identified in index cases and among the 2 pairs of parents.

Conclusion

In our cohort, 12.5% of early onset CRC patients have been identified as carriers of germline LP/P variants in known cancer predisposition genes. The 2 patients identified as carriers of germline variants in DNA mismatch repair genes did not have a family history suggestive of Lynch syndrome. Identification of 3 LP/P variants in *BRCA1/BRCA2* raises the question about a possible risk of CRC in HBOC syndrome.

This work was supported by a grant of the Swiss National Science Foundation.

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P42 - A GERMLINE SPLICING-VARIANT IN THE RPS20 GENE IS ASSOCIATED WITH A MULTI-TUMOUR PREDISPOSITION CHARACTERISED BY EARLY-ONSET COLORECTAL CANCER AND EXTRACOLONIC MALIGNANCIES

Fulvia Brugnoletti[1], Aurélie Ayme[1,2], Amandine Cadènes[1], Thomas Von Känel [3], Jean-Louis Blouin[4], Thomas Mckee[2], Petros Tsantoulis [1], Karl Heinimann[5], Pierre Olivier Chappuis [1,4]

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Background and aim

RPS20 has been proposed as a very promising colorectal cancer (CRC) predisposition gene with 4 families described today showing early-onset CRC associated with *RPS20* germline variants. Only in one report extracolonic manifestations were also described in carriers. Here we report a family with a very strong cancer history in which a *RPS20* germline splicing-variant was identified in 3 individuals showing early-onset multi-tumour phenotype.

Methods and Results

We performed germline genetic testing on 3 members (father and his 2 children) of a family followed in our Oncogenetic Unit from more than 20 years. All patients had a diagnosis of multiple tumors, including CRC before the age of 50. Genetic tests ranging from direct single-gene testing to next generation sequencing cancer panel were performed according to clinical practice at the time of evaluation.

In 2020, a gene-panel analysis including 23 known colorectal and breast cancer predisposition genes (*ATM, APC, BMPR1A, BRCA1, BRCA2, CHEK2, GREM1, MLH1, MSH2, MSH3, MSH6, MUTYH, NTHL1, PALB2, PMS2, POLD1, POLE, PTEN, RNF43, RPS20, SMAD4, STK11, TP53)* was performed on the daughter diagnosed with breast cancer at 31 and pMMR-CRC at the age of 45. This testing identified the heterozygous variant NM_001146227.3:c.178-3C>G in the *RPS20* gene. This variant is located at the splice acceptor site of exon 4 and is absent from currently used genomic databases (LOVD, HGMD, ClinVar, gnomAD). To elucidate the impact of this alteration, we performed complementary mRNA analysis by PCR and sequencing the

RPS20 RNA transcript. This showed that the c.178-3C>G variant leads to an in-frame loss of *RPS20* exon 4 and, at the protein level, to a loss of 52 amino acids (NP_001139699.1:p.(Thr60_Glu111del)).

Following these results suggesting the pathological potential of this variant, a segregation analysis was performed in the family: the *RPS20* splicing-variant has been identified in the DNA of the youngest daughter diagnosed with leg osteosarcoma at 9 and dMMR-CRC at 36 and in the father with pMMR-CRC at 26 and choroidal melanoma at 48. The variant was formally excluded in the unaffected mother.

Conclusion

Our study suggests that inactivating germline variants in *RPS20* gene may be associated with early-onset CRC and with multiple extracolonic malignancies. Future investigations are planned on tumour's material to better characterize the link between this *RPS20* variant and extracolonic cancers in this family.

P43 - MENTAL HEALTH AND UNMET NEEDS IN FAMILIAL ADENOMATOUS POLYPOSIS A SYSTEMATIC REVIEW

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Background and aim

Because of the nearly 100% risk for colorectal cancer without treatment, people with a pathogenic variant in the APC gene causing familial adenomatous polyposis (FAP) are under lifelong endoscopic surveillance and undergo prophylactic colectomy before the age of forty. Children of APC mutation carriers have a 50% risk to inherit the same disease. It can be a challenge to cope with the burden of FAP. The aim of this systematic review was to study the literature on the presence of mental issues and the support needs in FAP patients.

Methods

A search was performed in PubMed, EMBASE and Cochrane Library to retrieve English studies on mental health and FAP from 1947 to May 2024.

Results

Of the 1,954 identified papers, 39 met our criteria. Most papers did not show increased levels in mean scores of distress, anxiety or depression. However, subgroups with high levels of distress, anxiety and depression were identified, specifically at the time of genetic testing and bowel surgery. Associations between FAP and psychiatric disorders and lower cognitive

function were suggested. FAP patients experienced sufficient and adequate social support of their relatives, but a subset of patients does have a need for more and better professional mental care.

Conclusion

Overall, few mental problems are observed in FAP patients. However, subgroups that suffer from specific mental problems have been identified. As FAP is a rare disease and sample sizes were small, firm conclusions cannot be drawn. Large prospective studies, including specific questions on FAP over time are needed.

P44 - MULTIGENE PANEL TESTING IN YOUNG PEOPLE WITH DIGESTIVE CANCER: SEARCHING FOR UNDERLYING HEREDITARY SYNDROMES. FIRST EXPERIENCE IN A PUBLIC HOSPITAL IN ARGENTINA

Marina Antelo[1,2,3], Giuliana Testa[2,4], Lucia Stach[2,3], Juan Robbio[2,4], Florencia Piovaroli[2,4], Marcela Carballido[2,3], Guillermo Mendez[2,3], Julian Maquieira[2,3], Ana Oviedo[2,3], Enrique Roca[2], Mirta Kujaruk[5], Daniel Cisterna[6], Mariano Golubicki[2,4]

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Background and aim

Hereditary cancer syndromes infer high risks of developing cancer throughout life. Their identification have enormous implications for those affected, since it provides valuable prognostic and predictive information; and for their families, since it allows the detection of relatives at risk in whom surveillance from early ages and prophylactic surgeries reduce cancer mortality in up to 60%.

Method

Between July 2017 and June 2023, 2300 patients were attended at the weekly hereditary cancer genetic counseling clinic of the Hospital of Gastroenterology Bonorino Udaondo Oncology Section, in Buenos Aires, Argentina. From these, 490 had gastrointestinal cancer diagnosed at younger than 50^{yo}, and 245 were eligible for our study (mean age 36,98^{yo}): 219 (89%) had CRC, 13 (5,3%) had gastric cancer (GC), 8 (3,3%) had pancreatic cancer (PC), 1 (0,4%) had pancreatic NET, and 2 (0,8%) had gastric GIST. 194/209 non polyposis colorectal tumors were screened for MMR deficiency by MSI testing and/or IHC analysis. Blood underwent DNA extraction using standard methods and all patients underwent germline testing for 85 cancer susceptibility genes in our Laboratory or in commercial laboratories.

Results

Among 245 patients with early-onset digestive cancer, pathogenic or likely pathogenic cancer susceptibility gene mutations were found in 92 patients (37.6%): 70 (28.6%) in Lynch syndrome (LS) genes, 9 (3,6%) in colorectal polyposis genes (APC, biallelic MUTYH, BMPR1A), 13 (5,3%) in genes more traditionally associated with breast cancer, 2 (0,8%) in hereditary GIST genes, and 3 (1,2%) in other genes (CDKN2A, CFTR, MCM8). Sixty-three (66%) of the 95 patients with MMR-deficient CRC had Lynch syndrome, and seven (7,1%) of 99 patients

with MMR-proficient CRC had at least 1 mutation in a cancer susceptibility gene (BRCA1, BRCA2, ATM, CHEK2, PALB2 and biallelic MUTYH). Two of 8 (25%) patients with PC had a positive test (BRCA1 and CDKN2A) and none of 10 patients with GC had a positive study, despite the fact that 3/10 had clinical suspicion of Hereditary Diffuse Gastric Cancer. Only MMR-deficient CRC and first degree cancer family history significantly predicted a positive genetic (<0.001).

Conclusions

Because 1 of every 3 patients with early-onset digestive cancer has at least 1 pathogenic or likely pathogenic cancer susceptibility gene mutation, genetic counseling and testing with a broad multigene panel should be considered for all of them.

P45 - SIGNIFICANT ASSOCIATION BETWEEN BLOOD LEVELS OF SELENIUM, ZINC AND COPPER AND ALL CAUSE MORTALITY IN LYNCH SYNDROME CANCER PATIENTS

Magdalena Marciniak[1,2], Martyna Feherpataky[1], Wojciech Marciniak[2], Piotr Baszuk[1,2], Wiktoria Krauze[1], Roża Derkacz[2], Cezary Cybulski[1,2], Tomasz Huzarski[1,2,3], Jacek Gronwald[1,2], Tadeusz Dębniak[1], Jan Lubiński[1,2]

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Background and aim

Lynch Syndrome (LS) is caused by germline pathogenic mutation in one of the four MMR genes: MLH1, MSH2, MSH6 or PMS2. Mutation carriers have a cumulative lifetime risk of developing cancer that is many times higher than in the general population. Carriers of mutations in the MMR genes (mainly MLH1 and MSH2) have a particularly increased risk of developing colorectal (CRC) and endometrial cancer (EC) . It is estimated that the risk of CRC in men is ~64% and in women 42% and EC 45%. Despite high disease risk, LS patients have better prognosis. Recently we were able to show that mortality in cancer patients can be significantly dependent on levels of Selenium (Se), Zinc (Zn) and Copper (Cu) with tumors localized in breast, prostate, lung and/or larynx. The aim of herein studies was to asses all cause mortality depending on blood levels of Se, Zn and Cu in LS cancer patients.

Methods

The blood was obtained from 149 patients pathogenic with variants in MLH1, MSH2 or MSH6 gene (InSiGHT class 4 or 5) affected by at least one cancer (129 colorectal, 42 endometrial and 49 other cancers). The mean follow up time was 5,4 years. During the follow up - 28 deaths have been noted. Blood levels of Se, Zn and Cu have been measured using ICP-MS technology. Se, Zn, and Cu levels were divided into four groups (quartiles – Q) according to increasing levels. To estimate the potential role of Se, Zn, and Cu in survival among enrolled patients – univariable and multivariable COX regression models were calculated. For each element – the quarter with the lowest number of deaths was chosen as the reference group.

Results:

Lynch Syndrome patients with the lowest blood Se levels (≤83 µg/l) have significantly lower survival (in univariable and multivariable models respectively: HR=19.1; p=0.004 and HR=16.4; p=0.008) compared to patients with the highest blood Se levels (≥106 µg/l) **(Figure 1)**.

Study participants with the lowest blood Zn levels (≤5468 µg/l) also have lower survival (in univariable model: HR=4.45; p=0.021) compared to patients with the highest blood

Zn levels (≥6715 µg/l). For Cu, significantly lower survival (in univariable and multivariable analysis respectively: HR=25.3; p=0.002 and HR=29.0; p=0.002) was observed for patients with the highest blood Cu levels (≥1019 µg/l) compared to patients with the lowest blood Cu levels (≤823 µg/l). All the results are presented in **Table 1**.

Conclusions

Similarly to previous observations of different groups of tumors, cancer in patients carrying LS mutations are showing the strong association of all cause mortality with blood levels of Se, Zn and Cu.

Figure 1.

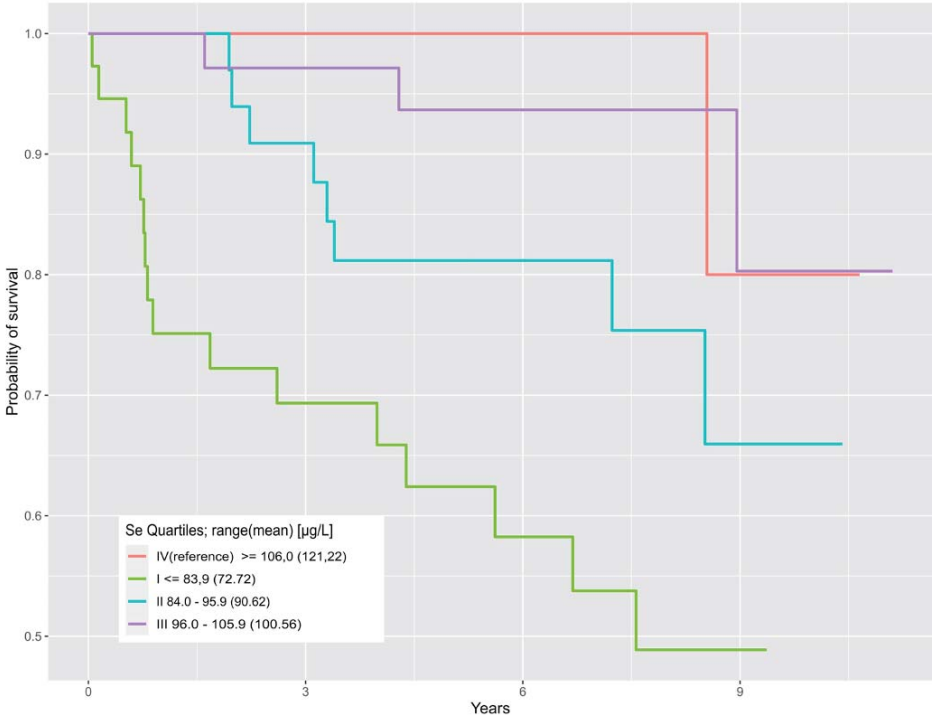
Probability of survival
1.0
0.9
0.8
0.7
0.6
0.5
0
3
6
9
Years
Se Quartiles; range(mean) [µg/L]
IV(reference) >= 106,0 (121,22)
I <= 83,9 (72.72)
II 84.0 - 95.9 (90.62)
III 96.0 - 105.9 (100.56)

Table 1. Overall survival among LS patients in association with micronutrients levels.

	Frequency			Univariable COX Regression			Multivariable COX Regression		
Characteristic (Quartile, Range, Mean)	**Overall** N = 149	**Alive** N=121	**Deceased** N=28	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***
Se_quartile									
IV(reference) ≥106.0 µg/l (121.22)	38 (26%)	37(31%)	1 (3.6%)	—	—		—	—	
I ≤83.9 µg/l (72.72)	37 (25%)	21 (17%)	16 (57%)	19.1	2.53-144	0.004	16.4	2.11- 128	0.008
II 84.0- 95.9 µg/l (90.62)	37 (25%)	29 (24%)	8 (29%)	7.60	0.95-60.9	0.056	10.1	1.23-82.6	0.031
III 96.0-105.9 µg/l (100.56)	37 (25%)	34 (28%)	3 (11%)	2.46	0.26- 23.8	0.4	2.10	0.21-21.4	0.5
Zn_ quartile									
IV(reference) ≥ 6715 µg/l (7275.75)	38 (26%)	35 (29%)	3 (11%)	—	—		—	—	
I ≤5468 µg/l (4700.78)	37 (25%)	25 (21%)	12 (43%)	4.45	1.25- 15.8	0.021	2.78	0.73-10.6	0.13
II 5469 - 6080 µg/l (5721.55)	37 (25%)	30 (25%)	7 (25%)	2.13	0.55- 8.27	0.3	2.22	0.52-9.53	0.3
III 6081 - 6714 µg/l (6326.30)	37 (25%)	31 (26%)	6 (21%)	1.94	0.48- 7.77	0.4	1.56	0.36-6.85	0.6
Cu_ quartile									
I(reference) ≤ 823 µg/l (780.58)	37 (25%)	36 (30%)	1 (3.6%)	—	—		—	—	
II 824 - 926 µg/l (880.30)	37 (25%)	34 (28%)	3 (11%)	3.53	0.37- 34.0	0.3	3.47	0.34-35.6	0.3
III 927 - 1014 µg/l (972.22)	37 (25%)	31 (26%)	6 (21%)	6.63	0.80- 55.2	0.080	6.86	0.73-64.1	0.091
IV ≥ 1015 µg/l (1148.49)	38 (26%)	20 (17%)	18 (64%)	25.3	3.36-190	0.002	29.0	3.57- 236	0.002

P46 - INCREASING CASCADE TESTING UPTAKE FOR HEREDITARY CANCER THROUGH A GENETIC REGISTRY IN SINGAPORE

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Background

Hereditary Cancer makes up around 5-10% of all cancers (1). A timely diagnosis is important, as not only do patients require long-term care from a young age, but their relatives also require management. The main approach to capture at-risk relatives is to carry out cascade testing **(Figure 1A)**. It involves genetic testing of relatives of the first detected carrier of a pathogenic variant in a given family i.e. the proband. The current standard of care for cascade testing is a patient mediated approach (2). Probands are advised to inform their family members and encourage them to undergo genetic testing. Family members must then present themselves to a clinic to be referred for genetic testing. Studies have shown that this patient-mediated approach is inefficient. In Singapore, cascade testing is around 10-15%, much lower than the 30% global average (3, 4). PRECISE, as part of Singapore's National Precision Medicine programme initiated a clinical implementation pilot that seeks to identify strategies for how cascade testing can be increased in a safe and cost-efficient manner (5). Here, we explore the feasibility of a digital genetic registry to increase cascade testing among family members of a proband in Singapore.

Method

We propose a digital pathway, via an app, for genetic testing in the form of a Genetic Registry. Probands sign up to be part of the registry after genetic testing identified them to

carry a pathogenic variant. Probands enter contact details of their relatives, which the app will contact and encourage to get tested.

Results

Discussions with stakeholders indicate that the first steps towards a genetic registry are to identify willingness and acceptability of the genetic registry in the Singaporean population. We are currently performing focus group discussions with up to 50 hereditary cancer patients and family members seen at the National Cancer Center Singapore. This will shape a questionnaire targeting up to 2000 Singaporeans. Further discussions with different stakeholders showed development of the app to cost several million dollars (SGD). A mockup was designed illustrating the app's layout, user interface and overall features. This includes different portals for patients and family members containing personalized risk management plans, new discoveries, and cancer education **(Figure 1B)**.

Conclusions

Digitalisation of the cascade testing process through a genetic registry will likely increase cascade testing. Cascade testing is deemed a cost-effective public health intervention for identifying individuals at risk. Surveillance and management of those unaffected at-risk individuals, if caught early, will result in improved patient outcomes, and further reduce the healthcare burden for the economy.

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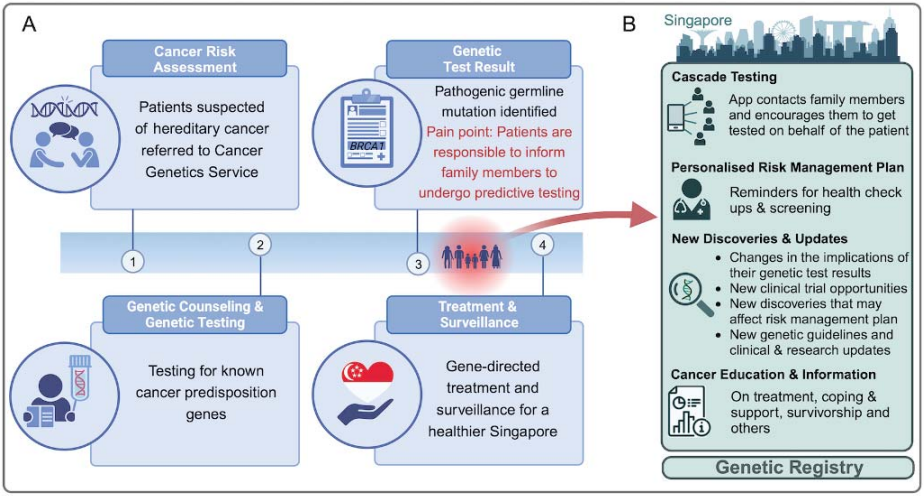
Figure 1.


A
Cancer Risk Assessment
Patients suspected of hereditary cancer referred to Cancer Genetics Service
Genetic Test Result
Pathogenic germline mutation identified
Pain point: Patients are responsible to inform family members to undergo predictive testing
BRCA1
1
2
3
4
Genetic Counseling & Genetic Testing
Testing for known cancer predisposition genes
Treatment & Surveillance
Gene-directed treatment and surveillance for a healthier Singapore
B
Singapore
Cascade Testing
App contacts family members and encourages them to get tested on behalf of the patient
Personalised Risk Management Plan
Reminders for health check ups & screening
New Discoveries & Updates
Changes in the implications of their genetic test results
New clinical trial opportunities
New discoveries that may affect risk management plan
New genetic guidelines and clinical & research updates
Cancer Education & Information
On treatment, coping & support, survivorship and others
Genetic Registry

P47 -LIQUID BIOPSY IN COLORECTAL CANCER - BEYOND THE TARGETED ANALYSIS OF SINGLE VARIANTS

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Background and aim

So far, the main focus in liquid biopsy diagnostics is on circulating tumor DNA (ctDNA) analysis, targeting actionable somatic hotspot variants to guide treatment decisions. Here, we developed a method for the untargeted analysis of ctDNA based on whole-genome sequencing (WGS), providing a promising tool for real-time monitoring of treatment response and the early detection of recurrence.

Method

We established LIquid biopsy Fragmentation, Epigenetic signature and Copy Number Alteration analysis (LIFE-CNA) using WGS with ~6x coverage in 259 plasma samples collected from healthy individuals and colorectal cancer (CRC) patients.

Results

Using 55 healthy controls, we established distinct cutoffs for the detection of ctDNA based on global and regional fragmentation patterns, transcriptionally active chromatin, and somatic copy number alterations. This enabled the accurate prediction of ctDNA in 81% of patients with localized and in 94% of patients with metastatic disease at the time of primary diagnosis. By adding a machine learning classifier to our workflow, which combines global and regional cfDNA fragmentation, we were able to increase the accurate prediction of ctDNA presence (93% of patients with localized and 94% of patients with metastatic disease at diagnosis). By following individual patients throughout their course of disease, we observed that changes in ctDNA signals reliably predicted response or progression up to 5 months prior to clinical manifestation in 77% or 100% of patients, respectively.

Conclusions

We developed and validated a sensitive and cost-effective method for untargeted ctDNA detection at the time of diagnosis or recurrence, as well as for treatment monitoring, expanding the advantages of liquid biopsy. Our approach is applicable to a wide range of cancer patients, and with minor adjustments in the bioinformatics analysis, LIFE-CNA could be easily extended to all tumor entities. The high sensitivity and cost-effectiveness of our approach form the basis for the implementation of LIFE-CNA into clinical practice.

Keywords

Liquid biopsy, ctDNA, WGS, colorectal cancer, machine learning.

Conflict of Interest

The authors declare no conflict of interest.

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P48 - CONEPIMUT-DB: AN INTERNATIONAL DATABASE TO IMPROVE CONSTITUTIONAL MLH1 EPIMUTATION DIAGNOSIS AND RESEARCH

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Background and aim

Constitutional *MLH1* methylation (epimutation) is a rare alternative cause of Lynch syndrome, accounting for 1-4% of colorectal and endometrial tumors displaying *MLH1* methylation (PMIDs 37433431, 36893489). Familial cases of constitutional *MLH1* epimutation have been reported, mainly linked in cis to genetic variants. However, most cases arise de novo and show intergenerational erasure. Some present with mosaicism. Therefore, molecular diagnosis in constitutional *MLH1* epimutation carriers, and risk assessment and counseling in relatives remain challenging. Here, we present ConEpiMut-DB (Constitutional Epimutation Carriers' database), a database for sharing information on carriers and their relatives.

Methods

An interest group on *MLH1* epimutation originated at InSiGHT 2022, with researchers from 21 institutions willing to cooperate. It was agreed to create a platform to collect data. The ConEpiMut-DB database has been designed using REDCap. It is promoted by Cedars-Sinai Medical Center (USA), Royal Melbourne Hospital (Australia), and Bellvitge Biomedical Research Institute (Spain), and works under the umbrella of the International Society for Gastrointestinal Hereditary Tumours (InSiGHT).

Results

ConEpiMut-DB is led by a steering committee (SC) who will evaluate and approve user registrations and research project proposals and oversee ethical-legal matters together with an advisory board. A database curator will ensure accurate recording and storage of data entered, and coordinate communications between the SC and the contributing centers. Registered users will be able to share and access information in a pseudonymised manner to protect patient privacy. Content is organised in four main blocks: patient demographics; clinical, pedigree and molecular diagnostic data; tumor pathology; genetic and epigenetic characterisation. Also, the database notifies of the option to share biological samples in a virtual or physical biorepository. As a pilot, previously published cases with constitutional *MLH1* epimutation are currently being collated in the database, along with a systematic review of clinicopathologic and molecular features.

Conclusions

ConEpiMut-DB is a joint effort to create a resource to aggregate valuable information on rare cases with constitutional *MLH1* epimutation, to leverage collaborative research, and ultimately improve clinical management of carriers and relatives. Interested researchers please contact epimutationsdb@idibell.cat.

P49 - CHROMATIN AND GENETIC CHARACTERIZATION OF PRIMARY CONSTITUTIONAL MLH1 EPIMUTATIONS

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Background and aim

Lynch syndrome (LS), characterized by an increased risk for cancer, is mainly caused by a germline pathogenic variant in a mismatch repair gene (*MLH1, MSH2, MSH6, PMS2*). Occasionally, LS is caused by a constitutional *MLH1* epimutation (soma-wide methylation of one allele of the *MLH1* promoter), which frequently arise de novo without an underlying cis-genetic cause and are reversible between generations. We aimed to characterize chromatin changes associated to primary constitutional *MLH1* epimutations to elucidate possible molecular mechanisms underlying them.

Methods

Four primary constitutional *MLH1* epimutation carriers heterozygous for promoter SNP rs180074, and 2 relatives carrying the methylation-associated allele in a non-methylated state were included in the study. Genetic alterations were analyzed by WGS. Transcriptome (RNA-seq), chromatin landscape (ATAC-seq, H3K27ac CUT&Tag) and 3D chromatin structure (UMI-4C), were studied in derived lymphoblastoid cell lines. Bioinformatic tools were used to scan transcription factors binding sites.

Results

The presence of rare variants in the differentially methylated region or shared variants within the *MLH1* locus were ruled out. MLH1 epimutant alleles presented a closed chromatin conformation and decreased levels of H3K27ac, as compared to the unmethylated allele. Moreover, the epimutant *MLH1* promoter exhibits a differential 3D chromatin landscape including loss and gained interactions with distal regulatory elements. In one case genetic variants inside differential contact regions were predicted to create new transcriptional repressor binding sites.

Conclusions

Primary *MLH1* constitutional epimutations present allele-specific differential interaction patterns with neighboring genes and regulatory elements. Further investigation is needed to elucidate the role of 3D chromatin changes in the origin and functional impact of *MLH1* epimutations.

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P50 - THE MLH1 GERMLINE VARIANT C.27G>A (P.ARG9=) IS AN EPIMUTATION-ASSOCIATED VARIANT WITH VARIABLY MOSAIC BUT HERITABLE CONSTITUTIONAL MLH1 METHYLATION IN LYNCH SYNDROME FAMILIES

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Background and aim

The *MLH1* exon 1 germline variant c.27G>A (p.Arg9=) has previously been reported in suspected Lynch syndrome (LS) cases [PMIDs 22878509, 29790873, 34711244], but is rare, synonymous and remains a variant of uncertain significance (VUS). We describe two new unrelated European suspected LS families with this VUS and sought clinicopathologic and molecular evidence for its pathogenicity and mechanism of action.

Methods

Detailed clinicopathologic, molecular genetic, and DNA methylation analyses were performed using custom-designed targeted assays and nanopore sequencing in available germline DNA and tumor and adjacent non-neoplastic tissue samples from members of two families with the c.27G>A VUS.

Results

One family from the UK and another from Spain carrying the *MLH1* c.27G>A VUS met Amsterdam I criteria for LS. Two siblings from the UK family with an extended maternal family history had both presented with colon cancer at 42y, each tumor displaying loss of MLH1/PMS2 and *MLH1* methylation. In the Spanish family, multiple members from three

generations had presented with colorectal cancer at ages 26-62y. Highly variable levels of mosaic constitutional *MLH1* methylation were detected in c.27G>A carriers, even within the same family. In the UK siblings, blood *MLH1* methylation levels were 6% in the sister but quasi-undetectable (≤1%) in the brother, whilst resected normal colon tissues had slightly higher levels at 8% in the sister and 6% in the brother. In three male carriers from three generations of the Spanish family (two affected, one unaffected at 34y), blood *MLH1* methylation levels ranged from 4%-16%. The constitutional *MLH1* methylation occurred exclusively on the variant c.27A allele in carriers from both families and was transmitted faithfully with the c.27A allele between generations. Long-range nanopore sequencing did not identify other candidate LS variants. Loss of heterozygosity of the unmethylated c.27G allele was observed as the "second hit" in one available tumor.

Conclusions

We show the c.27G>A variant is associated with dominantly heritable, mosaic constitutional *MLH1* methylation in two unrelated families, therefore represents a "secondary epimutation-associated" genetic variant. Our findings in these two new families extend on prior observations in other index cases with this variant. Collectively, these observational studies support a pathogenic role the *MLH1* c.27G>A variant.

P51 - AN 8-BASE MLH1 PROMOTER MICRODELETION, DEL C.-[52-59], IS LINKED TO A MOSAIC, AUTOSOMAL DOMINANT "SECONDARY" CONSTITUTIONAL MLH1 EPIMUTATION IN A LARGE PEDIGREE WITH SUSPECTED LYNCH SYNDROME

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Background and aim

Constitutional *MLH1* epimutation, whereby a single allele is inactivated by promoter methylation in normal tissues, is a rare cause for Lynch syndrome (LS). "Primary" epimutations have no apparent genetic basis and show null or Non-Mendelian inheritance. "Secondary" epimutations are autosomal dominant and caused by a genetic variant *in cis*. We investigated the cause for suspected LS in a large family with MLH1-deficient cancers whose germline multigene panel test (MGPT) returned negative results. We identified autosomal dominant, mosaic constitutional *MLH1* methylation linked to a promoter 8-bp microdeletion as causal.

Methods

Cancer-affected family members were referred for extended research-based testing by genetic counselors and invited their relatives to participate (Cedars-Sinai Medical Center Ethics Approval #00049624). Detailed methylation, allelic expression, and sequencing analyses were performed in fresh normal tissues (blood, saliva, buccals) from 12 family members using published methods. Allelic methylation sequencing was conducted using a custom-designed assay. Archived tumor and adjacent normal samples from both cousins, plus a deceased relative, were sequenced for "second hits".

Results

The family met Amsterdam I criteria for LS with a four-generation history of cancers. Two female cousins from the fourth generation, one with colon cancer at 31y the other with endometrioid ovarian cancer at 33y both showing MLH1-deficiency and *MLH1*-methylation, had each received negative germline MGPT results. *MLH1* methylation testing and promoter sequencing revealed constitutional *MLH1* methylation accompanied by a heterozygous

promoter 8-bp microdeletion, Del c.-[52-59], in both cousins and other unaffected relatives. The constitutional *MLH1* methylation segregated with the Del c.-[52-59] between generations and allelic methylation sequencing confirmed they were *in cis*. Methylation levels varied from 10% to 35% between tissue types and family members. Blood RNA analysis showed reduced expression of the variant haplotype (15% of the wildtype). Loss of heterozygosity of the unmethylated wildtype allele was observed in the colon tumors from one cousin and a deceased third-generation relative.

P52 - EXTENT OF INVESTIGATION AND MANAGEMENT OF CASES OF "UNEXPLAINED" MISMATCH REPAIR DEFICIENCY (U-DMMR)– A UK CANCER GENETICS GROUP CONSENSUS

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Background

Mismatch repair deficiency (dMMR) is a characteristic feature of cancers linked to Lynch Syndrome. However, in most cases, it results from sporadic somatic events rather than hereditary factors. The term "Lynch-like syndrome" has been used to guide colorectal cancer surveillance for relatives of individuals with a dMMR tumour when somatic and germline genomic testing is uninformative. As the assessment of MMR through immunohistochemistry and/or MSI is increasingly applied across various tumour types for treatment planning, dMMR is increasingly detected in tumours where suspicion of hereditary aetiology is low. Our objective was to establish current practices and develop national guidance for investigating, and managing relatives of, patients with cancers demonstrating unexplained dMMR.

Method

The UKCGG facilitated a virtual consensus meeting, involving key stakeholders from the UK, and co-ordinated pre-meeting surveys, structured discussions, and in-meeting polling to formulate best practice guidance.

Results

We identified variability in the availability of diagnostic technologies across specialist centres. It was agreed that equitable access to baseline testing is required, acknowledging the need for a pragmatic approach to investigating dMMR cancers not traditionally associated with Lynch syndrome. Factors such as family history, age, tumour type, protein loss pattern, and extent of the investigation were deemed crucial in guiding family management. The term "unexplained dMMR" (u-dMMR) was recommended over "Lynch-like syndrome."

Conclusions

Decisions regarding investigations and future cancer risk management in patients and relatives should be nuanced, considering factors like clinical suspicion of hereditary predisposition to allocate limited resources efficiently and avoid unnecessary investigations in low-suspicion families.

P53 - INFORMING RELATIVES AT-RISK ABOUT PREDISPOSITION FOR HEREDITARY CANCER: LONG TERM RESULTS OF A PROACTIVE AND PERSONALIZED APPROACH

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Background and aim

People who know they have a hereditary predisposition for cancer may achieve health gains with preventive measures. In 2019, a Dutch guideline was developed aiming to optimize the process of informing relatives in case of newly diagnosed high risk genetic disease. To implement this guideline, we started a pilot study with a family consultant to actively support cancer patients with hereditary predisposition to inform their at-risk relatives. The aim of this study was to compare the effect of this intervention to usual care without extra support on the percentage of referrals for genetic counseling of at-risk relatives.

