ABSTRACT

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Case report of a monoallelic germline MSH3 variant as the potential cause of early-onset MSI-H colon cancer

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Background Biallelic pathogenic germline variants in MSH3 have been described in a small number of individuals with adenomatous oligopolyposis of the colorectum, with polyps demonstrating MSI at dinucleotide repeats and elevated microsatellite alterations at selected tetranucleotide repeats (EMAST). MSH3 is thus thought to be an autosomal recessive cause of polyposis and colorectal cancer (CRC) susceptibility, though there is no known risk/phenotype currently associated with monoallelic/heterozygous germline variants. Here, we present a case of young-onset MSI-high colon cancer developing shortly after a normal colonoscopy, potentially caused by a monoallelic germline MSH3 variant.

Case presentation The patient is an otherwise healthy female who began colonoscopic surveillance at age 25, due to the family history of CRC (Figure). Colonoscopies at ages 25, 30, and 35 were normal, and follow-up was planned for age 40. At age 39, she was found to have a right-sided colon mass after presenting with abdominal pain and anemia. Right hemicolectomy revealed moderately-differentiated pT3N0 adenocarcinoma with intact IHC for MLH1/MSH2/MSH6/PMS2. Somatic sequencing determined the cancer to be MSI-high with 69.2 mutations/Mb with a predicted loss-of-function MSH6 c.3253_3254insC (p.Phe1088Leufs*5) variant and other missense MMR gene variants without predicted pathogenicity (MSH3 not evaluated on this assay). Germline testing identified a heterozygous “likely pathogenic” c.1764-1G > A MSH3 variant, expected to disrupt the intron 12 acceptor splice site; no other MMR gene variants were identified. Subsequent multigene germline testing on her father identified homozygous carriage of the c.1764-1G > A MSH3 variant. He had prior right-sided colon cancer at age 50 as well as colonic adenomatous oligopolyposis (exact number of polyps unknown) and prior duodenal adenomas.

Discussion The MSI-high findings and short-interval development of this cancer despite early-onset colonoscopic surveillance suggests that the heterozygous MSH3 germline variant may have played a causative role in carcinogenesis. Additional analysis is underway on the index patient’s colon cancer – including MSH3 IHC, MSH3 sequencing, EMAST evaluation, RNAseq, and whole exome sequencing – which could further clarify the etiologic role of this monoallelic germline MSH3 variant and the biology of such malignancies. This case highlights the need for further data on the clinical significance and possible phenotype of monoallelic germline MSH3 variants.
Family history documentation at the time of colonoscopy

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Background Obtaining a family history at the time of colonoscopy allows gastroenterologists to identify patients who would benefit from genetic screening. However, in practice, obtaining and applying this information to patient recommendations is observed to be inconsistent. Therefore, we sought to determine the baseline rate of family history documentation and compliance with appropriate referral to a hereditary cancer clinic among endoscopists in our own institution.

Methods Endoscopy reports were retrospectively reviewed for patients 18–89 years old who underwent a screening or surveillance colonoscopy between 12/2019–12/2020 at a tertiary care center. Data collected included demographics, family history documentation, procedural details and determination of criteria fulfillment for referral to hereditary cancer clinic. The data was stratified into two groups: physicians with designated focus in hereditary colon cancer (Hereditary Focus Group) and remainder of gastroenterology faculty (General Group).

Results 1222 consecutive colonoscopy reports were reviewed. 334 of the procedures were performed by the hereditary focus group and 887 were performed by the general group. The hereditary focus group was more likely to document family history at the time of colonoscopy (78.1% vs 68.3%, p = 0.001). Among those procedure reports where family history was not documented, the majority were surveillance colonoscopies in both hereditary focus group and general group (89.0% vs 82.6%). In the hereditary focus group 5.1% (n = 17) of patients met criteria for referral to hereditary cancer clinic, 42.1% (n = 7) were not referred despite meeting institutional criteria. In the general group, 2.4% (n = 21) of patients met criteria for referral and 61.9% (n = 13) were not referred despite meeting institutional criteria.

Conclusion Family history assessment at time of colonoscopy plays a critical role in allowing identification of high-risk individuals with genetic disposition for development of colorectal cancer. In our institution, the hereditary focus group had a higher rate of family history documentation compared to remainder of general faculty. However, both groups had substantial missed opportunities for referral to hereditary cancer clinic. Heightened awareness of proper referral criteria and standardization of family history documentation in colonoscopy reports may be potential modalities to achieve improvements in both these areas.

Diffuse gastric cancer in a family with a germline, pathogenic ATM variant

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Background Heterozygous, germline ATM pathogenic variants (PV) are associated with hereditary breast cancer. Literature supports there is likely an increased risk of pancreatic cancer and possibly other cancers including gastric cancer (1). Several studies examining individuals with diffuse gastric cancer identified heterozygous, germline ATM PV in their cohort (2,3). We report a case of a family with only diffuse gastric cancer that segregates with the ATM PV.

Case Presentation A 38-year-old male presented with a history of postprandial gastric pain for three months and three weeks of vomiting. EGD showed diffuse ulceration in the entire antrum and prepyloric region and an ulcerated mass-like lesion in the antrum. A biopsy noted poorly differentiated carcinoma. CT scan showed distended stomach with wall thickening involving antrum. He had a gastrectomy with Bilroth II anastomosis with biopsy of mesenteric lymph node and peritoneal nodule. Final pathology showed adenocarcinoma, diffuse type. Paired germline/somatic tumor testing identified a germline ATM PV (p.K750K), which is known to cause abnormal splicing. The patient completed 10 cycles of mFOLFOX7. He presented to Medical Genetics given his early-onset diffuse gastric cancer and family history of a paternal uncle with diffuse gastric cancer who was also previously seen in Medical Genetics. This paternal uncle was heterozygous for the same ATM PV. The family history was not significant for any other cancers (Fig. 1). Germline testing for the patient was performed using a 37-gene panel test (including CDH1 and CTNNB1) that included RNA analysis and confirmed the heterozygous, ATM PV found in the tumor. All other genes analyzed were negative.

![Fig. 1 Pedigree](image-url)
Discussion This case illustrates that an ATM PV may increase the risk of diffuse gastric cancer which recent studies have also suggested [1–4]. Confirming an association between an ATM PV and an increased risk of diffuse gastric cancer will have important, future screening implications. We cannot exclude the possibility that there may be another underlying genetic mechanism that is causing diffuse gastric cancer in this family beyond the ATM PV. Additional research will be helpful in further defining this cancer risk.

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Investigating the molecular mechanisms of adenomatous polyposis syndromes using 3D organoid models

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Background Duodenal polyposis and cancer have become leading causes of morbidity and mortality in patients with Familial Adenomatous Polyposis (FAP) and MUTYH-Associated Polyposis (MAP). However, the processes underlying duodenal tumourigenesis remain poorly understood and no suitable treatments are available. Previous work in our research group identified somatic mutations in 12 putative driver genes in the first reported whole exome sequencing (WES) analysis of FAP and MAP duodenal adenomas, but no adequate model existed to investigate the mechanisms of tumourigenesis further.

Methods Three-dimensional intestinal organoids have been established from patient-derived gastrointestinal (GI) endoscopy samples of FAP and MAP patients. WES, immunofluorescent confocal imaging of stem cell and differentiated cell markers, and flow cytometry were used to characterise these organoid lines and to investigate the functional effect of putative tumour drivers.

Results Thirteen organoid lines from 6 FAP patients (6 normal and 6 adenoma derived) and 1 MAP patient (1 normal derived) have so far been established in long term culture. Five of these lines have been expanded by Cellesce Ltd, to produce maximal material at a low passage number. These organoids have a cystic morphology and are comprised of cell types characteristic of the small intestinal epithelium. WES has shown that they are genetically similar to the matched original biopsy and remain genetically stable in culture.

Conclusions We have established duodenal organoid lines from FAP and MAP patient biopsies. Genetic and histological characterisation confirms that they contain all duodenal epithelial cell types, thereby histologically and genetically recapitulating human tumours. As they can be expanded long-term in vitro, use of these novel models to investigate tumour drivers will enable us to better understand disease progression and cancer risk in these patients.

Keywords adenomatous polyposis syndromes, FAP, MAP, 3D Organoids.
NTHL1-associated polyposis: A diagnostic dilemma solved by repeated multi-gene panel testing

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Background Biallelic germline pathogenic variants (mutations) in the NTHL1 gene cause autosomal recessive NTHL1-associated polyposis (NAP), which was first described in 2015. NAP is characterized by adult-onset colorectal adenomatous polyposis with an increased risk for colorectal cancer (CRC), similar to attenuated familial adenomatous polyposis (AFAP) and MUTYH-associated polyposis (MAP). Multiple other tumors have been reported, including endometrial, breast, bladder, meningiomas, basal cell carcinomas, and duodenal polyps, among others.

We present an individual with two CRCs and polyposis whose third round of genetic testing confirmed the diagnosis of NAP. We also discuss considerations for evaluation and testing.

Case presentation A 68-year-old male of European descent had rectal cancer at age 49 treated with low anterior resection and chemoradiation. Follow up colonoscopies revealed numerous adenomas. At age 57, he was again diagnosed with a microsatellite-stable sigmoid cancer and five tubular adenomas, one with high-grade dysplasia. He then underwent total proctocolectomy with ileostomy. Five years later, he had a clear upper endoscopy and ileoscopy, which were his last endoscopies. He has also had two sebaceous cysts.

He previously had negative genetic testing before NTHL1 was available—APC and MUTYH in 2010 and a 70-gene panel in 2016. In 2021, he had repeat testing with a 150-gene panel that included new CRC risk genes, which identified two NTHL1 mutations: c.268C>T (p.Gln90*) and c.649_650del (p.Val217trpfs*55).

His parents are deceased and were never tested, but neither had polyposis or CRC. His mother had multiple late-onset malignancies, including thyroid, uterine, breast, and metastatic spindle cell carcinoma. His sibling had a clear colonoscopy at age 47 and no cancer history. His two children had clear colonoscopies in their 30s.

Discussion We share this interesting case to highlight the importance of including NTHL1 on hereditary cancer panels. We also add phenotypic data about NAP and highlight the importance of offering updated testing to patients with an unexplained history as new genes are characterized. Since NAP is an autosomal recessive condition, it should be considered when personal history is suggestive even without additional family history. In these families, establishing the diagnosis is extremely useful in assessing CRC risks for relatives.
Risk and Screening of Carriers of the CDKN2A, p.I49T Hispanic Variant

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Background Southern California has a high proportion of Hispanic residents, representing 34–49% of the Orange/Los Angeles County areas. The regional Centers for Clinical Genetics and Genomics in Southern California provide population screening, risk assessment, and genetic testing programs to identify and counsel carriers of pathogenic variants. The CDKN2A: c.146 T > C (p.I49T) variant is characterized by most laboratories as a likely pathogenic, moderate-risk variant, and is found in 0.44% of Hispanic/Latin American individuals. Given the prevalence of this variant within an underserved population and absence of penetrance estimates and management guidelines, the objective of this study was to report findings of personal and family history and screening compliance.

Methods IRB exemption was granted to perform a retrospective chart review and regional data sharing to collate individuals and families found to carry the CDKN2A: c.146 T > C (p.I49T) variant. Information collected included: ancestry, age, personal and family history, genetic test results, management recommendations, and screening compliance.