Method

We performed a retrospective quantitative monocenter study of cancer patients with a newly diagnosed pathogenic DNA variant in the MLH1, MSH2, MSH6, PMS2, BRCA1 or BRCA2 gene. Patients diagnosed in the Radboud University Medical Center, Nijmegen, The Netherlands, with a positive DNA result between 15/9/2017 and 14/9/2019 were included in the control cohort (usual care), and between 15/9/2019 and 14/9/2021 in the intervention cohort (with support from a family consultant). The follow-up period was set at 2 years after the test result. Pedigrees were checked for first degree relatives with possible personal health gains. The percentage of these relatives per patient who were referred for genetic counseling was compared between cohorts.

Results

There was no statistically significant difference between control (N=91) and intervention cohort (N=86) in percentage of first degree relatives referred per patient (78% vs.78%, P= 0,42).

Conclusion

Customized support to cancer patients with hereditary cancer predisposition by a family consultant did not increase the percentage of at-risk relatives being referred for counseling on hereditary predisposition for cancer.

Keywords

Hereditary cancer, predisposition, informing relatives, relatives at-risk, personalized approach.

Table 1 Patient population characteristics based on proband

	Control cohort (N= 91)	Intervention cohort (N=86)	Total (N=177)	P-value
Age (Year) Mean (SD)	53 (12,0)	55 (13,0)	54 (12,7)	0,77
Sex *Female (N (%))*	73 (80%)	70 (81%)	143 (81%)	0,85
Category hereditary predisposition *Breast and ovarian cancer (N(%)* *Colon cancer (N (%))*	66 (73%) 25 (27%)	65 (76%) 21 (24%)	131 (74%) 46 (26%)	0,64[a]
Type of hereditary predisposition Breast and ovarian cancer				
BRCA1 (N (%))	25 (28%)	32 (37%)	57 (32%)	
BRCA2 (N (%))	41 (45%)	33 (38%)	74 (42%)	
Type of hereditary predisposition Colon cancer				
MLH1 (N (%))	2 (2%)	3 (4%)	5 (3%)	
MSH2 (N (%))	4 (4%)	1 (1%)	5 (3%)	
MSH6 (N (%))	8 (9%)	10 (12%)	18 (10%)	
PMS2 (N (%))	11 (12%)	7 (8%)	18 (10%)	

[a] Breast and ovarian cancer versus colon cancer.

Table 2 Referred at-risk relatives of proband (primary outcome)

	Control cohort (Proband N=91 # Family N= 332)	Intervention cohort (Proband N=86 # Family N= 319)	P-value
Total *N(%)*	264 (80%)	231 (72%)	0,03
Children *N (%)*	72 *(88%)*	56 (78%)	0,10
Siblings *N (%)*	133 (82%)	104 (65%)	<**0,001**
Parents *N (%)*	35 (76%)	24 (71%)	0,58
2nd degree relative *N (%)*	14 (42%)	21 (48%)	0,64
Percentage per proband referred at-risk family members[a]			
Mean (%)	**78%**	**78%**	**0,42**

[a] concerns the proportion of referred family members per proband.

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P54 - CLINICOPATHOLOGICAL FACTORS AND OUTCOMES OF 'IN HOUSE' GENETIC TESTING WITHIN A SPECIALIST HEREDITARY COLORECTAL CANCER REGISTRY

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Background and aim

Approximately 5-10% of colorectal cancer (CRC) is due to known Mendelian inheritance. The clinicopathological factors associated with outcomes of genetic testing is based on historical data and referral external to genetics services. This study explored the diagnostic yield and clinical impact of 'in house' mainstream constitutional genetic testing for hereditary CRC in a specialised National Bowel Hospital.

Methods

This study retrospectively reviewed clinical, pathological and genetic factors using prospectively collected data from the St Mark's Hospital Centre for Familial Intestinal Cancer (SMCFIC) registry. Consecutive patients at risk of hereditary CRC syndromes were tested between December 2021 and June 2023, largely selected according to UK National Genomic Testing criteria. The diagnostic yield and the impact of testing were evaluated.

Results

283 patients underwent genetic testing, 100 (35.3%) mainstreamed with CRC, 95 (33.6%) multiple polyps, 74 (26.6%) cascade testing (within families where the probands were known to the registry) and others including 'unaffected' patients with a relevant family history **(Figure 1)**. Variants were detected in 85/283 (30%) patients in known CRC predisposition genes. Of 86 patients undergoing genetic testing following universal CRC testing for Lynch syndrome (LS): 38 were dMMR, 48 were pMMR CRCs, and the yield of P/LP variants was 44.7% of dMMR tumours (23.7% *MLH1*, 10.5% *MSH2*, 5.3% *MSH6*, 5.3% *PMS2*, 55.3% no variant). An average of 6 first-degree relatives of each proband was invited for predictive testing and/or colonoscopy surveillance. Independent factors suggestive of LS included proximal colorectal cancer, any MSI-H histologic features, Amsterdam criteria family and having ≥1 first-degree relatives with CRC or any LS-related tumours **(Table 1)**. In 95 patients with multiple polyps, P/LP variants were detected in 14 (14.9%) patients, most commonly in MUTYH in 4(4.2%) patients. No germline P/LP variants were detected for 26 patients by the single indication of <10 adenomatous polyps.

Conclusion

Testing performed by our specialist hereditary CRC unit provides patients with high-yield, timely and effective genetic diagnosis, and directly linked comprehensive lifelong care. Clinicopathological factors suggestive of LS have been identified in a 'mainstream' testing population recently diagnosed with cancer. A larger dataset may provide further insight into predictive factors which may be relevant in this non-surveillance detected CRC patients.

Keywords

Hereditary Colorectal Cancer, Lynch Syndrome, Universal Tumour Testing.

Figure 1. Prevalence of P/LP/VUS Variant Detection.

Patient Categorisation	P/LP/VUS Variants
Mainstream cancer patients	27/100 (27.0%)
Patients with polyps	16/74 (21.6%)
Cascade testing	38/74 (51.3%)
Patients with non -CRC phenotypes	3/8 (37.5%)
Unaffected testing	1/3 (33.3%)
Carrier testing for partners of MUTYH	0/4 (0%)

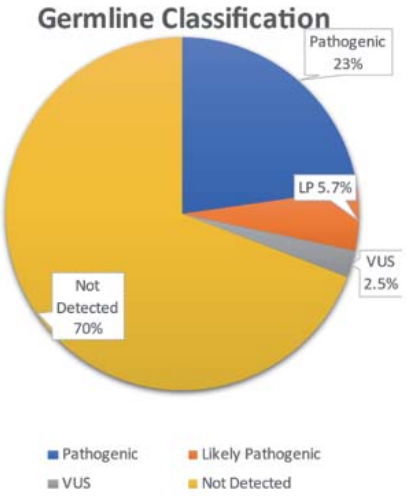

Table 1. Clinicopathological Characteristics of CRC Patients according to MMR Status.

Variables	dMMR	pMMR	P-value
Mean age of diagnosis	56.0±16.5	47.9±14.0	0.019*
Cancerlocalisation -Small bowel -Right colon -Left colon -Rectum -Synchronous cancer	 1 18 10 7 2	 0 9 14 20 2	0.034*
Stage at diagnosis - I - II - III - IV	 7 19 9 1	 13 8 17 6	0.009*
MSI-H histopathological features TIL, Crohn's-like lymphocytic reaction, mucinous/signet ring differentiation, or medullary growth pattern -Presence -Absence	 11 26	 5 41	0.030*

P55 - METHYLATION-SENSITIVE HIGH-RESOLUTION MELTING, A SIMPLE TECHNIQUE TO DETECT A RARE MECHANISM IN LYNCH SYNDROME: CONSTITUTIONAL HYPERMETHYLATION OF THE MLH1 GENE PROMOTER

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Background and aim

Rare patients with epimutation consisting in constitutional hypermethylation of the mismatch-repair (MMR) *MLH1* gene promoter (CHMP-*MLH1),* responsible for Lynch Syndrome (LS), have been reported. We herein present a retrospective study of 76 patients who exhibited *MLH1* gene promoter hypermethylation in tumour DNA. Our aim was to compare Methylation-Sensitive High-Resolution Melting (MS-HRM) to the more laborious though reference pyrosequencing (PS) method for ascertainment of CHMP-*MLH1* in those patients.

Patients & Method

The reference group (group 1) consisted of 31 patients with prior ascertainment of CHMP-*MLH1* status by PS (3/31 were positive for CHMP-*MLH1,* two of which at a very low allelic levels, *i.e.*, < 5%). The prospective group (group 2) consisted of 45 patients without any prior CHMP-*MLH1* analyses. CHMP-*MLH1* status was ascertained in both groups by MS-HRM from blood samples. Positive group 2 samples were reanalysed by PS. In addition, MS-HRM was performed from available buccal swabs (BS) in positive patients. The MS-HRM ascertainment threshold was defined using a dilution range.

Results

MS-HRM ascertainment threshold was defined as an epimutation frequency of 0.4%. In group 1, CHMP-*MLH1* was correctly ascertained by MS-HRM in the 3 patients identified by PS with epimutation frequency estimates of ≥50%, 6.25%, and 1.6%; all negative samples with PS were also negative with MS-HRM. In group 2, MS-HRM elicited 2 further CHMP-

MLH1 patients, eventually confirmed by PS. MS-HRM was also performed on DNA extracted from available BS of positive patients (4/5), allowing to detect very-low-level mosaics with epimutation frequencies of 1.6% and 0.4%. All positive patients fell in the predefined clinical settings: i, LS carcinoma < age 60, ii, rectal cancer at any age, iii, individual or familial aggregation of LS tumours, including advanced adenoma.

Conclusions

MS-HRM is straightforward and low-cost real-time PCR method that can be used routinely to ascertain CHMP-*MLH1*, with high sensitivity on both blood and BS samples. Although a rare genetic alteration, it is important not to overlook CHMP-*MLH1* for its consequences on patient care and genetic counselling. We suggest that patients exhibiting an MMR-deficient tumour with *MLH1* promoter hypermethylation should be tested for CHMP-*MLH1* if they meet the clinical criteria outlined above. Whether a cis-acting alteration is responsible for CHMP-*MLH1* in a subset of patients is a matter of further research.

P56 - RAPID TURNAROUND MULTIGENE PANEL TESTING: A PILOT STUDY TO ASSIST SURGICAL PLANNING IN COLORECTAL CANCER

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Background

Patients with a confirmed gene variant as an etiology for a colorectal cancer diagnosis are recommended to undergo extended resection rather than segmental resection. Standard return of results for multigene panel testing (MGPT) typically is greater than 21 days which delays the surgery date while waiting on results.

Purpose

To determine the feasibility of MGPT for diagnosis of hereditary colorectal cancer syndromes with results reported within 7 days of the sample's accession date.

Methods

Patients with colorectal cancer, who were to undergo surgical resection as first line treatment, were offered MGPT via a pilot program with goal of results reported within 7 days of the sample accession date. The pilot program was utilized at an outpatient clinic of a tertiary Cancer Center. All patients were offered genetic testing and consented by a genetic counselor or program assistant, and blood samples were collected and sent to the commercial laboratory. Basic patient demographics, family history of cancer, and tumor characteristics were recorded. MGPT results and the time for results return were collected.

Results

Ten patients were enrolled between September 2023 and December 2023. The mean age of patients was 52 years and seven patients were male. Two patients had a family history of CRC in a first degree relative. Nine patients had colon cancer and one patient had rectal cancer. The median time for return of results was 6.5 days, with eight results returned within seven days and two results returned on day eight. There were no pathogenic variants in genes that would impact surgical decision making or medical management. Four patients were identified to have a variant of uncertain significance.

Conclusions

Rapid turnaround of MGPT is feasible and can be used to expedite surgical decision making and facilitate earlier treatment. Although no differences were made in terms of the extent

of surgery in this small pilot study, the rapid return of results made the impact on surgical scheduling ia non-factor.

Keywords

Genetic testing, surgery.

Conflict of Interest

The authors have no conflicts of interest.

P57 - MicroRNA-137 REGULATES MSH2 AND INDUCE APOPTOSIS IN COLON CANCER CELL LINES

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Background and aim
The Lynch syndrome (LS) is associated to sequence variants in Mismatch Repair (MMR) genes. The MMR genes dysregulation of play a fundamental role in the pathogenesis of LS. In the previous study, we identified a sequence variant, the c*226A>G in 3'UTR of MSH2 gene, in some LS patients. Bioinformatic analysis identified a putative binding site for hsa-miR137 in the 3'UTR region of MSH2. This analysis was confirmed by luciferase assay that demonstrated the specific binding of mir-137 on the 3'UTR region. The aim of this study was to investigate a putative role of miR-137 in MSH2 gene expression regulation.

Methods
To this regard, we investigated this role in several colon cancer cell lines, SW480, HT29 and HCT116 by expression study. Furthermore, we performed the MTT assay in all cell lines and analysis of apoptosis by flow cytometry.

Results
The functional relevance of miR-137 in regulating the expression of MSH2 was established by over-expressing miR-137 in each used cell lines; this showed that MSH2 mRNA expression and protein levels were downregulate. Moreover, when the used cells lines were transfected with anti-miR-137 the production of mRNA and protein of MSH2 gene was increased, confirming a negative regulatory role for miR-137 on MSH2. Our data showed that the overexpression of Mir-137 significantly suppressed cell proliferation relative to each control group. Based on these results we also hypothesized a role MSH2 in apoptotic and tumorigenic processes. Therefore, we examined the apoptotic effects by treating the cells with Pre-miR adding cisplatin stimuli, comparing them with the respective controls. The data showed us a marked apoptosis rate when we treated cells with PremiR-137 under the pro-apoptotic stimuli.

Conclusions
Overall, our data showed that miR-137 negatively regulates the MSH2 gene, inhibits proliferation, and sensitizes colon cancer cells to apoptosis.

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P58 - RISK FACTORS AND CLINICAL CHARACTERISTICS OF EARLY-ONSET GASTRIC CANCER VS. LATE-ONSET GASTRIC CANCER: A CASE-CASE STUDY

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Background and aim

Early-onset gastric cancer (eoGC), defined as GC before 50, accounts for about 5% of GC and 3% are part of a hereditary syndrome. Its incidence is rising. eoGC was reported to present at an advanced stage at diagnosis, with a poor differentiation and a worse prognosis than in the elderly (late-onset GC, loGC), even though eoGC risk factors and characteristics remain unclear. Therefore, we compared eoGCs with loGCs in terms of clinicopathological characteristics and risk factors.

Methods

We conducted a retrospective case-case study of patients with eoGC and loGC diagnosed from 2015 to December 2022 at IRCCS San Raffaele Scientific Institute (Milan, Italy). We collected clinical data, tumor characteristics (including Helicobacter Pylori status, HP), family history of GC, smoking habit, alcohol intake, and BMI. We performed multi-gene panel testing on all eoGC.

Results

58 eoGC (51.7%M, 48.3%F; median age at diagnosis [IQR] 44.5y [39.00, 47.75]) and 290 loGCs (63.8%M, 36.2%F; median age at diagnosis [IQR] 70.33y [63.90, 75.00]) were enrolled. 19.2% eoGCs carried a germline pathogenic variant (4 CDH1; 2 TP53; 1 MMR; 1 ATM; 1 PTEN; 1 heterozygous MUTYH). Having a second-degree relative (SDR) with GC was significantly associated with eoGC (15.8% eoGCs vs. 4.6% loGCs, p = 0.015). eoGCs reported lower BMI (median 22 eoGC vs 24 loGC, p=0.016). We also found a statistically significant difference between groups in alcohol consumption (P = 0.042) and smoking status (P = 0.023). While eoGCs were mainly at the cardia (29.3% eoGCs vs. 1.7 % loGCs; p<0.001), loGCs were mostly at the antrum (19% eoGC vs. 36.9% oGCs; p=0.009).

eoGCs reported more dysphagia (30.9% eoGCs, compared to 4.9% loGCs, $p < 0.001$), while most patients in the loGC cohort had anemia (25.3% loGCs vs 7.3% eoGCs, $p = 0.002$) and asthenia (9.1% loGCs vs 0% eoGCs, $p = 0.012$). 48.3% eoGCs were diffuse-type adenocarcinoma (p=0.014), while 51% loGCs were intestinal-type ($p<0.001$). No significant differences were observed in terms of T and N status, while more eoGC were M1 (26.3% eoGCs vs. 9.7% loGCs, $p=0.001$) and presented at stage III-IV (56.9% eoGCs vs. 41.7% loGCs, $p=0.042$) at diagnosis.

Conclusion

19.2% patients with eoGC carry a germline pathogenic variant, suggesting that patients with eoGC should be evaluated for germline testing at diagnosis. eoGC more often is localized at the cardia with dysphagia and presents as diffuse-type adenocarcinoma at an advanced stage (III-IV) ad diagnosis. However, 35.6% patients with eoGC presents with early gastric cancer T1 (vs 24.1% loGCs), possibly implying that younger age does not entail worse outcomes.

P59 - A SNAPSHOT OF THE RECENT 100 SURGICAL CASES UNDERTAKEN AT A NATIONAL REFERRAL CENTRE FOR FAMILIAL INTESTINAL CANCERS: CELEBRATING 100 YEARS

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St Mark's the National Bowel Hospital

Background and aims

Patients with polyposis syndromes frequently require surgical intervention, typically risk reduction gastrointestinal tract surgery, to prevent cancer and manage polyposis-related complications. This study provides a snapshot of the last 100 surgical cases performed at the world's oldest Polyposis Registry, which celebrates its 100th centenary in 2024.

Methods

A retrospective analysis of 100 consecutive surgeries in patients with polyposis conducted between May 2021 and November 2023 was undertaken. Upper GI tract surgery and intestinal transplantation are not included as these are performed in regional centres. We evaluated patient demographic data, genetic diagnosis, genotype and phenotype characteristics, surgery performed, and histopathological findings. Postoperative complications were categorized using the Clavien Dindo Classification, and postoperative outcomes and follow-up data were analysed.

Results

There were 100 surgical procedures performed on 96 patients, with 57% of them being male. The median age at surgery was 42 (range 17 -89) years. The most frequently performed surgical procedure was near-total colectomy with ileo-distal sigmoid anastomosis (NTC-IDSA), followed by restorative proctocolectomy (RPC) and ileostomy closure. The majority of diagnoses were familial adenomatous polyposis (FAP, 62%), Peutz-Jeghers syndrome (PJS, 11%), and MutYH-associated polyposis (MAP, 11%). Only one patient (1%) experienced a Clavien-Dindo grade III postoperative complication, and no Clavien-Dindo grade IV or V complications were observed. 15% of patients had a histologically confirmed malignancy.

Conclusion

Centralising and coordinating specialist care for patients with inherited GI cancer syndromes leads to accrual of expertise and a safe surgical experience for patients with low complication rate. The registry remains pivotal in offering these patients and their families complex lifelong care.

Keywords

Polyposis syndromes, surgical intervention, genetics, cancer prevention, centralizing specialist care.

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P60 - EVIDENCE FOR GERMLINE SUSCEPTIBILITY GENES FOR SERRATED POLYPOSIS SYNDROME

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Background and aim

Serrated Polyposis Syndrome (SPS) is characterised by multiple serrated polyps in the colon,

a familial component and increased risk of colorectal cancer (CRC). Identifying inherited susceptibility to SPS remains elusive despite reports of several candidate genes. The aim of this study was to identify susceptibility genes in a large, selected cohort of SPS.

Methods

505 SPS cases, including 24 relatives from 11 families, were selected from the Genetics of Colonic Polyposis Study for whole genome or exome sequencing (WGS/WES) based on one or more of the following criteria: young age at SPS diagnosis, high serrated polyp count, CRC diagnosis or family history of SPS. Predicted pathogenic variants (PPV) were defined by REVEL>0.6 or SpliceAI>0.5 and gnomAD AF<0.05% and prioritised in three *Tiers* of genes: *1)* known hereditary CRC/polyposis genes (n=20); *2*) known hereditary cancer genes (n=84) and *3*) candidate genes for SPS from the literature (n=29). The frequency of PPVs in candidate SPS genes were compared between SPS cases and the non-cancer, Non-Finnish European-gnomAD reference data (v2.1) using Fisher's exact test. To identify novel candidate genes, loss of function (LoF) variants that segregated in the familial SPS cases were prioritised for further analysis.

Results

Of the 378/505 SPS cases with WGS/WES analysed to date (median age at SPS diagnosis=30 (IQR=25) years, median serrated polyp count of 34 (IQR=16), 66% were females, 97% white European, and 15% developed CRC. Pathogenic variants were identified in *APC*, *MLH1*, *MSH2*, *MSH6 (Tier 1*), and *BRCA1*, *BRCA2*, *FLCN*, *TGFBR2, BARD1, EXO1, LZTR1 (Tier 2*) genes.

For the Tier 3 genes, PPV carriers in 2x*RNF43*, 9x*CFTR*, 2x*EPHB2*, 1x*FBLN2*, 1x*INO80*, 1x*PDLIM2*, 1x*RBL1*, 1x*TMEM43*, 1x*ULK4*, 1x*WNK2* and 1x*ZNRF3* were identified. None occurred in the familial SPS cases. Of these Tier 3 genes, only *RNF43* showed statistical enrichment of PPVs in SPS compared with PPVs observed in gnomAD (OR=17.02[1.9-66.4], p=0.007) although there were only 2 *RNF43* carriers with SPS identified.

15 LoF variants that segregated with SPS in the familial cases were identified representing novel candidate SPS genes.

Conclusion

This large genetic study of young SPS people with high serrated polyp count and strong familial component found limited evidence to support the testing of 29 candidate genes, with only *RNF43* showing an association with SPS. The analysis of familial cases may reveal additional candidate genes for SPS.

Keywords

Serrated Polyposis Syndrome, whole exome sequencing, candidate genes, *RNF43*, *WNK2*.

P61 - STUDYING THE DEVELOPMENT AND CLONAL HETEROGENEITY OF GASTRIC INTESTINAL METAPLASIA IN HUMAN BIOPSY-DERIVED ORGANOIDS

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Gastric stem cells are responsible for maintaining a healthy gastric epithelium. A single gastric stem cell proliferates and differentiates to form a gland. Genetic and epigenetic changes can interfere with this tightly regulated process and result in inappropriate activation of pre-malignant phenotypes. Evidence from our team suggests that intestinal metaplasia, the replacement of patches of normal gastric mucosa by pre-malignant intestinal-like epithelium, is driven by individual gastric stem cells switching their normal gastric differentiation lineage to an intestinal-like lineage.

Our aim is dissect the evolutionary processes behind metaplasia, which drive further progression to this intestinal precursor and drive further progression to cancer. I have developed a translational pipeline to establish patient-matched organoids. I have been able to derive, establish and culture patient-matched gastric, gastric intestinal metaplasia (GIM) and duodenal organoids from biopsy samples of 8 patients. Prelimary data from rt-PCR and confocal microscopy shows how the organoids are representive of their tissue of origin: the gastric organoids appropriately express MUC5AC and a lack of CDX2 and MUC2, whilst the duodenal organoids suitablely exhibit the reverse expression. The GIM organoids have mixed expression of these markers, suggesting an incomplete metaplastic phenotype **(Figure 1)**. I have begun methylation analysis of the organoids utilising a multi-package R stream to study the differentially methylated states between phenotypes. Preliminary data through DNA methylation analysis that the organoids can be clustered together based on their phenotype, opposed to patient of origin **(Figure 2)**.

At present I have shown that I have developed strong models of normal gastric, duodenal and GIM organoids that are representative of the tissue of origin. Now, I am ready to begin the most meaningful experiments of the PhD carrying out future experiements such as:

- To complete methylation analysis to study differential methylation between the normal and metaplastic conditions.
- Using thiol-reactive organoid barcoding in situ (TOBis) and cytometry by time-of-flight (CyTOF), to demonstrate the post-translational signalling profile of GIM.
- Chromatin profiling of the organoids to interrogate the effect of epigenetic control on GIM development.
- DNA sequencing of the organoids to assess the mutations present in GIM organoids and the mutational burden present in each organoid phenotype.

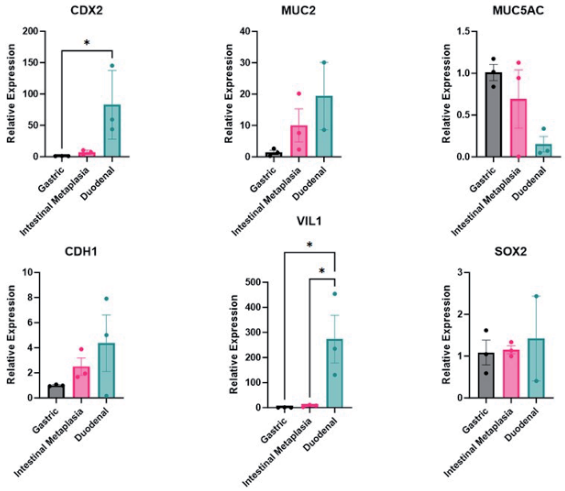

Figure 1: RT-qPCR results shows the difference gastric and intestinal-specific transcript expression between the different organoid lines. 3 wells of each ID8 organoid phenotype were grown and harvested before RNA was extracted and converted to cDNA. qPCR was performed. One-way ANOVA test was performed(n=3).

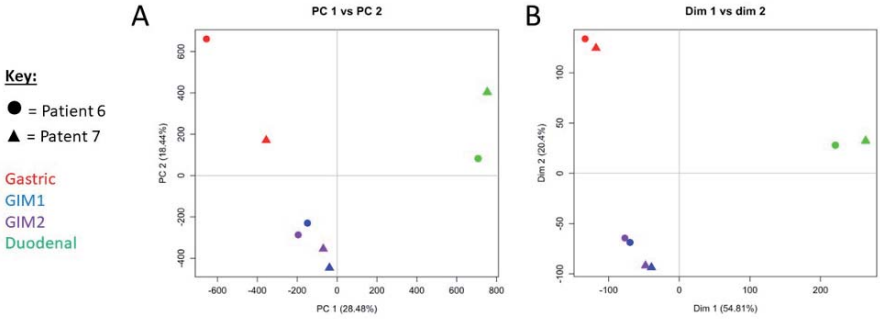

Figure 2: PCA and MDS Plots using non-normalized data demonstrate how the methylation profiles of the organoids allow them to cluster together based on phenotype. Two patient had four organoid lines derived from biopsy samples that were later sent for methylation analysis on Epic arrays. Graphs were generated using R. (A) The graphs on the left-hand side of the screen are PCA plots. (B) The graphs on the right-hand side of the screen are MDS plots.

P62 - A LOW-INFLAMMATORY DIETARY INTERVENTION IN INDIVIDUALS WITH FAMILIAL ADENOMATOUS POLYPOSIS: METABOLOMICS ANALYSIS

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Background and aim

Familial adenomatous polyposis (FAP) is an autosomal-dominant hereditary condition associated with germline mutations in the Adenomatous Polyposis Coli gene. Patient management involves prophylactic surgery and lifelong endoscopic surveillance. Diet is a major concern for FAP individuals who report multiple alterations of bowel function postoperatively. We hypothesized that a low-inflammatory Mediterranean diet may improve bowel function, reduce markers of local and systemic inflammation and, in the long term, adenoma recurrences. In the present study we aimed to identify bio-active compounds modulated by the dietary intervention that may be effective in regulating inflammation.

Method

We conducted a one-arm 3-month low-inflammatory Mediterranean dietary intervention on 30 individuals with FAP (aged>18 years), included in our Institutional endoscopic surveillance programme, who underwent rectum-sparing prophylactic colectomy. Blood and stool samples were collected at baseline (T0) and at the end of the dietary intervention (T1). We performed untargeted metabolomics analysis by HPLC-HRMS on participant's serum samples. Raw data were processed by Compound Discoverer software to identify the features detected in mass spectrometry. Univariate and multivariate analyses were applied to select the metabolites modulated by diet.

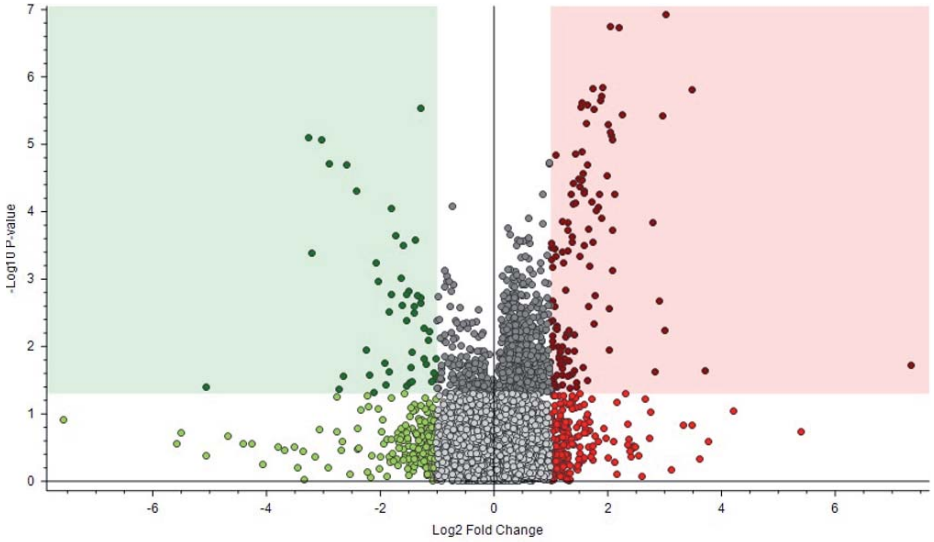
Results

We found 121 features whose levels increased after the dietary intervention, having a fold change > 2 and a p-value < 0.05 (**Figure 1**, green area). Also, the levels of 49 features decreased, having a fold change < 0.5 after the intervention and a p-value < 0.05 (Figure1, pink area). Consistently with the design and purpose of the dietary intervention, we observed a reduction of metabolites linked to animal-derived foods (Acylcarnitine, 2-HydoxyMiristic acid), and an increase of metabolites linked to plant-derived foods (Astaxanthin, 3-Hidroxybenzoic acid) and gut microbiota activity (Methyl indole-3-acetate, Indole-3-acetic acid), which could be suggestive of an improvement in the inflammatory profile.

Conclusions

These preliminary results are encouraging regarding the possibility of assessing the patients' compliance with diet using metabolomics analysis, which also helps in understanding the mechanisms by which diet modulates inflammation.

Figure 1.



P63 - RISK FACTORS FOR THE DEVELOPMENT OF DESMOID TUMOR AFTER PREVENTIVE COLORECTAL SURGERY IN FAMILIAL ADENOMATOUS POLYPOSIS INDIVIDUALS: A MONOCENTRIC RETROSPECTIVE COHORT STUDY

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Background and aim

Desmoid tumor (DT) is the primary cause of death in patients with familial adenomatous polyposis (FAP) related to *APC* gene, along with duodenal cancer, since the introduction of colorectal cancer (CRC) preventive surgery. This study aimed to identify risk factors for DT in FAP individuals

Method

The dataset was maintained prospectively and reviewed retrospectively. Two hundred and nine patients with FAP underwent proctocolectomy or total colectomy with rectal sparing to treat or prevent a CRC from 2000 to 2023 at the Fondazione IRCCS Istituto Nazionale dei Tumori of Milan. Patients were regularly followed-up at the outpatient clinic according to the National Comprehensive Cancer Network guidelines for FAP. Descriptive statistics and frequency distribution tables were used to summarize the study sample; associations between DT presence and clinical, pathological and genetic characteristics were assessed using Chi-square or Fisher exact test. The pattern of Desmoid-free survival (DFS) was assessed by resorting to univariate Cox regression model.

Results

One hundred and eleven (53%) patients were male. The median age was 25 years-old (range: 7 – 77). One hundred and nine (53%) had a mutation between codon 713 and 1440. One hundred fifty-four patients (73.7%) underwent a total colectomy and 55 (26.3%) a

proctocolectomy. 159 (76%) patients were treated laparoscopically. The 94% of patients did not experience post-operative complication. The specimen exam revealed adenocarcinoma in 31 (15%) patients, 43 (20%) high grade dysplasia and, 135 (65%) low-grade dysplasia. Twenty-three (11%) patients developed intra-abdominal DT after abdominal surgery [**Table 1**]. The median follow-up was 85 months (range: 49 – 136 months). Preliminary results showed that DT was significantly associated with codon ($p = 0.014$), histology of cancer at surgery ($p = 0.003$), surgical procedure (proctocolectomy vs. total colectomy; $p = 0.013$), type of surgery (laparoscopic vs open approach; $p = 0.004$). The surgical procedure and approach seem to be also associated with DT occurrence when evaluated in terms of DFS.

Conclusions

Preliminary results showed some association between DT presence and clinical, pathological and genetic characteristics, as well as a higher probability of DT development for open surgery and proctocolectomy procedure.

Keywords

Desmoid tumor, familial adenomatous polyposis, risk factors, genotype-phenotype correlation.

Table 1. Clinical, pathological and genetic characteristics of study patients according to intra-abdominal DT presence.