Results We identified 33 carriers of the p.I49T variant, 30 of whom (90.9%) reported full/partial Mexican ancestry. Two carriers of an additional, high-risk, variant were excluded. Of the 31 remaining, 3 (9.7%) had pancreatic cancer, 1 (3.2%) had melanoma, 4 (12.9%) reported family history of pancreatic cancer, 3 (9.7%) reported family history of melanoma, and 25 (80.6%) reported no family history of pancreatic cancer or melanoma. The average age was 47.3 (range 21–79) and sex ratio was 90.9% female to 9.1% male. Of the 22 (84.6%) identified greater than 6 months ago, 21 were advised to initiate screening, and 14 of 21 (66.7%) had not completed any melanoma or pancreas screening. Due to increasing population screening programs, 7 (21.2%) were identified within the last 6 months. (See Table 1).

Table 1 Screening compliance amongst CDKN2A p.I49T carriers by time since recommendation

| Test result disclosure and recommendation to initiate screening was: | Melanoma screening initiated per total recommended | Pancreas screening initiated per total recommended | No screening (pancreas or melanoma) initiated per total recommended |
|---|---|---|---|
| Greater than 12 months ago | 0/3 (0%) | 0/1 (0%) | 4/4 (100%) |
| Between 6–12 months ago | 6/17 (35.3%) | 4/13 (30.8%) | 10/17 (58.8%) |
| Less than 6 months ago | 0/7 (0%) | 0/3 (0%) | 7/7 (100%) |
| Total | 6/27 (22.2%) | 4/17 (23.5%) | 21/28 (75.0%) |

Conclusions The p.I49T carriers in this study exhibited reduced screening compliance compared to past studies. Ethnic health disparities, limited variant-specific information, particularly for variants identified in non-white populations, and the absence of personal or family history of associated cancers likely contribute to reduced compliance. Further studies are needed to provide accurate risk assessment and management information, to increase male carrier identification, and to improve meaningful communication and screening compliance.

Wnt pathway components in the predisposition to serrated polyposis

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Extensive efforts have been made to elucidate the inherited factors that predispose to serrated/hyperplastic polyposis (SP), a heterogeneous disease associated with a significant personal and familial CRC risk. Germline RNF43 pathogenic variants have been causally linked to the disease, explaining <2% of SP cases. We aimed to identify additional inherited risk factors by performing exome sequencing in 44 non-related SP cases followed by a pathway-centered analysis. We selected rare, predicted damaging variants affecting genes involved in pathways or processes relevant in colorectal carcinogenesis and hereditary cancer, including DNA repair, BMP/TGF-β and Wnt pathways. Mutational burden analysis comparing the frequency of predicted pathogenic variants in cases vs. controls (gnomAD, non-Finnish Europeans) identified significant differences in Wnt pathway components: allele frequency in cases was 50% (44/88), compared to 36.12% (42,692/118,190) in controls (p = 0.007). Differences were not observed when analyzing DNA repair and TGF-β pathway components. Focused on the genes involved in Wnt signaling, we identified 44 rare, predicted damaging variants in 34 Wnt-related genes. Of the 34 genes, 11 harbored significantly more predicted damaging variants in SP cases than in controls. Analysis of the 11 candidate SP genes was performed in further 98 SP and 105 adenomatous polyposis patients obtained through SOLVE-RD project. These genes showed an enrichment of Wnt mutated alleles in both polyposis groups, similar to what had been observed in the original SP cohort. The same analysis performed in 1,006 familial/early-onset CRC patients compared to controls discarded the association of germline (predicted) damaging variants in Wnt components in nonpolyposis phenotypes. Functional analyses are currently being performed to evaluate the effect of the identified variants on Wnt signaling by using a TOP/FOP luciferase reporter assay.
Differences between inherited and acquired polymerase proofreading deficiencies in cancer

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Introduction Pathogenic variants in the exonuclease domain of POLE and POLD1 affect their proofreading activity and promote tumorigenesis. Inherited polymerase proofreading deficiency cause an autosomal dominant cancer- and polyposis-predisposing syndrome. Somatic POLE exonuclease domain mutations are identified in 7–15% of endometrial cancers, 0.5–8% of colorectal cancers, and more rarely in other tumors. Proofreading-deficient tumors present their own characteristic mutations that define a good prognosis and response immune checkpoint inhibitors. Here we aimed to determine the differences between somatic and germline variants to improve their predictive value in the clinical practice.

Methods We carried out a systematic search for variants described in the literature and public databases and classified them according to the ACMG/AMP guidelines adapted to POLE and POLD1 (Mur et al. 2020). Variants classified as pathogenic were analyzed according to their nature, location in the 2D and 3D structure of the protein, mutational characteristics of the tumors, and clinical phenotypes.

Results 18 germline pathogenic variants were identified in 206 carriers from 58 families and 19 somatic variants in 238 tumors (TCGA and COSMIC). Of the 27 variants, 10 were found in the two groups (somatic and germline), while the other 17 were exclusive to one or the other group. While POLE p.P286R, p.V411L, and p.A456P are recurrent somatic mutations that hardly ever occur in the germline, POLE p.L424V and POLD1 p.S478N are recurrent germline variants. Somatic pathogenic variants in POLD1 are very rare, and usually occur together with microsatellite instability (mismatch repair deficiency). The distribution of the variants, both somatic and germline, occurs in the Exo motifs or in their flanking regions, and almost all of them are in direct or close contact to the DNA (DNA binding cleft). Variants in the N-terminal of the exonuclease [Exo I/II] seemed to be more pathogenic than those located in the C-terminal [Exo III/IV/V] (p = 0.0005). When classic somatic mutations occur in the germline, the patient’s phenotype is extremely aggressive and precocious, mimicking the constitutional mismatch repair deficiency syndrome (CMMRD).

Conclusions There are relevant differences between somatic and germline exonuclease domain mutations that may determine the clinical and molecular characteristics of tumors.

Disclosures None.

Mosaic STK11 mutation in the context of a clinical diagnosis of Peutz-Jeghers syndrome and Ashkenazi Jewish ancestry: genetic counseling opportunities and challenges

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Background Peutz-Jeghers syndrome (PJS) is a cancer predisposition condition associated with transient mucocutananeous hyperpigmentation and an increased risk for benign and malignant tumors of the gastrointestinal tract, breast, and reproductive organs due to mutations in STK11. Mosaicism is a phenomenon resulting from development of a genetic mutation in an embryo and leading to the presence of multiple cell lines. There are limited case reports of mosaic PJS.

Case presentation A 35-year-old Ashkenazi Jewish (AKJ) female with a history of three Peutz-Jeghers type polyps of the small bowel and one “lip freckle” which appeared in childhood and faded in adulthood. She denied any family history of cancer or other clinical features of PJS. At her pre-test genetic counseling consultation, she met clinical diagnostic criteria for PJS. Genetic testing identified a low-level mosaic pathogenic mutation in STK11 and two AKJ founder mutations in FANCC and APC.

Discussion This case represents an important minority of persons with a clinical diagnosis of PJS in the context of an apparently de novo, mosaic mutation in STK11. This patient’s STK11 mutation was found at the lowest level detectable by the laboratory and it was reported that it likely would have been missed had a thorough personal and family history not been provided to the laboratory. This highlights the vitality of provision of detailed medical history information at the time of test requisition and the contribution of genetic counselors as members of a medical team to meeting this need.

Further, this patient was aware prior to her initial consultation that persons of AKJ ancestry have a higher frequency of certain genetic conditions. However, this patient demonstrated significant difficulty in understanding the distinction between the general concept of increased frequency of certain genetic conditions in persons of AKJ ancestry, the AKJ founder mutations for which she was identified as a carrier, and her mosaic STK11 mutation which is unrelated to her ancestry. This case illustrates the need for comprehensive pre- and post-test genetic counseling to review complex genetic test results in the context of personal and family history and appropriately manage medical care based on this information.
An international study of duodenal disease in MAP: incidence of polyposis, cancer, and next steps

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Results We recently published the initial findings in 394 patients with genetically confirmed MAP who had undergone one or more upper GI endoscopies (Gastroenterology 2021 Feb;160(3):952–954). 57/394 MAP patients had duodenal disease at index endoscopy (14.5%) at a median age of 51 years (range: 37–81): this was Spigelman stage I in 35 patients (61.4%), stage II in 11 (19.3%), stage III in 10 (17.5%) and stage IV in 1 patient (1.8%). The risk of duodenal polyposis at index endoscopy in MAP depended on genotype, with Y179C homozygotes at highest risk. 202 patients had follow up endoscopies (1463 follow up years). Stage IV disease remained uncommon (1.5% at a median age of 55 years) but three patients (1.4%) developed duodenal cancer, all apparently without prior stage IV disease. Solitary polyps, high grade dysplasia in small adenomas (< 10 mm) and a lack of ampullary disease may also be characteristic of duodenal disease in MAP.

Conclusions Initial cross-sectional analysis suggests duodenal disease in MAP progresses differently to FAP. A better understanding of duodenal disease progression in MAP requires the study of more patients, information on procedure quality, and longer prospective follow up. Our study may enable identification of better markers of disease progression risk and clarify whether and how guidance on surveillance and intervention should be revised. We invite new collaborators to join the group to address these challenges.

Universal tumor screening for Lynch syndrome on colonoscopic tumor biopsy impacts surgical treatment decisions

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Background Universal tumor screening (UTS) for Lynch syndrome (LS) on colorectal cancer (CRC) can be performed on diagnostic colonoscopy biopsies or resected surgical specimens. An advantage of UTS on biopsies is the chance to provide preoperative genetic counseling/testing (GC/T) and make a diagnosis so those with LS can make informed surgical decisions regarding extent of resection. We evaluated the utilization of UTS on biopsy, the percentage of patients with deficient mismatch repair (dMMR) who underwent GC/T preoperatively, and whether surgical decision-making was impacted.

Methods On 1/01/2017, we changed our UTS process to include preoperative genetic counseling/testing (GC/T) and make a diagnosis so those with LS can make informed surgical decisions regarding extent of resection. Among 1,144 CRC patients, 58 (5%) were dMMR and did not have MMR IHC. Nearly half (28/58; 48.3%) of dMMR cases were diagnosed on biopsy. Of those 28, 14 (50%) underwent GC/T at some point, 7 (25%) had GT results prior to surgery. The main reason for not performing dMMR IHC was not occurring outside our health system. Among 1,144 CRCs, 58 (5%) were dMMR and did not have MLH1 promoter hypermethylation when indicated. Nearly half (28/58; 48.3%) of dMMR cases were diagnosed on biopsy. Of those 28, 14 (50%) underwent GC/T at some point. 7 (25%) had GT results prior to surgery (3 already knew they had LS). Of the 6 patients who underwent surgery thus far, 5 had the LS diagnosis included in preoperative decision-making; three elected a more extensive surgery after a risk/benefit discussion with their surgeon. Overall, 5/28 (17.9%) dMMR patients identified on biopsy made an informed surgical decision based on their diagnosis of LS.
Conclusions When applied, UTS on biopsy was effective at increasing the ability to allow for LS patients to have informed surgical decision making. Many challenges remain in using biopsies for LS screening to help inform surgical procedure. To fully see the impact, uptake of this change would also need to occur at referring hospitals and there is room for improvement at our institution including patient follow-up and coordination of pre-surgery genetics consultations.

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APC mosaicism testing in milder polyposis phenotypes reveals pks + *E. coli* bacteria as a possible additional explanation for the development of colorectal adenomas

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**Background** Mosaic mutations in the APC gene have been identified as a common cause (25%) for unexplained polyposis in patients with > 20 adenomas. The frequency of APC mosaicism remains unknown in milder phenotypes.