	Intra-abdominal DT (n = 23)		No intra-abdominal DT (n = 186)		All (n = 209)	
	n	%	n	%	n	%
Sex						
Female	12	52.17	86	46.24	98	46.89
Male	11	47.83	100	53.76	111	53.11
Age *Median (range)*	27 (14–61)		25 (7–77)		25 (7–77)	
BMI *Median (range)*	23 (16–35)		22 (15–40)		22 (15–40)	
Histology of cancer						
Adenocarcinoma	9	39.13	22	11.83	31	14.83
LGD	5	21.74	38	20.43	43	20.58
HGD	9	39.13	126	67.74	135	64.59
Type of surgery						
Laparoscopic approach	12	52.17	147	79.03	159	76.08
Open approach	11	47.83	39	20.97	50	23.92
Surgical procedure						
Proctocolectomy	11	47.83	44	23.66	55	26.32
Total colectomy	12	52.17	142	76.34	154	73.68
Number of abdominal surgeries						
One surgery	14	63.64	148	80.87	162	79.02
Two surgeries	8	36.36	35	19.13	43	20.98
Post-operative complication						
No	21	91.30	175	94.09	196	93.78
Yes	2	8.70	11	5.91	13	6.22
Codon						
0 – 543	4	19.05	60	32.61	64	31.22
543 – 713	0	0.00	21	11.41	21	10.24
713 – 1140	13	61.90	96	52.17	109	53.17
1440 – 2843	4	19.05	7	3.81	11	5.37

DT, desmoid tumor; BMI, body mass index; LGD, low-grade dysplasia; HGD, high grade dysplasia.

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P64 - FACTORS INFLUENCING COLORECTAL SURVEILLANCE ADHERENCE IN LYNCH SYNDROME: A RETROSPECTIVE MONOCENTRIC STUDY

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Background and aim

Lynch syndrome (LS) leads to an increased risk of developing cancer, predominantly colorectal (CRC) and endometrial. Early detection and intervention are crucial for improving LS patients' outcomes. This study aimed to assess adherence to recommended surveillance programs and identify factors influencing compliance in this population.

Method

We retrospectively analyzed data from a specialized cancer center's Hereditary Digestive Tumors Registry. Patients were categorized based on their adherence to endoscopic surveillance, defined as having had at least one colonoscopy every three years. Survival rates and factors associated with adherence were examined.

Results

Among 397 analyzed LS patients, 76.8% demonstrated commendable adherence by completing the endoscopic surveillance program. This adherence translated to a survival rate of 83.5% at 240 months (**Figure 1**), highlighting the benefits of consistent surveillance for long-term outcomes. Genetic counseling emerged as a significant factor in promoting adherence ($p<0.001$). Patients who received genetic counseling at diagnosis were more likely to adhere to the surveillance program. Additionally, higher education levels were associated with increased adherence. No significant differences in adherence were observed across different gene mutations, although a trend towards higher adherence in *MSH2* carriers compared to *MLH1* carriers was noted. Distance from the referral center negatively impacted endoscopic adherence (**Table 1**).

Conclusions

Regular adherence to surveillance programs is critical for improving survival in LS patients. Genetic counseling and education play key roles in enhancing adherence and positive attitudes towards screening. Future research should focus on personalized risk stratification, early detection strategies, and addressing geographic disparities in care access to ensure optimal outcomes for all individuals affected by LS.

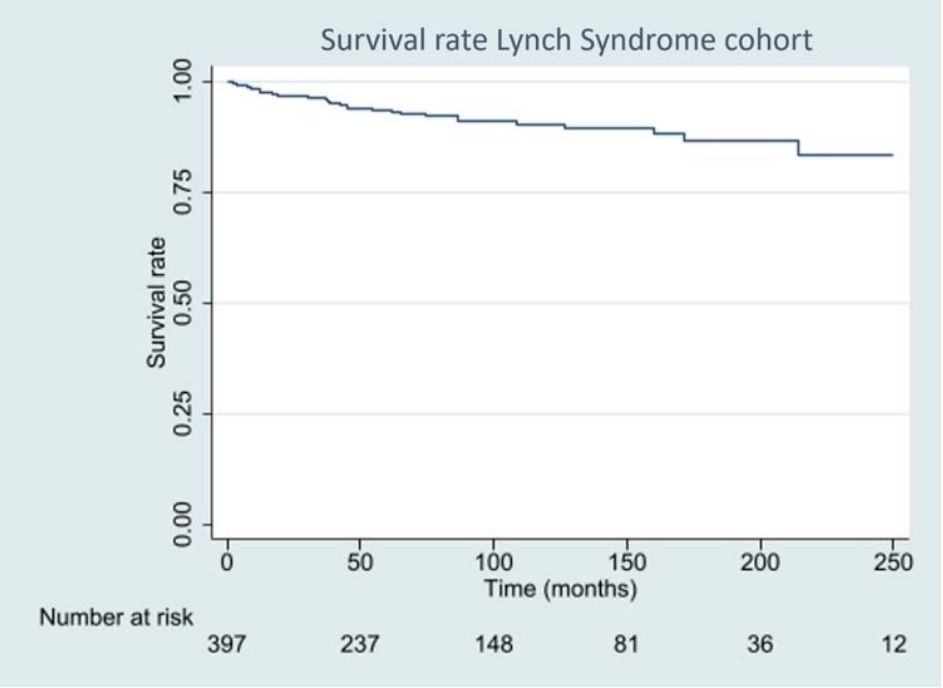
Keywords

Lynch syndrome, surveillance, adherence, genetic counseling, survival.

Table 1. Factors associated with full surveillance adherence and endoscopic compliance at multivariable binary logistic regression. OR=odds ratio, CI=confidence interval, B=logistic regression coefficient; SE=standard error, n=number, CRC=colorectal cancer. ® P<0.05.

	Univariable P-value	Multivariable analysis			
		OR (95% CI)	B	SE	P-value
Female gender, n (%)	0.603	-	-	-	-
Age < 40 years, n (%)	0.393	-	-	-	-
Genetic counselling, n (%)	**0.000**	**2.401 (1.377-4.184)**	**0.876**	**0.283**	**0.002**
Personal history of CRC, n (%)	0.070	-	-	-	-
Personal history of other tumors, n (%)	0.646	-	-	-	-
Family history of CRC, n (%)	1.000	-	-	-	-
Family history of other tumors, n (%)	0.210	-	-	-	-
Higher education, n (%)	**0.008**	**1.810 (1.131-2.897)**	**0.593**	**0.240**	**0.013**
Employed, n (%)	0.395	-	-	-	-
Out of region, n (%)	**0.047**	**0.609 (0.373-0.993)**	**-0.496**	**0.250**	**0.047**
Has sons, n (%)	0.421	-	-	-	-
Genetic mutations					
MLH1	0.748	-	-	-	-
MSH2	**0.049**	0.705 (0.427-1.162)	-0.350	0.255	0.170
MSH6	**0.048**	1.635 (0.676-3.956)	0.492	0.451	0.275
PMS2	0.612	-	-	-	-
EPCAM	1.000	-	-	-	-

Figure 1.


Survival rate Lynch Syndrome cohort
Survival rate
0.00
0.25
0.50
0.75
1.00
0
50
100
150
200
250
Time (months)
Number at risk
397
237
148
81
36
12

P65 - BLOOD MICROSATELLITE INSTABILITY TO DETECT PRE/CANCEROUS COLORECTAL LESIONS IN LYNCH SYNDROME PATIENTS

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Background and aim

Lynch Syndrome (LS) is the most frequently inherited condition characterized by an increased risk of developing colorectal cancer and other malignancies. It is caused by a germline mutation in one of the genes involved in the DNA mismatch repair (MMR) mechanism and the pre/cancerous lesions occurred in LS patients are all characterized by microsatellite instability (MSI). Close endoscopic surveillance, starting from the early twenties, with colonoscopies scheduled every 1-2 years, stands as the sole viable approach to mitigate morbidity and mortality for individuals with LS. Nevertheless, the invasive nature of endoscopy is frequently met with resistance from patients. In this respect, a low-invasive test able to detect the presence of early lesions would be essential to may overcome these limitations.

Method

Taking advantage of an endoscopic surveillance program, we retrospectively assessed the instability of 5 microsatellites (BAT26, BAT25, NR24, NR21 and Mono27) in liquid biopsies collected at baseline and possibly at two further endoscopic rounds. For this purpose, we tested a new multiplex drop-off digital polymerase chain reaction (dPCR) assay, reaching mutant allele frequencies (MAFs) as low as 0.01%.

Results

A total of 78 plasma samples at the three time-points from 18 patients with baseline (pre) cancerous lesions and 18 controls were available for molecular analysis. Of these, 25 were collected in the presence of a colonoscopy-detected lesion: 14 low grade dysplasia, 8 high grade dysplasia and 3 adenocarcinomas. No main differences in the distribution of sex, age

and germline mutations in MMR genes were observed between patients with and without endoscopically detected lesions at baseline. On the other hand, the MAF values of BAT26, BAT25 and NR24 were significantly higher in samples from patients with endoscopically detected lesions. When all markers were combined to determine MSI in blood (bMSI), this test was able to discriminate lesion-bearing patients with an AUC of 0.80 (95%CI: 0.66; 0.94). In longitudinal samples, when lesions were detected only at baseline, a decrease of bMSI values was observed in 8 out of 10 (80%) patients. Conversely, in all the 7 patients with metachronous lesions, bMSI values showed a not well defined trend over time.

Conclusions

MSI by liquid biopsy is a feasible tool which may help in stratify patients risk, tailor the surveillance program and increase patient acceptance.

Keywords

LS; biomarker.

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P67 - EVALUATION OF THE QUALITY OF LIFE IN PATIENTS WITH GENETIC DIAGNOSIS OF LYNCH SYNDROME: THE EXPERIENCE AT THE FONDAZIONE IRCCS ISTITUTO NAZIONALE DEI TUMORI – MILANO

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Background and aim

In recent years, there has been a growing interest in monitoring treatment effect and burden of disease on quality of life (QoL) of cancer patients. Lynch Syndrome (LS) is an autosomal dominant inherited disease increasing the risk to develop colorectal, gynaecological or other minorly cancers. The aim was to investigate the QoL in LS patients by evaluating associations with demographical and clinical characteristics and comparing our QoL results with those related to healthy adults collected in an Italian representative cohort and also explore their cancer worries.

Method

The LS patients were registered and prospectively monitored at the Fondazione IRCCS Istituto Nazionale dei Tumori of Milan. QoL was investigated with the SF-36 questionnaire and concern about cancer with the Cancer Worry Scale Revised for Genetic Counseling questionnaire (CWS-GC) adapted for LS. The questions of SF-36 were coded and transformed in eight positively health scale domains and then grouped in two indices using the U.S. general population coefficients. The associations were assessed using Wilcoxon or Kruskal-Wallis test.

Results

The 223 patients analysed had a median age of 51 years (range:19-78yrs); 128 were female and 60% had already experienced a LS tumor, which was more spread in the colon (67%).

The SF-36 domains medians ranged from 57.95 for vitality to 89.12 for physical functioning, role-emotional functioning had the highest variability. Physical component index (PCS) achieved a median score of 53.49, slightly higher than one of mental component index (MCS) (46.35). For PCS all age categories were found to be different from 75+ yrs old group (p:0.006). MCS was significant in the comparison 55-64 vs 45-54 yrs (p:0.03). A significant association with gender was observed for MCS (p:0.008) and PCS was associated with tumor history (p:<.001). All eight SF-36 domains had medians lower than those of healthy adults of normative population. Regarding cancer worries, 39% of who already experienced a tumor and 49% of who didn't were "*quite*" worried about possibility of a Lynch tumor.

Conclusions

Cancer history, age and gender impact on the QoL perception of patients. Specifically, female and older patients with a previous cancer history showed a lower mental and physical score when compared to the counterpart. Patients with LS also fell worst respect healthy normative population in all QoL aspects.

Keywords

Quality of life, Lynch Syndrome, SF-36.

P68 - CURRENT WORK OF THE APC SUBCOMMITTEE OF THE INSIGHT - CLINGEN HEREDITARY CRC / POLYPOSIS VARIANT CURATION EXPERT PANEL: INTERPRETATION OF SELECTED APC VARIANTS BASED ON THE APC-SPECIFIC ACMG/AMP VARIANT CLASSIFICATION CRITERIA

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Background and aim

The Hereditary CRC/Polyposis Variant Curation Expert Panel was recently established by InSiGHT and ClinGen. As part of the ClinGen process, the *APC* subcommittee (*APC* VCEP) developed gene-specific ACMG/AMP variant classification criteria (Spier & Yin et al. 2023; https://cspec.genome.network/cspec/ui/svi/doc/GN089). After approval of the *APC* VCEP in December 2022, the ongoing variant assessment and curation was implemented to continue long-term variant evaluation by use of the specified criteria.

Method

As part of the validation process, the gene-specific criteria had been applied to 58 *APC* variants covering a wide range of scenarios (pilot variants). In addition, 20 variants with

borderline evidence levels between uncertain significance (VUS) and (Likely) Pathogenic (LP/P) were selected by the *APC* VCEP for intensive re-evaluation (promising variants). These variants were the subject of further in-depth data mining including a survey of clinical and RNA data among *APC* VCEP members. All variants were first evaluated by a group of 11 biocurators, afterwards reviewed and discussed by expert members in virtual VCEP meetings, and finally made publicly available via ClinVar and the ClinGen Evidence Repository.

Results

Of 78 evaluated variants, 87% with a previous established classification in ClinVar were confirmed (14/15 (Likely) Benign [LB/B], 27/32 (Likely) Pathogenic [LP/P]). About half of the 31 previous VUS in ClinVar were reclassified: 10 as LB/B (32%) and 6 as LP/P (19%). Out of the 20 promising variants, 13 (65%) were evaluated as LP/P and 7 (35%) as VUS. The classification for 5 of the promising variants changed compared to the previous evaluations in ClinVar based on additional RNA analysis and clinical data (2 VUS were upgraded to LP and 3 LP/P variants were downgraded to VUS). The most challenging/interesting variants will be discussed in detail.

Conclusions

So far, 78 variants have been approved by the *APC* VCEP. The application of the *APC* specifications has led to the reclassification of ~50% of VUS into a clinically relevant pathogenicity class, which is a particular encouraging result given the large number of *APC* VUS listed in ClinVar awaiting reclassification. For selected promising variants a more comprehensive and valid evaluation could be achieved particularly with RNA and clinical data. The *APC* VCEP will continue to interpret prioritised lists of conflicting variants / VUS to improve clinical utility.

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P69 - CONSTITUTIONAL PROMOTER METHYLATION OF *LTBP4* AND *BRCA1* IN UNSOLVED FAMILIAL AND EARLY-ONSET COLORECTAL CANCER PATIENTS

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Research initiatives aimed to identify hereditary nonpolyposis mismatch repair (MMR)-proficient colorectal cancer (CRC) genes through genomic analyses have been mostly unsuccessful. Constitutional monoallelic inactivation of hereditary cancer genes through promoter methylation is seldom responsible for cancer predisposition; having been reported in Lynch and other cancer syndromes. Here we aimed at exploring the involvement of constitutional promoter methylation as a mechanism of gene inactivation in genetically unsolved familial and/or early-onset MMR-proficient CRC cases.

Bisulfite-treated peripheral blood DNA from 46 unrelated CRC patients diagnosed before age 50 and/or with family history of CRC was analyzed with the Illumina Infinium MethylationEPIC array. To identify patient-specific methylation patterns, CpGs beta-values of each patient were compared with those of the other analyzed individuals using Qlucore.

Monoallelic methylation of *LTBP4* CpG island (CGI) 102 was detected in a patient diagnosed with CRC at age 46. No additional epimutants were identified among 230 additional patients. CGI 102 constitutes a promoter that regulates two *LTBP4* transcripts which are the second and third most expressed transcripts in normal colon. CGI 102 is frequently found methylated in sporadic CRCs, and this methylation correlates with global *LTBP4* RNA downregulation in CRC. Supporting the role of *LTBP4* inactivation in CRC predisposition, mice KO for *LTBP4* develop colorectal tumors (PMID: 12208849). No deleterious *LTBP4* germline variants were identified among 50 familial and/or early-onset CRC patients and 632 patients with metastatic CRC. No association of germline *LTBP4* predicted deleterious missense variants with CRC occurrence was observed.

Mosaic methylation of the *BRCA1* promoter was identified in blood DNA of a patient diagnosed with CRC at age 37. Constitutional *BRCA1* promoter methylation is associated with increased risk of breast and/or ovarian cancer. Whether the development of the early-onset CRC was driven by the BRCA1 promoter methylation remains to be explained. A deep learning classifier for pathology trained to detect homologous recombination deficiency in tumors is currently being applied.

Our findings suggest that constitutional monoallelic methylation of *LTBP4* CGI 102 causes increased risk of early-onset CRC. We have also identified a mosaic constitutional methylation of the *BRCA1* promoter in a patient diagnosed with early-onset CRC.

P70 - IMMUNE PROFILING IN ADENOMAS FROM PATIENTS WITH FAMILIAL POLYPOSIS SYNDROME

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Background

Familial adenomatous polyposis (FAP) and MUTYH-associated polyposis (MAP) are genetic disorders characterised by the development of multiple pre-cancerous adenomatous polyps within the colorectum and duodenum, and consequently increases in the likelihood of developing colorectal and/or duodenal cancer. However, little is known about the underlying molecular mechanisms responsible for adenoma and cancer development in these conditions. Exogenous factors and endogenous processes related to the gastrointestinal microenvironments, including changes in immune system regulation, are known to play a role in the development of sporadic-CRC and other familial CRCs. These mechanisms have not been investigated in FAP and MAP but are expected to play a similar role in adenoma development and progression to cancer in these patients. This study aims to use genomic datasets from duodenal and colorectal normal mucosa and adenomas, to better understand the molecular and immunological events leading to tumorigenesis.

Methodology

RNAseq data from duodenal and colorectal adenomas and normal mucosa will be analysed to determine changes in the immune landscape during adenoma development in the two gastrointestinal regions; identifying differentially expressed genes and dysregulated pathways associated with altered immune signalling and regulation.

Results

Analysis of differentially expressed genes and volcano plotting revealed several significantly up-regulated and down-regulated immune regulatory genes ($P < 0.05$, Fold-change > 1.5) within colorectal and duodenal MAP or FAP. Noteworthy findings include the up-regulation of activation markers (including *CD44*) within colorectal MAP adenomas, and down-regulation of T cell markers within colorectal MAP adenomas and duodenal FAP adenomas.

Conclusion

In this study, we have identified distinct differences in the expression of immune regulatory genes and abundance of immune cell types within FAP and MAP colorectal and duodenal adenomas. These findings will contribute towards our understanding of the role of the immune system in adenoma development in these patients.

P71 - CHARACTERIZATION OF THE SPLICING VARIANTS C.423-12A>G AND C.423-14A>G IN APC GENE

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Background and aim

Familial adenomatous polyposis (FAP) is an autosomal dominant condition caused by germline pathogenic variants (PVs) in the *APC* gene. The majority of *APC* PVs are predicted to result in truncated protein products. PVs predicted to result in APC aberrant splicing have rarely been reported. In some cases, the clinical significance of *APC* variants is difficult to predict: these variants are classified as variants of uncertain significance (VUSs). The clinical significance of the VUSs can be defined only after evaluating additional evidence, such as population frequency, tumor characteristics, *in silico* predictions, and functional studies.

We report the re-characterization of two germline heterozygous variants located in the intron 4 of *APC* gene identified in two Italian families with FAP phenotype.

Methods

In silico predictions were made with Alamut™ Visual Plus version 1.2.1 software. The resulting predictions were confirmed through RNA Extraction and Reverse Transcriptase-PCR (RT-PCR) using primers located in *APC* exon 4 and exon 6. The amplified fragments were checked on agarose gel and then characterized by Sanger sequencing. Transcripts from variants carriers were compared to at least three controls for RT-PCR and at least one control was included for sequencing analysis. The evaluation of the capacity to produce a functional transcript was performed with a full-length PCR (FL-PCR).

Results

In silico prediction of the two VUS c.423-12A>G and c.423-14A>G suggested the possible activation of a criptic acceptor site in intron 4 of the *APC* gene that could result in altered sequence and formation of a non-functioning protein. *In vitro* analyses confirmed *in silico* predictions: the variant c.423-12A>G cause the insertion in the cDNA of the last 11 nucleotides of the intron 4; also the c.423-14A>G cause the insertion of the last 13 nucleotides of the

intron 4. Therefore, both variants produce also a little quote of transcripts with the deletion of exon 5 that lead to premature termination codon. The FL-PCR indicates that the variants alleles are not able to produce a functional transcript.

Conclusions

According to ACMG/AMP criteria, our clinical and molecular characterization of the two variants provides evidence of pathogenicity. The reclassification of VUSs is crucial to allow appropriate management for patients and their families.

Keywords

FAP, genetic testing, variants reclassification, personalized medicine.

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P72 - SYSTEMIC MARKERS FOR PREDICTING AND MONITORING RESPONSE TO IMMUNE CHECKPOINT BLOCKADE THERAPY IN PATIENTS WITH ADVANCED MICROSATELLITE-UNSTABLE GASTROINTESTINAL CANCERS

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Background and aim

Cancers presenting with microsatellite instability (MSI), which is associated with high immunogenicity mediated by frameshift peptide (FSP) neoantigens, are susceptible to immune checkpoint blockade (ICB) therapy. However, a relevant proportion of MSI cancer patients do not durably respond to ICB and reliable biomarkers for therapy response prediction as well as therapy monitoring are still lacking. In this study we analyzed systemic FSP-specific immune responses and MSI status of extracellular vesicles (EV) for predicting and monitoring treatment response in ICB-treated MSI cancer patients.

Method

IFN-γ ELISpot and PEPperCHIP® peptide microarray were used to analyze systemic T cell and antibody responses against FSPs in 52 peripheral blood samples from 19 patients with advanced MSI gastrointestinal adenocarcinomas before and during ICB therapy. The applied FSP panel consisted of up to 40 recurrent FSPs (rFSPs) originating from putative driver mutations and with widespread occurrence in MSI tumors. Plasma samples from 18 patients and 30 healthy controls were utilized for precipitation-based EV isolation and EV DNA was analyzed using four diagnostic microsatellite markers.

Results

Future ICB responders displayed significantly higher rFSP-specific T cell responses at baseline compared to non-responders. However, immune responses during therapy were not correlated with the patients' clinical course. In 7/10 patients the MSI phenotype was successfully identified in EV DNA obtained prior to therapy start. In six of those patients EV-associated MSI signals were lost typically within the first three months of treatment, which was generally associated with a favorable treatment response and an objective response in three patients.

Conclusions

Future ICB therapy response in patients with advanced MSI adenocarcinoma was associated with higher rFSP-specific T cell responses before ICB initiation. Favorable response to ICB therapy was further associated with a switch from the EV-associated MSI status from MSI to non-MSI, warranting further exploration of EV DNA for liquid biopsy-based therapy monitoring.

Keywords

Biomarkers, extracellular vesicles, tumor neoantigens, immune checkpoint blockade, microsatellite instability.

P73 - ROLE OF A HEREDITARY-FAMILIAL CANCER REGISTRY IN THE GENETIC CHARACTERIZATION AND FOLLOW-UP IN CHILEAN FAMILIES WITH LYNCH SYNDROME

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Background and aims

Colorectal cancer (CRC) is the third cause of death from cancer in Chile. Approximately 3-5% of cases correspond to Lynch syndrome (LS), due to a pathogenic variant in the DNA mismatch repair genes (MLH1, MSH2, PMS2 and MSH6) with autosomal dominant inheritance. In addition to CRC, LS predisposes to the development of multiple malignant neoplasms, this is why suspected patients should be referred to a hereditary-familial cancer registry (HFCR) for genetic and clinical counseling, to improve the management, monitoring, and identification of family members at risk. To characterize the first cancer diagnosed in patients with LS and its relationship with age, sex, and mutated gene.

Methods

From HFCR, 47 families with LS were identified, with 303 patients carrying a pathogenic variant (index cases and relatives), who have been followed for an average of 8.2 years. The variables were analyzed: sex, type of cancer, age at diagnosis, mutated gene.

Results

Of the 47 families, 23 carry a pathogenic/likely pathogenic variant in the MLH1 gene (49%), followed by MSH2 (28%), MSH6 (15%) and PMS2 (8%). A total of 303 carriers were identified, 50.2% are men, 235 (77%) have developed at least one neoplasm, with a total of 341 cancers registered. In men, the vast majority were diagnosed with CRC as their first neoplasm (72%) on average at 42 y.o., followed by gastric cancer (6.1%; 52.7 y.o.); in women it was CRC (48%) on average at 42 y.o., followed by gynecological (21.5%; 47.9 y.o.) and breast (7.4%; 59 y.o.) cancer. The most common stage of CRC was II and III (40% each). Patients with mutated MLH1 demonstrated a higher frequency of CRC as their first neoplasm than patients with mutated MSH2 (72.9% and 49.1%), whose average age was 41 and 42 y.o. respectively. Carriers of MSH6 and mutated PMS2 also had CRC, whose ages at diagnosis were later (61.5 and 53 years).

Conclusion

In summary, most families with SL are explained by mutations in the MLH1 and MSH2 genes. Differences by gender show that CRC is the first neoplasm diagnosed in both sexes, however 1 in 3 women debut with gynecological or breast cancer. Regardless of the mutated gene, CRC was the first most frequent tumor, followed by gynecological cancers, especially for MSH2. Patients with MLH1/MSH2 report earlier cancers, while MSH6/PMS2 report later cancers. These results demonstrate the importance of multidisciplinary care.

Keywords

Lynch syndrome, Hereditary-familial cancer registry, Genotype-phenotype correlation.

P74 - IMMUNE DEFICIENCY INCREASES TUMOR BURDEN IN A LYNCH SYNDROME MOUSE MODEL

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Background and aim

Tumors arising in the context of Lynch syndrome show pronounced immunogenicity. Moreover, recent data suggest that the immune milieu in the normal mucosa could modify cancer risk in Lynch syndrome scenario. However, it is unknown, to what extent the immune surveillance controls the outgrowth of tumors in Lynch syndrome. To determine the influence of the adaptive immune system during Lynch-associated tumorigenesis, we crossed Msh2loxP/villin-Cre (VCM) Lynch mice with immunodeficient Rag2 knockout mice generating immunodeficient Lynch mice (VCMR-/-). Lynch mice heterozygous for Rag2 (VCMR+/-) were used as an additional immunocompetent control.

Method

VillinCreMsh2LoxP/LoxP (VCMR+/+) mice were crossed with Rag2-/- mice to generate immunodeficient Lynch mice (VCMR-/-) and Lynch mice heterozygous for Rag2 (VCMR+/-). Presence of the VillinCre transgene, loxP sites and Rag2 knockout was confirmed by PCR. Mice were sacrificed at the age of 7, 9 and 11 months to assess MMR-deficient intestinal tumorigenesis. The intestinal tract was removed and tissue Swiss rolls were prepared. Lesions were identified in the scanned tissue sections, categorized in 3 groups (dysplastic crypts, adenoma, carcinoma) and the area [mm2] of the lesions was determined using the Quantitative Pathology and Bioimage Analysis (QuPath) software (version 0.3.1). Tumor burden for each mouse was calculated by the sum of the area of all identified lesions in the intestine was added up.

Results

A significant difference between the size of different lesion categories was observed for all three mouse strains. Dysplastic crypts presented with the smallest size, followed by adenomas and invasive carcinomas which presented with the greatest size. Overall tumor burden was elevated in the immunodeficient group. When separating the identified lesions by category, 9 and 11 months old immunodeficient Lynch mice presented with a significantly increased tumor burden for invasive carcinomas ($p=0.0278$ and $p=0.0112$).

Conclusions

Immunodeficient mice presented with higher tumor burden of invasive carcinomas and developed tumors at an earlier age. These findings indicate that lack of immune surveillance during Lynch syndrome-associated tumorigenesis may favor outgrowth of invasive carcinoma in the intestinal tract.

Keywords

Immune surveillance, Lynch syndrome, cancer risk.

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P75 - COMPREHENSIVE CHARACTERISATION OF TWO LYNCH SYNDROME FAMILIES HARBOURING CONSTITUTIONAL MLH1 METHYLATION SHOWING DIFFERENT INHERITANCE PATTERNS

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Background and aim

Constitutional *MLH1* epimutation is a rare cause of Lynch syndrome, which accounts for 1-4% of colorectal and endometrial tumours displaying *MLH1* methylation. Most of the reported cases arise de novo and are frequently erased in the offspring. Few cases showing autosomal dominant inheritance of the constitutional *MLH1* methylation have been linked to in cis genetic variants (secondary epimutations). In rare cases, non-Mendelian patterns of inheritance have been reported (PMIDs 17301300, 26181641).

Here, we aimed to characterise two unrelated Lynch syndrome families showing constitutional *MLH1* methylation inheritance.

Methods

MLH1 promoter methylation in blood was evaluated by pyrosequencing and MS-MCA. Methylation-associated haplotype was investigated by microsatellite analysis. One constitutional *MLH1* methylation carrier from each family was selected for analysis. Genetic variants were analysed by linked-read whole genome sequencing (lrWGS) and targeted long-read PacBio sequencing of *MLH1*. Genomic rearrangements and insertion of mobile elements were inferred using data from lrWGS, PromethION, and Bionano Optical Mapping. Bioinformatic tools were used to analyse variants effect on transcription factors (TFs) binding.

Results

In family 1 constitutional methylation is found in 3 individuals and exhibits an autosomal dominant inheritance. In family 2 only the index case and her maternal aunt harboured the *MLH1* epimutation, showing a non-Mendelian inheritance pattern. A distinct methylation-associated haplotype was identified in each family.

Rare variants in *MLH1* CpG island and coding region were not identified. Structural variants were not detected within *MLH1*. Rare variants predicted to affect splicing were not identified in neighbouring genes *EPM2AIP1* and *LRRFIP2*. One rare variant in family 1 and four in family 2 in intergenic regions annotated as enhancers by ENCODE were predicted to affect TFs binding.

Conclusions

Through an exhaustive genetic analysis, we have ruled out the presence rare variants, large rearrangements, and insertions in the regions where variants associated with secondary epimutations have been previously described. Our results may suggest other regions and/or non-genetic mechanisms involved in the inheritance of constitutional *MLH1* epimutations.

P76 - DEVELOPING AN IPSC-BASED MSH2-DEFICIENT IN VITRO MODEL AS A PLATFORM TO CHARACTERIZE EARLY MOLECULAR EVENTS DRIVING BRAIN GLIOMAS IN CONSTITUTIONAL MISMATCH REPAIR DEFICIENCY (CMMRD) SYNDROME

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Introduction and aim

Constitutional mismatch repair deficiency (CMMRD) is a rare childhood-onset cancer predisposition syndrome caused by biallelic germline mutations in mismatch repair (MMR) genes. High-grade gliomas are the most frequently diagnosed tumours in CMMRD. However, the mechanisms underlying this malignant transformation are still poorly understood. Thus, novel tools for the study of cancer development in CMMRD are needed.

We aim to develop a biallelic MMR-deficient iPSC-derived neural progenitor cell (NPC line) that allows the characterization of early molecular events contributing to glioblastoma development in CMMRD.

Methods

A pure biallelic mutated clone as confirmed by western blot was obtained after CRISPR / Cas9 editing system with a gRNA targeting exon 3 of MSH2. Mutational burden and MSI were analyzed by TrueSight Oncology 500 Assay and a high-sensitivity MSI-based NGS tool, respectively. Wild-type (WT) cells and an MSH2-mutated clone were treated with an alkylating agent (MNNG). WT and MSH2-deficient iPSC lines were differentiated in a stepwise manner to obtain NPCs. Neural rosettes were harvested and plated. NPCs were identified by the co-expression of SOX2 and PAX6 markers by immunofluorescence and RT-qPCR. To monitor the acquired mutational load, WES, highly sensitive MSI assessment and RNA-seq analyses will be performed in each differentiation stage.

Results

IPSC clones harbouring biallelic MSH2 frameshift mutations were isolated. As a consequence of MMR-deficiency, microsatellite instability and resistance to apoptosis were evidenced in the MSH2-deficient clone compared to WT iPSC. When differentiated to NPCs, both WT and biallelic MSH2 mutated clone showed similar morphology and both lines co-expressed neural progenitor markers such as SOX2 and PAX6.

Conclusion

The generated CRISPR/Cas9 edited *MSH2*-deficient hiPSC clone demonstrated resistance to apoptosis and the ability to differentiate towards NPCs. This model will allow the study of early molecular events driving brain carcinogenesis in CMMRD.

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P77 - PERFORMANCE OF GERMLINE GENETIC TESTING IN PATIENTS WITH PANCREATIC DUCTAL ADENOCARCINOMA ≤60 YEARS

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Background and aim

A diagnosis of pancreatic ductal adenocarcinoma (PDAC) at a young age in the absence of other personal or family history does not justify germline genetic testing. The aim of this study is to evaluate the performance of genetic testing in patients with PDAC onset ≤60 years, regardless of personal and/or family history of other neoplasms.

Methods

Multicenter study with retrospective and prospective inclusion carried out in three Spanish hospitals over a period of 8 years. Germline genetic testing was performed in all patients with PDAC diagnosed ≤60 years with the TruSight-cancer multigene panel (Illumina version 2) of Next-Generation Sequencing using the MiSeq platform.

Results

Finally, 129 patients with PDAC ≤60 were included, with an average age of 53 years (Interquartile range (IQR) 49-58), of which 47 (36.4%) were women and 86 (66.7%) were current or former smokers.

Fifteen (11.6%) individuals met criteria for genetic testing based on European guidelines (family or personal history of other neoplasms that already required the exclusion of a hereditary syndrome). Of these 15 patients, 6 (40%) had a germline pathogenic variant (GPV): 1 in *TP53*, 1 in *ATM*, 1 in *BRCA1*, 1 in *PALB2* and 2 in *BRCA2*.

Of the remaining 114 (87.7%) individuals (only with the "age ≤60 years" criterion), 8 (7%) had a GPV: 5 in *ATM*, 2 in *BRCA2*, and 1 in *KIF1B*.

Conclusions

More than 10% of patients with PDAC diagnosed ≤60 are associated with a hereditary syndrome, of which more than 50% would not have been diagnosed with the traditional criteria. These results support performing germline genetic testing in all patients with PDAC ≤60 years.

P79 - THE GERMLINE MLH1 C.-42C>T VARIANT IS ASSOCIATED WITH MONOALLELIC MLH1 PROMOTER METHYLATION IN MLH1-DEFICIENT COLON AND ENDOMETRIAL CANCER

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Background and aim

Epigenetic silencing of one allele of *MLH1* via constitutional methylation (*MLH1* epimutation) predisposes to MLH1-deficient Lynch-type cancers. The germline *MLH1* c.-42C>T promoter variant has previously been found in (suspected) Lynch syndrome cases with MLH1-deficient colorectal and endometrial cancers, suggesting it is a pathogenic variant. This study describes the clinicopathologic and molecular characterisation of tumour and non-neoplastic tissues in two new index cases carrying *MLH1* c.-42C>T with the aim of determining if this variant confers pathogenicity via "secondary" *MLH1* methylation.

Methods

Genetic testing for suspected Lynch syndrome identified two index cases with the *MLH1* c.-42C>T germline variant from Australia (A) and the USA (B). Tumour molecular characte-

risation included MMR immunohistochemistry and multigene panel sequencing to identify somatic *MLH1* mutations (2nd hit). Droplet digital PCR (ddPCR) was used to assess *MLH1* promoter methylation in tumour and non-neoplastic tissues (blood, saliva, buccal, and/or tumour-adjacent normal).

Results

Both index cases were of European heritage. Proband A (male) developed an MLH1-deficient, *BRAF* p.V600 wildtype, and CIMP-negative colon cancer at 61yrs and had a positive family history of colorectal cancer. *MLH1* methylation (43.2%) was detected in his tumour and sequencing identified a somatic 2nd hit in *MLH1* (c.1122_1126dup p.Asp376Valfs*27 mutation). *MLH1* methylation was detected by ddPCR in normal colonic mucosa adjacent to the tumour (3.7%) and at even lower levels in the blood (0.07%) and saliva (0.09%), affecting ~1/1000 to 3.7% of non-neoplastic cells.

Proband B had no family history of cancer but developed MLH1-deficient endometrial cancer at 38yrs. The tumour showed loss of heterozygosity of the wildtype *MLH1* c.-42C allele and monoallelic methylation of the variant c.-42T allele. The ddPCR of normal endometrium, saliva and buccal samples showed no evidence of *MLH1* methylation.

Conclusion

The germline *MLH1* c.42C>T variant is associated with monoallelic *MLH1* methylation of the variant T allele in the tumour and a second somatic hit inactivating the wildtype allele, resulting in biallelic inactivation of *MLH1* and loss of MLH1 protein. Despite the absence of, or very low-level mosaic *MLH1* methylation in non-neoplastic tissues, both cases developed *MLH1*-methylated cancers via a molecular mechanism distinct from the biallelic *MLH1* methylation commonly observed in sporadic cancers.

P80 - THE LANDSCAPE OF BENIGN GASTROINTESTINAL LESIONS IN ADULTS WITH PTEN HAMARTOMA TUMOUR SYNDROME

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Background and aim

Colorectal cancer (CRC) surveillance with regular colonoscopies is advised for PTEN Hamartoma Tumour Syndrome (PHTS) patients from age 40 years. The hereditary risk of CRC in PHTS patients is in recent studies estimated as < 1% below age 50, approximately 2% at age 60 and 5% at age 70. Benign lesions are common, but not well described in larger cohorts. We aimed to determine the occurrence of benign gastrointestinal (GI) lesions in adult PHTS patients.

Method

Findings were retrieved from two groups: colonoscopy and pathology findings of PHTS patients with CRC surveillance in our expertise centre (group A) and a cohort of PHTS patients who received genetic testing at our centre from whom records were obtain from pathology reports (group B). These groups partly overlap.

Results

In group A (n= 37, median age 47 years), 33 patients (89%) developed one or more colorectal lesion upon colonoscopy. A total of 29 adenomas were detected in 13 patients (35%, of which 1 advanced) at a median age of 52 years, of which 79% located in the proximal colon. The adenoma detection rate was 23 per 100 colonoscopies. Hamartomas of no special type were detected in 16 patients (43%) and ganglioneuromas in 15 patients (41%). Hyperplastic and sessile serrated polyps of < 10 mm without dysplasia were detected in 7 patients (19%). Male patients, who were slightly older at the last colonoscopy, had significantly higher detection rates of hamartomas NST (53% versus 19%) and ganglioneuromas (42% versus 29%). No statistically significant or clinically relevant differences between females and males were found for any of the other colorectal lesions.

In group B (n=379, median age 38 years), 199 patients (53%) had one or more GI lesions removed. They were of different pathology: 55 adenomas (15%), 33 hamartomas (9%), 34 ganglioneuromas (9%), 71 hyperplastic, juvenile and/or sessile serrated lesions (19%). There were no significant differences observed in frequency or age of onset between sexes. Number of lesions detected by colonoscopy but left *in situ* are unknown.

Conclusions

The majority of adult PHTS patients, both males and females, develop multiple benign gastrointestinal lesions varying from adenomas to hamartomas and ganglioneuromas. For the gastroenterologist performing the colonoscopies it is the challenge to distinguish between benign lesions without malignant potential and those with malignant potential. Therefore we recommend colonoscopy by PHTS experts.

P81 - IDENTIFYING THE UNMET NEEDS OF YOUNG ADULTS WITH GASTROINTESTINAL (GI) CANCE

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Background and aim

Globally, incidence of gastrointestinal (GI) cancer is decreasing however, it is increasing in young adults (YA), aged between 25-39. Cancer remains the leading cause of disease-related death in YA in the Western world. As epidemiological and preventative research into this age group matures, we seek to identify the unique challenges and psychosocial factors experienced by this group of patients faced with GI cancers.

Methods

As part of a larger study in YAs, 7 patients with GI cancers were recruited with 4 participating in a focus group and 3 via semi-structured interviews. The study invited YA diagnosed with cancer in the preceding 5 years. The interviews and focus groups were recorded and anonymised transcripts provided. These were analysed using inductive thematic analysis with two professionals (one clinical, one nonclinical), results were appraised by YA patient representatives.

Results

Distinct themes recognised within this group of patients:

Early identification of young adults at risk: due to current national screening (UK age 50) these patients were not offered routine screening for CRC. The currently identified risk factors for all adults include alcohol, tobacco, obesity, diabetes and sedentary behaviour however this cohort of patients may not have had time to accrue this damage.

Delay of diagnosis: due to non-specificity of symptoms delay to diagnosis can be caused by both patient and healthcare provider factors seeking to rule out other more common causes.

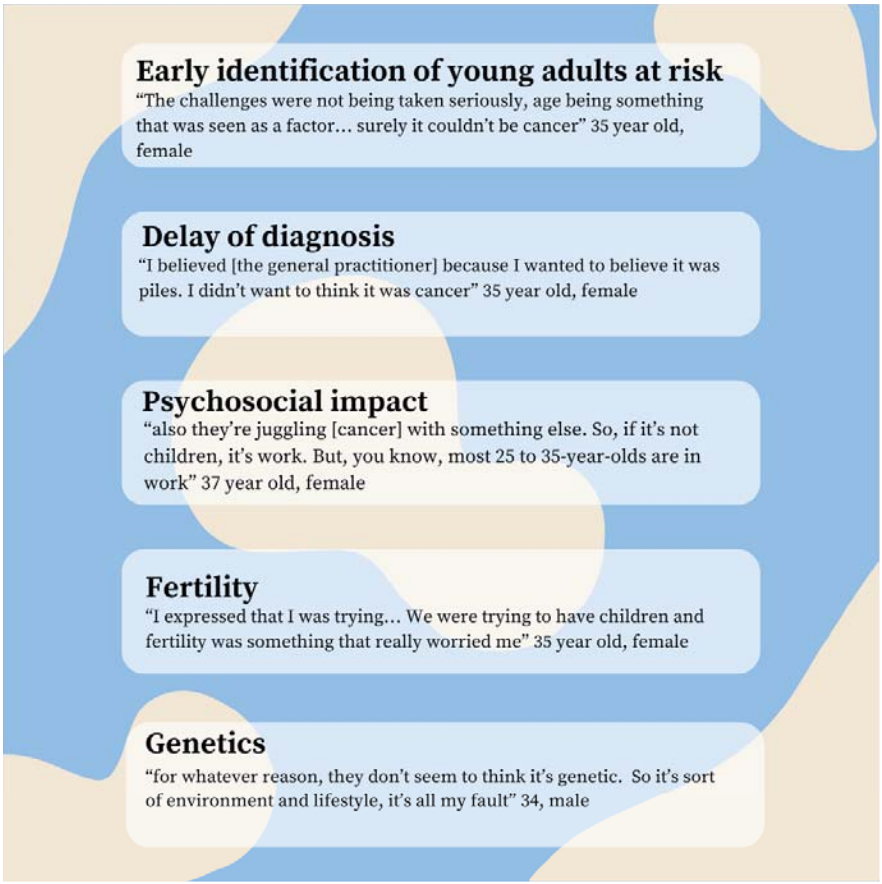
Psychosocial issues: symptoms in this group include diarrhoea, sleep disorders and sexual dysfunction. These may impact day-to-day function and mental health. YAs felt their "normal" lives were on hold compared to peers.

Fertility: priority is often given to anti-neoplastic treatment but, impact on fertility can be a distressing sequelae of cancer and treatment. Discussion around fertility preservation is increasingly important as methods improve as well as survival from cancer treatment.

Genetics: global guidelines suggest colorectal cancers should be tested for mismatch repair deficiency which can be hereditary, caused by a Lynch syndrome. YA should be referred to genetics so appropriate recommendations can be given for screening of relatives and offspring. Patients can assume that lack of finding an identified genetic cause infers lifestyle or behaviour caused the disease.

Conclusions

Delivery of appropriately bespoke services is key in tailoring care needs to young adults with cancer. This research supports the specific needs of young adults with GI cancer in requirement of better screening, awareness, clinical trial development and psychosocial support.



P82 - MULTIPLE COLORECTAL ADENOMAS – ONGOING TIGHT SURVEILLANCE IS REQUIRED

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Background and aim

Patients with multiple colorectal adenomas (MCRA) but without a monogenetic abnormality present a frequent dilemma, as there is little data on this. We aim to provide phenotypic description of patients with MCRA referred to a national registry, to examine the polyp, cancer and colonoscopy burden over time.

Methods

Adults managed by the NZ Familial GI Cancer Service between 1988 and 2020 were screened. Inclusion criteria: ≥10 adenomas at a single colonoscopy, or ≥20 cumulative adenomas over multiple colonoscopies; and negative gene testing for APC and MYTH mutations. Data was retrieved from a prospectively entered database including demographics, polyp findings, surgery information and personal/family history of colorectal cancer (CRC) at any age.

Results are expressed in medians and IQR.

Results

A total of 110 patients with MCRA met study criteria, with a total 716 colonoscopies; median follow up was 7.7 (3.3-15.3) years from first procedure. Demographics are in **Table 1.** Diagnosis of MCRA, as per above criteria, was made at index colonoscopy for 40 (36.4%) patients; the remaining 70 after a median of 5 (3-7) examinations. 91.8% (101/110) of patients had a mixture of adenomas and serrated polyps, and 23.6% (26/110) fulfilled WHO 2019 criteria for serrated polyposis syndrome.

During surveillance (n=97), median interval between examinations was 14 (11.0-18.0) months. Median number of adenomas per surveillance procedure was 4.0 (2.0-5.5). Polyp burden **(Figure 1)** did not significantly decrease over time, although one patient had >100 polyps during surveillance. Of all adenomas detected pre-diagnosis, 26.0% (588/2259) were advanced (≥10mm; villous; or high grade dysplasia), vs. 7.9% (141/1791) during surveillance ($p< 0.01$). CRC occurred in 24.5% (27/110) of patients at presentation, vs. 7.2% (7/97) during surveillance ($p< 0.01$), after a median interval of 26.5 (13.2-35.0) months from last colonoscopy. Pre-diagnosis, 26 colonic surgeries were performed in as many patients. A further 12 had surgery during surveillance – for CRC (7), polyp burden (4), and large polyp (1).

Conclusion

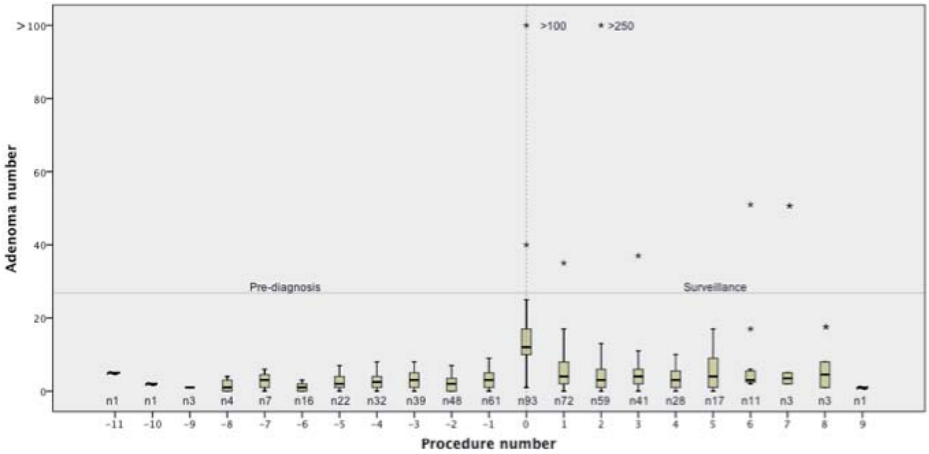
Majority of patients with MCRA, in addition to numerous adenomas, also have serrated polyps. Less than half had a family history of CRC. Polyp burden remained stable over time. Standard surveillance intervals reduced the number of advanced adenomas, but did not completely prevent CRC or surgical outcomes; tight adherence to annual surveillance is recommended for patients with MCRA.

Table 1. Demographics and clinical characteristics of patients with MCRA .

Total no. of patients	**n = 110**
Male sex, no. (%)	72 (65.5)
Ethnicity, no. (%)	
European	85 (77.3)
Maori	2 (1.8)
Asian	4 (3.6)
Other	19 (17.3)
Index colonoscopy indication, no. (%)	
Symptomatic	83 (75.5)
Screening positive FIT	8 (7.3)
Family history	19(17.3)
Other / no indication stated	22(20)
CRC in a first-degree relative at any age, no. (%)	39 (35.5)
Median age first adenoma, years (IQR)	56.0 (49.0-64.0)
Median age polyposis diagnosis, years (IQR)	64 (56-71)

Figure 1. A box-plot analysis of adenoma numbers detected from the first procedure to the last.

Colonoscopy procedure 0 indicates examination at which diagnosis of multiple colorectal adenoma, as per study criteria, was made in patients who subsequently had negative gene testing. n is the number of patients who had a colonoscopy at that time point. Horizontal line represents the median number of adenomas; box shows the inter-quartile range (IQR); whiskers show either 1.5*IQR from the bottom and top of the box or the minimum and maximum data values; * represents extreme outliers.



P83 - OVARIAN CANCER BURDEN IN LYNCH SYNDROME PATIENTS IN SLOVENIAN POPULATION

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Background and aim

Patients (pts) with pathogenic/likely pathogenic variants (P/LPV) in Lynch syndrome (LS) genes have increased risk of endometrial (EC) and ovarian cancer (OC). Up to 20% of LS pts are diagnosed with synchronous EC and OC (SEOC). Limited studies of SEOC in LS identified both cases of synchronous primary OC and EC and cases of clonally related metastases, mostly from primary EC. Our aim was to assess OC and SEOC burden in LS.

Methods

We retrospectively analysed genetic testing results and clinicopathologic features in 3 different cohorts. The first included unselected pts with OC and germline/tumour next-generation sequencing multi-gene panel (NGS-MGP) testing performed at the Institute since 2014. In the second we included all consecutive newly-diagnosed OC pts with tumour NGS-MGP tests results available since tumour-first strategy was implemented in 2021. In the third we included all female carriers of germline P/LPV in LS associated genes in the Slovenian Registry of tested individuals from hereditary cancer families. We excluded pts with tubal cancer or primary peritoneal serous carcinoma.

Results

In 4/1084 pts with clinical diagnosis of OC, germline P/LPV in LS genes were identified **(Table 1)**. Pts 1 and 2 had synchronous uterine and ovarian involvement: in the first clinical diagnosis of OC with metastases to uterus was made, and SEOC in the other. In 134 consecutive OC pts with tumour NGS-MGP testing, no P/LPV in LS genes were identified. In 9 of these tumours, high TMB was detected: 3 had somatic P/LPV in *POLE* and one had MLH1 promotor hypermethylation (a patient with clinical diagnosis of SEOC although NGS-MGP data suggested clonal relatedness). In a third cohort of 98 female with LS, in addition of 4 pts with clinical diagnosis of OC **(Table 1)**, 4 pts had clinical diagnosis of EC with metastases to the ovary. In 51 (54%) non-OC female with LS, adnexectomy was previously performed, either therapeutically (38) or prophylactically (13).

Conclusions

Primary OC in LS were rare in our cohorts, which could be due to low prevalence of P/LPV in high-risk LS genes in our population and/or adnexectomies. In pts with LS and synchronous uterine and ovarian involvement, tumours' molecular and clinicopathologic features should

be interpreted in a multidisciplinary setting to establish correct clinical diagnoses/staging as this informs prognosis and tailors therapy, especially in view of possible immunotherapy in mismatch repair deficient EC.

Keywords

Lynch syndrome, ovarian cancer, synchronous endometrial and ovarian cancer.

Table 1. Clinicopathologic and molecular features of OC in LS pts

No.	Clinical diagnosis (stage; age)	Histopathology report (year)	Germline P/LPV	Tumour NGS: TMB, P/LPV (allele frequency %)	MMR IHC
1.*	Ovarian cancer (FIGO IIB; 41)	high-grade serous carcinoma with clear cells in an ovary and multiple foci in endometrium; IHC staining suggests origo in the ovary or uterus (2014)	*MSH2*:c.1015C>T p.(Gln339*)	/	/
2.*	Ovarian cancer (FIGO IA; 48) Endometrial cancer (FIGO IB; 48)	clear-cell adenocarcinoma G2 of the right ovary; synchronous endometrioid G2 and clear-cell adenocarcinoma G2 of the endometrium (2000)	*MSH2*:c.1015C>T p.(Gln339*)	/	/
3.	Ovarian cancer (FIGO IA; 52)	high-grade endometrioid adenocarcinoma of the left ovary, less likely high-grade serous (2023+)	*PMS2*:c.88C>T p.(Gln30*)	TMB 152/Mb *IDH2* PV (21) *RB1* (29) *SDHB* PV (28) *TP53* PV (44)#	Loss of PMS2 expression
4.	Ovarian cancer (FIGO IV; 54)	+high-grade serous carcinoma of ovaries (2019)	*PMS2*:c.400C>T p.(Arg134*)	/	/

*mother and daughter;
#germline variant in *PMS2* was not identified in the tumour due to technical limitations of the test;
+revision of the original report from 2005;
MMR: mismatch repair; IHC: immunohistochemistry; /: not performed.

P84 - STUDYING GENOMIC EVOLUTION IN COLONIC CELL LINE MODELS OF MISMATCH REPAIR DEFICIENCY

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UCL Cancer Institute

Background and aim

Mismatch repair deficiency (MMRd) occurs in ~15% of colorectal cancers (CRC). Its causes can be sporadic or familial (Lynch syndrome). MMRd results in a high burden of single nucleotide variants and indels and microsatellite instability (MSI). MMRd CRC demonstrate profound clonal complexity and evolvability under immune selection. High mutational load translates into elevated neoantigen numbers which is thought to be the basis of their excellent response to immune checkpoint inhibitors (ICIs). However, for reasons yet unclear, ~50% of MMRd CRC do not respond to ICIs. Thus, improved biomarkers for patient stratification are required. The aim of my project is to understand genomic differences between MMRd genotypes that likely contribute to the variable clinical outcomes.

Methods

I used CRISPR-Cas9 to knock out four genes implicated in MMR (MLH1, MSH6, MSH3, MBD4) in different combinations in a human colonic epithelium cell line (HCEC). I validated the gene knockouts through Sanger sequencing and Western blotting. I subjected the cell lines to two rounds of subcloning and 3-4 weeks of mutation accumulation, followed by WGS at 30x. I investigated cell doubling time using live-cell fluorescent imaging and flow cytometry. I probed MMR gene and protein expression levels using qPCR and Western blots, respectively.

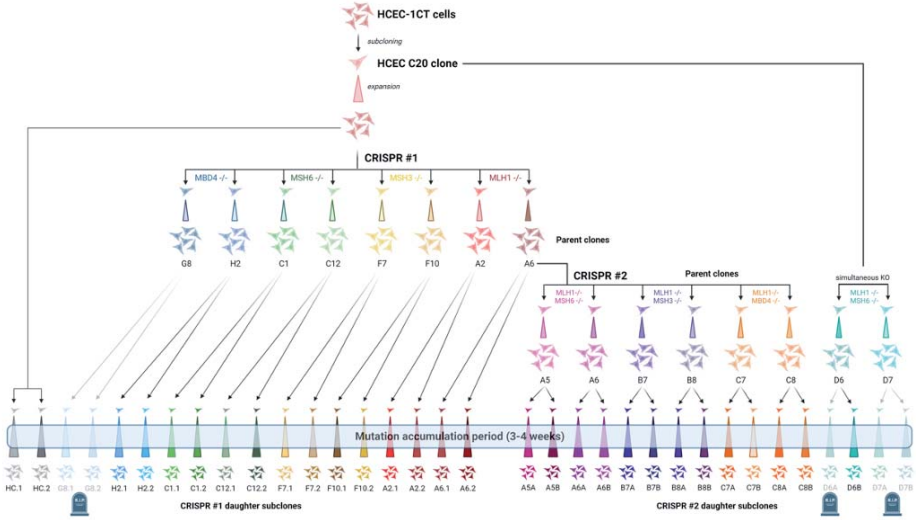
Results

I produced 29 colonic epithelial cell lines that model eight MMRd genotypes observed in clinical setting. I showed that their cell cycle profiles and cell doubling times are similar, irrespective of the genotype. So far, limited evidence from qPCR and Western blot experiments suggests that knockout of one MMR gene in cells does not significantly affect expression levels of other MMR genes. I obtained good-quality WGS data that I am currently preparing to analyse to derive mutation rates and mutation signatures of individual MMRd genotypes.

Conclusions

I have established a suite of valuable cell models to study genomic impact of MMRd. My WGS dataset obtained through carefully planned rounds of cell line subcloning will provide detailed insights into the burden and types of mutation caused by various forms of MMRd. I am planning to further interrogate mutation distribution across the genome using CUT&TAG

and investigate the adaptability of MMRd cell lines to stress, e.g., temozolomide treatment. Overall, my work will add to the view of MMRd as a multifaceted genetic phenomenon.



P85 - RECURRENT VARIANT IN THE 3'UTR OF THE MSH6 GENE IN FOUR UNRELATED ITALIAN FAMILIES WITH LYNCH SYNDROME PHENOTYPE

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Pathogenic variants (PVs) in *MSH6* account for 12%-35% of Lynch syndrome (LS). Despite classification guidelines for genetic variants proposed by different scientific societies, the finding of variants of uncertain significance (VUS) in MMR genes of LS represents a diagnostic dilemma in genetic counseling for the assessment of cancer surveillance in patients and healthy relatives. We present here clinical and molecular data on six different patients from four urelated families, carrying the c.*23_*26dup *MSH6* variant. Although this variant is reported in ClinVar database as VUS, a previously published study proposed that it might act as marker of a disease-associated *MSH2/MSH6* haplotype and it was suggested that it should be managed as pathogenic.

IHC of MMR proteins was performed in four endometrial tumours and one colorectal cancer (CRC); the CRC sample was also investigated for microsatellite instability (MSI). Based on tumour test results, the four probands carrying the c.*23_*26dup *MSH6* variant were offered germline NGS analysis of a panel compriseing the 4 LS genes. Variant analysis was subsequently performed in 2 affected relatives.

All tested patients were found to be heterozygotes for the c.*23_*26dup variant. In our cohort, endometrial cancer was diagnosed in all 4 female carriers, with a median age of 45.7 years. All endometrial cancers showed lack of expression of MSH2 and MSH6 proteins. CRC was diagnosed in all two male carriers, at age of 62 and 32, respectively. IHC and MSI showed lack of expression of MSH2 and MSH6 and MSI-H status in one CRC sample tested.

The clinical phenotypes of c.*23_*26dup *MSH6* carriers in these four families are highly suggestive of LS. As previously suggested, the penetrance is significantly high for gynaecological tumours, including early-onset endometrial cancer. However, molecular tests could not exclude the presence of undetectable *MSH2* variants and the hypothesis that it may represent a marker of a large rearrangement or a deep intronic variant within *MSH2* still requires experimental validation. A founder effect for c.*23_*26dup *MSH6* identified in families from northern Italy was previously suspected. Considering the similarity of the clinical phenotypes between the families from this study and those previously reported, it is possible all these pedigrees share a common PV, although the families here described are from other parts of Italy. Alternatively, it is possible that the *MSH6* variant is pathogenic.

P87 - A COMPARATIVE ANALYSIS OF LAPAROSCOPIC AND ROBOTIC PROCTOCOLECTOMIES WITH ILEAL POUCH-ANAL ANASTOMOSIS FOR ADENOMATOUS POLYPOSIS PATIENTS

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Background and aim

Colorectal cancer (CRC) ranks third in incidence and second in mortality among all malignancies worldwide. Around 35% of all CRC cases exhibit a familial clustering, with 6–10% attributed to hereditary CRC, categorized into Non-Polyposis and Polyposis syndromes. The most common adenomatous Polyposis syndrome is Familial Adenomatous Polyposis (FAP), associated with a lifetime risk of CRC approaching 100%. Less common due to the recessive inheritance is MUTYH-associated Polyposis with a risk of approximately 50%. For endoscopically uncontrollable colonic polyposis, risk-reducing surgery is often indicated. In the case of extensive rectal polyposis preferred treatment is minimally invasive prophylactic proctocolectomy with ileal pouch-anal anastomosis (IPAA), aiming for rapid recovery and desmoid tumor risk reduction. Robotic surgery offers benefits, but the limited operative field of the most widely used system (Intuitive daVinci) is often cited as a counterargument for surgeries involving multiple abdominal quadrants, resulting in limited data on robotic IPAA. This study aims to prove the feasibility of robotic (ROB) IPAA and compare it with laparoscopic (LAP) IPAA.

Method

A retrospective analysis was conducted at our institution involving 43 patients (29 LAP and 14 ROB) who underwent proctocolectomy with IPAA for polyposis syndromes (s. **Table 1**).

Results

Operative time war significantly longer in ROB (498 min.) compared to LAP (382 min., $p < 0.001$). However, ROB demonstrated a significantly shorter length of stay on a general ward (14 days, $p = 0.034$) and total length of stay (15 days, $p = 0.03$) compared to LAP. Additionally, blood loss was significantly reduced in ROB (15 ml) compared to LAP (293 ml). Complications, the rate of conversion, stay in Intensive Care Unit and readmission rates did not show significant differences between the two groups.

Conclusion

This study highlights the trade-offs between laparoscopic and robotic approaches in proctocolectomies with IPAA for polyposis patients. Although ROB experienced a longer operative time, the benefits of a shorter hospital stay and reduced blood loss may contribute to the overall efficiency and patient satisfaction. Importantly, comparable complication rates and readmission rates suggest that both approaches are equally safe. Surgeons should consider a robotic approach, when institutional resources are available. Further prospective studies are warranted to validate these findings.

Table 1.

	ROB	LAP
Patients in total	14	29
Mean age (years)	31.8 ± 13.7	34.4 ± 13.1
Gender	8 ♀ / 6 ♂	13 ♀ / 16 ♂
Body mass index (kg/m²)	22.4 ± 5.4	23.3 ± 5.1
Mutation		
FAP	13	20
aFAP	1	4
MUYTH	0	5

P88 - OLIGOPOLYPOSIS

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Background and aim

Colonic adenomatous polyposis phenotype of 10-100 adenomas known as oligopolyposis. We aimed to characterize the clinical, genetic and outcomes of patients with oligopolyposis, compared between those with pathogenic mutation and without mutation.

Methods

Retrospective study included patients with cumulative 10-100 adenomas in colonoscopy in a single center. Clinical, genetic and outcomes were collected.

Results

155 patients were identified with oligopolyposis, genetic testing with multi-gene panel was performed among 85 (55%) patients, founder or family mutation was performed among 7 (4.5%) patients and among 63 (40.5%) patients no genetic investigation was performed. Pathogenic polyposis related mutations were found among 14 (16%) of 85 patients who underwent genetic investigation. 7 (50%) mutations were found in APC gene and 7 (50%) were found in MUTYH gene.

No significant differences between carrier and non-carriers in age and gender, 64.3±15.6 vs 67.1±10.3, p=0.401, females 57.1% vs 32.4%, p=0.079. Significant higher rate of Arab ethnicity found among carriers (35.7% vs 4.2%, p< 0.001). No significant difference was found regarding the family history of polyps, 14.3% vs 11.4%, p=0.763. Colorectal cancer was found to be the first presentation among 2 (14.3%) of the carriers and among 5 (7%) of non-carriers. Colonic surgeries were reported among 4 (28.6%) of the carriers compared to 13 (18.6%) of the non-carriers. No significant rates of colorectal cancer or death were found among carriers compared to non-carriers.

Conclusions

Only small part of patients with oligopolyposis patients diagnosed with polyposis related mutation, with significant ethnic difference in mutation frequency rate but no significant differences in the clinical features, colorectal cancer rate or death.

P89 - HEALTHCARE UTILIZATION AMONG INDIVIDUALS DIAGNOSED WITH LYNCH SYNDROME THROUGH A UNIVERSAL GERMLINE GENETIC TESTING PROGRAM

Heather Hampel, Ilana Solomon, Michael Garcia, Marilan Luong, Joseph D. Bonner, Lawrence A. Shaktah, Anuja Chitre, Lauren Gima, Christine Homg, Gregory Idos, Sidney Lindsey, Kevin McDonnell, Wai Park, Katherine Roth, Elise Sobotka, Yi Xiao, Stanley Hamilton, Stephen B. Gruber, Stacy W. Gray

City of Hope National Medical Center, Duarte, CA, USA

Background and aim

Most individuals with Lynch syndrome (LS) are unaware of their risk. Criteria-based testing will not close this gap as it fails to identify ~50% of mutation carriers. To identify all patients with inherited cancer risk, the City of Hope's INSPIRE study offers germline sequencing at no cost for 155 cancer gene panel and the ACMG 59 actionable gene panel. The aim of this study was to evaluate healthcare utilization before and after a diagnosis of Lynch syndrome through a universal testing protocol.

Methods

Patients enrolled in the INSPIRE study from 7/9/2020 – 6/23/2023 were found to have a pathogenic/likely pathogenic variant (P/LPV) in a LS gene were included. To assess healthcare utilization, we utilized an informatics approach followed by medical record abstraction (MRA). Enterprise data warehouse queried for codified data elements prior to and post genetic testing (*post testing interval: 1-33 months*). MRA was conducted after query to assess the strengths and limitations of the informatics approach. Healthcare utilization (i.e., procedures, imaging) were binned as guideline vs not guideline related based on concordance with NCCN. Stratified analysis was applied to evaluate sex-specific clinical management guidelines.

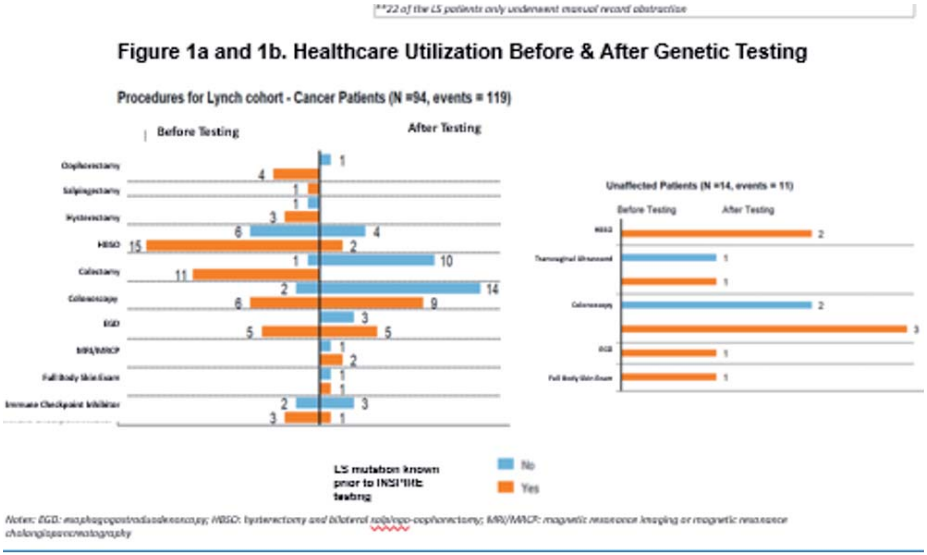
Results

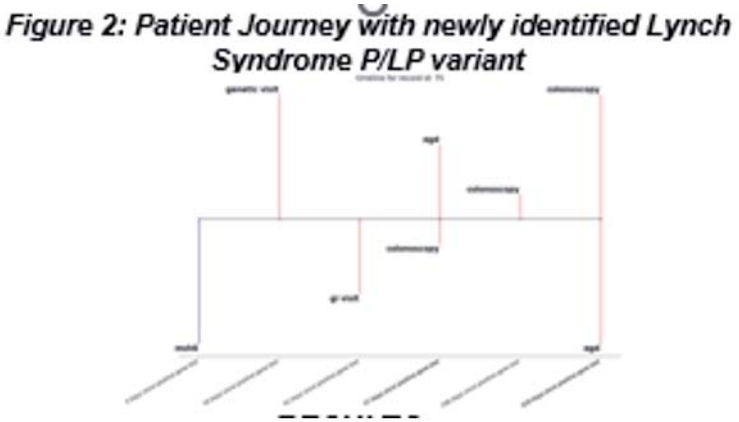
Of 16,883 patients enrolled in the study, 13,946 (82.6%) underwent germline GT. 108 (0.77%) had a P/LPV in a LS gene. The demographic distribution of LS patients was white (n=73, 67%), Asian (n=17, 16%), other (n=18, 17%) with 27% (n=29) having Hispanic ethnicity. Most were female (n=73, 67%)and the mean age was 55 years. Eighty-seven percent (n=94) had cancer, including 61% (n=57) with LS-associated cancers; colorectal 17%, esophageal/stomach 5%, endometrial 7%, bladder/urinary tract 5%, ovarian 6%, and prostate 5%.