**Methods** To test for APC mosaicism, we analyzed the APC gene in multiple lesions of patients with unexplained colonic polyposis using Next Generation Sequencing. Additionally, patients with milder phenotypes, e.g. > 20 adenomas at age > 70, were included.

**Results** The mosaicism detection rate was 11% (29/271) in the entire cohort, 4.5% in patients with < 10 adenomas (2/44) and 7.4% in those with 10–20 adenomas (9/122). Stratified for age, 2.5% (1/40) of patients aged > 70 showed with a mosaicism. Besides these “true” mosaicism cases, 23% (61/271) of patients showed a so-called hybrid mosaicism, where multiple, but not all lesions share an identical variant.

Interestingly, 39% (24/61) of hybrids have a specific APC splice variant c.835-8A > G in multiple lesions. Together with other recurring APC variants, this variant was compatible with the recently described mutational signature caused by colibactin, a compound produced by pks + *E.coli*. The possible influence of colibactin needs further exploration. Therefore, we are now performing additional analyses like Whole Genome Sequencing.

**Conclusions** Our results indicate that APC mosaicism also plays a role in milder polyposis phenotypes. Furthermore, a substantial proportion of our cohort had a hybrid mosaicism of which the clinical consequences are not yet clear. In some patients, the presence of pks + *E.coli* might be the explanation for the development of polyps.

High colorectal neoplasia detection rate in first colonoscopy in Lynch syndrome

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**Introduction** Currently published data on colon neoplasia detection rates in Lynch syndrome is distorted by the inclusion of patients with previous neoplasia and multiple past procedures. Our aim was to assess the results of first ever colonoscopy performed on patients with Lynch syndrome.

**Methods** A retrospective review of patients followed in the Hereditary and High-Risk GI Clinic from 8.2014 through 12.2020 was performed. Inclusion criteria included a pathogenic/likely pathogenic variant in a mismatch repair gene with a subsequent first ever colonoscopy performed at our center. All colonoscopies were performed by a single endoscopist (PPS). Standard definitions for advanced adenomas and sessile serrated adenomas (SSA) were utilized. Exclusion criteria included previous colonoscopy or multiple hereditary cancer syndromes. Comparisons were done by Fisher’s exact test.

**Results** There were 73 patients that met criteria, with the majority of patients having variants in *MSH6* (31, 42.5%) and *PMS2* (23, 31.5%) with *MSH2* (13, 17.8%) and *MLH1* (6, 8.2%) less frequent. Further demographic details are available in Table 1. The median age at first colonoscopy was 35 (IQR 29.5–45.5). The mean Boston bowel prep score was 8.4 and the mean withdrawal time on procedures without intervention was 11.3 min. There were 33 patients (45.2%) with any adenoma or SSA on first colonoscopy, including 25 (34.2%) with adenoma and 12 (16.4%) with SSA. Colorectal cancer was found in 2 (2.7%), with advanced adenomas (10, 13.7%) and advanced SSA (7, 9.6%) commonly identified (Table 1). The adenoma detection rate (ADR) was significantly higher in those over 50 years of age (p = 0.04, Figs. 1, 2) and in men (p = 0.04). ADR was similar across mismatch repair genes (p = 0.64).

**Discussion** The high detection rate of colorectal neoplasia and especially advanced neoplasia in Lynch syndrome patients undergoing first colonoscopy highlights the importance of prompt cascade testing to allow for early initiation of colonoscopy. Our results show that ADRs higher than previously published rates are achievable across mismatch repair genes and ages. However, higher ADR targets in patients over age 50 and in men should be considered as colonoscopy quality metrics are established for Lynch syndrome.

**Table 1** Characteristics of Lynch syndrome patients receiving first colonoscopy

| Characteristic | n = 73 |
|---------------|-------|
| Age (median [IQR]), years | 35 [29.5—45.5] |
| Women | 50 (68.5%) |
| Body mass index (median [IQR]) | 28 [24.0—32.2] |
| Race | |
Table 1 continued

|                          | n = 73 |
|--------------------------|--------|
| Caucasian                | 69 (94.5%) |
| African American         | 1 (1.4%)  |
| Asian American           | 2 (2.6%)  |
| Other                    | 1 (1.4%)  |
| Mismatch repair gene mutation |      |
| MLH1                     | 6 (8.2%)  |
| MSH2                     | 13 (17.8%) |
| MSH6                     | 31 (42.5%) |
| PMS2                     | 23 (31.5%) |
| Aspirin use (daily)      | 23 (31.5%) |
| History of cancer        | 20 (27.4%) |
| Family history colorectal cancer |     |
| First degree relative    | 22 (30.1%) |
| Second degree relative   | 39 (53.4%) |
| Colonoscopy results      |        |
| Any adenoma or serrated adenoma | 33 (45.2%) |

**Fig. 1** Adenoma detection rate by age

**Fig. 2** Colonoscopy findings

**Ileal pouch-anal anastomosis is more “desmoidogenic” than ileorectal anastomosis in patients with familial adenomatous polyposis**

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**Background** Desmoid disease is a leading cause of morbidity and mortality in patients with familial adenomatous polyposis (FAP). Abdominal desmoid disease usually follows total proctocolectomy with ileal pouch anal anastomosis (TPC/IPAA) or total abdominal colectomy with ileorectal anastomosis (TAC/IRA). Sex, extraintestinal manifestations, and a 3’ mutation location have been identified as risk factors, but surgical risk factors are poorly understood. We hypothesize that pouch construction creates a higher risk of desmoid formation due to increased stretch of the small bowel mesentery. This study aims to investigate the surgical risk factors for desmoid formation.

**Methods** A single-institution, prospectively-maintained, hereditary colorectal cancer registry was queried for patients with a diagnosis of FAP who underwent either IRA or IPAA between the years 1995 and 2015. Patients who were referred to the registry following index surgery performed elsewhere were excluded, as were patients with desmoid disease diagnosed prior to colon resection. Post-operative development of symptomatic desmoid disease served as the primary endpoint. This was defined by any desmoid disease diagnosed on investigation of patient symptoms of any severity, excluding only diagnoses made purely incidentally. Demographics, presence of extraintestinal manifestations, genotype, family history of desmoid disease, and surgical details were included in analysis. Univariable and multivariable analyses were conducted to determine risk factors.

**Results** 345 patients met inclusion criteria. 172 (49%) patients underwent proctocolectomy/ileoanal pouch, while 173 (51%) underwent total colectomy/ileoanal anastomosis. Overall, 100 (28.9%) developed symptomatic desmoids following surgery. On univariable analysis open surgery and pouch surgery were associated with desmoid development, along with extracolonic manifestations, family history of desmoids, mutation location, and high desmoid risk score. On multivariable analysis, proctocolectomy with pouch was most strongly associated with desmoid disease (p < 0.01, OR 6.49; Table 1). Other associations with desmoid formation were family history of desmoid disease and desmoid risk factor score.

**Conclusions** Polyposis patients who underwent total proctocolectomy with pouch by any approach had significantly greater risk of developing desmoid disease than total colectomy with ileorectal anastomosis, even when accounting for other risk factors (Table 3).
Table 1 Multivariable logistic regression model for desmoid formation (N = 282)

| Risk factor                          | Desmoid formation |
|--------------------------------------|-------------------|
|                                      | Odds Ratios       | 95% Confidence Interval | p value |
| Height (per 10 cm increase)          | 1.50              | 1.09–2.06               | 0.01    |
| Supernumerary teeth (Yes vs. No)     | 0.49              | 0.18–1.36               | 0.17    |
| Family history of desmoids (Yes vs. No) | 4.67              | 1.43–15.24              | 0.01    |
| Desmoid risk factor score (per score increase) | 1.73              | 1.15–2.62               | 0.01    |
| Surgical procedure (TPC/ IPAA vs. TAC/IRA) | 6.49              | 3.21–13.10              | < 0.001 |
| Surgical approach (Open vs. Laparoscopic) | 1.48              | 0.77–2.82               | 0.24    |

Therapy associated polyposis: an acquired and under-recognized polyposis syndrome

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Background Therapy associated polyposis (TAP) remains an ill-defined and under-recognized diagnosis. In their largest multi-institutional cohort of 34 patients, Biller et al. defined TAP as patients with > 10 gastrointestinal polyps without a known causative germline alteration or hereditary colorectal cancer predisposition syndrome, who had a history of prior treatment with chemotherapy and/or radiotherapy for childhood and young adulthood cancer (CYAC). There is ample literature to support increased risk of adenoma formation in CYAC but frank polyposis with tens of hundreds of polyps and their natural history is not well studied.

Case presentation Here we present an interesting patient who was incidentally diagnosed with therapy associated polyposis. This is a 43 year old female who was diagnosed with Hodgkin’s Lymphoma at age 16 and underwent radiation (to the neck, chest and upper abdomen) and chemotherapy (adriamycin, bleomycin, vinblastine, procarbazine.) She remains in remission with good health. She reported dysphagia and constipation for which she underwent endoscopy for the first time at age 43. Endoscopy showed esophagitis and incidentally she was noted to have numerous colonic polyps of size 5 mm to 10 mm. 25 of those polyps were removed with snare and biopsy forceps. Pathology showed tubular adenoma, sessile serrated adenoma and hyperplastic polyps. She has had a total of 4 colonoscopies with removal of approximately 145 polyps of size 2–5 mm and 13 larger polyps of size 10–13 mm. No polyps were noted in the stomach or duodenum. She is considering a subtotal colectomy in the near future.

Germline genetic testing with a 51-gene panel was negative. She has no Ashkenazi Jewish ancestry and no family history of colon polyposis. The only relevant family history was colon cancer in her grandmother in her 70s.

Discussion We write this case report to increase awareness of this acquired polyposis syndrome. TAP should be considered in patients who develop significant polyposis without known causative germline alteration but who have had prior treatment for a CYAC. The Children’s Oncology Group guidelines recommend colonoscopy for CYAC survivors who received abdominopelvic radiotherapy either at age 30 or 5 years after radiotherapy, whichever occurs later, and continue every 5 years.

Improving identification of patients meeting Lynch Syndrome testing criteria in endoscopy

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Introduction ACG guidelines recommend assessment of family history for all patients and referral for genetic testing when indicated. However, the fast-paced nature of clinic visits and high turnover rate of endoscopy units pose significant barriers to this assessment.

Methods We developed a one-page, six-question survey designed to evaluate risk for LS based upon the National Comprehensive Cancer Network (NCCN)-2020 criteria for LS genetic testing. This survey was administered to patients in the waiting room prior to routine outpatient endoscopy at a tertiary GI referral practice over a four-month period from January through April 2021, and was completed on a voluntary basis.

Results A total of 1029 patients completed the survey, and 227 patients (22.1%) screened positive for one of the NCCN LS genetic testing criteria. Additionally, 69 of 227 (6.7% of all patients) met more than one LS testing criteria. Chart review was performed for all patients who screened positive. Of these positive screens, 83 patients had previously been referred for genetic counseling, 16 were known to have LS, and 16 were known to have another genetic cancer predisposition syndrome (e.g. BRCA1, BRCA2).

Conversely, 144 patients (63.4% of positive screens) had no prior genetic counseling and were therefore eligible for a referral for genetic counseling and testing.

Table 1 Clinical, genetic, endoscopy and histology features of the cohort associated with clinically significant progression of the ampullary adenoma

| Factor | Unadjusted analysis* | Adjusted analysis† |
|--------|----------------------|-------------------|
|        | Hazard Ratio | 95% CI | P-value | Hazard Ratio | 95% CI | P-value |
| Age at |          |        |        |          |        |        |
| Ampullary adenoma detection | 1.022 | 1.00–1.04 | 0.04 | 1.022 | 1.00–1.04 | 0.04 |
| FAP diagnosis | 0.999 | 0.97–1.02 | 0.92 | 1.00 | 0.97–1.02 | 0.92 |
| Gender: male vs. female | 1.683 | 0.95–2.98 | 0.08 | 1.91 | 1.02–3.6 | 0.04 |
| Race: non-White vs. White | 2.584 | 1.02–6.57 | 0.05 | 2.584 | 1.02–6.57 | 0.05 |
| Cigarette smoking: reformed vs. active | 1.200 | 0.48–2.97 | 0.69 | 1.200 | 0.48–2.97 | 0.69 |
| Cigarette smoking: never vs. active | 0.802 | 0.35–1.83 | 0.60 | 0.802 | 0.35–1.83 | 0.60 |
| Cholecystectomy: yes vs. no | 2.670 | 1.53–4.66 | < 0.01 | 2.670 | 1.53–4.66 | < 0.01 |
## Abstracts

### Table 1 continued

| Factor | Unadjusted analysis* | Adjusted analysis† |
|--------|----------------------|-------------------|
|        | Hazard Ratio | 95% CI | P-value | Hazard Ratio | 95% CI | P-value |
| Personal history of colorectal cancer | 1.341 | 0.57–3.15 | 0.50 |  |
| Personal history of extra-colonic cancer | 2.082 | 1.10–3.93 | 0.02 | 2.6 | 1.3–5.2 | < 0.01 |
| Family history of colorectal cancer | 0.841 | 0.33–2.14 | 0.72 |  |
| Family history of non-colorectal cancer | 1.158 | 0.28–4.82 | 0.84 |  |
| Years since colectomy to ampullary adenoma detection | 1.016 | 0.99–1.04 | 0.21 |  |
| Chemoprevention use: any vs. none | 0.901 | 0.51–1.59 | 0.72 |  |
| Endoscopic appearance of papilla: abnormal vs. normal | | | |  |
| Tubulovillous + Villous vs. villous | 0.924 | 0.33–2.57 | 0.88 |  |
| Size of duodenal polyps | | | |  |
| 5–10 vs. 1–4 | 1.049 | 0.56–1.95 | 0.88 |  |
| > 10 vs. 1–4 | 1.837 | 0.81–4.15 | 0.14 |  |
| Number of duodenal polyps | | | |  |
| 1–4 vs. 0 | 3.494 | 0.98–12.35 | 0.05 |  |
| 5–20 vs. 0 | 2.223 | 0.66–7.50 | 0.19 |  |
| > 20 vs. 0 | 2.987 | 0.85–10.4 | 0.08 |  |
| Duodenal polyp histology: tubulovillous + villous vs. villous | 1.559 | 0.55–4.36 | 0.39 |  |
| Spigelman stage (baseline) | 0.924 | 0.33–2.57 | 0.88 |  |

*Unadjusted Cox PH regression model used; †Multivariable Cox PH regression model was used, the model included gender, papillary appearance at AA detection, cholecystectomy and extra-colonic malignancy. The hazard ratio for the individual variables was calculated after adjusting for the other three variables in the model. AFAP: attenuated familial adenomatous polyposis; IFAP: intermediate familial adenomatous polyposis; PFAP: profuse familial adenomatous polyposis; SFAP: severe familial adenomatous polyposis.

Finally, 85% of patients found the questionnaire to be easy or somewhat easy to answer.

**Conclusion** This brief self-administered six-question survey identified 22% of the patients presenting for routine outpatient endoscopy as meeting NCCN criteria for genetic testing for LS. Interestingly, the majority (63%) of these patients had no prior referral for genetic testing.

These data suggest that screening of individuals in the endoscopy waiting room is feasible and acceptable to patients, with no disruption to the busy workflow.

It is critical for gastroenterologists to incorporate such innovative workflow to identify these high-risk individuals and to refer them for genetic testing, thereby aiding in CRC prevention for them and for their family members.

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### The natural history of ampullary adenomas in familial adenomatous polyposis: a long-term follow-up study

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**Background and aims** Ampullary adenomas (AA), common in familial adenomatous polyposis (FAP) are precursors to ampullary carcinoma. We assessed the natural history of AA and factors associated with clinically significant progression (CSP) of them.

**Methods** Consecutive FAP patients with AA and 2 esophagogastroduodenoscopies (EGD) were identified from a hereditary gastrointestinal cancer registry. We assessed the incidence of CSP (increase in size to > 10 mm, or development of advanced histology) of AA. Clinical, endoscopic, and pathologic features between patients with CSP and non-progressors were compared.

**Results** 165 patients with AA were included. Over median follow-up of 7.6 years, 50 (30.3%) patients developed CSP. Of the 165, 27 (16.3%) progressed to AA > 10 mm, 12 (7.3%) progressed to advanced histology, and 11 (6.6%) progressed both in size and histology. Two (1.2%) patients developed ampullary cancer. Male gender, abnormal appearance of the papilla at initial AA detection, prior cholecystectomy, and personal history of extra-colonic malignancy were associated with CSP (Table 1). Neither Spigelman stage (Fig. 1) nor APC pathogenic variant were associated with CSP. Intervention specifically for AA and not duodenal polyposis was performed in 20% patients including endoscopic papillectomy in 29 and duodenectomy in 4 at a median observation of 6.3 years.

**Conclusions** A majority of FAP patients with AA did not experience CSP or require AA resection over 8 years of surveillance. Patients...
with CSP are at a high risk of progression to cancer and warrant closer monitoring. Ampullary cancer was rare. Male gender, abnormal appearance of the papilla at AA detection, cholecystectomy and a history of extra-colonic malignancy are associated with CSP. Our findings favor endoscopic surveillance of AA over expedited resection for most patients with FAP.

**Fig. 1** (a) Kaplan Meier cumulative incidence plot showing clinically significant progression in the study cohort. 1b: Kaplan Meier incidence plot showing clinically significant progression as per the Spiegelman Stage at the time of ampullary adenoma detection

**3D patient-derived intestinal organoid models for familial polyposis**

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### Introduction

Juvenile polyposis syndrome (JPS) is an autosomal dominant syndrome associated with germline pathogenic variants (PV) in the SMAD4 and BMPRIA genes in 60% cases. The syndrome predisposes to hamartomatous GI polyps especially in the colon. Also, it increases the risk of developing colonic and gastric cancer. However, true rate of gastric cancer in JPS and genotype–phenotype correlation is unknown. This information may help individualize surveillance endoscopy intervals in these patients. Hence, we conducted a systematic review and meta-analysis to assess the occurrence of gastric cancer in patients with JPS.

### Methods

We searched Medline, Embase and Scopus databases for keywords ‘Juvenile polyposis syndrome, juvenile polyps, stomach cancer, and hereditary cancers.’ Studies from January 1974 to May 2021 were screened. JPS was diagnosed based on phenotype: > 3 colonic juvenile polyps (JP), or multiple JP in other parts of the GI tract or family history of JPS and any number of JP. Studies reporting upper GI manifestations in JPS patients were considered eligible for inclusion. The primary and secondary outcomes were to assess the occurrence of gastric cancer in all patients with JPS and the correlation with the underlying PV, respectively.

### Results

Nine studies including 553 patients met inclusion criteria (Table 1). 254 (45.9%) patients had a SMAD4 PV, 154 (27.8%) had BMPRIA PV, 93 (16.8%) had no identifiable PVs and 52 (9.4%) patients were untested. The pooled occurrence of gastric cancer was 4.5% (95% CI: 1.9, 7.1; I^2: 35.2%). The median age at gastric cancer diagnosis was 42.5 years (range: 29–57.6 years). In studies that did not report the genetic testing, gastric cancer occurred in 7.8% (95% CI: 0, 16.3; I^2: 0) patients.

In patients with known PVs, gastric cancer was seen only in patients with SMAD4 (11.5%, 95% CI: 3.5, 19.6; I^2: 67.1%). There was an overall moderate risk of bias in the studies.

### Discussion/Conclusion

There is an increased risk of gastric cancer in patients with JPS especially in patients with underlying SMAD4 PV. Considering the absence of PV testing in a significant proportion of the patients with JPS, the role of PV like BMPRIA needs to be studied in further large-scale studies.

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**Table 1** Characteristics of the included studies

| Author, Country, Year | Study risk of bias** | Patients with JPS | Occurrence of gastric cancer |
|------------------------|----------------------|-------------------|-----------------------------|
|                        | Total No. PV detected (%) | SMAD4 PV (%) | BMPRIA PV (%) |
|                        | Age diagnosis (median) | Only occurred in patients with SMAD4 PV (%) | SMAD4 PV (%) |

**Note:** All No PV detected, No (%); BMPRIA PV, No (%); NR, not reported.

*Multicenter studies; †Age at any cancer diagnosis, not specific to gastric cancer. [Includes patients from the same family.] §Patients were not tested for pathogenic variants, none of these patients developed gastric cancer. The risk of bias of individual studies was assessed using the validated criteria by Hoy et al.**

**Discussion/conclusion:** There is an increased risk of gastric cancer in patients with JPS especially in patients with underlying SMAD4 PV. Considering the absence of PV testing in a significant proportion of the patients with JPS, the role of PV like BMPRIA needs to be studied in further large-scale studies.
Background Familial adenomatous polyposis (FAP) and MUTYH-associated polyposis (MAP) are inherited genetic syndromes in which colorectal and duodenal polyposis and cancer are the most significant manifestations. Duodenal adenomas from FAP and MAP patients carry somatic mutations in cancer driver genes, as well as in APC and MUTYH, which could also be considered for targeted therapy approaches. Although there are several models of FAP and MAP both in vitro and in vivo; there are no 3D cell models that genetically and phenotypically recapitulate duodenal adenomas from FAP and MAP patients. Thus, development of patient-derived model systems to investigate the pathophysiology and treatment of duodenal polyposis is a high priority.

Methods This study aimed to determine the suitability of 3D patient-derived intestinal organoids as models for drug development in FAP and MAP patients. Normal duodenal mucosa and duodenal adenoma biopsy samples from FAP and MAP patients were obtained at surveillance endoscopy. Intestinal crypts (duodenal, ileal and colon) were dissected out from the whole biopsy and organoid lines were established in Matrigel (Corning), overlaid with Intesticult (Stem Cell), and cultured at scale (CellCes Ltd). CellTiter-Glo® Luminescent Cell Viability was used to measure the viability of the organoids after drug treatment with clinically approved drugs.

Results Sulindac (40 to 1.25 mM) and erlotinib (10 to 0.3125 mM) as single agents or in combination (at a 4:1 ratio) had an effect on the viability of both normal and adenoma lines in a dose dependent manner. Combination treatment of sulindac and erlotinib significantly affected cell viability in adenoma lines in comparison to sulindac as a single agent.

Conclusion Establishing and utilising patient derived organoids from intestinal normal mucosa and adenomatous tissues as an ex-vivo model may provide a much valuable tool for preclinical assessment of drug treatment and precision medicine approaches in polyposis syndromes.