Sixty percent of all LS patients had procedures, imaging, and/or therapy potentially related to their LS diagnosis. As expected, among patients with a new diagnosis of LS, utilization of colonoscopy, EGD, MRCP/EUS, and transvaginal ultrasound increased after testing. Seventeen (18%) cancer patients with Lynch syndrome received Immune Checkpoint Inhibitor therapies.

Conclusions

We found high levels of relevant healthcare utilization for LS gene mutation carriers in the context of universal GT. An automated informatics approach to assess healthcare utilization needed to be augmented with MRA. We found high levels of relevant healthcare utilization for LS patients in the context of universal GT.





POSTERS

P90 - PREVALENCE AND PENETRANCE OF SPINK1 PATHOGENIC VARIANTS: A BURDEN TO PATIENTS AND PROVIDERS

Kathryn Reyes, Jasmine Alcantara, Anuja Chitre, Christina Fujii, Lauren Gima, Christin Rybak, Duveen Sturgeon, Joseph Bonner, Joanne Jeter, Wai Park, Kevin McDonnell, Stephen Gruber, Stacy Gray, Gregory Idos, Heather Hampel

City of Hope National Medical Center

SPINK1 is associated with hereditary chronic pancreatitis. N34S (c.101A>G; p.Asn34Ser), a common *SPINK1* variant, is reported to be present in 1-2% of the general population. Most studies exploring *SPINK1* were conducted in populations affected by pancreatitis and pancreatic cancer. There is a paucity of data regarding the penetrance of pancreatitis among individuals with heterozygous SPINK1 variants in an unselected population.

Results of patients undergoing germline multigene panel testing (MGPT) through the INSPIRE study at City of Hope from 7/1/2020 – 8/30/2023 were reviewed. Medical record abstraction was performed for patients with variants identified in *SPINK1* to assess for personal or family history of pancreatitis or pancreatic cancer and other risk factors.

17,549 individuals have undergone MGPT. 240 unique patients had heterozygous variants identified in *SPINK1* (1.4%). The majority (235; 97.9%) had the N34S increased risk allele. Nine patients had a personal history of pancreatitis (9/240; 3.8%), of which all had the N34S variant. Two of the 9 patients had necrotizing pancreatitis, one of which had a 54-pack year smoking history and the other who had gallstones likely contributing to their pancreatitis. Another 2 patients had chronic pancreatitis, one of which had a germline likely pathogenic variant in another pancreatitis-associated gene. Among the 5 patients with isolated episodes of acute pancreatitis; 3 were associated with a concomitant pancreatic cancer diagnosis (1 PDAC, 1 PNET, and 1 solid pseudopapillary carcinoma), 1 was associated with cholelithiasis, and 1 was associated with widely disseminated metastases. Thus overall, only 1 patient with a *SPINK1* variant within our cohort had pancreatitis without an identifiable precipitating event.

SPINK1 N34S variants are common in the general population and were infrequently (<5%) associated with pancreatitis or pancreatic cancer in our study cohort. Some propose that the *SPINK1* N34S variant should be viewed as a disease modifier, rather than conferring an increased risk for pancreatitis on its own, especially when additional risk factors for pancreatic inflammation, such as environmental or lifestyle factors (like alcohol or tobacco consumption), are present. However, the necessity of evaluating or monitoring for pancreatitis and pancreatic cancer based on the presence of the SPINK*1* N34S remains a subject of ongoing discussion.

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P91 - COMPREHENSIVE GUIDELINES FOR THE DIAGNOSIS, COUNSELLING, SURVEILLANCE AND CLINICAL MANAGEMENT OF PEOPLE WITH CONSTITUTIONAL MISMATCH REPAIR DEFICIENCY: A JOIN EFFORT FROM ERN GENTURIS AND THE EUROPEAN CONSORTIUM CARE FOR CMMRD

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[8] *Ern Genturis*

Constitutional mismatch repair deficiency (CMMRD) firstly described 25 years ago confers an extraordinarily high and lifelong cancer risk, including hematologic, brain, and gastrointestinal tract malignancies, and is associated with several non-neoplastic features. Our understanding of this condition has improved and novel assays to assist CMMRD diagnosis have been developed. Surveillance protocols need adjustment taking into account recent observational prospective studies assessing their effectiveness. Response to immune checkpoint inhibitors and the effectiveness and toxicity of other treatments have been described. An update and collation of the different guidelines on diagnosis and clinical management of CMMRD into one comprehensive guideline was needed.

Seventy-two expert members of the European Reference Network GENTURIS and/or the European care for CMMRD consortium and one patient representative developed recommendations for CMMRD diagnosis, genetic counselling, surveillance, quality of life, and clinical management based on a thorough literature review and a modified Delphi process.

Recommendations for the diagnosis of CMMRD provide indication criteria and strategies for testing, and define criteria for diagnosis. Recommendations for surveillance cover each CMMRD-associated tumour type and contain information on starting age, frequency, and surveillance modality . Recommendations for clinical management cover cancer treatment, management of benign tumours or non-neoplastic features, and chemoprevention. Recommendations also address genetic counselling and quality of life.

Based on existing guidelines and all currently available data, we present 82 recommendations to improve and standardise the care of CMMRD patients in Europe. These recommendations are not meant to be prescriptive and may be adjusted based on individual decisions.

P92 - STUDY OF THE MESSENGER RNA/CIRCULAR RNA COUPLE: NEW BIOMARKER OR PREDISPOSING FACTOR FOR COLORECTAL CANCER?

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Background and aim

In oncogenetics, despite the widespread use of gene panels and even genome analyses, roughly 30% of suspected predispositions to colorectal cancer (CRC) are explained. New hypotheses are needed to decipher this missing heritability. Given the prominent role of messenger RNAs (mRNA) in genetic diseases and the competition between mRNAs and circular RNAs (circRNA) in terms of production from their host genes, the following hypothesis was made: mRNAs and circRNAs are co-regulated and a physiological mRNAs-circRNAs equilibrium is mandatory.

Methods

We embarked upon the study of the couple mRNA/circRNA in the French multicenter ODCC collection (Oligogenic Determinism of Colorectal Cancer), comprising DNA and RNA samples extracted from whole blood for 716 CRC patients and 249 matched controls [1]. Patients were selected based on early onset CRC and/or the presence of multiple primary tumors and/or an affected 1st-degree relative, but without any constitutional mismatch repair genes pathogenic variant.

Simultaneous characterization and quantification of mRNAs and circRNAs for a panel of 23 genes involved in CRC predisposition was carried out using the novel SEALigHTS technique (Splice and Expression Analyses by exon Ligation and High Throughput Sequencing) [2] allowing the exploration of all exon-exon junctions, thanks to probes designed at exon extremities. Briefly, after reverse transcription and probe hybridization on complementary DNA, neighboring probes are ligated and the number of ligations quantified using unique molecular identifiers and sequencing.

Results

In addition to the detection of mRNAs canonical and alternative junctions, we described the landscape of circRNAs for these 23 genes i.e. 258 circular junctions including 64 novel ones not yet listed in the circRNA databases. The circRNA/mRNA ratios were then calculated for all genes. A disequilibrium was evidenced as patients exhibited a 1.5-fold higher circRNA/mRNA ratio as compared to controls ($p<2x10-16$), irrespective of RNA quality, age of cancer onset or gender. This increase in the ratio was particularly true for the POLD1 gene, which presented the highest level of circular RNAs (1.5-fold higher, $p=5.2x10-8$).

Conclusion

Overall, this large RNA collection allowed us to evidence a circRNA/mRNA disequilibrium for CRC patients. It remains to be determined if this is a cause or a consequence of a yet hidden anomaly but in any case, our findings open new avenues in CRC genetics.

1. Baert-Desurmont et al. Clinical relevance of 8q23, 15q13 and 18q21 SNP genotyping to evaluate colorectal cancer risk. European Journal of Human Genetics 2016; 24: 99–105.
2. Levacher et al. Disequilibrium between BRCA1 and BRCA2 Circular and Messenger RNAs Plays a Role in Breast Cancer. Cancers (Basel) 2023;15:2176. PMID: 37046838

Keywords

Oncogenetics, hereditary colorectal cancer, mRNA, circular RNA, SEALiGHTS.

P93 - DO EPCAM FULL DELETIONS HAVE A ROLE IN LYNCH SYNDROME?

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Background/Objectives

Pathogenic variants of the *EPCAM* gene are associated with different clinical conditions. Loss of function variants usually cause autosomal recessive congenital tufting enteropathy, while partial deletions of the 3' exons are involved in 1%-3% of Lynch Syndrome (LS) cases. The latter association is due to a secondary effect of the *EPCAM* deletion on the function of the downstream LS gene MSH2, which is silenced by hypermethylation.

Methods

We studied 3 unrelated subjects with a complete *EPCAM* deletion using highresolution oligonucleotide Array-CGH (Agilent 2x400k), SALSA MLPA® P003 MLH1/MSH2 D1- 0718 and SALSA MLPA® P072- MSH6/MUTYH D1-0120.

Results

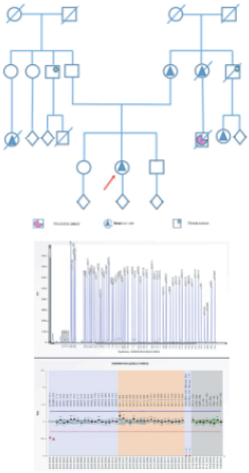
The EPCAM deletion was ascertained incidentally in the 3 subjects, who were 74, 49, and 38 years old, respectively. The first proband underwent NGS analysis (SOPHiA® HCS panel) because of breast cancer. The second subject discovered the deletion incidentally following prenatal array-CGH. The third also performed array-CGH following the finding of a deletion in his son with intellectual disability. MLPA showed that in all cases the deletion included the upstream region of *MSH2* but didn't contain the core promoter region of the *MSH2* gene. All patients underwent colonoscopy, with negative results, and had negative cancer family history. One patient had a history of chronic diarrhea and malabsorption.

Conclusion

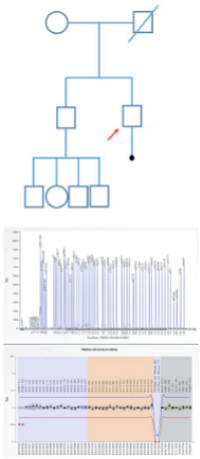
These findings support the notion that monoallelic deletions of the entire *EPCAM* gene are unlikely to cause LS. Further confirmation can be provided by follow-up of these patients and molecular studies of bowel tissue, in *MSH2* methylation analysis.

Keywords
Lynch Syndrome, *EPCAM*, oncogenetics.

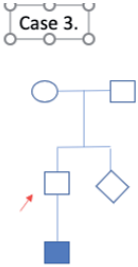
Case 1.



Case 2.



Case 3.



P94 - THE BRAZILIAN GENOME MAP ONCOLOGY SUBPROJECT (BGMONCOLOGY): HISTORY, GOALS, AND PROGRESS TO DATE

Fabio Oliveira Ferreira, Edenir Inez Palmeiro, Luciana Rodrigues Carvalho Barros, Pablo Domingos de Nicola, Vanessa Figueiredo Monteleone, Natalia Campacci, Erika Maria Monteiro dos Santos, José Pereira de Souza, Paulo Thiago de Souza Santos, Gisele Medeiros, Bruna Palma da Matta, Cristina da Silva Sabato, Natalia Costa Braz, Tamara Soares Guerra, Carla Vanessa Quirino, Gabriele Perroni dos Santos, Andreuza Juliana Lopes, Bruna Lorena Alves, Delciany Mylla de Aguiar, Giselly Lopes Chagas, Janaina Silva da Costa, Jéssika Fontes Lima, Maira Jaqueline de Souza, Samara Régia Oliveira, José Fernando Bastos de Moura, Edilmar de Moura Santos, Augusto Modesto de Souza Neto, Luissa Hikari Hayashi Araujo, Paulo Carrara de Castro, Hilka Flávia Barra do Espírito Santo Alves Pereira, Cristina de Deus Anjos Tavares Sampaio, Juliana Ramos Chaves, Victor Yuri de Oliveira Mattoschoell, Alecsander Alves da Silva, Talita Reimão Badesso Fonseca, Silvana Soares dos Santos, Luciana Sacilotto, Luanda Guimaraes da Silva, Andre Luiz Serra, Giovanna Tiemi Goto, Fernanda Cascapera, Cristiane da Fonseca Amancio, Lucas Campacci, Fernando Alves Alencar de Moura, Dante Gambardella, Rozana Ciconelli, Mariana Beraldo da Costa Saad, Gustavo Cardoso Guimaraes, Benedito Mauro Rossi.

Financial support

Ministry of Health – Federal Government - Brazil

PROADI

Brazilian Genome Map Coordinator Center – A Beneficência Portuguesa de São Paulo – Brazil

Background

The Brazilian Genome Map Oncology Subproject (BGMOncology) represents a pioneering initiative investigating the intersection of ethnic diversity and cancer distribution in Brazil. The nation's extensive ethnic diversity leads to significant variations in cancer incidence and distribution, influenced by genetic, environmental, and social factors. Cancer stands as a primary cause of morbidity and mortality in Brazil, disproportionately affecting vulnerable populations due to socioeconomic disparities. In response, the BGMOncology project was launched to analyze the genetic diversity within the Brazilian population, focusing specifically on breast, prostate, and colorectal cancers.

Method:

The project employed meticulous prospective data collection methods encompassing clinical, epidemiological, familial, pathological, and molecular data from patients and their relatives. Nine cancer treatment centers representing different regions of Brazil were included, with

Hospital BP - A Beneficência Portuguesa de São Paulo serving as the coordinating center. The sole inclusion criterion was the confirmed diagnosis of breast, prostate, or colorectal cancer in patients above 18 years of age without prior treatment for the current tumor. Germline and somatic whole-genome sequencing (WGS) libraries were sequenced using the Illumina© platform. Primary sequencing read files were generated on the Illumina© Dragen platform. Variant classification was conducted using the Franklin program by Genoox, following the major international guidelines Additionally, the project emphasized education and awareness through the Brazilian Genome Map Professional Qualification Subproject (BGMPQ), conducting training sessions for healthcare professionals to enhance genetic counseling practices and knowledge.

Results

By August 2023, the project had enrolled 275 patients and 515 relatives. Of these, 171 patients underwent germline WGS, including 88 cases of breast cancer (51.5%), 37 cases of prostate cancer (21.6%), and 46 cases of colorectal cancer (26.9%). Nineteen pathogenic variants (PV) or likely pathogenic variants (LPV) were identified in 18 patients (10.5%), with 16 in genes associated with increased cancer risk and 3 in genes related to secondary findings. Additionally, 26 relatives of 13 probands with positive findings were assessed, with 11 (42.3%) carrying PV or LPV, although none were diagnosed with cancer. Among the 171 patients who underwent germline WGS, 141 received definitive reports and genetic counseling. Notably, BGMPQ's educational efforts impacted 474 participants from 23 federal states, positively influencing genetic counseling practices and enhancing understanding of genetics and genomics topics.

Conclusion

BGMOncology signifies a significant advancement in comprehending the genetic factors influencing cancer susceptibility in Brazil. Ongoing analyses correlating demographic, epidemiological, clinical, pathological, and familial data with molecular findings are underway and are poised to make significant contributions to the understanding of breast, prostate, and colorectal cancers in the country. The project's holistic approach, amalgamating advanced genomic analysis with educational initiatives, significantly contributes to cancer research and healthcare practices in Brazil.

Keywords:

Ethnic diversity, Genetic factors, Environmental factors, Social factors, Cancer distribution, Socioeconomic vulnerability, Precision health, Whole Genome Sequencing (WGS).

Figure 1. PROADI-SUS – Brazilian Genome Map Subproject Oncology (BGMOncology): Comprehensive flowchart illustrating the steps of molecular analyses, bioinformatics processing, seamless integration flow, and systematic data storage.

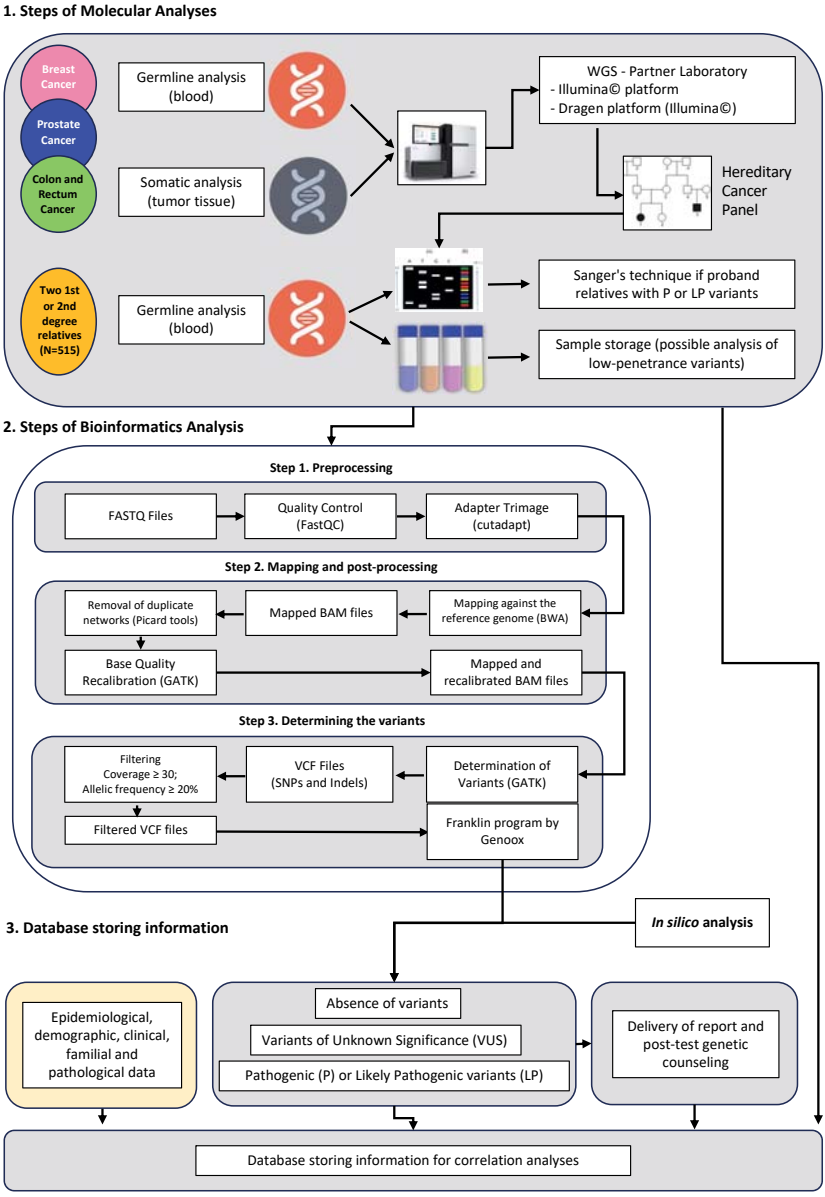
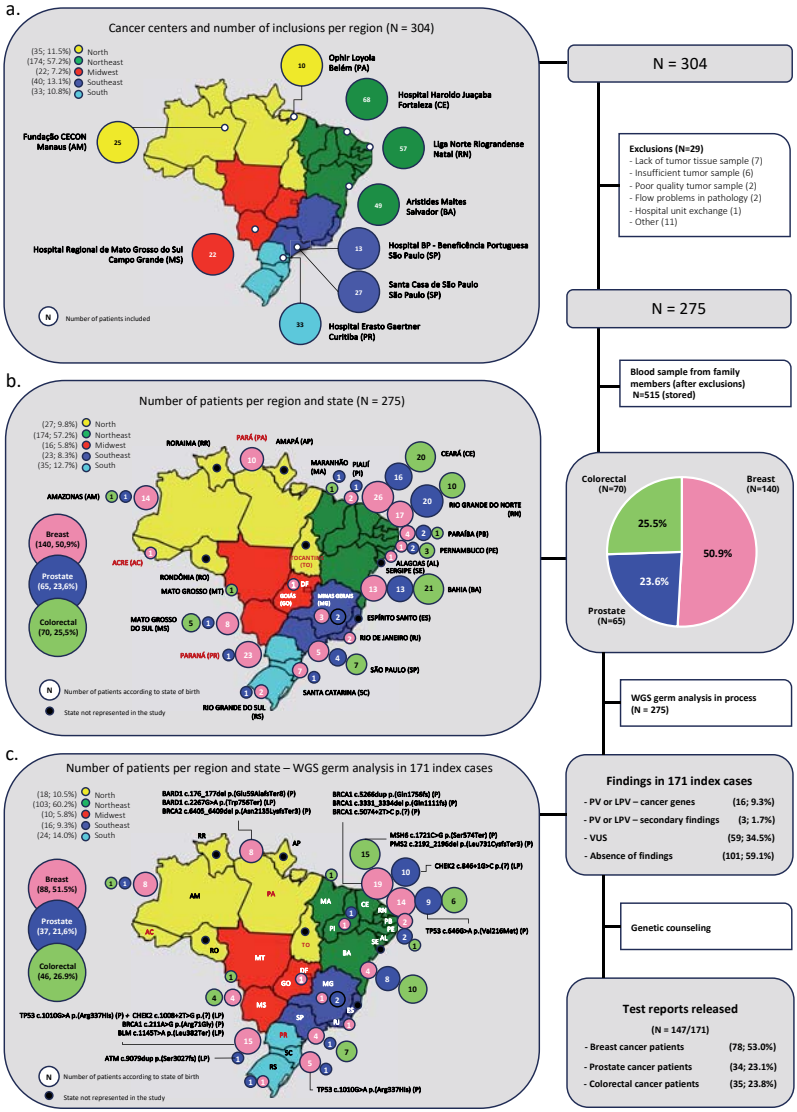


Figure 2. PROADI – SUS – Brazilian Genome Map Subproject Oncology (BGMOncology): a. Cancer centers and respective number of inclusions; b. Number of patients included according to the type of tumor (breast, prostate or colon and rectum) by Federative State of birth; c. Pathogenic or Likely Pathogenic germinative variants found according to the type of tumor (breast, prostate or colon and rectum) and Federative State of birth – partial analysis in 171 patients and test reports released.



P95 - THE CLINGEN-INSIGHT MUTYH VARIANT CURATION EXPERT PANEL: LESSONS LEARNED AND A CALL TO ACTION

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Background and aim

The ClinGen-InSiGHT Hereditary Colon Cancer/Polyposis Variant Curation Expert Panel (VCEP) was formed in 2021 to create variant curation specifications for the genes definitively associated with these conditions. The *MUTYH* subcommittee is nearing the release of gene-specific recommendations for ACMG/AMP variant classification. *MUTYH*-associated polyposis (MAP) is an autosomal recessive disorder caused by constitutional biallelic pathogenic variants in the base excision repair gene *MUTYH*. *MUTYH* encodes a glycosylase that identifies and excises adenines mispaired with the oxidation product 8-oxo-deoxyguanosine, which, if left incorporated, lead to somatic G>T transversions. We aimed to develop ACMG criteria for *MUTYH* based on a literature review of functional, clinical, and computational fields.

Method

Subcommittees with clinical, computational, and functional expertise had monthly calls and performed extensive literature reviews. These were supplemented by calls with the full VCEP to define the specifications of all ACMG criteria for *MUTYH*.

Results

We first identified nomenclature discrepancies. Confusion exists in the use of the Matched Annotation from NCBI and EMBL-EBI (MANE) versus other *MUTYH* transcripts. The *MUTYH* MANE Select transcript, NM_001048174, encodes a 521 AA protein. However, transcript NM_001128425 encodes a 549 AA protein and is the most used by clinical labs. We successfully had transcript NM_001128425 recognized as MANE Plus Clinical, harmonizing historical data with clinical reports. We established specific phenotypic descriptions of affected individuals. These are needed since MAP has a diverse phenotype, with a variable large bowel adenoma burden, age of onset, extracolonic features, and association with colorectal cancer. We assigned the strongest level of evidence to somatic genomic analysis for G>T transversions which provides strong support for a MAP diagnosis. "Well-established" functional assays to apply criteria PS3 and BS3 are lacking due to suboptimal use of controls and inattention to statistical principles to establish the strength of evidence.

Conclusions

Improved communication among academics, clinicians, and clinical labs is needed to address gaps in understanding *MUTYH* transcript expression, functional studies, and clinical features. We have identified gaps in these fields in the hopes of galvanizing these communities to generate the needed data for this routinely assessed cancer susceptibility gene.

P96 - OPTIMAL 25-HYDROXYVITAMIN D STATUS IS ASSOCIATED WITH A LOWER RISK OF COLORECTAL NEOPLASMS IN INDIVIDUALS WITH LYNCH SYNDROME

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Wageningen University & Research

Background and aim

Individuals with Lynch syndrome are at high risk of developing colorectal neoplasms, defined as colorectal adenoma and colorectal cancer. Disparities in neoplasm risk among individuals with the same mutation suggest the influence of lifestyle factors. Previous research in the general population found that higher concentrations of 25-hydroxyvitamin D are associated with a decreased risk of colorectal neoplasms. However, the association between 25-hydroxyvitamin D concentration and colorectal neoplasm development in individuals with Lynch syndrome remains unknown. Therefore, this study aimed to investigate this association.

Methods

The study population included 1556 individuals with Lynch syndrome from two prospective cohort studies: GEOLynch (the Netherlands) and the Colon Cancer Family Registry (USA, Canada, and Australia). A multivariable Cox proportional hazards regression model with age as the time axis was used to estimate the hazard ratios (HRs) and 95% confidence intervals (CIs) for the association between 25-hydroxyvitamin D concentration and colorectal neoplasm risk. The fully adjusted model included age, sex, education level, ethnicity, study centre, season of blood collection, smoking status, body mass index, physical activity, and number of colonoscopies.

Preliminary results

During a median follow-up of 8.0 years (interquartile range: 4.0-13.0), 356 individuals with Lynch syndrome developed a colorectal neoplasm. After adjusting for confounding variables, optimal concentrations (≥75 nmol/L) were associated with a decreased risk of colorectal neoplasms (HR: 0.77; 95% CI: 0.60-0.99) when compared to sufficient concentrations (50–74 nmol/L). While severely deficient concentrations showed an HR of 1.06 (95% CI: 0.64-1.75) and those with deficient concentrations an HR of 1.09 (95% CI: 0.83-1.43), both in comparison with sufficient concentrations.

Conclusions

This study suggests that maintaining optimal 25-hydroxyvitamin D concentrations may reduce the risk of colorectal neoplasms in individuals with Lynch syndrome.

Keywords

Lynch syndrome, 25-hydroxyvitamin D, colorectal neoplasm, colorectal adenoma, colorectal cancer, mismatch repair.

P97 - IMPORTANCE OF ACCURATE EPCAM DELETION CHARACTERIZATION TO PREVENT MISDIAGNOSIS OF LYNCH SYNDROME

Carolyn Horton[1], Felicia Hernandez[1], Ashley Marsh[1], Rebecca Mar-Heyming[1], Jessica Grzybowski[1], Marcy Richardson[1], Daniel Kosteck[1], Christopher Lau[1], Elizabeth Chao[1,2], Steven Harrison[1,3], Heather Hampel[4]

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[2] *University of California, Irvine, School of Medicine*

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[4] *City of Hope National Medical Center*

Background and aim

Gross deletions involving the 3' end of *EPCAM* cause Lynch syndrome (LS). Prior to the introduction of NGS, *EPCAM* deletion screening was typically performed by one MLPA kit throughout the country, in which the 5'-most probe resides in exon 3. Therefore, it can be unclear whether some deletions encompass the full gene. However, full *EPCAM* deletions may not be disease-causing so accurate differentiation of deletion size has significant clinical implications.

Methods

We reviewed cases with a gross deletion of *EPCAM* identified at a single laboratory from 2011-2021 to determine how many had a known or possible full *EPCAM* deletion detected via MLPA or microarray. Amsterdam criteria II (AC) and revised Bethesda criteria (BC) were assessed in families with full and partial deletions. Data presented herein are exempt from IRB review.

Results

A total of 503 cases were identified that included an *EPCAM* deletion, 373 of which also included *MSH2*. Isolated *EPCAM* deletions were identified in 129 individuals from 91 unique families. In most families (79.1%; 72/91), MLPA indicated a definitive partial *EPCAM* deletion based on retention of the exon 3 probe. In 9 additional families (9.9% of 91), 5'UTR coverage from a microarray was available and identified a full *EPCAM* deletion. Deletion size could not be determined in 10 remaining families (11.0%). Therefore, 20.9% of families with *EPCAM* deletions identified at our laboratory may not have LS due to a known or possible full gene deletion. When clinical history was evaluated, no families with a known full gene deletion met AC and 1 met BC while 44.7% (n=21) and 89.3% (n=42) of those with a known partial deletion met AC or BC, respectively.

Conclusions

This study identifies a need for re-evaluation of a subset of individuals with *EPCAM* deletions reported through clinical testing. There was a stark difference in phenotype between those

with a known full deletion compared to a known partial deletion, in which no individuals with full deletions met AC or BC. This supports findings that full *EPCAM* deletions are not pathogenic. Accurate characterization of *EPCAM* deletions is critical to prevent misdiagnosis of LS.

P98 - A JOINT EFFORT FOR SPECIFYING SMAD4 SPECIFICATIONS TO THE ACMG/AMP VARIANT CLASSIFICATION GUIDELINES

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Background and aim

Juvenile polyposis syndrome (JPS) and Hereditary Haemorrhagic telangiectasia (HHT) represent different phenotypes that can both manifest in patients with *SMAD4* pathogenic variants. Due to the disparate clinical specialties involved in their management, two distinct Variant Curation Expert Panels (SMAD4/JPS InSiGHT VCEP and SMAD4/HHT VCEP) emerged to tackle their genetic underpinnings, which coincide in the *SMAD4* gene, though only <2% of HHT cases are due to defective *SMAD4*. Clinical features of HHT are easy to miss in patients with gastrointestinal (GI) syndromes and therefore the prevalence of *SMAD4* carriers is often underestimated. Most publications are from GI clinics and not HHT clinics. A unified approach for defining ACMG/AMP criteria specifications for *SMAD4* is required to better define its clinical variability. Here we report on the initial work of the InSiGHT ClinGen SMAD4/JPS VCEP which is currently focussing on SMAD4.

Method

With the support and input of the Hereditary Haemorrhagic Telangiectasia VCEP, we have commenced a joint effort involving clinical experts in both HHT and JPS, including genetic counsellors, functional assay researchers, molecular geneticists, and bioinformaticians. To our knowledge, this is the first time that two VCEPs have combined their expertise in different clinical fields to harness the same gene.

Results

The InSiGHT VCEP, together with members from the existing HHT VCEP has decided to specify the ACMG/AMP criteria to classify *SMAD4* variants. Initial progress has seen agreement on defining JPS and HHT as a combination syndrome and the decision to collaborate on *SMAD4* variant interpretation recommendations. The VCEP structure is divided into groups for clinical and functional/*in silico* assay expertise.

Conclusion

VCEPs combining distinct clinical fields can be structured to pursue the specification of ACMG/AMP criteria for improved outcomes. This VCEP structure will be proposed to ClinGen for approval. In the future, we aim to move this work forward with other hamartomatous polyposis genes including *BMPR1A* and *STK11*. Interested researchers are welcome to contact the curator johnpaul @variome.org.

Keywords

Variant classification.

P99 - DEVELOPMENT OF AN AI ALGORITHM FOR THE DETECTION OF MSI STATUS IN NEW ZEALAND COLORECTAL CANCER PATIENTS

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Microsatellite instability (MSI) involving deficient DNA mismatch repair (dMMR) is the hallmark of Lynch Syndrome colorectal cancer (CRC). MSI occurs in 10-20% of CRC due to either Lynch Syndrome or sporadic tumours with hypermethylation. In addition to identifying Lynch Syndrome, identifying MSI/dMMR status is increasingly important in the era of personalized medicine, as MSI/dMMR status is predictive of prognosis and response to chemo- and immunotherapy. As a result, universal screening of CRC for MSI/dMMR status is now recommended. Currently tumour screening occurs in most centres using manual immunohistochemistry.

Recent developments in the field of artificial intelligence(AI), have shown the ability to recognize tumour molecular subtypes from analysing tissue histomorphology patterns with real potential to improve the accuracy and efficiency of diagnosis.

Aim

This research aims to utilise digital pathology and computer vision to develop an AI deep learning algorithm for the detection of MSI/dMMR status from whole-slide images of histological stained CRC cancer cases.

Method

Whole-slide FFPE images of colorectal cancer cases (n=461) with MSI status information were downloaded from The Cancer Genome Atlas (TCGA). These cases were randomly split, 70:30 ratio, into a training and test dataset. An AI algorithm using a deep learning convolutional neural network (Resnet18) was developed using transfer learning methods to predict MSI status. To test the AI algorithm performance and translatability to a New Zealand population,

an independent dataset of digitally scanned whole-slide FFPE slides from New Zealand CRC patients (n=500) is under recruitment.

Results

Preliminary results from training an AI algorithm Resnet18 model upon the TCGA dataset returned AUROC performance levels ranging between 0.68 – 0.71. Comparable AUROC performance levels of 0.59 for the AI algorithm were observed when tested on the initial 250 cases from the New Zealand cohort. Further training and optimisation of the AI algorithm is continuing to improve performance levels.

Conclusion

This study showed the potential of using digital pathology and AI to detect MSI status from histological stained FFPE CRC patient whole-slide images. To our knowledge, this is the first study to utilise AI to detect MSI status on a New Zealand cohort of CRC patients. Larger whole-slide image datasets with MSI status information will allow further refinement and increase accuracy of the algorithm.

P100 - SERUM RAMAN SPECTROSCOPY IN THE COLORECTAL SURVEILLANCE OF LYNCH SYNDROME

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Background and aim

Lynch syndrome (LS) is the most common colorectal cancer (CRC) predisposition syndrome. LS mutation carriers are subject to regular colonoscopy surveillance, which is not without risk. Raman spectroscopy is a vibrational spectroscopic technique which provides a biochemical fingerprint of a given sample. The aim of this study is to determine if differences exist in the serum of individuals with LS and if Raman spectroscopy can be used to detect these changes.

Method

Individuals with a known diagnosis of LS presenting for surveillance colonoscopy were recruited. A single serum sample was obtained from each patient and analysed with the Raman spectrometer using the 785nm laser line. Spectral data was then pre-processed and analysed and correlated to the diagnosis at the time of colonoscopy. Significant peak differences between sporadic and Lynch cancers were matched and corresponding metabolites identified.

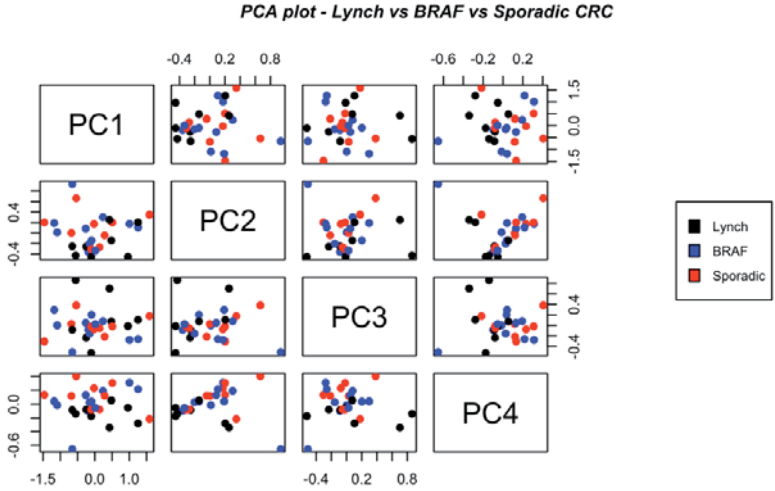
Results

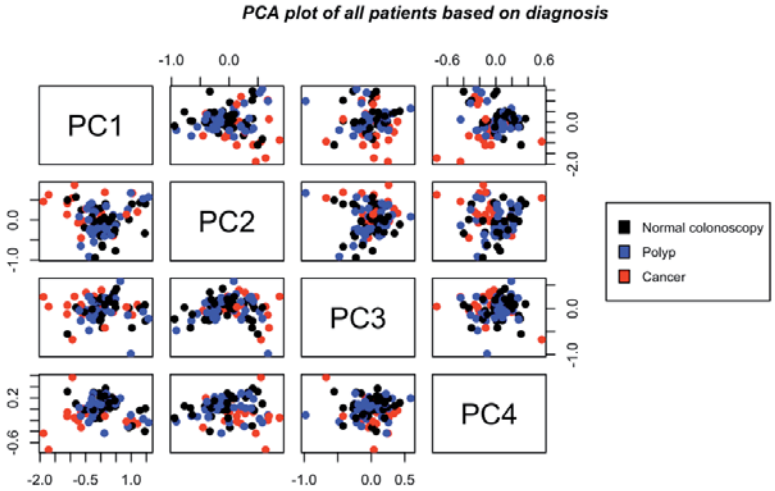
A total of 80 participants with LS were recruited. 35 participants had a normal colonoscopy and polyps were detected in 36 participants. 9 participants were diagnosed with CRC at the time of colonoscopy. All were right sided tumours. There were no obvious differences seen on principal components analysis of the spectral data based on constitutional mutation type (i.e. gene). Some separation of CRC spectra was seen in PC1 compared to polyps and carriers with a normal colonoscopy. The samples from patients with CRC were propensity matched for site and stage with available serum from participants with sporadic cancers without *BRAF* mutations (n=11) and from those with *BRAF* mutations (n=11). There were significant spectral differences in individuals with LS CRC compared to individuals with sporadic CRC without *BRAF* (p= 0.00017) or those with *BRAF* mutations (p=0.001). There were no significant differences between the spectra of participants with sporadic CRC and *BRAF*-related CRC (p=0.360). Significant differences between the Lynch CRC and sporadic

non-*BRAF* mutated sporadic CRC were seen in regions corresponding to proline, GABA, sarcosine, phenylalanine, alanine, tryptophan and fructose.

Conclusion

There are differences in the circulating metabolome of individuals with Lynch-related CRC compared to sporadic CRC (with or without *BRAF* mutation). Serum Raman spectroscopy has the potential to be used in the context of LS surveillance. Further work is required to validate its use in a clinical setting.





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P101 - EVALUATION OF THE UTILITY OF UPPER ENDOSCOPY FOR SURVEILLANCE OF UPPER GASTROINTESTINAL LESIONS IN PATIENTS WITH LYNCH SYNDROME

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Background and aims

Lynch Syndrome (LS) predisposes individuals to the development of various tumors, including gastric and small intestine cancers. Currently, there is no consensus on surveillance for upper gastrointestinal (UGI) precursor lesions and cancer. Our aim is to describe the UGI endoscopic findings in patients with LS.

Methods

We performed a retrospective, descriptive, and observational study at the Puerta de Hierro University Hospital. We included patients diagnosed with LS undergoing follow-up in our center who had undergone at least one esophagogastroduodenoscopy (EGD). Patients with a prior diagnosis of UGI adenocarcinoma (2 cases) were excluded.

Results

A total of 124 patients were analyzed, of which 85 (68.54%) had undergone at least one EGD. H. pylori infection was identified in 26 of them (34.7%). 45 (52.94%) presented pathological findings **(Table 1),** with chronic gastritis being the most common (17.65%). Preneoplastic or neoplastic lesions were found in 19 patients (22.35%), including one diagnosed with early-stage duodenal adenocarcinoma and another with duodenal adenoma with high-grade dysplasia. The most frequent preneoplastic finding was chronic gastritis with intestinal metaplasia (12.94%). No statistically significant differences were found between the mutated gene and the prevalence of endoscopic lesions, as well as between a family history of UGI cancer and the occurrence of preneoplastic or neoplastic lesions.

Conclusion

Over half of LS patients who underwent EGD presented some pathological lesion, with approximately a quarter having preneoplastic or neoplastic lesions. These results support the utility of performing surveillance with upper endoscopy for all LS patients to detect pathological lesions in the early stage.

Table 1. Clinical and pathologic findings on EGD.

FINDING	n	%
Normal	40	47.06
Peptic esophagitis	3	3.53
Hyperplastic polyp	4	4.71
Fundic gland polyps	3	3.53
Chronic gastritis	15	17.65
Atrophic gastritis	1	1.18
PRENEOPLASTIC LESIONS		
Gastric Intestinal Metaplasia	11	12.94
Gastric Low-grade dysplasia	1	1.18
Esophageal papilloma	2	2.35
NEOPLASTIC LESIONS		
Gastric Low-grade dysplasia with visible lesion	1	1.18
Duodenal Low-grade dysplasia	1	1.18
Duodenal High-grade dysplasia	1	1.18
Duodenal Adenocarcinoma	1	1.18
Neuroendocrine tumor	1	1.18
Total	**85**	**100.00**

P102 - FROG, THE FRENCH ONCOGENETICS DATABASE: A VARIANT CURATION TOOL TO IMPROVE THE DIAGNOSIS OF HEREDITARY TUMORS

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Background and aim

In France the diagnosis of cancer predispositions, including inherited colorectal cancers, is coordinated by a network of clinicians, genetic counsellors and biologists, the Groupe Génétique et Cancer (GGC). This network has defined different gene panels adapted to clinical context. The analysis of these panels confirms the cancer predisposition with the identification of a germline pathogenic variant (PV) in 10 to 20% of cases. We estimate that further 15% are carrying a variant of unknown significance (VUS). For over 20 years, GGC curation groups have been sharing their expertise to improve and harmonize the interpretation of these VUS. Thanks to this background, we decided to collect genetic variants identified in oncogenetic laboratories of the GGC in a national database called FrOG.

Methods

FrOG is governed by a consortium agreement, coordinated by UNICANCER, that brings together 21 healthcare institutions. We developed an IT tool hosted by an accredited health data provider for collecting, curating and sharing patient data via a website (frog-db.fr). A patient information leaflet is given during the oncogenetic sessions to explain the patients' rights and his/her benefits to participate in the FrOG programme. When the NGS analysis is done, new variants and associated phenotypic data are deposited by authorised contributors in pseudo-anonymised form in a secure repository. Curators can then select and review variants or data newly submitted, directly available for the lab members of the consortium.

Results

FrOG-db currently offers interpretation of 13350 variants of 13 genes identified in 47481 patients. Focusing on gastro-intestinal hereditary tumors, the database integrates data for 3400 variants in the MLH1, MSH2, MSH6, PMS2, APC, MUTYH and CDH1 genes. These variants can be classified as 52% pathogenic or likely pathogenic, 28% VUS and 6% unclassified. Our next goal is to collect variant calling files (VCF) from whole gene panels simultaneously with the initiation of new curation groups specific to juvenile polyposis and polymerases genes.

Conclusion

The FrOG database now become an essential tool for the molecular diagnosis of patients performed by oncogenetics laboratories of the GGC network. The website is used several hundred times a day to assess variants. The FrOG programme contributes to improve patient care ensuring rapid, reliable and harmonized genetic analyses.

Feel free to click on the following link to watch a brief video: https://www.youtube.com/watch?v=tZs6y324GSk.

P103 - THE C.386A>C P.(ASN129THR) VARIANT IN SMAD4 IS LIKELY TO BE PATHOGENIC, CAUSING JUVENILE POLYPOSIS SYNDROME

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Background

Juvenile Polyposis Syndrome (JPS) is a rare autosomal dominant hereditary disorder characterized by the development of multiple hamartomatous gastrointestinal polyps. Here, we present a case of JPS with a mosaic variant in SMAD4.

Methods

Exome sequencing TRIO analysis, using germline DNA from the biological mother and father along with the index case (IC).

Results

A 46-year-old male with no family history of cancer presented with chronic iron deficiency anemia and was diagnosed with massive gastric polyposis (≥100 polyps). In addition, for the last 14years, he has undergone surveillance colonoscopies with the removal of multiple polyps throughout the colon (16 adenomatous, 4 hamartomatous, and 19 inflammatory polyps). At the age of 59, the patient presented with impaired anemia, and upper endoscopy showed a nonresectable giant polyp. He underwent a total gastrectomy, revealing numerous polyps occupying the entire gastric mucosa, including a 5 cm gastric hyperplastic polyp with high-grade dysplasia and focal adenocarcinoma. TRIO analysis identified the c.386A>C p.(Asn129Thr) variant in the SMAD4 gene at an allele frequency (AF) of 22%, suggesting its mosaic origin. Subsequently, the variant was found in heterozygosity in the IC's son, who exhibited two subcentimeter polyps in the colon and seven inflammatory gastric polyps with gastric inflammatory areas and hyperplasia, suggesting that the c.386A>C p.(Asn129Thr) variant in SMAD4 segregated with the phenotype. Conclusion: Our study provides evidence supporting the classification of the c.386A>C p.(Asn129Thr) variant in SMAD4 as a likely pathogenic variant. This finding contributes to improved accuracy in the diagnosis and genetic counseling of JPS.

Keywords

Hamartomatous gastrointestinal polyps, JPS, SMAD4.

P104 - MUTATIONAL SPECTRUM OF LYNCH SYNDROME IN THE MID-SOUTH OF ISRAEL

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Background and aims

Lynch syndrome stands as a prevalent hereditary cancer syndrome with elevated risks for various cancers. Mutations in mismatch repair (MMR) genes including MLH1, MSH2, MSH6, and PMS2 underlie this syndrome. Israel's diverse population is composed of approximately 73.9% Jews and 21% Arabs. The Jewish population is comprised of various ethnicities arising from communities worldwide. To date, 13 recurring mutations were identified in the MMR genes in Lynch patients in Israel. Identification of Lynch subjects commences usually in tumor specimens of patients using immunohistochemistry (IHC). For negative staining, a two-step workup starting with a targeted panel of the recurring Israeli pathogenic variants, and if negative- an NGS (Next Generation Sequencing) based multi cancer gene panel is performed. Understanding incidence and spectrum of mutations within specific populations facilitates diagnostics and cascade testing. This study aimed to characterize the mutational spectrum of Lynch carriers in the mid-south of the country, emphasizing potential novel founder alterations.

Methods

Data of carriers diagnosed through genetic institutes in two medical centers within a close geographical area between 2012 and 2023 were meticulously reviewed and analyzed.

Results

A total of 166 carriers representing 76 families were identified. 74 families of Jewish descent, one of an Arab Moslem descent and one of a European non-Jewish background. Among these, 53% carried founder mutations, while 32 families harbored private mutations. Two seemingly unrelated families shared a common PMS2 mutation and descent thus hinting a potential founder alteration. An additional two families with a common MSH2 mutation raised uncertainty regarding a common descent. Founder mutations were prevalent in 60%

of MSH2 families, 48% of MSH6 families, 25% of MLH1 families, and 40% of PMS2 families. Surprisingly, 44% of Ashkenazi Jewish families did not carry founder mutations.

Conclusions

A decade of genetic counseling and testing for Lynch syndrome in Israel's mid-south revealed a diverse mutation spectrum. While founder mutations played a significant role in Lynch pathogenesis, nearly half the families exhibited private mutations. Further analysis is imperative to determine whether founder germline testing should precede IHC in this population. Population level analysis is needed to confirm whether the two common mutations identified in this cohort are founder alterations.

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P105 - INVESTIGATING ENVIRONMENTAL INFLUENCES OF POUCH NEOPLASIA IN FAMILIAL ADENOMATOUS POLYPOSIS USING MULTIOMICS STUDIES

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Aim

In familial adenomatous polyposis (FAP) pouch neoplasia develops progressively over time; by 15 years, 60-75% of patients have developed adenomas, predominantly in the lower pouch body. No genotype-phenotype association has been demonstrated. Thus, we aimed to investigate environmental influences on pouch neoplasia.

Methods

Mutational signature analysis was performed on normal appearing mucosa and adenomas from the pre-pouch ileum, upper pouch and lower pouch. 16S rRNA gene sequencing was used to analyse microbiome composition from samples obtained from pouch effluent, ileostomy effluent and pouch mucosa. Metabolomic investigations included untargeted UPLC-MS analysis of pouch effluent and pouch tissue and targeted UHPLC-MS/MS bile acid analysis of bile and plasma samples.

Results

Mutational signature analysis (n=10; 5 male) found some genomes with a higher burden of neoplasia, to have a higher presence of signature 18 which can occur from changes induced by reactive oxygen species, however this was not a consistent finding.

Metataxonomic studies found differences between pouch effluent (n=32) and ileostomy effluent (n=9) on alpha and beta diversity, and differential taxonomic abundance analysis e.g. *P. copri*, *B. caccae*, and *P. dorei*. Sub-analysis of pouch effluent found Amplicon Sequence Variants (ASV) to be significantly differentially abundant according to adenoma count, history of advanced adenoma, Spigelman stage and pouch age. We observed significant differences in beta-diversity testing, in lower pouch mucosa (n=35) from participants with a

pouch created 10 years to those created 20 years. Adenoma count, history of an advanced adenoma and the pouch age were all associated with significantly different ASVs on differential abundance analysis.

Targeted analysis of plasma samples (n=55) found an association between bile acid profile and pouch adenoma number. Untargeted analysis of pouch tissue (n=36) found lipid perturbations associated with pouch location. Heme levels (which have been linked to bile metabolism) were found to be affected by pouch age.

Conclusion

Locoregional variation in mutational processes within the pouch supports the hypothesis of environmentally driven mutagenesis. The microbiome, bile acids and lipids have been identified as possible factors associated with pouch neoplasia. Further studies are warranted to better understand these factors and their role in pouch neoplasia and to develop new therapeutic strategies.

P106 - IS LESS REALLY ENOUGH? EXTENT OF COLORECTAL CANCER RESECTION IN LYNCH SYNDROME

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Background and aim

The risk of developing metachronous colorectal cancer (CRC) depends significantly on the type of surgical treatment chosen and the underlying pathogenic germline variant (PGV) in Lynch syndrome (LS) patients. According to a 2020 guideline by the British Society of Gastroenterology, the decision to opt for partial colectomy or extended colectomy in LS carriers with CRC should take into consideration factors such as the risk of metachronous

CRC, the functional consequences, patient's age and personal preferences. A recent study showed no significant risk difference in high-risk PV carriers between partial and extensive colectomy diagnosed in the year 2000 or later, suggesting that improved surveillance might make partial colectomy sufficient for these patients.

Methods

For this retrospective cohort study, carriers of LS with CRC having partial or extended colectomy and having at least one follow-up colonoscopy thereafter were identified in the registry of the German Consortium for Familial Intestinal Cancer. PV carriers in *MLH1*, *MSH2*, and *EPCAM* were classified as high-risk, *MSH6* and *PMS2* as low-risk. Cox regression analysis was performed to assess the risk of metachronous CRC in four subgroups based on pathogenic variant (high-risk vs low-risk) and the extent of surgery (extensive colectomy vs partial colectomy), adjusting for sex and age at diagnosis of primary CRC. Observation ended at time of last documented colonoscopy or metachronous CRC.

Results

A total of 585 PV carriers diagnosed with CRC in the year 2000 or later, who underwent partial or extensive colectomy and had at least one follow-up colonoscopy after surgery were enrolled into the analysis (mean age at first CRC 42.9 years [SD 10.3]; 262 [44.7%] female). Seventy-five (12.8%) of the patients developed metachronous CRC (median time from primary to metachronous CRC 5.0 years [IQR 3.3-7.1]. Among high-risk patients (n=483), metachronous CRC occurred significantly more often in patients with partial colectomy (64 of 440; 14.5%) compared to patients with extensive colectomy (1 of 43; 2.3%), corresponding to a hazard ratio of 7.59 (95% CI 1.04 – 55.07; p=0.045) see **Figure 1**.

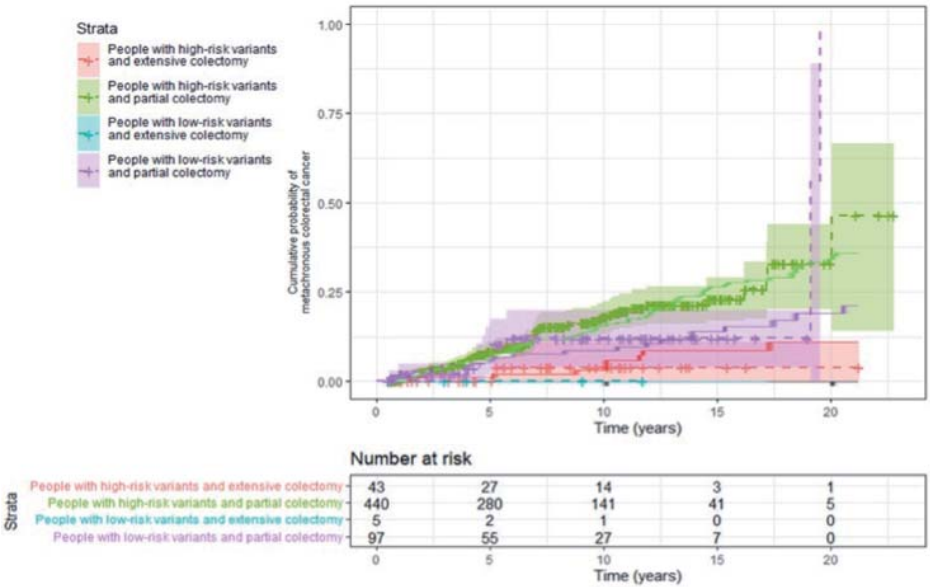
Discussion

In CRC patients with high-risk PV, an individualized decision on the extent of surgery should be made. Our data on the risk of metachronous CRC in this patient group makes an important contribution to informed decision-making.

Keywords

Metachronous CRC, Lynch syndrome.

Figure 1. Probability of metachronous colorectal cancer between Lynch syndrome patients according to high-risk or low-risk variants with either prior extensive or partial colectomy in Germany (dotted line) compared to the Netherlands (solid line).



P107 - MULTIPLE PRIMARY NEOPLASIA IN EARLY-ONSET COLORECTAL CANCER: DESCRIPTIVE AND COMPARATIVE ANALYSIS AND THEIR RELATIONSHIP WITH LYNCH SYNDROME

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Background and aims

Multiple primary neoplasia (MPN) is the appearance of 2 or more neoplasms in the same individual, which can occur synchronously or metachronously (throughout the follow-up), being more common in hereditary forms of cancer. CRC in individuals younger than 50 years-old (known as early-onset CRC; EOCRC), shows a remarkable increasing incidence, but also particularities compared with CRC at other ages. Our aims were to characterize this topic in EOCRC and their association with Lynch syndrome (LS).

Method

We selected consecutive patients with at least 3 years of follow-up enrolled in the Spanish EOCRC consortium (SECOC). Clinicopathological, familial and follow-up data were collected. MPN were defined as: 1) Synchronous CRC (SCRC), with 2 or more CRCs diagnosed within a 6-month period after primary CRC diagnosis; 2) Metachronous CRC (MCRC), diagnosed in a period longer than 6 months; and other MPNs (the EOCRC and other different than CRC). All patients underwent Microsatellite instability analysis, and LS, when appropriate. Finally, a comparative analysis was developed to characterize all the cases showing MPNs compared with those EOCRC which did not.

Results

Three hundred and twenty-four patients were included, with a mean age of onset of 43, with similar proportions of tumor location in right, left colon, and rectum. Forty per cent of

the cases were sporadic, and only 30% showed CRC family history. There were 14 SCRC, 4 MCRC and other 15 MPNs diagnosed (10% globally). The most frequent MPNs, apart from CRC were breast (3), gastric (2), appendiceal (2) and testes (2). Fifty-five cases (17%) out the 324 showed MSI, being 29 defined as LS. Three SCRC, 2 MCRC and one other MPNs were defined as LS, being the proportion of MPN withing LS cases of 6 out of 29 (21%). Remarkably MPNs cases showed comparatively younger age and higher proportion of right colon neoplasia.

Conclusions

Although the proportion of MPNs in EOCRC is not higher than that of CRC in general, the need for complete colonoscopy at diagnosis and lifelong follow-up in these patients is mandatory. In the case of LS this is more remarkable, due to the higher frequency, and even the possibility of more extensive surgeries to prevent MCRC. It is necessary to explore other possible cancer susceptibility genes in other cases of MPNs to define a more individualized management.

Keywords

Early-onset; colorectal cancer; Lynch syndrome; Multiple primary neoplasia; synchronous; metachronous.

P108 - HEREDITARY COLORECTAL CANCER - FIRST REPORT ON MUTATIONAL PROFILE IN SERBIA

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Background

Hereditary colorectal cancer (CRC) accounts for approximately 5-10% of all newly diagnosed cases. It is characterized by the presence of a germline mutation in one of the high-risk genes. Lynch syndrome (hereditary non-polyposis colorectal cancer-HNPCC) and Familiar adenomatous polyposis (FAP) account for 3-5% and 1% of all newly diagnosed CRC cases, respectively.

Method

In Serbia, genetic counseling and testing for hereditary CRC have been available since 2018 at the Institute for Oncology and Radiology of Serbia (IORS), specifically at the Department of Genetic Counseling for hereditary cancers. So far, 111 families suspected of having hereditary CRC have undergone testing. Patients eligible for genetic testing were selected according to the Amsterdam criteria and/or the Bethesda guidelines. A certain number of patients who have not been previously diagnosed with malignant disease were referred to genetic counseling because of a diagnosed colon polyposis or because of a positive family history of CRC.

Results

Genetic testing was conducted on 93 families until now, and pathogenic or likely pathogenic variants were identified in 26 of them (*MLH1*-8, *MSH2*-4, *MSH6*-1, *APC*-6, *CHEK2*-4, *MUTYH*-3).Three of the detected variants were found for the first time in *MLH1*-2 and *APC*-1 genes. To our knowledge, these variants have not been reported in the literature and/or genetic databases so far. The same *MLH1* gene mutation c.392C>G (p.Ser131Ter) was detected in three young non-related CRC patients. Additionally, all four patients with detected *CHEK2* mutation had the same c.470T>C (p.Ile157Thr) variant, while two from three patients with detected mutation in *MUTYH* gene had c.1103G>A (p.Gly368Asp) heterozygous (monoallelic) variant. Variants of unknown significance (VUS) were found in *MLH1, MSH2, MSH6, PMS2, APC, CHEK2*, and *MUTYH* genes in 15 patients.

Conclusions

The analyzed group of patients indicates that Lynch syndrome is present in 14% of the tested families, while FAP is present in 6.5% of them. Based on the current hereditary CRC mutational profile in Serbia, further population screening is crucial as a promising approach for disease prevention and identification of founder mutations.

Keywords

Genetic counseling,Lynch syndrome, FAP.

Acknowledgements

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P109 - THE DIVA PROJECT: RETROSPECTIVE DEEP INTRONIC VARIANT ANALYSIS OF 2000 PATIENTS SUSPECTED OF GASTRO-INTESTINAL TUMORS PREDISPOSITION

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Background and aim

Gastro-intestinal tumors predisposition is confirmed with the identification of a germline pathogenic variant (PV) in 20% of cases. Routine diagnostic focuses on coding regions and exon-intron junctions bypassing the non-coding. To fill the gap, UTR and intronic regions are covered in our optimized panel[1]. DIVA consists of retrospective analysis of NGS data from 2000 undiagnosed patients to identify UTR and deep intronic PV. Here is the pilot study on 483 patients.

Methods

Our diagnostic gene panel explores *MLH1, MSH2, MSH6, PMS2, APC, MUTYH, BMPR1A, SMAD4, POLE, POLD1, PTEN* and *STK11*. Retrospective NGS data processing considered heterozygous and homozygous variants. To search for uORF we used UTR annotator and Sutr modules in VEP, and for splicing anomalies we retained variants filtered at a 0,2 threshold by SpliceAI tool and all positive hits given by SPiP tool. We filtered with a MAF below 1% (popMax gnomAD), removed known variants in splice regions and benign variants in ClinVar. Then, the impact of candidate variants was studied at RNA level by SEALiGHTS method[2].

Results

For the 483 patients, the retrospective analysis of UTR and deep intronic regions revealed 21 rare variants including 17 unique variants: one 5'UTR variant in *PTEN* and 16 deep intronic variants in *APC, BMPR1A, MSH2, MSH6, PMS2, PTEN, POLE*, and *STK11*. Among these, 3 recurrent variants in *APC, PTEN,* and *STK11* with frequencies in gnomAD higher than those associated with the relevant pathologies that may suggest a modifier effect. Of the remaining 13 deep variants, we identified a published PV [3] *MSH2*:c.2458+976A>G, confirmed at

RNA level by SEALiGHTS analysis in a patient with MSI and loss of MSH2 protein expression colorectal cancer (CRC) at 52 y; 5 variants in *APC*, *BMPR1A*, *MSH6*, and *PMS2* suspicious of pathogenicity, absent from ClinVar, in patients with a concordant phenotype; the remaining 7 variants showed moderate probabilities of impacting splicing.

Conclusion

Finally this relevant strategy validated by the lack of increased diagnostic yield in oncogenetics through WES/WGS, rely on the use of an optimized panel and a straightforward annotation pipeline. The ongoing DIVA allows retrospective exploration of patients suspected of unexplained predisposition to CRC. We indicate that 3% of these patients may benefit from additional investigation involving targeted RNA analysis, specifically focusing on deep variant. This approach could lead to new diagnoses.

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P110 - OUTCOMES OF UNIVERSAL TUMOR SCREENING, GENETICS REFERRAL AND GERMLINE TESTING FOR ENDOMETRIAL CANCERS IN A DIVERSE PATIENT COHORT

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Background and aims

Universal tumor screening with immunohistochemistry (IHC) or microsatellite instability testing is recommended for all endometrial cancers (ECs). Some patients with abnormal tumor screening results warrant referral for genetic counseling (GC) and germline testing (GT). This study aimed to determine: 1) outcomes of universal tumor screening of ECs, and 2) rates of genetic counseling referrals and germline testing in a diverse cohort at an academic center.

Methods

Retrospective chart review of ECs diagnosed between 1/2018-1/2023 was performed. Data on patient demographics, EC subtype, IHC, referral for genetic counseling and germline test results were recorded. Fisher's exact test was performed to determine differences between IHC testing, mismatch repair status and promoter MLH1 (p*MLH1*) hypermethylation rates by demographic factors.

Results

Overall, 904 EC patients were identified; the majority self-reported race as Non-Hispanic white (56.9%) or Black (27.8%). IHC testing was performed in 583 (64.5%) tumors and varied by subtype (**Table 1**). A flow diagram of testing outcomes is shown in **Figure 1**. Of tumors tested, 141 (24.2%) had abnormal IHC. Of these, 116 (82.3%) had *MLH1/PMS2* loss of which 93 (80.2%) underwent p*MLH1* hypermethylation testing. Twenty-eight (4.8%) patients were eligible for germline testing of which 19 (67.9%) were referred for genetic counseling. Of those referred, 15 (78.9%) patients had genetic testing and 9 (60%) were positive for >1 pathogenic variant (**Figure 1**). Overall, 9/583 (1.5%) of EC patients were diagnosed with Lynch syndrome. No statistically significant differences in rates of IHC testing, IHC results, p*MLH1* hypermethylation, or GC referral rates were noted across racial groups.

Discussion

Nearly a third of ECs did not have universal tumor screening. Abnormal IHC was found in a 25% of tumors with most due to p*MLH1* hypermethylation. Of eligible patients, a third were

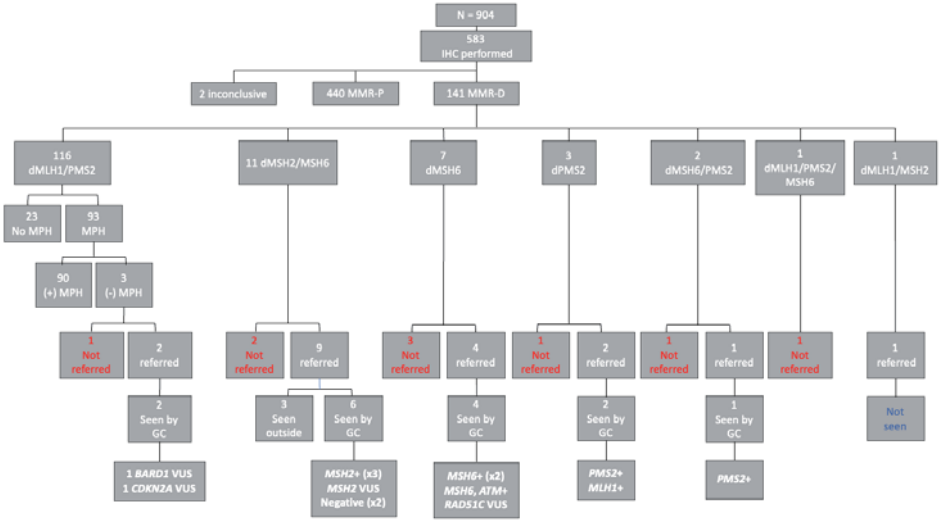
not referred for genetic counseling. Lynch syndrome was diagnosed in 1.5% of EC patients, less than the expected 3%. Universal tumor screening missed patients likely due to factors along the continuum from tumor to germline testing. Assessment of universal tumor screening in a larger, diverse EC cohort is warranted to confirm these results.

Table 1.

Demographics	N = 904 (%)	
Age at Diagnosis (median, range/IQR)	65	21-94/59-72
Race American Indian/ Alaskan Native Asian/ Middle Eastern Black or African-American More than one race Native Hawaiian/ Other Pacific Islander Unknown/patient declined White	 2 (0.2%) 16 (1.8%) 252 (27.9%) 22 (2.4%) 1 (0.1%) 58 (6.4%) 553 (61.2%)	
Ethnicity Non-Hispanic Hispanic Unknown/declined	 801 (88.5%) 54 (6.0%) 49 (5.4%)	
Histologic Subtype Carcinosarcoma Clear Cell De-differentiated Endometrioid Malignant/mixed Mullerian Mixed Other* Serous Stromal Sarcoma	Total (n=904) 52 (5.8%) 31 (3.4%) 12 (1.3%) 579 (64.1%) 12 (1.3%) 61 (6.7%) 18 (2%) 132 (14.6%) 7 (.8%)	IHC performed (n=583) 32 (61.5%) 25 (80.6%) 10 (83.3%) 393 (67.9%) 1 (8.3%) 41 (67.2%) 6 (33.3%) 75 (56.8%) 0 (0%)

*epithelioid, mesonephric-like, neuroendocrine, POLE Ultra-Mutated, SCC, fibroblastic sarcoma, embryonal rhabdomyosarcoma, unknown

Figure 1.