Saturation-scale functional evidence supports clinical variant interpretation in Lynch Syndrome

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Background Lynch Syndrome (LS) is a cancer predisposition syndrome affecting more than 1 in every 300 individuals worldwide. Tumorigenesis in LS is driven by germline loss-of-function variants in DNA mismatch repair (MMR) genes. Clinical genetic testing for LS can be life-saving but is complicated by a large burden of variants of uncertain significance (VUS), especially missense changes. We previously applied deep mutational scanning (DMS) to measure functional effects for > 94% of the 17,746 possible missense variants in the key LS gene MSH2 (Jia et al., AJHG, 2021).

Methods DMS data were overlaid on clinical databases comprising > 15,000 individuals with MMR gene variants detected by paired tumor and germline testing at a clinical genetic testing laboratory. To gauge the strength of evidence provided by the DMS, we curated a list of MSH2 germline missense variants previously classified as Pathogenic (N = 23) or Benign (N = 26) as controls. To avoid redundant application of evidence, these validation variants’ classifications were derived without use of any functional data. Subsequently, we applied the DMS to 720 standing missense VUS, obtained from a distinct cohort from the original validation set.

Results Our functional DMS measurements agreed with the clinical interpretation for all 49 variants. Following recommendations for application of the functional evidence criterion using the ACMG/AMP variant interpretation framework (Brnich et al., Genome Med, 2019), our MSH2 function map can be used with PS3/BS3 evidence codes given its observed strong concordance with these prior clinical interpretations. For the 720 VUS, while most of these are predicted to have a neutral impact on gene function, 29 (4.0%) scored as high-risk**.

Table 1 Univariate and multivariable predictors of advanced colorectal neoplasia (compared to no neoplasia) in Veterans undergoing colonoscopy age < 50

| Patients with ACN n (%) or mean (SD) | Univariate analysis | Multivariate analysis* |
|-------------------------------------|--------------------|------------------------|
| Odds ratio (95% CI) | p | Odds ratio (95% CI) | p |
| **Age group** | | | |
| 20–29 | 4 (1.7%) | Reference | Reference |
| 30–39 | 31 (4.4%) | 3.13 (1.09–9.01) | **0.034** | 3.22 (1.10–9.43) | **0.033** |
| 40–44 | 31 (7.5%) | 7.22 (2.50–20.87) | < **0.001** | 4.81 (1.58–14.64) | **0.006** |
| 45–50 | 75 (10.9%) | 13.40 (4.81–37.32) | < **0.001** | 10.73 (3.73–30.87) | < **0.001** |
| **Sex** | | | |
| Female/Other | 19 (5.0%) | Reference | Reference |
| **Male** | 122 (17.0%) | 1.81 (1.09–3.00) | **0.021** | 2.88 (1.47–5.64) | **0.002** |
| **Body mass index** | | | |
| 30.7 (6.83) | 1.05 (1.02–1.08) | < **0.001** | 1.02 (0.98–1.06) | **0.291** |
| **Prior non-gastrointestinal cancer** | | | |
| No | 129 (6.6%) | Reference | Reference |
| Yes | 12 (13.3%) | 2.5 (1.27–4.90) | **0.008** | 3.65 (1.49–8.92) | **0.005** |
| **Diagnostic indication** | | | |
| Low risk** | 19 (3.8%) | Reference | Reference |
| High-risk*** | 82 (9.0%) | 2.65 (1.57–4.45) | < **0.001** | 2.33 (1.36–4.00) | **0.002** |

*AUC 0.755
**Peri-operative evaluation, change in bowel habits, constipation, diarrhea, other
***Abnormal imaging, Positive FIT/FOBT, Bleeding, Iron-Deficiency
SD: Standard Deviation; CI: Confidence Interval
deleterious in our function map, consistent with previously published rates among other cancer predisposition genes. We are pursuing reclassification for these variants, combining functional evidence with family history and tumor characteristics consistent with Lynch Syndrome. Additionally, in addition to retrospective VUS reclassification, to date these functional data have enabled resolution of five newly detected missense MSH2 VUSs.

**Conclusions** High-throughput assays for mismatch repair loss of function provide a scalable method for VUS resolution and serve as strong evidence criteria for variant classification.

**Rates & risk factors for advanced colorectal neoplasia and anticipated yield of average-risk screening in veterans under age 50**

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**Background** Colorectal cancer (CRC) incidence and mortality in patients age <50 is increasing. Recent guidelines recommend decreasing the average-risk screening age from 50 to 45. The rates and risk factors for advanced colorectal neoplasia (ACN) (advanced polyps or adenocarcinoma) and the yield of screening average-risk Veterans <50 is unknown. Our aim was to describe the rate of and risk factors for ACN in Veterans age <50 and to estimate the yield of expanding screening to average-risk Veterans age 45–49.

**Methods** We conducted a single-center retrospective cohort study including Veterans age 20–49 undergoing colonoscopy for any indication from 2012–2019. We collected Veteran- and colonoscopy-level data and reported rates of non-advanced and ACN. We assessed risk factors for ACN with univariate and multivariable analysis. We assessed the expected yield of expanding screening to average-risk Veterans by reporting neoplasia rates in average-risk equivalent Veterans. We defined this as Veterans 45–49 undergoing colonoscopy for low-risk diagnostic indication (diarrhea, constipation, change in bowel patterns, peri-operative evaluation). These rates were compared to those with ACN in 50–54 year-old Veterans who underwent average-risk screening colonoscopy.

**Results** Of 2,030 Veterans age 20–49 who underwent colonoscopy, 141 (6.9%) had ACN and 831 (40.9%) had non-advanced neoplasia. Multivariable analysis identified older age (OR 10.73, 95% CI 3.73–30.87, 45–49 vs. 20–29), male sex (OR 2.88, 95% CI 1.47–5.64), prior non-gastrointestinal cancer (3.65, 95% CI 1.49–8.92) and high-risk diagnostic indication (bleeding, iron deficiency) (OR 2.33, 95% CI 1.36–4.00) as independent risk factors for ACN (Table 1). Approximately 50% of ACN found in the 45–49 year old average-risk equivalent group were located in the right colon. There was no difference in rate of ACN between average-risk equivalent Veterans 45–49 and average-risk Veterans 50–54 (8.5% vs. 8.8%, p = 0.922) (Figure).

**Conclusions** Male Veterans with high-risk symptoms and prior non-gastrointestinal cancer should be prioritized for colonoscopy. The rate of ACN in average-risk equivalent Veterans 45–49 was comparable to average-risk Veterans 50–54, supporting expanding screening to younger populations. High rates of right-sided ACN suggest colonoscopy as a favored screening modality; however, further study is required to determine optimal screening modality in this patient population.

**Adenomas risk in Lynch syndrome compared to populations with average and familial risk of colorectal cancer**

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Methods Multicentre retrospective study with inclusion of LS colonoscopy surveillance. We selected the first colonoscopy from non-index cases and compared adenomas characteristics, prevalence and age of detection between genes, adjusted by age and gender. We then compared them with a retrospective familial risk cohort (FR)(n = 1,099)[1 FDR < 60-years and/or ≥ 2 FDR with CRC] and an average risk population (n = 3,043). Advanced adenomas (AA) were defined as: ≥ 10 mm in size, high-grade dysplasia, and/or villous component.

Results We included 937 LS carriers, 567(60.5%) women, 318 (33.94%) MLH1, 313 (33.4%) MSH2, 221(23.6%) MSH6 and 85(9.1%) PMS2. The median age was significantly different between genes: MLH1:38(30–45), MSH2:36(28–47), MSH6:42(35–54), PMS2:44(35–55), p < 0.000. No differences were found in the prevalence of adenomas and AA (MLH1:17.3% adenomas/6% AA; MSH2:15.7%/6.7%; MSH6:18.6%/5.9% and PMS2:15.3%/3.5%; p = 0.809 and p = 0.751); although the age of onset was earlier in MLH1/MSH2 carriers (MLH1: adenomas 45(38–56)/AA 50(40–57); MSH2:48(36.5–58)/48(39–50); MSH6:57(41.5–65.5)/57(47–65); PMS2:56(51–66);p = 0.001; p = 0.001 and p = 0.018).

In the FR and AR cohorts, the ages at first colonoscopy were significantly older than in LS (FR:59(55–64) and AR:51(44–59) vs SL; p = 0.000). Between FR and LS cohorts, no differences were found in the prevalence of adenomas and AA (27.5% vs 16.9%, p = 0.897 and 6% vs 8.4%, p = 0.199). MLH1/MSH2 carriers presented adenomas and AA earlier(p < 0.002) but no differences on the age of onset were found between MSH6/PMS2 carriers and FR individuals(p = ns). Between AR and LS, we found no differences in the prevalence of adenomas (16.9% vs 32.5%, p = 0.471), although AR individuals were significantly older (50(39.75–59.25) vs 60(55–65), p = 0.000). The prevalence of AA was higher in AR individuals compared to MLH1/MSH2 (AR 10%, p = 0.016), but the age of detection in the AR cohort was older (60 years (56–65); p = 0.000). However, no differences on AA prevalence nor age of diagnosis were detected when compared to MSH6/PMS2 carriers (p = ns).

Conclusions Although the prevalence of adenomas and AA in LS is similar across genes, the age of onset is older in MSH6 and PMS2 carriers, resembling AR and FR populations. These results support personalized CRC surveillance strategies based on the affected gene.

Comparison of novel healthcare delivery models on the uptake of genetic education and testing in families with a history of pancreatic cancer: The GENetic Education, Risk Assessment, and TESting (GENERATE) Study

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Background Roughly 7–10% of patients with pancreatic ductal adenocarcinoma (PDAC) have a deleterious germline variant. Although identification of germline variants in family members has implications for cancer surveillance, early detection, and interception, cascade genetic testing rates are low. The GENetic Education, Risk Assessment, and TESting (GENERATE) study evaluates novel methods of providing genetic education and testing for individuals at risk for hereditary PDAC.

Methods Eligible participants had: a first- or second-degree relative with a PDAC diagnosis and a known familial germline variant in APC, ATM, BRCA1, BRCA2, CDKN2A, EPCAM, MLH1, MSH2, MSH6, PALB2, PMS2, STK11, or TP53 (Known Familial Mutation
(KFM); or were first-degree relatives of PDAC patients (no KFM). Participants were recruited through six academic centers, patient advocacy organizations, and online outreach. Enrollment occurred through the study website (www.GENERATEstudy.org). Study participation was remote, including at-home genetic testing. Participants were cluster randomized at the family level. Arm 1 included remote genetic education via a live interactive session with a genetic counselor on a video-based telemedicine platform (Doxy.me) plus testing through Color Genomics. Arm 2 included remote genetic education via a pre-recorded video plus testing through Color Genomics.

**Results** Between 5/8/2019–6/01/2021, 423 families were randomized, comprising 595 participants. Recruitment occurred via healthcare providers (n = 128, 21.5%), family members (n = 271, 45.5%), and friends, advocacy groups, and online outreach (n = 223, 37.5%). Participants were referred from six GENERATE academic centers (n = 270, 45.4%) and other institutions (n = 325, 54.6%). Study participants were 52.5 years on average, primarily identified as White (n = 577, 97%), and from the Northeast (n = 184, 30.9%). 296 participants were randomized into Arm 1 and 299 into Arm 2. To date, 527 (88.6%) participants have ordered genetic testing; 253/296 (85.5%) in Arm 1 and 274/299 (91.6%) in Arm 2 (p = 0.049, generalized mixed-effects model) (Table 1). 82 PDAC associated pathogenic variants were identified (Table 2).

**Conclusions** Remote methods of genetic education and testing are successful alternatives to traditional cascade testing, with genetic testing rates nearly 90%. Participant follow-up will assess if satisfaction with decision making, cancer-risk distress, knowledge gained, family communication, and uptake of surveillance were impacted by the mode of pre-test genetic education delivery.