MMR-D= mismatch repair deficient, MMR-P= mismatch repair proficient, MPH= MLH1 promoter hypermethylation testing.

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P111 - GENOTYPE-PHENOTYPE CORRELATION IN CHILEAN PATIENTS WITH FAP: IDENTIFICATION OF A RECURRENT APC DUPLICATION A

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Background and aims

Familial adenomatous polyposis (FAP) is mainly caused by germline mutations in APC. Several studies have described a genotype-phenotype correlation, like the grade of severity and/or the presence of extracolonic manifestations. In our registry, since 2004, a recurrent duplication of exons 1-3 has been identified, even more frequently than the two hotspots (codons 1061 and 1309). This duplication has been described once in a Brazilian FAP family; however, the pathogenic effect is unknown. This study aimed to demonstrate the pathogenicity of this duplication and describe its clinical presentation.

Methods

In our registry, we identified 99 unrelated FAP families with genetic study, of which 61 carry an APC mutation, 10 of these carry an exons 1-3 duplication. Clinical characteristics such as polyps' number, age at diagnosis of PAF and/or colorectal cancer (CRC), and extracolonic manifestations were registered.

Results

A total of 43 different APC mutations were identified in 61 FAP families, mainly point mutations (95.4%), and only two large deletion/duplication (4.6%). Seven are novel mutations. The most frequent was a duplication of exons 1-3 identified in 10 FAP families (16.4%), followed by mutation hotspot at codon 1309 (8.2%). RT-PCR analysis confirmed that this duplication corresponds to an in tandem repeat of exons 1-3 within the APC that includes 18 nucleotides of the 5'UTR, which produce an early stop codon (p.L143Kfs*15).

A comparison between patients carrying exons 1-3 duplication and other APC mutations, showed that patients with the duplication had: more frequently an attenuated phenotype (26% vs 4.6%, p=0.0074), fewer cases of CRC (38% versus 47%), older age at diagnosis of FAP (43y.o. vs 30.8y.o) and CRC (46y.o. vs 40.6y.o.) and less extracolonic manifestations (36% vs 56.6%). Desmoid tumors were not reported in patients with the duplication, while 23.7% of patients had been diagnosed in the other group (p=0.0083). Finally, studies of STR markers to demonstrate a common ancestor were unsuccessful and more studies are required to demonstrate if it corresponds to a founder mutation.

Conclusion

We demonstrated the pathogenic effect of a recurrent duplication in FAP Chilean families. This duplication, localized in the 5' extreme of APC, is associated with a more attenuated FAP phenotype. Our study supports the importance of genetic study to determine a predisposition, besides the endoscopic study, especially in attenuated cases.

Keywords

APC, duplication, genotype-phenotype correlation, familial adenomatous polyposis.

P112 - A DEEPER DIVE: PUSH-ENTEROSCOPY IN LYNCH SYNDROME MANAGEMENT

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Background and aim

Besides colorectal cancer, Lynch syndrome (LS) also includes a variety of extracolonic malignancies such as gastric cancer (life-time risk 13%) or small bowel cancer (life-time risk 8%). Up to 47% of small-bowel cancers occur in the duodenum and up to 33% in the jejunum, which is missed by current screening modality. Our study aims to analyze the prevalence of jejunal cancer in a large cohort of patients with LS and to evaluate the efficacy of jejunal examination via push-enteroscopy as a potential screening method. Methods: Patients with a proven pathogenic germline variant (PGV) in a DNA mismatch repair gene were included in a surveillance program at our National Center of Hereditary Tumor Syndromes, where all patients are followed prospectively after signing an informed consent. We assessed endoscopic findings using standard esophagogastroduodenoscopy (EGD) compared to push enteroscopy using a colonoscope at our center. The same endoscope was used for the following colonoscopy. Additionally, we extracted patient data from the German Consortium for Familial Intestinal cancer (GCFIC) with jejunal cancer with respect to PGV, previous cancer history, gender and age.

Results

The GCFIC has 2859 patients enrolled with pathogenic variants in MLH1, MSH2, MSH6, PMS2, or EPCAM. Jejunal cancers were documented in 36 (1.3%) patients, predominantly in male patients (78%) and high-risk PVs (89%). The age of onset ranged from 31 to 76 years (median age 50.6 y). Between April 2013 and July 2022, a total number of 499 EGDs were done in 213 patients (range 1-13; and beetween August 2022 and August 2023, a total number of 156 push enteroscopies were done in 150 patients In both cohorts, almost half of the patients had a positive history of colorectal cancer, and no differences were observed concerning PGV, age or gender.

Using standard EGDP111 - **(Table 1)**, Helicobacter pylori infection was detected in 24 (5%) cases, in 52 (10%) patients' intestinal metaplasia was found. Using push-enteroscopy, Helicobacter pylori infection was detected in 5 (3%) cases, in 31 (20%) patients' intestinal metaplasia was found and two adenomas. Additionally, we detected five jejunal adenomas (three advanced adenomas) **(Table 2)**.

Conclusion

This prospective endoscopic study shows that surveillance of the upper GI tract identifies clinically relevant results in a large proportion of LS patients. The use of push enteroscopy does not hamper endoscopic detection of relevant lesions.

Table 1. Results of the endoscopic examinations by either esophagogastroduodenoscopy or Push-enteroscopy

	Esophagogastroduodenoscopy (n=499)	Push-enteroscopy (n=156)
Female (%)	268 (54%)	86 (57%)
Age, years	47.4 (+/- 12.3; range 18-78)	48.2 (+/- 13.7; range 20-84)
Number of examinations/pat.	2.4 (range 1-13)	1 (range 1-2)
Esophagus		
Barrett without dysplasia	26 (5%)	6 (4%)
Barrett with dysplasia	3 (0.006)	-
Eosinophilic esophagitis	-	1 (0.6%)
Stomach		
Gastritis	315 (63%)	136 (87%)
Helicobacter pylori	24 (4.8%)	5 (3%)
Intestinal Metaplasia	52 (10%)	31 (20%)
Adenoma/dysplastic fundic gland polyp	7 (1%)	2 (1%)
Gastric cancer	5 (1%)	-
Duodenum		
Adenoma	8 (1%)	1 (0.6%)
Duodenal cancer	3 (0.6%)	-
Ampullary cancer	2 (0.5%)	-

Table 2. Patient characteristics of detected jejunal adenoms using Push-enteroscopy

Sex	Age	Gene	Previous CRC	Synchronous/metachronous small bowel adenoma	Size (mm)	Histology	Dysplasia
Male	65	MSH6	Y	Y	15	Tubular	Low-grade
Male	66	MSH6	Y	Y	12	Tubular	Low-grade
Male	50	MLH1	Y	Y	15	Tubular	High-grade
Male	38	MLH1	Y	Y	1	Tubular	Low-grade
Female	34	MSH2	N	N	6	Tubular	Low-grade

P113 - TARGETED AND UNTARGETED METABOLOMIC PROFILING OF PLASMA AND BILE IN PATIENTS WITH FAMILIAL ADENOMATOUS POLYPOSIS

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Background and aims

The understanding of the metabolic profile of patients with familial adenomatous polyposis (FAP) and their role in duodenal disease is limited. We investigated the metabolic profile of plasma and bile from patients with and without FAP according to burden of duodenal disease.

Methods

Blood (plasma) and bile were collected from patients undergoing surgery for FAP, ulcerative colitis (UC) or laparoscopic cholecystectomy (LC). Details of age, gender, *APC* mutation, desmoid disease, Spigelman stage (SS), surgery and past medical history were collated.

Targeted samples were processed using liquid chromatography mass spectrometry (LC-MS) and analysed using MetaboAnalyst. Untargeted samples were processed using ultra-performance liquid chromatography mass spectrometry (UPLC-MS) and analysed using R/Bioconductor package structToolbox.

Results

Targeted analysis of 125 plasma samples (98 FAP, 23 LC, 4 UC) identified a statistically significant increase in nine bile acids following colectomy. All patients undergoing colectomy had an increase in the concentration of the primary bile acid, cholic acid and its conjugates and a reduction in the secondary bile acid deoxycholic acid and its conjugates. No difference in the bile acid concentration according to SS was observed. Analysis of 37 bile samples (19 FAP, 15 LC, 3 UC) identified a significant increase in the concentration of four bile acids in all patients undergoing colectomy but no difference according to SS.

Untargeted analysis of plasma from 106 patients (84 FAP, 22 LC) using a paired t-test identified 373 statistically significant differences in metabolites in the plasma of patients with and without FAP. Patients with FAP had a higher concentration of metabolites involved in lipid metabolism (i.e. ceramides, sphingolipids, triacylglycerides, ubiquinones).

Statistically significant differences were seen in metabolites according to age and gender but not SS. No statistically significant differences were identified in the bile or duodenal tissue of patients with or without FAP according to age, gender or SS in untargeted analysis.

Conclusions

Our data are the largest cohort to describe the metabolomic profile of bile and plasma from patients with and without FAP. Primary bile acids and their conjugates are significantly increased following colectomy but are not associated with the severity of duodenal disease. 373 metabolites differ significantly in patients with and without FAP. Lipid metabolism is significantly perturbed in patients with FAP.

P114 - COLD SNARE POLYPECTOMY IN THE MANAGEMENT OF DUODENAL ADENOMA IN FAMILIAL ADENOMATOUS POLYPOSIS

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Introduction

Duodenal adenomas are frequent manifestations in individuals affected by familial adenomatous polyposis (FAP), being present in 30–90% of FAP patients. Due to the specific risk of neoplastic transformation associated with duodenal adenomas, careful surveillance and management are essential. The Spigelman index (SI) score system, introduced in 1989, is utilized to predict the risk of duodenal cancer in FAP patients. Patients with a Spigelman score of IV face a higher risk of duodenal cancer and are advised to undergo pancreaticoduodenectomy. Endoscopic resection of duodenal adenoma can prevent the occurrence of duodenal cancer and recently the use of cold snare polypectomy has been proposed for the prevention of advanced duodenal lesions.

Aim

The aim of this study is to conduct a prospective evaluation of the safety and feasibility of cold snare polypectomy for the treatment of duodenal adenomas in patients with FAP.

Material and method

From September 2022 to October 2023, we prospectively enrolled all patients with FAP who underwent upper gastrointestinal endoscopy for the surveillance of gastric and duodenal lesions. The diagnosis of FAP was established based on genotyping information and/or clinical observations of colorectal polyposis. We aimed at identifying and removing all significant adenomatous duodenal lesions, defined as lesions exceeding 5 mm in diameter. For lesions ranging from 5 to 10 mm, we performed cold snare en-bloc polypectomy, while lesions exceeding 10 mm underwent cold snare piece-meal polypectomy. Upper GI endoscopies were conducted with conscious sedation or deep sedation as necessary. Follow-up endoscopy appointments were scheduled at 6-12 months based on the pathology report. Data on patient characteristics, lesion characteristics, details of endoscopic treatment, adverse events, pathologic findings, and Spigelman index (SI) were collected and evaluated.

Results

Overall, 230 upper gastrointestinal endoscopies were conducted in patients with FAP. Among these, 14 individuals (7 females, median age 53 years) had relevant duodenal involvement. All patients had previously undergone total colectomy. A total of 48 lesions were removed over 16 sessions of endoscopic resections. No significative adverse events were reported. Histology reports indicated low-grade tubular adenoma in 3 cases, low-grade tubular-villous adenoma in 7 cases, and high-grade tubular-villous adenoma in 5 cases. One patient, having a SI of IV at the index upper endoscopy had evidence of adenocarcinoma at histology and was referred for surgery. Follow-up endoscopy data were available for 33 % of patients. Follow-up examination showed stable SI in all cases without evidence of advanced lesions or significant fibrosis.

Conclusions

Cold snare resection proves to be a safe and feasible and feasible technique for the endoscopic management of duodenal adenomas in patients with FAP. Even if more extensive studies are necessary to delineate the role of cold snare polypectomy in FAP, it appears that repeated endoscopic treatments may play a role in enhancing disease control.

P115 - LARGE-SCALE SEQUENCE ANALYSES TO IDENTIFY NOVEL COLORECTAL POLYPOSIS PREDISPOSITION GENES

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Background

Colorectal polyposis (CP), when left unmonitored, can develop into colorectal cancer. Many cases of CP are of an unknown genetic basis and the aim of my research is to identify novel predisposition genes.

Method

226 unrelated individuals with CP (104 from the Genetic mechanisms in polyposis of the bowel study and 122 from the 100k Genomes Project), who had previously tested negative for constitutional mutations in *APC* and *MUTYH*, underwent whole exome or genome sequencing. After excluding other known predisposition genes, potentially novel candidate genes were identified as those with variants in 2 or more unrelated index cases under either of two models: (i) an autosomal recessive model with subsequent exclusion of genes where biallelic loss of function (LOF) variants are tolerated (i.e. found in healthy controls), and, (ii) an autosomal dominant LOF model with subsequent exclusion of genes that tolerate LOF based upon GnomAD LOEUF scores >0.35.

Results

We found 4 cases with previously missed pathogenic *APC* variants, one with biallelic pathogenic *MUTYH* variants, one with biallelic pathogenic *MBD4* variants, one with an *AXIN2* variant and one with a *POLE* variant of unknown significance. Under a recessive model, 142 genes were potential novel candidate genes but all tolerated biallelic LOF so were excluded. Under a dominant model, 1368 genes were potential candidates but only 80, present in 179 cases, were intolerant to LOF variants and warrant further investigation.

Conclusion

Monogenic CP traits attributable to novel genes are likely to be very rare and our analyses of candidate genes are ongoing.

P116 - OUR EXPERIENCE SCREENING LYNCH SYNDROME BY MMR PROTEINS IMMUNOHISTOCHEMISTRY IN NON-COLORECTAL AND NON-ENDOMETRIAL CANCERS

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Background and aim

MMR proteins immunohistochemistry (IHC) has been proven useful to screen for Lynch Syndrome (LS) in patients with colorectal cancer (CRC) and endometrial cancer (EC). Recommendations and guidelines on when to consider screening for LS in non-colon and non-endometrial malignancies are limited (PMID 34247540). In our institution, MMR IHC universal testing in CRC and EC was implemented in 2022 but MMR IHC testing in non-CRC/non-EC is not systematized, i.e. requested by oncologists to guide therapy or under high LS suspicion. We aim to preliminary assess the performance of MMR IHC in non-CRC/non-EC in the identification of LS carriers.

MATERIALS AND METHODS

Our in-site database was interrogated to identify cancer patients recruited between Nov 1998 and March 2023 who fulfilled the following criteria: diagnosed with any tumour tested for MMR IHC *(MLH1* promoter methylation, if indicated) and germline LS genetic testing subsequently performed. Sensitivity (SE), specificity (SP), positive predictive value (PPV) and negative predictive value (NPV) for IHC screening regarding each tumour was estimated. Germline genetic testing was assumed as the reference test. Descriptive analysis and estimations were done with Excel.

RESULTS

643 probands were identified. As expected as per local clinical practice, CRC and EC were most common MMR IHC screened tumours (n=607). Among patients with non-CRC/non-EC (n=36), most common screened tumours were Sebaceous Adenomas (SAd) (n=9), small bowel (SBC) (n=8), ovarian (OC) (n=6), gastric (GC) (n=4), upper urinary tract (UTC) (n=3) and bile duct (BDC) cancers (n=2). LS was confirmed in 11/36 non-CRC/non-EC: 1

out of 9 SAd *(EPCAM carrier* patient), 4 out of 8 SBC patient (1 *MLH1,* 1 *MSH2* and *2 PMS2),* 4 out of 6 OC (2 *MLH1,* 1 *MSH6* and 1 *PMS2)* and 1 out of 3 UTC patient *(MSH6). SE,* SP, PPV and NPV was calculated for SAd, OC and SBC (Table 1). IHC MMR screening for SBC showed the highest PPV and NPV. Indeed, 37,5% (3/8) of SBC showed MMR IHC loss but 50% (4/4) had LS (1 false negative with MMR IHC). Concerning clinical criteria,1/1 LS families having SAd fulfilled Bethesda criteria (1 *EPCAM). Interestingly,* 2/4 LS families having SBC (1 *MSH2* and 1 *PMS2)* and 1/4 LS families with OC (1 *MSH6)* did not fulfill Amsterdam I/II or Bethesda criteria and would have been missed.

CONCLUSIONS

MMR IHC as a tool to screen non-CRC and non-EC should be explored in larger series. In concordance with previous studies (PMID 33199489, 33046565, 34944998), LS tumour screening in small bowel cancer is warranted.

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SE, SP, PPV and NPV was calculated for SAd, OC and SBC (Table 1). IHC MMR screening for SBC showed the highest PPV and NPV. Indeed, 37,5% (3/8) of SBC showed MMR IHC loss but 50% (4/4) had LS (1 false negative with MMR IHC).

Concerning clinical criteria,1/1 LS families having SAd fulfilled Bethesda criteria (1 *EPCAM*). Interestingly, 2/4 LS families having SBC (1 *MSH2* and 1 *PMS2*) and 1/4 LS families with OC (1 *MSH6*) did not fulfil Amsterdam I/II or Bethesda criteria and would have been missed.

MMR IHC	SE	SP	PPV	NPV
OC (n=6)	1	--*	0,67	--*
SBC (n=8)	0,75	1	1	0,8
SAd (n=9)	1	--*	0,1	--*

Table 1. MMR IHC performance in terms of sensibility (SE), specificity (SP), positive predictive value (PPV) and negative predictive value (NPV) in different tumour types. *Not evaluable

P117 - PREIMPLANTATION GENETIC TESTING (PGT) FOR BRCA1/2 LYNCH SYNDROME CARRIERS: LESSONS FROM A LARGE COHORT IN A CENTRALIZED UNIT

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Background

PGT for prevention of cancer in Lynch carriers became recently optional yet debatable. We present the experience of a centralized unit performing PGT for carriers of Lynch syndrome pathogenic variants.

Method

We collected data regarding patients who underwent PGT genetic counseling for Lynch syndrome as well as for BRCA1/2 between 2006–2022 in Shaare Zedek Medical Center.

Results

Overall, 24 couples underwent counseling regarding PGT for preventing fetus with their pathogenic variants (PV): 13 (54%) were MSH2 carriers, 6 (25%), 3 (12.5%) and 2 (8.3%) were carriers of MLH1, MSH6 and PMS2, respectively.

Of the carriers, 14 (58.3%) were female. All patients presented a family history of cancer, mostly significant, one PMS2 carrier had a sister with CMMRD. Four carriers had cancer prior to PGT counseling: CRC at age 23 and 28, uterus cancer at age 34 and urinary bladder cancer a in situ in the thirties.

The majority (62.5%) did not have children, 25% couples had fertility problems.

At the same period, 179 couples, which one spouse had a BRCA1/2 PV underwent PGT. Although the usage of PGT among BRCA1/2 carriers was higher, the same carriers' characteristics were observed: familial and personal cancer history, female/males and fertility problems rate.

Conclusions

Application of PGT for Lynch syndrome is less acceptable compared with BRCA1/2. Carriers for genes with higher penetrance, severe family history and having no children are seemed to be promoting factors for PGT. professionals involved in the process should be aware and address the specific issues and concerns of this group regarding medical, emotional and moral aspects.

P118 - MISSENSE MUTYH VARIANTS FROM CLINVAR, GNOMAD, AND LOVD: RECONCILING DISCREPANCIES AMONG DATABASES

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Background and aim

Pathogenic biallelic variants in the *MUTYH* base excision repair gene can lead to *MUTYH*-associated polyposis (MAP), with colorectal polyposis and an increased risk of colorectal cancer. Currently, there are over 1000 unique *MUTYH* variants within publicly available genomic databases. However, the amount of data and the reference transcript used varies by database. Thus, obtaining a comprehensive understanding of *MUTYH* variants requires reconciliation of multiple genomic databases.

Method

We collected unique missense variants from 3 public databases: ClinVar, Genome Aggregation Database (gnomAD), and the Leiden Open Variation Database (LOVD). Variants belonged to one of five categories: Pathogenic, Likely Pathogenic, Variant of Unknown Significance (VUS), Benign, and Likely Benign. Variants with no assertion criteria and those with conflicting interpretations of pathogenicity were removed.

Results

Databases recorded variant position based on three transcripts: NM_0122222.3 (a1), NM_001128425.2 (a5), and NM_001048174.2 (β3). The majority of variants in gnomAD are recorded on α1, while LOVD records variants exclusively on α5. To harmonize the data, we used Clustal Omega to generate DNA and protein alignments of the 3 transcripts, and then converted variant positions to β3, the MANE Select transcript. We mapped the variants to identify any patterns across known functional domains. Because different databases used different transcripts, there were significant discrepancies in clinical classification and mutation type for some variants. Out of 354 recorded missense variants in gnomAD, 2.8% (n=10) are synonymous mutations in the MANE Select transcript; 0.8% (n=3) do not have existing locations in the MANE Select; and 0.6% (n=2) are in transcripts that are not the most biologically relevant for MAP. Of 366 recorded missense variants in LOVD, 7.9% (n=29) are not missense mutations in the MANE Select transcript. In addition, 12.7% (n=45) missense variants are unique to gnomAD; these variants remain unclassified and do not meet the BA1 allele filtering frequency threshold (a stand-alone criteria for establishing benignity).

Conclusion

These discrepancies in *MUTYH*, the 2nd most common cause of hereditary polyposis and a gene that has been studied fairly intensively, indicates that all existing genomic databases must be carefully curated, and highlights the need to standardize information in variant databases to optimize clinical interpretation.

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P119 - THE BALEARIC EXPERIENCE: A RETROSPECTIVE EVALUATION OF MULTI-GENE PANEL TESTING AND PATIENT REFERRAL FOLLOWING THE ESTABLISHMENT OF A MULTIDISCIPLINARY AND INTERTERRITORIAL COMMITTEE FOR HEREDITARY COLORECTAL CANCER DIAGNOSIS

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Background aim

The patient selection for hereditary colorectal cancer syndrome (HCCS) genetic testing requires the individual phenotype recognition and the compilation of detailed personal and three-generation family health histories. The geographical complexity of the Balearic Islands, comprising: Mallorca, Menorca, Eivissa and Formentera, poses challenges in implementing unified and standardized procedures for patients and samples. Moreover, establishing multidisciplinary and interterritorial committees can enhance the exchange of scientific information on clinical cases. To address this, last year, we formed a committee on Hereditary Digestive Cancer Syndrome consisting of Digestive Health Specialists from each health area and Clinical Laboratory Specialists in Molecular Genetics from Hospital Universitari Son Espases (HUSE).

In this retrospective study, we present the results of patients suspected of HCCS from January to June 2023 after the committee's establishment.

Methods

Blood samples from suspected HCCS patients across various health areas were sent to HUSE, where DNA was extracted and sequenced using the TruSight Hereditary Cancer (Illumina®) panel on the Illumina MiSeq platform in the Molecular Diagnostics and Clinical Genetics Unit (UDMGC). The TruSight Hereditary Cancer panel includes genes associated with all well-known cancer susceptibility syndromes, so we specifically analyzed a virtual sub-

panel of genes related to HCCS: *APC, BMPR1A, EPCAM, MLH1, MSH2, MSH6, MUTYH, NTHL1, PMS2, POLD1, POLE, SMAD4, TP53*. Pathogenic, likely pathogenic and variants of unknown significance found in these genes were reported. Additionally, pathogenic and likely pathogenic variants in ACMG secondary

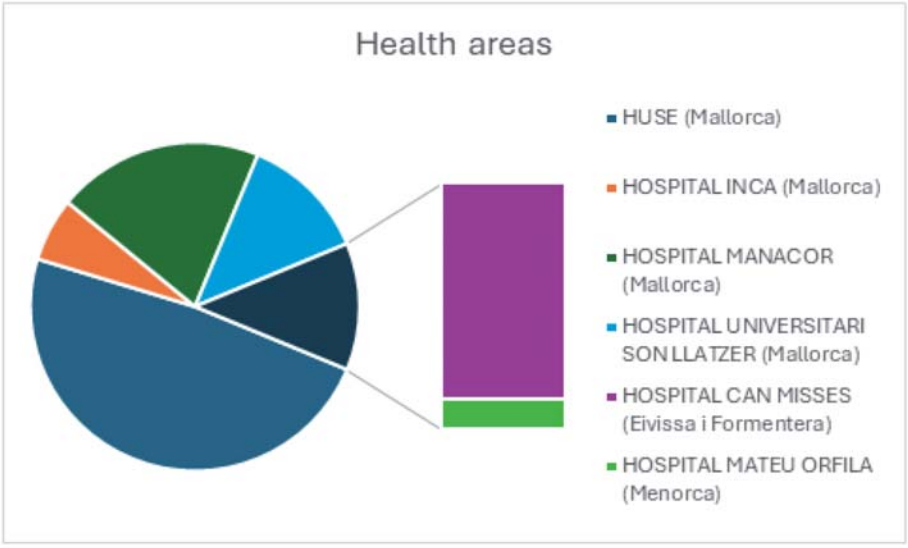
finding panel genes were also reported. Multiplex ligation-dependent probe amplification (MLPA) was also conducted to detect copy number variation (CNV).

Results

We sequenced samples from 63 patients, including 27 males and 36 females aged between 22 and 83 (mean: 59.7 ± 13.7). In 16 patients, we identified at least one variant related to their clinical phenotype, and no CNVs were detected by MLPA. The most frequently mutated gene was *MUTYH*, and a pathogenic variant in *BRCA1* and *BRCA2* genes were identified as a secondary finding in two patients.

Conclusion

We strongly endorse the establishment of multidisciplinary and interterritorial committees in regions facing similar geographical constraints to standardize protocols and procedures, ensuring equitable access to all patients regardless of their origin.



Most patients (87%) were from Mallorca, followed by the Eivissa i Formentera health area (11%) and Menorca (2%).

	Gene	NM	Variant	Consecuence	Zigosity	Classificacion	Inheritance	Syndrome
1	MSH2	NM_000251	c.2459G>A (p.Gly820Asp)	Missense	Het	VUS	AD	HNPCC
2	MUTYH	NM_001048174	c.1103G>A (p.Gly368Asp)	Missense	Het	P	AR	FAP-2
		NM_001128425	c.1187G>A (p.Gly396Asp)					
	MUTYH	NM_001048174	c.1353_1355del (p.Glu452del)	Frameshift	Het	P		
		NM_001128425	c.1437_1439del (p.Glu480del)					
3	MUTYH	NM_001048174	c.1103G>A (p.Gly368Asp)	Missense	Het	P	AR	-
		NM_001128425	c.1187G>A (p.Gly396Asp)					
4	POLD1	NM_002691	c.3321G>C (p.Trp1107Cys)	Missense	Het	VUS	AD	PPAP
5	MUTYH	NM_001048174	c.1103G>A (p.Gly368Asp)	Missense	Het	P	AR	-
		NM_001128425	c.1187G>A (p.Gly396Asp)					
	MUTYH	NM_001048174	c.1103G>A (p.Gly368Asp)	Missense	Het	P	AR	-
6		NM_001128425	c.1187G>A (p.Gly396Asp)					
	BRCA2*	NM_000059	c.262_263delCT (p.Leu88AlafsTer12)	Frameshift	Het	P	AD	HBOC
7	BRCA1*	NM_007294	c.3607C>T (p.Arg1203Ter)	Nonsense	Het	P	AD	HBOC
8	APC	NM_000038	c.5816A>T (p.Asp1939Val)	Missense	Het	VUS	AD	FAP
9	MUTYH	NM_001048174	c.452A>G (p.Tyr151Cys)	Missense	Het	P	AR	-
		NM_001128425	c.536A>G (p.Tyr179Cys)					
10	POLE	NM_006231	c.3185A>C (p.Glu1062Ala)	Missense	Het	VUS	AD	PPAP
11	MUTYH	NM_001048174	c.1103G>A (p.Gly368Asp)	Missense	Het	P	AR	FAP-2
		NM_001128425	c.1187G>A (p.Gly396Asp)					
	MUTYH	NM_001048174	c.452A>G (p.Tyr151Cys)	Missense	Het	P		
		NM_001128425	c.536A>G (p.Tyr179Cys)					
12	MSH6	NM_000179	c.1367G>A (p.Trp456Ter)	Nonsense	Het	P	AD	HNPCC
13	MUTYH	NM_001048174	c.1192C>T (p.Arg398Cys)	Missense	Het	VUS	AR	-
		NM_001128425	c.1276C>T (p.Arg426Cys)					
	MSH6	NM_000179	c.3720dupA (p.Cys1241MetfsTer34)	Frameshift	Het	P	AD	HNPCC
14	MUTYH	NM_001048174	c.901G>A (p.Val301Met)	Missense	Het	VUS	AR	-
		NM_001128425	c.985G>A (p.Val329Met)					
15	MUTYH	NM_001048174	c.1103G>A (p.Gly368Asp)	Missense	Het	P	AR	-
		NM_001128425	c.1187G>A (p.Gly396Asp)					
16	SMAD4	NM_005359	c.1375G>A (p.Ala459Thr)	Missense	Het	VUS	AD	JPS

*Listed on ACMG secondary findings. Het: heterozygous, P: pathogenic, VUS: variant of uncertain significance, AR: autosomal recessive, AD: autosomal dominant, HNPCC: Hereditary Nonpolyposis Colorectal Cancer, FAP-2: Familial Adenomatous Polyposis-2, HBOC: hereditary breast and ovarian cancer, PPAP: Polymerase Proofreading-Associated Polyposis, FAP: Familial Adenomatous Polyposis, JPS: Juvenile Polyposis Syndrome.

P120 - A QUALITY IMPROVEMENT INITIATIVE TO LEVERAGE TUMOR SEQUENCING RESULTS OF GASTROINTESTINAL CANCERS FOR POTENTIAL GERMLINE REFERRALS

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Background

Tumor sequencing can incidentally identify potential germline findings. In patients where germline cancer risk is confirmed, about half do not have a personal/family history (phx/fhx) of cancer that implies hereditary cancer risk. This suggests a role for tumor sequencing to identify patients with hereditary cancer risk who may not otherwise undergo germline analysis. We implemented an initiative to increase referrals for germline testing for this indication.

Method

An educational initiative about identification of potential germline findings from tumor sequencing reports was presented at gastrointestinal tumor boards at an academic medical center in January 2023. It focused on recognition of a "germline banner" on laboratory reports indicating that a variant found during tumor sequencing may be germline in origin and highlighted this as an indication for germline testing referral. We compared referral rates for this indication between the year prior (PreI) and the year following (PostI) delivery of the educational initiative using results from tumor sequencing of gastrointestinal cancers at our center for patients tested at a laboratory that includes germline banners.

Results

119 reports resulted PreI and 145 resulted PostI (patient characteristics shown in Table 1 and Figure 1). 16 (13.4%) of PreI reports had potential germline findings flagged and 10 (6.9%) PostI. After exclusion of patients referred to genetics for other phx/fhx indications, 0/8 (0%) were referred for germline testing based on reported potential germline findings PreI and 1/3 (33.3%) were referred PostI; no significant difference was found between referral rates PreI and PostI (p=0.28). Our analysis was limited by the low number of patients with potential germline findings flagged, particularly as our center has a robust genetics referral process and 15/26 (57.7%) of patients with a potential germline finding on their report were referred for germline testing for other indications prior to tumor sequencing.

Conclusions

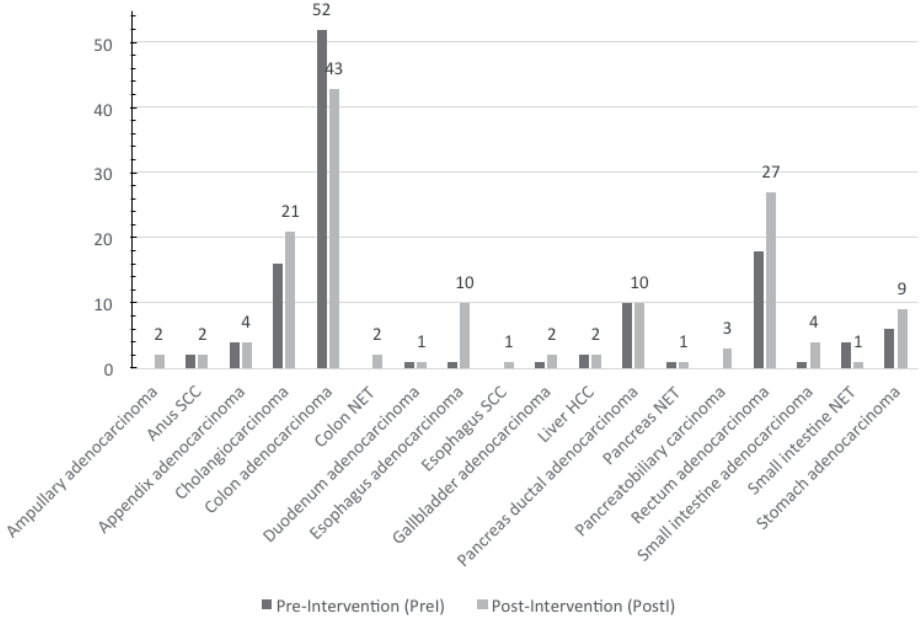
Tumor sequencing offers an opportunity to capture patients with hereditary cancer risk who may not meet classic phx/fhx criteria for germline testing. An initiative at our center was im-

plemented to assist oncology providers in recognizing these referrals. There was no significant difference in the referral rates of patients with this referral indication PreI and PostI, indicating a different approach to identifying these patients may be needed.

Table 1. Characteristics of patients with gastrointestinal cancers undergoing tumor sequencing.

Item	Pre-initiative cohort (PreI) (n=119)	Post-initiative cohort (PostI) (n=145)
Sex assigned at birth		
Female, n (%)	63 (52.9%)	53 (36.6%)
Male, n (%)	56 (47.1%)	92 (63.4%)
Age, mean (range)	61.3 yr (30-86 yr)	59.8 yr (23-89 yr)
Sample type		
Tumor, n (%)	100 (84.0%)	126 (86.9%)
Liquid biopsy, n (%)	19 (16.0%)	19 (13.1%)
Potential germline finding identified, n (%)	16 (13.4%)	10 (6.9%)

Figure 1. Frequency of primary cancer sites for patients with gastrointestinal cancers undergoing tumor sequencing.



P121 - A NEW GERMLINE CTNN1A TRUNCATING VARIANT IN A HDGC/ ILBC FAMILY OF PORTUGUESE DESCENT

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Background

Hereditary Diffuse Gastric Cancer (HDGC) is an autosomal dominant cancer-predisposing syndrome characterized by a high risk of diffuse gastric cancer (DGC) and invasive lobular breast cancer (ILBC). HDGC is mainly associated with *CDH1* germline pathogenic variants, encoding E-cadherin, a cell adhesion molecule implicated in epithelial *adherens junction*. Loss-of-function *CTNNA1* variants, encoding for αE-catenin, have been recently associated with non-*CDH1* HDGC and a DGC cumulative risk for *CTNNA1* variant carriers ranging from 65 to 83% at 40 years. Catenins play an important role in cell adhesion by connecting cadherins located on the plasma membrane to the actin filaments inside the cells, therefore mediating the linkage of cadherins to the cytoskeleton at *adherens junction*. Loss-of-function *CTNNA1* variants, such as nonsense (with the exception of variants localized in the last/NMD-incompetent exon), splicing or frameshift variants, are clinically actionable in HDGC families carriers. Missense *CTNNA1* variants have been associated, for their part, with butterfly-shaped pigment dystrophy.

Methods

One hundred and ten DGC/ILBC index-cases were analyzed by next-generation sequencing at the Molecular Cancer Genetics Lab of Strasbourg University Hospitals, from April 2020 to January 2024. An hybrid capture-based target enrichment system including *CDH1* and *CTNNA1* genes (Agilent SureSelect^{XTHS} reagent, high-sensitivity DNA library preparation kit and customs probes) was analyzed on a MiSeq sequencing platform (Illumina). Testing was performed after obtaining written consent from all legal major patients. Only variants localized in exons +/-50bp and found in ≤10% of a non-cancer control population (gnomAD v2) were considered.

Results

Among our cohort, a woman of Portuguese descent diagnosed with ILBC at 48, was identified heterozygous for NM_001903.5(*CTNNA1*): c.518T>G p.(Leu173Ter). Her mother, previously diagnosed with DGC at 60, was also found to carry the variant. This rare nonsense variant induces the loss of the dimerization and alpha-actin binding domains, and was classified deleterious following ACMG PVS1, PP2, PM3 criteria.

Conclusion

Current knowledge supports that *CTNN1A* pathogenic variants, albeit their rare occurrence, are clinically actionable in non-*CDH1* HDGC families. This new case underlines the importance of *CTNNA1* analyze when considering HDGC families.

Keywords

Diffuse gastric cancer, invasive lobular breast cancer, *CTNNA1* gene.

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P122 - MOLECULAR DIAGNOSIS OF HEREDITARY NON-POLYPOSIS COLORECTAL CANCER (HNPCC) AND HNPCC-LIKE FAMILIES: REVISING STANDARD MULTIGENE PANELS TO INCLUDE HOMOLOGOUS RECOMBINATION-MEDIATED DNA DAMAGE REPAIR GENES, ESPECIALLY ATM

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Background

Hereditary nonpolyposis colorectal cancer (HNPCC) is a major cause of hereditary colorectal cancer and families are clinically defined by the fulfilment of the Amsterdam criteria (AC). An important subgroup of HNPCC is Lynch syndrome, characterized by germline mutations in mismatch repair genes. However, in the majority of the cases, it is not possible to identify the genetic cause within the standard colorectal cancer (CRC) multigene panel test approach. Recent studies have identified germline pathogenic mutations in a few cancer susceptibility genes, in patients diagnosed with CRC, however it is important to establish their relevance in specific clinical groups of familial CRC to clarify the need to broaden the standard multigene panels to incorporate new genes.

Aim

To assess the contribution of pathogenic mutations in genes associated with cancer predisposition, in HNPCC and in patients with familial history of CRC or other cancers from the Lynch syndrome spectrum that fulfill the fourth or the fifth Bethesda criteria (HNPCC-like families).

Methods

Retrospective analysis of 104 HNPCC and 207 HNPCC-like index patients from a unique reference center, who underwent routine NGS germline mutation analysis for Lynch syndrome, using a multigene panel in a Miseq platform.

Results

With this retrospective analysis we identified pathogenic germline mutations in 22/311 (7%) index patients [6/104 (6%) HNPCC and 16/207 (8%) HNPCC-like]. In the HNPCC group, we observed statistically significant differences between the ACI and ACII patient subgroups (1/58 ACI vs 5/46 ACII, p=0.05). Half of the mutations were identified in genes belonging to the homologous recombination (HR)-mediated DNA damage repair (HR-DNA) pathway (11/22) with the majority of them being identified in the ATM gene [6/11 (55%)]. Other genes with pathogenic mutations included other DNA repair genes, mainly NBN and BRIP1.

Conclusions

This study has contributed to clarifying the molecular cause of a large number of HNPCC and HNPCC-like families. Our results suggest that germline mutations in HR-DNA repair genes may have a role in a subset of these families, especially in the ATM gene. We also suggest that the inclusion of these genes should be considered in routine multigene panels applied to these specific groups of CRC families, considering the relevance for their clinical management and possible therapeutical intervention (PARP inhibitors).

Keywords

HNPCC, ATM gene, HR-DNA repair genes.

P123 - MULTIVARIATE MODEL PREDICTING RISK OF COLORECTAL CANCER IN JEWISH AND NON-JEWISH CARRIERS OF APC I1307K

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APC I1307K is a well-described founder mutation that confers moderately increased risk of colorectal cancer (CRC). While the average overall relative risk of CRC among carriers of any copy of the APC I1307K allele is 1.9, multivariate adjusted models incorporating modifiable risk factors that also account for the per allele dosage of APC I1307K in Jewish and non-Jewish carriers have not been described. Here we analyze data from the Molecular Epidemiology of Colorectal Cancer study, a population-based study of incident cases of CRC, incorporating risk factors into a multivariate model that includes age, sex, ethnicity, APC I1307K, family history of CRC, aspirin use, statin use, body mass index, physical activity, and diet (>5 daily vegetable servings). Data were analyzed from 6,006 cases and 5,023 controls. The per allele odds ratio that APC I1307K conferred on risk of CRC after adjustment only for matching variables (age, sex, ethnicity) was 1.94 (95% CI 1.63 - 2.30). Ancestry/ethnic specific risks are incorporated into the model, with lower relative risks among non-Jewish carriers (1.03, 95% CI 0.54-2.02), and higher risks among Sephardi Jews (3.56, 95% CI 1.59-9.02) than Ashkenazi Jews (1.93, 95% CI 1.60 - 2.33). The average per-allele adjusted relative risk from APC I1307K as calculated from the full multivariable model is 2.12 per allele (95% CI, 1.74 - 2.59). The ratio of odds ratios for Jewish and non-Jewish carriers is significantly different, even after adjustment (p=0.003), although the confidence intervals for estimated relative risk each included a two-fold increase risk among Ashkenazi Jews, Sephardi Jews, and non-Jewish carriers. Using our adjusted, multivariate logistic regression model, risks of CRC can be calculated for individuals with zero, one, or two copies of the APC I1307K allele in Jewish and non-Jewish women and men between ages 18-90+ by applying baseline risks from population-based incidence data in a way that now incorporates modifiable risk factors.

P124 - THE YIELD OF UROLOGIC SURVEILLANCE IN SUBJECTS WITH LYNCH SYNDROME

Maya Aharoni Golan[1], Yaniv Yaniv Shilo[2], Elizabeth E. Half[3], Tom Konikoff[4], Rachel Gingold-Belfer[4], Naim Abu Freha[5], Shira Shur[6], Rakefet Chen-Shtoyerman[7], Yael Goldberg[8], Daniel Halstuch[9], Zohar Levi[4]

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Introduction

Subjects with Lynch syndrome (LS) are at increased risk for urinary tract cancers (UTCs) including renal pelvis, ureter, and bladder cancer. However, data on the efficacy and the optimal surveillance strategy are lacking. We aimed to assess the efficacy of surveillance strategies for UTCs in subjects with LS.

Methods

We included all subjects with LS who were diagnosed with UTC and were followed in high-risk cancer clinics in several centers in Israel. UTCs included upper tract urothelial carcinoma (UTUC) and lower tract urothelial carcinoma (LTUC). All available surveillance strategies including urine analysis (UA), urine cytology (Ucyt), and urinary ultrasound (RUS) performed up to 1 year before diagnosis were recorded. Efficient screening was defined as a pathologic screening test at the time of UTC diagnosis.

Results

A total of 15 patients with LS were included in our analysis; 73% were diagnosed with UTUC, 53% were male, median age 61 years (IQR 55-66), 87% had pathogenic MSH2 mutation, 80% had a LS- associated cancer, 40% were diagnosed with UTC before LS diagnosis, 40% were past or present smokers, 13% have a first degree relative with UTCs and 26% reported any relative with UTCs. Nine patients were diagnosed with UTC after LS diagnosis (median 10 years [IQR 7-13]). Of them, four subjects were asymptomatic and were diagnosed by active urologic surveillance; all had microhematuria, 75% had pathologic RUS findings and 50% had atypia in Ucyt. Among all subjects with UTCs, we found that UA was pathologic in 73%, Ucyt in 27%, and RUS in 47%.

Conclusion

Our study suggests that urologic surveillance in subjects with LS is feasible and efficient. The most common pathogenic gene was MSH2, however, UTCs are not diagnosed exclusively in this population. Microhematuria and RUS are reasonable surveillance strategies while Ucyt was an unsatisfying screening modality.

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P125 - RE-ANALYSIS FOR THE GENETIC DIAGNOSIS OF ADENOMATOUS POLYPOSIS SYNDROMES AND OTHER COLORECTAL (CRC) SYNDROMES SUGGEST THAT AN INCREASED RISK FOR POLYPOSIS AND/OR CRC SHOULD BE CONSIDERED IN NTHL1 MONOALLELIC CARRIERS, AT LEAST FOR SPECIFIC MUTATIONS

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Background and aims

Whereas mutations in *APC* and *MUTYH* have been known for several years as responsible of adenomatous polyposis syndromes (APS), novel genetic causes have more recently been detected that have shed light on the broader spectrum of these syndromes, such as mutations in *POLE*, *POLD1*, *NTHL1*, *AXIN2, RNF43* or *MSH3*genes. Since 2020 we included these new genes in an extended multigene panel in order to improve the genetic diagnosis of APS families and to evaluate the relevance of these genes in our cohort.

Moreover, since *NTHL1* biallelic mutations are often associated with few polyps and/or colorectal cancer (CRC), we aimed to broad the spectrum beyond the polyposis phenotype to identify new hereditary CRC cases associated with *NTHL1* deficiency.

Methods

Mutation analysis was performed by next-generation sequencing using a custom panel, including the APS extended multigene panel and increased *APC* sequencing coverage to enhance the detection of somatic mosaicisms, in APS families from a unique reference Centre: 266 APS index patients (2020-2023) and 44 APS index patients that were so far re-analysed (previously to 2020). *NTHL1* mutations were evaluated, retrospectively, in 1047 patients from other CRC syndromes.

Results

In the group of 266 APS patients, in addition to *APC* and *MUTYH* mutations, we detected pathogenic germline mutations in *NTHL1* (2), *RNF43* (2), *MSH3* (1) and *CHEK2* (1) genes. The re-analysis of 40 APS patients without pathogenic germline mutations in *APC* and *MUTYH*, allowed the genetic diagnosis of 4 patients: 2 *APC* somatic mosaicisms and 2 with *NTHL1* mutations.

Overall, *NTHL1* pathogenic germline mutations were detected in 4/306 (1,3%) APS patients, one in homozygosity. In the group of 1047 patients from other CRC syndromes, we detected *NTHL1* pathogenic mutations in 6 index patients (0,6%), one in homozygosity. Two of the *NTHL1* pathogenic mutations (c.115+1G>A and c.526-1G>A) were significantly more frequent in our total cohort in comparison with control populations **(Tables 1, 2)**.

Conclusions

A retrospective analysis allowed that former families may benefit from new genetic analysis that was not available before, as well as the discovery of *APC* somatic mosaicisms.

Our results point out the importance of analysing *NTHL1* gene in CRC families, even in the absence of colorectal polyposis, and suggest that an increased risk for polyposis and/or CRC should be considered in NTHL1 heterozygous individuals, at least for specific mutations.

Keywords

NTHL1, adenomatous polyposis, germline mutations, colorectal cancer risk.

Table 1. Clinical information of patients carrying the *NTLH1* mutations c.115+1G>A and c.526-1G>A.

***NTHL1* mutation**	**Clinical information**
c.115+1G>A (homozygous)	Rectum carcinoma at age 44 and bilateral breast carcinoma (right at age 45 and left at age 49). Central nervous system tumours in paternal uncles. Mother with 5 stomach polyps and father died at age 42.*
c.115+1G>A (heterozygous)	Sigmoid carcinoma at age 56. Father with CRC at age 87, paternal grandfather with stomach carcinoma at age 50, mother with cervix carcinoma at age 60 and maternal aunt with stomach carcinoma at age 60.*
c.526-1G>A (heterozygous)	Mucinous CRC at age 58 (microsatellite stability). Mother with CRC at age 64 and maternal grandmother with gastric carcinoma at age 69.*
c.526-1G>A (heterozygous)	Colorectal cancer and 20 tubular adenomas with low grade dysplasia (TALGD) at age 58, 16 hiperplastic polips and 2 adenomas with high grade dysplasia at age 60 and prostate carcinoma at age 67. Father with CRC at age 87,brother with 2 adenomas at age 60 and son with adenomas at age 33.
c.526-1G>A (heterozygous)	Ten TALGD at age 77. Father with CRC at age 79 and mother with ovarian carcinoma (not confirmed)

* without information regarding the presence of polyps

Table 2. Allele frequencies of germline *NTHL1* mutations in individuals suspect of HPAS and other CRC hereditary syndromes compared with control populations.

***NTHL1* mutation (NM_00002528.7)**	**Allele frequency in our cohort**	**Allele frequency in non-Finnish European (gnomAD)**	**p Value**	**Allele frequency in total population (gnomAD)**	**p Value**
c.115+1G>A	3/2706	25/1169094	$P=3,8X10^{-5}$	36/1577246	$P=4,4X10^{-5}$
c.244C>T p.(Gln82Ter)	6/2706	2084/1180000	p=0,49	2568/1613200	p=0,33
c.526-1G>A	3/2706	40/1179946	$P=1x10^{-4}$	59/1611400	$P= 2x10^{-4}$

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P126 - EARLY-ONSET COLORECTAL CANCER PATIENTS PRESENT DISTINCT TUMOR AGGRESSIVENESS AND CLINICAL FEATURES ACCORDING WITH AGE AT DIAGNOSIS, FAMILY HISTORY AND PRESENCE OF GERMLINE MUTATIONS IN DNA REPAIR GENES

Carolina Bandeira[1], Maria Inês Francisco[1], Patrícia Loureiro[1], Bruno Jordão[1], Pedro Carvalho[2], Isadora Luz[2], Sofia Freire[3], Paula Campos[3], Maria Joana Parreira[3], Isabel Ponte[2], Cristina Mantas[1]

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Background and aims

Colorectal cancer (CRC) is the third most diagnosed and the second most deadly cancer in general population. The incidence of early-onset CRC (EO-CRC), which includes patients diagnosed under the age of 50, has been increasing dramatically over the last few decades. The younger affected population tends to have more advanced stages, poor response to therapy and a worse prognosis, thus emerging the need to investigate novel susceptibility genetic variants for monitoring disease progression and support therapeutic decisions.

Methods

With the aim of identifying inherited mutations underlying increased susceptibility for EO-CRC, 108 patients (≤ 50 years) who had previously undergone Next-Generation Sequencing-based germline genetic diagnosis were analyzed in a retrospective study. To carry out the germline analysis, a custom multigene panel (Agilent) was designed and implemented in house, which includes all genes presently predisposing for CRC and selected cancer susceptibility genes. The genetic variants of interest were selected based on their ClinVar pathogenicity classification and population frequency.

Results

Hence, 238 variants from 70 different genes were identified in 95 patients. Of these, 9 corresponded to pathogenic/likely pathogenic variants and 229 to variants of uncertain significance, 57 of which were considered as putative damaging according to different software (Figure 1). Interestingly, most mutations were identified in DNA repair genes and these appear to be associated with the development of synchronous colorectal cancer (p=0.023). Also, a significant correlation was found between patients aged 20-30 and T4 tumor (p=0.028). Notably, liver metastases seemed to be more prevalent in patients aged 30-

40 (p=0.008) and lung metastases in patients aged 45-50 (p=0.008). Moreover, metastatic disease was inversely correlated with a history of polyps (p=0.017). Cancer family history in 1-4 non-1st-degree relatives appeared to be associated with the presentation of metastases at diagnosis (p=0.003) and development of metachronous lesions (p=0.017). Interestingly, these significant correlations were not found with 1st-degree relatives.

Conclusion

This study allowed the characterization of this young population in terms of germline defects and clinical features and shed light to the distinct etiologies of EO-CRC and the identification of specific traits that may be associated with greater tumor aggressiveness.

Keywords

Early-onset colorectal cancer, germline genetic diagnosis, pathogenic/likely pathogenic variants, variants of uncertain significance, DNA repair genes.

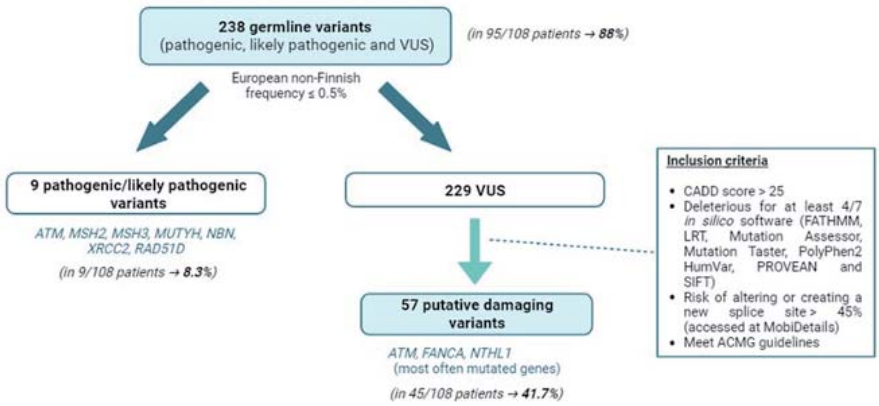

Figure 1. Representative scheme of the results of germline mutation analysis in Early Onset Colorectal Cancer patients using a custom multigene next generation sequencing panel and the criteria used for selection of putative damaging variants (Created with Bio.Render.com).

P127 - HISTOLOGICAL CHARACTERIZATION OF EARLY HEREDITARY DIFFUSE GASTRIC CANCER FOCI

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Background and aim

Characterizing early hereditary diffuse gastric cancer (HDGC) foci is important to stratify patients for endoscopic surveillance versus referral for curative gastrectomy. Previously we proposed to differentiate HDGC into three subtypes based on endoscopy and histology: type 1, T1a lesions which seem to behave indolent; type 2, T1a/b lesions that show signs of deeper invasion and/or atypia; type 3 advanced gastric cancer. In this study we examined T1a lesions of gastrectomy specimens to determine whether we can distinguish type 2 lesions.

Methods

Collection of 74 T1a HDGC foci of 22 gastrectomy specimens of persons with a confirmed *CDH1* germline pathogenic variant. Categorization of the foci based on morphology (H&E), P53 and Ki-67 immunohistochemical stain. Annotation of the foci into three different cell clusters: classical signet ring cells, smaller and slightly irregular signet ring cells, and poorly differentiated cells.

Results

In 5 of the T1a lesions there was profound atypia (all 5), diffuse or irregular P53 overexpression (n=3) and increased proliferation based on Ki-67 staining (all 5). In 18 cases there was atypia but less profound, these were difficult to classify as either type 1 or type 2. In most lesions (n=51), the histology with low proliferation and wild type P53 fulfilled the classification of (indolent/ well-differentiated) type 1 lesions. The 18 indeterminate cases showed increased amounts of smaller and poorly differentiated cells. Using the histological categorization (51 for type 1 and 5 for type 2), we observed on a cellular level that early HDGC foci showed non-significant differences between the number of small signet ring cells. The amount of classical signet ring cells (53 vs 19%) and the amount of poorly differentiated cells (18 vs 52%) were significantly different between type 1 and type 2 lesions respectively ($p< 0,001$ using independent-samples t-test).

Conclusions

The distribution of different cell types in biopsies of early HDGC may aid in stratifying which person with HDGC may safely continue endoscopic surveillance. Future work will focus on a larger set of HDGC foci including additional immunohistochemical biomarkers, Delphi consensus meetings and on deep learning for multi-class cell detection in H&E-stained slides which will aid the pathologist by classifying early HDGC.

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Keywords

Hereditary diffuse gastric cancer, CDH1, endoscopy, histology, surveillance.

P128 - LYNCH SYNDROME CAUSED BY DI-GENIC EVENTS – INHERITED MSH6 PATHOGENIC VARIANT AND MOSAIC MLH1 METHYLATION

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Background

Lynch syndrome (LS) is a cancer predisposition syndrome, associated with increased risk mainly for mismatch repair deficient (MMRd)-colorectal and endometrial cancer. LS results from a monoallelic germline mutations in one of four MMR-genes: *MLH1, MSH2, MSH6 and PMS2*. Inactivation of the MMR genes can also be mediated by epigenetic mechanisms, mainly *EPCAM* deletions or *MLH1* methylation. We here report on a patient with co-occurrence of inherited *MSH6* pathogenic variant and *MLH1* germline methylation.

Patient and Methods

A 28 y/o female presented with MMRd right CRC. She reported on a neurofibroma previously removed from her skull and had five café au lait spots. Family history was significant for cancer on both sides. Molecular analysis of the tumor included MMR immunohistochemistry, MSI analysis and NGS. Germline analysis included multigene panel testing and MLH1 promoter methylation analysis by MS-MLPA, done on two independent DNA extractions.

Results

Tumor molecular analysis showed loss of MLH1 and PMS2 expression with intact MSH2 and MSH6, . Tumor molecular profile showed MSI-H, mutation burden of 22 (m/mb), wild-type BRAF, and *MSH6* c.755C>G;p.Ser252* variant in 46% of the reads. There were no pathogenic variants in *MLH1, PMS2 and NF1*. Germline testing revealed the *MSH6* c.755C>G;p.Ser252* variant, inherited from the proband's father, along with increased *MLH1* methylation, indicating mosaic germline methylation.

Conclusion

The discrepancy between tumor IHC for MMR and germline status was explained by an epigenetic event of *MLH1* methylation. The combined comprehensive tumor-germline analysis contributed to understanding of the underlying genetic diagnosis.

P129 - CHALLENGES OF PMS2 SCREENING: TECHNICAL PROCEDURES TO VALIDATE THE PRESENCE OF PMS2 VARIANTS

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Background and aim

In the era of next generation sequencing technology (NGS), the molecular screening for *PMS2* is challenging due to the presence of a large family of pseudogenes that are highly homologous. Fourteen pseudogenes overlap with some or all of exons 1–5, whereas the *PMS2CL* pseudogene shares up to 98% of genetic sequences for PMS2 exons 9 and from 11 to 15, with identical coding sequences for exons 12 and 15. Long-range PCR method using described by Vaughn and collaborators (2010) is routinely performed together with MLPA to evaluate the presence of *PMS2* variant in patients with tumors deficient for PMS2. In this work, we present additional strategies to confirm *PMS2* alteration in which neither long-range PCR nor MLPA could ensure the location of the variant detected.

Method

After routine genetic study (NGS, Long-range PCR and MLPA) we detected 6 patients that require additional experiments. All of these patients presented isolated loss of PMS2. Patients 1 to 3 presented an unique deletion of PMS2 exon 14, patient 4 showed a duplication of exons 7 to 11, patient 5 carried a deletion encompassing exons 14 and 15, and patient 6 presented a pathogenic variant in exon 12 in which long-range PCR was not conclusive. For copy number variants (CNVs) specific primers were used in order to confirm their presence by PCR and/or Sanger sequencing. For patient 6, RNA was extracted and cDNA was sequenced.

Results

All patients with single deletion of exon 14 were confirmed using the primers described in Clendenning and collaborators (2013) identifying a recurrent deletion of approximately 2kb (c.2276-113_2245+159del). The duplication of exons 7 to 11 was confirmed using the combination of specific primers revealing the presence of an inverted tandem duplication of these exons. In patient 6, RNA assay suggested the presence of a *PMS2- PMS2CL* hybrid allele. Patient 5 remains under assessment.

Conclusions

Although NGS has become a routine diagnostic tool in medical genetic, short read based NGS may fail to align sequences to the correct reference gene, leading to false negative

or false positive variant calls. Therefore, all *PMS2* variants require addition confirmation with specific approaches. Interestingly, the fact that all 6 patients showed isolated PMS2 loss which indicates that it could represent a highly indicator of a germline pathogenic variant.

P130 - SOMATIC PANEL NGS OF DMMR COLORECTAL CANCER REVEALS A HIGH FREQUENCY OF DIFFERENT LIKELY GERMLINE VARIANTS IN YOUNG INDIGENOUS AFRICAN PATIENTS

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Background and aims

Mismatch repair deficient (dMMR) colorectal cancer (CRC) is thought to be more prevalent in sub-Saharan Africa. Recognising dMMR CRC is important for prognostic and predictive purposes, as well as for triaging patients to Lynch syndrome screening programmes. This disease, however, remains poorly studied in the indigenous African population in sub-Saharan Africa. Our aim was to categorise the somatic *MLH1* variants in a cohort of dMMR CRCs using panel based NGS.

Methods

The clinicopathological features of 18 suitable cases of dMMR CRC with MLH1/PMS2 loss and negative BRAF V600E by immunohistochemistry were selected and evaluated by a consultant histopathologist. DNA was extracted from representative FFPE tissue blocks. NGS was performed using a 27 gene panel on the Ion GeneStudio S5 Prime. Variants with allele frequency > 5% and depth > 500 were manually annotated and called by evaluating VCF files and searching the publicly availably ClinVar and ACMG databases.

Results

The median age at diagnosis was 46 years (range 27-59). There were 8 males and 10 females. Fifteen cases (83%) occurred in the right colon and 3 were left-sided. Fifteen of the 18 cases (83%) harboured pathogenic or likely pathogenic variants in *MLH1*. Eight of these (53%) had a variant allele frequency suggesting a germline mutation. Four variants occurred in splice regions of *MLH1* (27%). Three novel variants were identified (c.1731+5G>T, c.83C>G, c.2017_2038del). c.1528C>T, a nonsense variant common in our mixed ancestry population, was only detected in 2 cases. One *POLD1* and no *POLE* exonuclease domain pathogenic variants were detected.

Conclusions

Our findings show good correlation (83%) between loss MLH1/PMS2 protein expression and somatic pathogenic variants in MLH1. The high frequency of possible germline variants in our cohort, and the spectrum of variants encountered, provides supportive evidence for reflex up front genetic counselling and NGS to screen for Lynch syndrome in our setting.

P131 - ADVANCING GENOMIC MEDICINE IN DEVELOPING COUNTRIES: A PARADIGM SHIFT ILLUSTRATED BY THE LYNCH SYNDROME EXPERIENCE IN SOUTH AFRICA

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Background and aim

Genomic medicine faces formidable challenges in developing countries, characterized by competing health priorities, scarcity of genetic expertise and diagnostics, and a dearth of evidence demonstrating tangible health benefits. Over two decades, our pioneering efforts in South Africa have focused on the ascertainment of Lynch syndrome (LS) within a single province, offering a transformative model for the introduction of genomic medicine in resource-limited settings, with incremental innovations that may be implemented in similar settings. This presentation delineates our journey from empirical risk assessment to the implementation of next-generation sequencing (NGS) and the integration of a locally devised scoring model for LS identification, alongside the establishment of genetic counseling services towards improved outcomes in a developing world context.

Method

Patients meeting the Bethesda guidelines for colorectal cancer (CRC) were recruited from the Surgical Gastroenterology Unit at Groote Schuur Hospital. A locally developed scoring instrument, utilizing clinical and histopathological criteria, was employed to triage CRC patients, with those scoring ≥8 out of 10 points triaged for NGS. Cost-benefit analysis compared symptomatically managed CRC patients with those receiving presymptomatic management, revealing the economic advantages of predictive testing and management.

Results

The archive encompasses data from 2696 patients across 1018 families, with 243 families revealing disease-causing germline mutations. Ongoing cascade management involves clinical surveillance of mutation-positive subjects, demonstrating clear survival benefits, and reduced mortality and morbidity. Cost-benefit analysis underscores the net positive impact of adopting this schema for LS ascertainment and presymptomatic management in our setting.

Conclusions

Lynch syndrome, as a prototypical hereditary condition, epitomizes the efficacy of disease-causing mutation ascertainment, cascade screening, and clinical surveillance. Our program

significantly reduces mortality and morbidity and also demonstrates substantial cost benefits. Beyond economic gains, the profound impact lies in sustaining those at the highest risk, keeping them disease-free and economically active. This paradigm shift is an exemplary template for global organizations like the World Health Organization, advocating the integration of genomic medicine in developing countries.

Keywords

Genomic medicine, Improved ascertainment, Economic benefit.

P132 - HUMAN LEUKOCYTE ANTIGEN-ALLELIC VARIATIONS MAY INFLUENCE THE AGE AT CANCER DIAGNOSIS IN LYNCH SYNDROME

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Background and aim

Lynch syndrome (LS) is an inherited cancer predisposition disorder associated with an elevated risk of various epithelial cancers. Despite sharing the same pathogenic variant (PV), Lynch syndrome variant heterozygotes (LSVH) exhibit considerable phenotypic variability in cancer risk. The role of Human Leukocyte Antigen (HLA) in modifying cancer risk prompted our investigation into whether HLA variations act as genetic modifiers influencing age at cancer diagnosis in a unique cohort of LSVH carrying a PV in the *hMLH1* gene in South Africa.

Method

Within our extensive LS cohort, 426 individuals carried the same *hMLH1* PV (*MLH1*:c.1528C>T). We selected 100 individuals with the greatest diversity in age at cancer diagnosis and the oldest unaffected individuals for high-throughput HLA genotyping of 12 HLA class I and II loci using next-generation sequencing. Statistical analyses employed Kaplan-Meier survival analyses with Logrank tests and Cox proportional hazards.

Results

Following the robust application of statistical correction methods, six HLA alleles (3.2%) were significantly associated with a young age at cancer diagnosis. Notably, *HLA-B*15:17* and *HLA-DPB1*55:01* correlated significantly with very young colorectal cancer (CRC) diagnosis (Mean age: 21y [17-25]; HR = 71.59; q<**0.001** and Mean age: 25y; HR = 54.05; q<**0.001**, respectively). Four *HLA-DPB1* alleles showed significant associations with a younger age at cancer diagnosis (*HLA-DPB1*04:02, -DPBI*20:01*, *-DPB1*55:01*, and *-DPB1*296:01*).

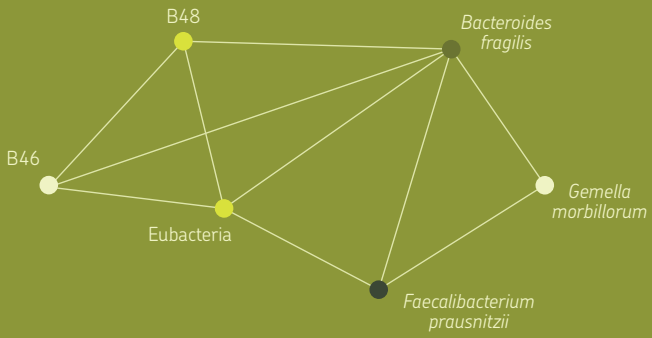
Conclusions

HLA allele variations may influence the age at cancer diagnosis in LSVH with the same PV. Pending validation in a larger cohort, these high-risk HLA alleles could enhance cancer risk prediction models for personalized cancer screening in LSVH.

Keywords

Lynch syndrome, colorectal cancer, germline pathogenic variant *MLH1*:c.1528C>T, Human leukocyte antigen (HLA), genetic risk modifiers.

GOODGUT



RAID-CRC

Non-invasive faecal microbial test as a key tool to increase Colorectal Cancer screening efficiency by 12-15%[1]

[1]Malagón M, Oliver L, Ramió-Pujol S, Guardiola J, Balaguer F. Returning to endoscopy normality through the support of a new non-invasive faecal test based on microbial signatures. Digestive and Liver Disease. 2021; 53(12):1666-1668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