Table 1 Demographics and Uptake of Genetic Testing of Randomized Study Participants in the Doxy.me + Color Genomics arm and Color Genomics only arm

| Age (years) | Doxy.me + Color Genomics Arm N = 296 | Arm 2: Color Genomics Only N = 299 |
|-------------|--------------------------------------|-----------------------------------|
| Mean ± SD   | 53.2 ± 13.9                          | 51.8 ± 14.8                       |
| Range       | 19 – 85                              | 18 – 90                           |
| Sex         |                                      |                                   |
| Male        | 101 (34.1%)                          | 111 (37.1%)                       |
| Female      | 195 (65.9%)                          | 188 (62.9%)                       |
| Racial background |                                  |                                   |
| White/Caucasian | 288 (97.3%)                       | 289 (96.7%)                       |
| Black/African American | 3 (1.0%)            | 2 (0.7%)                         |
| American Indian/Alaskan Native | 0                               | 0                                 |
| Asian/Asian-American | 2 (0.7%)                      | 2 (0.7%)                         |
| Native Hawaiian/other Pacific Islander | 0                           | 1 (0.3%)                         |
| Two or more races | 3 (1.0%)                     | 4 (1.3%)                          |
| Unknown     | 0                                    | 1 (0.3%)                          |
| Ethnic background |                                  |                                   |
| Hispanic or Latino | 7 (2.4%)                   | 4 (1.3%)                          |
| Non-Hispanic or Latino | 282 (95.3%)        | 293 (98.0%)                       |
| Unknown     | 7 (2.4%)                             | 2 (0.7%)                          |
| Geographic Location* |                                  |                                   |
| Northeast   | 90 (30.4%)                           | 94 (31.4%)                        |

**Table 2 Variants Detected in the Doxy.me + Color Genomics arm and Color Genomics only arm Among randomized participants who obtained genetic testing**

| Overall KFM | Non-KFM | Overall KFM | Non-KFM |
|-------------|---------|-------------|---------|
| Overall number of variants detected (n = 115) | 57 | 38 | 19 | 58 | 44 | 14 |
| Pathogenic PDAC associated variants detected (N = 82) | 42 | 36 | 6 | 40 | 38 | 2 |

**Table 2 continued**

| | Doxy.me + Color Genomics Arm N = 296 | Arm 2: Color Genomics Only N = 299 |
|-------------------------|--------------------------------------|----------------------------------|
| **Midwest**             | 71 (24.0%)                           | 83 (27.8%)                       |
| **South**               | 86 (29.1%)                           | 72 (24.1%)                       |
| **West**                | 49 (16.6%)                           | 50 (16.7%)                       |
| Referring Institute     |                                      |                                  |
| GENERATE institution**  | 130 (43.9%)                          | 140 (46.8%)                      |
| None of these institutions | 166 (56.1%)                     | 159 (53.2%)                      |
| How did you hear about the study? [multiple choices] |                                  |                                  |
| From a healthcare provider | 65 (22.0%)                      | 63 (21.1%)                       |
| From a family member    | 135 (45.6%)                          | 136 (45.5%)                      |
| Other***                | 116 (31.2%)                          | 107 (35.8%)                      |
| Uptake of genetic testing‡ | 253 (85.5%)                     | 274 (91.6%)                      |

*Northeast = Maine, New Hampshire, Vermont, Massachusetts, Connecticut, Rhode Island, New York, New Jersey, Pennsylvania; Midwest = North Dakota, South Dakota, Nebraska, Kansas, Minnesota, Illinois, Missouri, Iowa, Wisconsin, Michigan, Ohio, Indiana; South = Tennessee, Kentucky, Texas, Oklahoma, Arkansas, Louisiana, Mississippi, Alabama, Georgia, Florida, South Carolina, North Carolina, West Virginia, Virginia, District of Columbia, Maryland, Delaware; West = Washington, Oregon, California, Nevada, Arizona, New Mexico, Colorado, Utah, Idaho, Wyoming, Montana, Hawaii, Alaska

**Referring GENERATE sites include Dana-Farber Cancer Institute, Johns Hopkins University, Mayo Clinic, MD Anderson Cancer Center, University of California, San Diego, and Weill Cornell

***Other includes patient outreach through friends, advocacy groups, internet campaigns, and social media

‡ Between-group comparison of the uptake of genetic testing using a generalized mixed-effects model: Odds Ratio 0.56 (0.95CI; 0.32 to 1.00, p = 0.049)
Results

Thirty-four individuals were identified to have a deletion involving EPCAM; 12 individuals from nine families with 3' EPCAM deletions and 22 individuals from 17 families with EPCAM-MSH2 deletions. 3' EPCAM deletions were identified via a next-generation sequencing (NGS) panel in nine cases and targeted variant testing (TVT) in the remaining three. EPCAM-MSH2 deletions were identified via NGS in 18 cases and TVT in the remaining four.

Conclusion While CRC was the most commonly reported cancer in both cohorts (7/12, 58.3% 3' EPCAM; 11/22, 50.0% EPCAM-MSH2). In the 3' EPCAM cohort there was one report of a sebaceous carcinoma and small bowel cancer, and five individuals with adenomatous polyps. Other LS-associated cancers were not reported and infrequently reported in family histories. In contrast, the EPCAM-MSH2 cohort reported several other cancers: gynecological (n = 3), prostate (n = 1), breast (n = 1), and skin (n = 2). Adenomatous polyps and sebaceous neoplasms were also reported. The provided family histories showed a preponderance of typical LS cancers.

Table 2 continued

|                     | Doxy.me + color genomics arm | Color genomics only arm |
|---------------------|-----------------------------|-------------------------|
|                     | Overall | KFM | Non- | Overall | KFM | Non-KFM |
| PALB2 (n = 6)       | 2       | 2    | 0    | 4       | 4    | 0        |
| PMS2 (n = 2)        | 2       | 2    | 0    | 0       | 0    | 0        |
| STK11 (n = 1)       | 1       | 1    | 0    | 0       | 0    | 0        |
| Other pathogenic variants* (n = 13) | 5  | 2    | 3    | 8  | 3 | 5 |
| Low penetrance variants† (n = 20) | 10 | 0    | 10   | 10 | 3 | 7 |
| Double variant carriers‡ (N = 6) | 3  | 2    | 1    | 3  | 3 | 0 |

* There were no pathogenic variants detected in APC, EPCAM, MSH6 and TP53
† Includes low penetrance variants APC I1307K (4), CHEK2 Ile1577Thr (4), and monosomic MUTYH (12), which are not believed to be associated with PDAC susceptibility.
‡ Includes 1 participant carried ATM/BRA2, 1 carried ATM/BRA2/MUTYH, 1 carried MUTYH/BRIP1, 1 carried MUTYH/BRIP1, and 2 carried MUTYH/BRIP1, Pathogenic variants in CHEK2, BRCA2, BRCA1, and RAD51D are not believed to be associated with PDAC susceptibility. BRIP1 may play a role in PDAC susceptibility.
§ Includes low penetrance variants APC I1307K (4), CHEK2 Ile1577Thr (4), and monosomic MUTYH (12), which are not believed to be associated with PDAC susceptibility.

A comparison of phenotypes associated with 3' EPCAM deletions versus combined EPCAM-MSH2 deletions

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Background Approximately 1%-3% of Lynch syndrome (LS) is due to germline deletions of the 3' end of EPCAM resulting in epigenetic silencing of MSH2. The EPCAM deletion can extend into the 5' end of the MSH2 gene resulting in a combined EPCAM-MSH2 deletion. The associated phenotype and cancer risks associated with confined 3' EPCAM deletions were initially reported as different from EPCAM-MSH2 deletions. However, recent updates to NCCN LS cancer risks and management strategies combine these deletions with respect to cancer risk and preventative recommendations. We aimed to determine if a difference in phenotypes exists between 3' EPCAM versus EPCAM-MSH2 deletions.

Methods We retrospectively reviewed clinical histories of individuals identified to have pathogenic/likely pathogenic (PV) 3' EPCAM and EPCAM-MSH2 deletions who underwent genetic testing for hereditary cancer syndromes from 2013 through May 2021.

Results Thirty-four individuals were identified to have a deletion involving EPCAM; 12 individuals from nine families with 3' EPCAM deletions and 22 individuals from 17 families with EPCAM-MSH2 deletions. 3' EPCAM deletions were identified via a next-generation sequencing (NGS) panel in nine cases and targeted variant testing (TVT) in the remaining three. EPCAM-MSH2 deletions were identified via NGS in 18 cases and TVT in the remaining four. Colon cancer (CRC) was the most commonly reported cancer in both cohorts (7/12, 58.3% 3' EPCAM; 11/22, 50.0% EPCAM-MSH2). In the 3' EPCAM cohort there was one report of a sebaceous carcinoma and small bowel cancer, and five individuals with adenomatous polyps. Other LS-associated cancers were not reported and infrequently reported in family histories. In contrast, the EPCAM-MSH2 cohort reported several other cancers: gynecological (n = 3), prostate (n = 1), breast (n = 1), and skin (n = 2). Adenomatous polyps and sebaceous neoplasms were also reported. The provided family histories showed a preponderance of typical LS cancers.

Risk perceptions and adherence to preventive screening in patients with Lynch Syndrome (LS)

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Background LS is a common hereditary cancer predisposition syndrome affecting ~ 1/300 individuals. Patients affected by LS have increased lifetime risk of colorectal cancer (CRC), endometrial, and multiple other cancers. The variable penetrance of LS means some patients manifest multiple cancers over a lifetime, while others never develop cancer. Additionally, screening recommendations for patients with LS are complex, minimally evidence-based, and ever-changing. These factors impact patient perceptions of risk and disease severity, perceived screening burden, and screening adherence. Understanding risk perceptions and screening behaviors among LS patients supports effective counseling and development of more acceptable prevention interventions for this population.

Methods LS patients from Fox Chase Cancer Center (FCCC) Risk Assessment Program registry were recruited to the PreventLYNCH study (IRB 20-8014). Following electronic informed consent, a link to a REDCap-administered survey was provided (active 1/2021 to 6/2021). Surveys queried demographics, items specific to LS diagnosis, screening behaviors, perceptions of screening intervals, and beliefs regarding LS severity. Factors hypothesized to be relevant in predicting screening behaviors were assessed using stratified analyses (Stata 11SE). Chi-square tests reported as odds ratios and odds trends (trend) for categorical variables assessed associations.

Results In total, 116 participants (104 FCCC patients, 12 family members) completed the survey out of ~ 219 LS patients invited to participate (response rate ~ 48%). Participants were 76% female and 96% self-identified as white. Mean age was 52.7 years. Familial mismatch repair deficiency among participants included: MLH1 (15.5%), MSH2 (27.6%), MSH6 (27.6%), and PMS2 (25.9%) (4 did not respond). In total, 72% of participants had a personal history of (at least one) cancer and 72% had a family cancer history in a close relative. Table 1 shows associations of LS patient characteristics with disease and susceptibility perceptions; Table 2 shows rates and...
associations of patient characteristics with LS and age-appropriate cancer screening.

**Conclusion** Results suggest that among LS patients, personal cancer history and familial cancer history have strong but often non-overlapping impact on disease and susceptibility perceptions. Beyond CRC screening where rates are universally ≥95%, LS patients with personal cancer history are most likely to pursue cancer screening options and maintain strict screening intervals.

### Table 1

| Lynch syndrome is a severe genetic condition | Lynch syndrome is a serious threat to my health | What is your What is your chance of developing a new Lynch cancer in the next 5 years? |
| --- | --- | --- |
| Age (> = 50 vs < 50) | NS | NS | NS |
| Sex (F vs M) | NS | NS | NS |
| MLH1/MSH2 vs MSH6/PMS2 | NS | NS | NS |
| Personal history cancer (Yes vs No) | + 0.07 | + 0.0002 |
| Family history cancer (Yes vs No) | + 0.02 | + 0.05 | NS |
| Years (> = 5 vs < 5) since LS diagnosis | NS | NS | + 0.07 |

| Screening test | Subgroup | Yes have had this test (%) | How often I undergo this test (%) |
| --- | --- | --- | --- |
| Colonoscopy | Overall rate | 96.6 | Yearly 60.5, Every few years 36.8 |
| Male | 96.4 | 60.7 | 35.7 |
| Female | 96.6 | 60.5 | 37.2 |
| Age < 50 | 93.9 | 58.3 | 37.5 |
| Age ≥ 50 | 98.5 | 62.1 | 36.4 |
| MLH1/MSH2 | 96.8 | 75.5 | 20.4 |
| MSH6/PMS2 | 96.0 | 45.9 | 52.5 |
| + Personal Hx | 96.4 | 66.7 | 30.9 |
| -Personal Hx | 97.0 | 45.5 | 51.5 |
| Family Hx | 97.5 | 62.5 | 35.0 |
| -Family Hx | 97.0 | 54.8 | 41.9 |
| Overall rate | 84.5 | 19.6 | 69.2 |
| Male | 78.6 | 28.0 | 56.0 |
| Female | 86.4 | 17.1 | 73.2 |
| Age < 50 | 83.7 | 15.6 | 73.3 |
| Age ≥ 50 | 85.1 | 22.6 | 66.1 |
| MLH1/MSH2 | 80.0 | 27.1 | 58.3 |
| MSH6/PMS2 | 87.1 | 10.9 | 80.0 |
| + Personal Hx | 84.3 | 20.8 | 68.8 |
| -Personal Hx | 84.9 | 16.7 | 70.0 |
| + Family Hx | 84.0 | 19.7 | 71.1 |
| -Family Hx | 84.4 | 20.7 | 71.1 |
| Overall rate | 84.5 | 19.6 | 69.2 |
| Male | 78.6 | 28.0 | 56.0 |
| Female | 86.4 | 17.1 | 73.2 |
| Age < 50 | 83.7 | 15.6 | 73.3 |
| Age ≥ 50 | 85.1 | 22.6 | 66.1 |
| MLH1/MSH2 | 80.0 | 27.1 | 58.3 |
| MSH6/PMS2 | 87.1 | 10.9 | 80.0 |
| + Personal Hx | 84.3 | 20.8 | 68.8 |
| -Personal Hx | 84.9 | 16.7 | 70.0 |
| + Family Hx | 84.0 | 19.7 | 71.1 |
| -Family Hx | 84.4 | 20.7 | 71.1 |
| Overall rate | 84.5 | 19.6 | 69.2 |
| Male | 82.1 | 50.0 | 38.5 |
| Female | 60.2 | 0.014 | 55.6 |
| Age < 50 | 57.1 | 40.5 | 26.2 |
| Age ≥ 50 | 71.6 | 64.3 | 19.6 |
| MLH1/MSH2 | 70.0 | 62.8 | 18.6 |
| MSH6/PMS2 | 62.9 | 48.1 | 25.0 |
| + Personal Hx | 73.5 | 63.0 | 19.2 |
| -Personal Hx | 45.5 | 0.002 | 28.0 |
| Family Hx | 66.7 | 51.4 | 25.7 |
| Overall rate | 82.8 | 63.0 | 27.8 |

Table 2 Cancer screening uptake and interval in LS patients
Lynch Syndrome patients’ perceptions of current prevention options and novel approaches to cancer prevention

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Background LS is a common hereditary cancer predisposition syndrome affecting ~1/300 individuals. LS patients have increased lifetime risk of colorectal, endometrial, and multiple other cancers. Beyond colonoscopy and aspirin chemoprevention, prevention options for LS patients remain largely unsupported by high-level clinical trial data. Several LS prevention studies are ongoing or under development, but whether patients will participate in trials or adopt novel prevention options remains uncertain. Understanding LS patients’ preferences towards cancer prevention research and options can aid in developing highly effective and impactful interventions.

Methods LS patients from Fox Chase Cancer Center (FCCC) Risk Assessment Program were recruited to the PreventLYNCH study (IRB 20–3014). Following electronic informed consent, a link to a REDCap-administered survey was provided (active 1/2021–6/2021). Surveys queried perceptions of current and novel prevention modalities (e.g. perceived inconvenience, prevention benefit/reassurance, side effect concerns, likelihood of study participation). Screening colonoscopy and daily aspirin chemoprevention served as benchmarks to gauge relative preferences for 10 novel options for prevention [Fig. 1]. Factors hypothesized as relevant in predicting behaviors and preferences were assessed using stratified analyses (Stata 11SE).

Results Altogether, 116 participants (104 FCCC patients, 12 relatives) completed the survey from ~219 LS patients invited to participate (response rate ~48%). Participants were 76% female and 96% self-identified as white. Mean age was 52.7 years. Familial mismatch repair deficiency included: MLH1 (15.5%), MSH2 (27.6%), MSH6 (27.6%), or PMS2 (25.9%) (4 non-response). Colonoscopy uptake was high (96%), while daily aspirin uptake was low: 35% (33/95) patients took daily aspirin—76% (25/33) took aspirin specifically for LS prevention (Fig. 1). Substantial subgroup variability was observed in behaviors and preferences for current and novel prevention options [Fig. 2].

Conclusion LS patients have highly varied prevention behaviors and preferences. Despite high convenience and high acceptability of side effects, uptake of aspirin is low, likely secondary to low perceived reassurance of protective benefits. Novel approaches to prevention offering nutritional feedback and/or exercise-based interventions may be more acceptable to LS patients than vaccine, gene-therapy, or immunotherapy.

Fig. 1 Colonoscopy and aspirin uptake among patients with LS

![Fig. 1 Colonoscopy and aspirin uptake among patients with LS](image-url)
Dietary risk factors and colorectal neoplasia in patients with Lynch syndrome (LS)

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Background Patients with Lynch syndrome (LS), an autosomal dominant inherited cancer syndrome caused by pathogenic germline variants in DNA mismatch repair genes, have an increased risk of colorectal cancer (CRC). Diets high in alcohol, red and processed meats and low in calcium, dairy, and fiber intake (including leafy vegetables and whole grains) have been associated with increased risk for sporadic CRC. We sought to investigate whether dietary risk factors may also affect risk for colorectal neoplasia (CRN) in patients with LS.

Methods Fourteen patients with genetically confirmed LS were enrolled in the University of Michigan Family Microbiome Initiative from 2014 to 2021 through Hereditary cancer clinic. At baseline, demographics and dietary information and were collected using a general questionnaire and a food frequency questionnaire that captured their general food intake over the past year. Subjects submitted stool samples for microbiome analysis. We investigated the differences in several food groups and Healthy Eating Index (HEI), a score used to approximate food-based diet quality. In order to compare subjects with and without a history of colorectal neoplasia, we used Wilcoxon’s ranks test for continuous variables and Fisher’s exact test for categorical variables.

Results Of the 14 subjects the median age at enrollment was 52 years (IQR: 38–64) and 75% were female. Four (27%) individuals were diagnosed with adenomas with a median age of diagnosis at age 60. There were no differences in age, body mass index (BMI), energy intake, alcohol, red meat, processed meat,
dairy, vegetables, dietary fiber consumption and HEI score according to CRN diagnosis.

**Conclusion** Although we did not find an association between dietary factors and risk for colorectal neoplasia in patients with LS, we recognize this analysis was limited by the small sample size. We plan to increase enrollment of LS and future studies will correlate microbiome composition with diet to identify potential markers to predict risk for CRN in LS (Table 1).

### Table 1 Characteristics of patients with history of colorectal neoplasm and no history of colorectal neoplasm at baseline

| Characteristic            | Without Hx of CRN | With Hx of CRN | p-value |
|---------------------------|-------------------|----------------|---------|
| Age                       | 57 (35, 60)       | 60 (52, 69)    |         |
| BMI                       | 28 (22, 32)       | 28 (22, 6,780) | > 0.9   |
| Female sex                | 9 (82%)           | 3 (75%)        | > 0.9   |
| Tobacco use               | 0.090             |                |         |
| Non-smoker                | 8 (73%)           | 1 (25%)        |         |
| Former smoker             | 3 (27%)           | 1 (25%)        |         |
| Smoker everyday           | 0 (0%)            | 1 (25%)        |         |
| Exposed to second hand smoke | 0 (0%)          | 1 (25%)        |         |
| Total dietary fiber, g/day| 20 (12, 23)       | 11 (10, 15)    | 0.3     |
| Calcium, mg/day           | 676 (540, 985)    | 684 (532, 751) | 0.6     |
| Dairy, g/day              | 420 (293, 810)    | 234 (104, 408) | 0.2     |
| Total yogurt, g/day       | 6 (0, 97)         | 20 (9, 67)     | 0.8     |
| Red meat, g/day           | 180 (29, 220)     | 182 (130, 243) | 0.5     |
| Processed meat, g/day     | 132 (91, 184)     | 79 (73, 113)   | 0.6     |
| Alcohol, g/day            | 0 (0, 3)          | 1 (0, 12)      | 0.7     |
| Energy, kcal/day          | 1,583 (1,254, 2,021) | 1,215 (885, 1,523) | 0.2 |
| N3-PUFA                   | 1.34 (0.88, 1.63) | 1.10 (0.88, 1.50) | 0.9 |
| N6-PUFA                   | 13.4 (9.0, 1.63)  | 9.5 (8.6, 11.2) | 0.8 |
| HEI score                 | 67 (52, 76)       | 71 (66, 74)    | 0.6     |
| Total Vegetable, servings/day | 0.95 (0.70, 1.80) | 1.73 (0.92, 2.61) | 0.6 |

1 Median (Interquartile range [IQR]); n (%)  
2 Wilcoxon rank sum test; Fisher’s exact test; Wilcoxon rank sum exact test  
3 CRN colorectal neoplasia  
4 BMI body mass index  
5 HEI Healthy eating index

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**Patients with GIST evaluated in a hereditary risk program**

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**Background** GISTs are most often sporadic, resulting from somatic (non-inherited) mutations in the KIT or PDGFRA genes. GISTs which test negative for these by IHC or molecular analysis are considered “wild-type GISTs” resulting in a referral for a genetic evaluation. Additional clinical features for genetics referrals include 3 or more primary GISTs in an individual or 3 or more close relatives with GISTs. Tumor genomic analysis has become an important tool for identifying mutations in individuals who may have an inherited predisposition to GISTs. Germline mutations in KIT, PDGFRA, NF1 and the SDH genes increase the risk for GISTs and have implications for screening for additional cancers as well as cascade testing for family members.

**Methods** A cross-query of the FCCC tumor registry and IRB approved Risk Assessment Program (RAP) registry databases searched for consented individuals with either a GIST or a germline mutation in KIT, PDGFRA, NF1, SDHA/B/C/D. The age, gender, c-KIT status, tumor location and family history of individuals with both a GIST and germline mutation were reviewed.

**Results** Approximately 55 patients with GISTs were seen annually at Fox Chase Cancer Center. Of the 34 genetics referrals, 16 proceeded with germline genetic testing and germline mutations were identified in 7 individuals including: KIT with a CHEK2(1), NF1 (1), SDHA (2) one with a CHEK2, MLH1 (1), PMS2 with a VUS in SDHA(1), MUTYH monoallelic (1). The mean age of diagnosis was 35. The majority were c-KIT positive. Four tumors were located in the stomach, 2 were small bowel and one was para-esophageal. Four had additional malignancies. Only the proband with a KIT germline mutation had a family history of a GIST, also early onset, and the proband had multiple GISTs.

**Conclusions** Our data suggest early age of diagnosis as the strongest predictor for a germline mutation. Family history and c-KIT status were unreliable predictors. Tumor genomic analysis is currently the most efficient way to identify individuals who may carry germline mutations related to GISTs (Table 1).
Table 1 ATM mutations detected in colorectal tumors from patients with known pathogenic germline ATM mutations

| Patient | Germline Mutation | ATM Mutation in Tumor | VAF of Mutation in Tumor | Somatic Mutation in Tumor | ATM Mutation in Tumor | VAF of Mutation in Tumor | Tumor Content | Tumor ATM Tumorigenesis? |
|---------|-------------------|-----------------------|---------------------------|---------------------------|-----------------------|---------------------------|---------------|--------------------------|
| 1       | p.E2052K, c.6154G > A* | 50%                  |                           |                           | p.R2227C, c.6679C > T  |                           |               |                          |
| 2       | p.F2799K, 8395_8404del | 24%                  |                           |                           |                       |                           |               |                          |
| 3**     | p.R2993*, c.8977C > T | 49%                  |                           |                           |                       |                           |               |                          |
| 4       | p.Q522H, c.1564_1565del | 79%                  |                           |                           |                       |                           |               |                          |
| 5       | p.S1905*, c.5712del   | 79%                  |                           |                           |                       |                           |               | 60%                      |
| 6**     | p.V2424G, c.7271 > G  | 82%                  |                           |                           |                       |                           |               |                          |
| NA      | 60%                | Yes                   |                           |                           |                       |                           | 60%           |                          |
| NA      | 70%                | Yes                   |                           |                           |                       |                           | 70%           |                          |

*Germline classification is conflicting depending on laboratory (ranging to variant of uncertain significance to Pathogenic)

**VAF was identical in germline and tumor samples at 24%. Reference allele bias accounts for the lower than expected VAF.

***Patient 3 also carried a germline pathogenic NBN Slavic Founder mutation, p.K219Nfs*16, c.657_661del.

****Patient 6 also carried a germline pathogenic CHEK2 p.T367Mfs*15, c.1100delC mutation.

VAF = variant allele fraction

NM_000051.3 is the ATM reference transcript used for mutation annotations

Germline pathogenic variant/likely pathogenic variant incidence in individuals with colorectal cancer stratified by age at diagnosis

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Background Colorectal cancer (CRC) is the fourth most common cancer in the United States, with the incidence increasing among individuals under the age of 50. Current National Comprehensive Cancer Network (NCCN) guidelines recommend germline genetic testing for all patients diagnosed with CRC at or after age 50, supporting the current NCCN genetic testing recommendation of germline genetic testing for all patients diagnosed with CRC under age 50.

Methods From January 01, 2017 through December 31, 2019, germline genetic testing of various hereditary cancer genes was performed in 98 participants diagnosed with CRC. All 98 participants met with a certified genetic counselor for pre-test genetic counseling. Participants were retrospectively stratified into cohorts based on age of CRC diagnosis: individuals diagnosed with CRC before age 50 (n = 54, 55.10%) and individuals diagnosed with CRC at or after age 50 (n = 44, 44.90%). Participants diagnosed under age 50 were further subcategorized as participants who were diagnosed between ages 45 and 49 (n = 18), participants who were diagnosed between ages 40 and 44 (n = 16), and participants who were diagnosed under age 40 (n = 20).

Results The incidence of PV/VLPs identified in these cohorts was analyzed. The identified PV/VLPs were categorized as Lynch Syndrome (LS)-associated PV/VLPs, Hereditary Breast and Ovarian Cancer (HBOC)-associated PV/VLPs, or other PV/VLPs. Among the 54 participants diagnosed with CRC under age 50, 11 PV/VLPs were identified (20.37%). The PV/VLP rate in this cohort was similar to the cohort of patients diagnosed at or after age 50 (n = 10, 22.72%); however, the incidence of PV/VLPs identified in LS-associated genes was increased among the cohort of individuals diagnosed with CRC prior to age 50 (45.45%) as compared to the cohort of individuals diagnosed at or after age 50 (30.00%).

Discussion Our study found an increased incidence in LS-associated PV/VLPs among cohorts of patients diagnosed with CRC under age 50, supporting the current NCCN genetic testing recommendation of germline genetic testing for all patients diagnosed with CRC under age 50.

Support for ATM gene as a driver of colorectal cancer

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Background Germline ATM pathogenic variants are associated with increased risk for breast cancer and pancreatic cancer in the heterozygous state. Previous studies have suggested a possible association with colorectal cancer (CRC) risk, although this is controversial.

Methods We evaluated 1,703 unscreened population-based CRC patients enrolled in the Ohio Colorectal Cancer Prevention Initiative (OCCPI, 2013–2016) with either multigene panel germline and/or tumor sequencing results. We assessed germline and somatic ATM likely pathogenic or pathogenic variants (PV) via next-generation sequencing and large rearrangement analysis. To determine if somatic ATM PV occurred by chance alone, we compared the rate of somatic ATM PV among the patients with germline ATM PV to the rate of somatic ATM PV among CRCs without germline ATM PV that were not MSI-high, POLE-ultrahypermutated, and with tumor content > 20%.

Results We identified 13 (0.8%) CRC patients with confirmed (9) or suspected (4) germline PV in the ATM gene, compared to 0.5% in the general population using gnoMAD v2.1.1 (RR 1.5, 95% CI 0.9–2.7, p = 0.13). ATM PV clustered in the FAT and kinase domains. We had paired tumor sequencing for 6 confirmed germline cases and all 6 (100%) showed a second somatic ATM PV; 3 sequence changes and 3 with loss of heterozygosity (LOH) of the ATM locus (Table 1). Among the 154 CRC cases without a germline ATM PV but with tumor sequencing results, 66 (30%) had a somatic ATM PV or LOH of the ATM locus. The difference was statistically significant (Table 2; p = 0.0009).

Conclusion While we did not find substantially increased prevalence of germline ATM PV in our CRC cohort, our data support that a subset...
of CRCs are caused by germline ATM mutations with acquired second-hits in the ATM gene. More studies are warranted to determine if ATM PV are associated with increased risk of CRC. We hypothesize that a subset of specific ATM PV in certain domains may be associated with higher CRC risk.

Table 2  Tumor 2nd Allele Inactivation in CRC Tumors of Germline ATM Carriers vs. Controls

| ATM LOH or Cases with Germ-Mutation in line ATM mutations | line ATM mutations* |
|----------------------------------------------------------|---------------------|
| Tumor?                                                    |                     |
| Yes                                                      | 6                   |
| No                                                       | 0                   |
| %                                                       | 100%                |
|                                                          | 30%                 |

Fisher exact test, p = 0.0009

*Controls consisted of consecutive OCCPI CRC patient tumor samples sequenced on the same platform as cases
56 controls had LOH, and 17 had an ATM mutation. 7 controls had LOH and an ATM mutation

Novel method for variant reclassification and extreme cascade testing among Lynch syndrome families utilizing a public genealogical database

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Background Cascade testing for cancer predisposition is the process of identifying and testing at-risk relatives after identification of a pathogenic variant in a proband. This allows for cancer prevention in unaffected relatives. Standard practice for genetic counselors includes ascertaining a three-generation pedigree and educating the proband to inform relatives of their risk and options for testing. However, this approach typically leads to only 3–4 relatives being tested. We sought to increase uptake of cascade testing by identifying distant relatives via public genealogical database, which also facilitated germline variant reclassification for one family.

Methods 3310 colorectal cancer (CRC) patients and 342 endometrial cancer (EC) patients were enrolled into the Ohio Colorectal Cancer Prevention Initiative (2013–2016). Those with mismatch repair-deficient tumors underwent germline multigene panel testing, among others. Two seemingly-unrelated probands (1. CRC 42, absent MSH2/6, 2. EC 44, absent MSH6) were identified as having a variant of uncertain significance in MSH6 (c.1109 T > C, p.L370S). Both received genetic counseling and three-generation pedigrees were obtained (and showed no relationship connection). However, both probands were from the same small town in Ohio. The genetic counselor used public genealogical database, Ancestry.com, with available birth/death certificates, marriage certificates, and census records to link the probands.

Results The probands linked to common ancestors from the 1800s, confirming they were 3rd cousins (Fig. 1). Co-segregation analysis using Thompson et al. 2013 full-likelihood method (PMID 12,900,794) showed a 2328:1 likelihood supporting pathogenicity. This led to reclassification of the VUS as pathogenic. Once linked, 6 additional branches of 3rd cousins were identified, and ultimately thousands of additional distant at-risk relatives. To date, 148 relatives of the two probands have undergone cascade testing (52 positives) and another branch segregating the variant was identified. Ninety-two tested individuals were greater than 3rd-degree relatives to at least one of the probands, and many were not on the original pedigree(s).

Conclusion Utilizing public genealogical databases for families with the same rare variant can significantly increase cascade testing and assist in variant reclassification by identifying the furthest common ancestor. Opportunities for future research should consider how to alert distant relatives of their risk.

Fig. 1 Segregation of MSH6 (c.1109 T > C, p.L370S). Shaded individuals tested positive for the mutation or are confirmed obligate carriers.
Facilitating communication about Lynch Syndrome within families: development and assessment of an informational resource

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Background In the past decade, many centers have implemented universal screening for Lynch Syndrome (LS) and established multidisciplinary clinics for annual follow-up, generating tremendous potential for cancer prevention. However, alerting at-risk individuals to genetic testing and cancer prevention strategies is reliant on communication within families and there is a paucity of data assessing communication tools used to facilitate this. We developed and evaluated an informational resource to facilitate this communication.

Methods We developed a written informational resource in consultation with the Hereditary Cancer Clinic and Hereditary Gastrointestinal Cancer Clinic in Manitoba, the Hereditary Cancer Program in British Columbia, and patient partners with LS. Content was guided by literature search and existing clinical communication tools. We recruited individuals with LS through clinics at a large urban teaching hospital in central Canada and at-risk relatives through snowball sampling. We distributed an online survey to evaluate the informational resource with Likert scale questions assessing the informational content, perceived clarity, comprehension, convenience, feelings provoked, and reactions triggered. We compared the responses between those who shared the resource with relatives and those who did not and used multivariable logistic regression to identify predictors of use. We also performed in-depth individual interviews as part of this mixed methods study (presented separately).

Results The new informational resource was graded at an eighth-grade reading level according to four common readability scales. A total of 92 participants provided feedback on the informational resource through the online survey (response rate 71%). Respondents strongly agreed the informational resource was easy to understand and 67% used or intended to use the resource to inform their relatives. The informational content, perceived clarity, comprehension, and convenience were highly rated. Among those who did not share the informational resource, 88% indicated their relatives were previously informed. Logistic regression analysis revealed that individuals age 45 or older were about four times more likely to share information about LS with their relatives. A need for increased access to reputable online resources was identified.

Conclusions We developed and assessed an informational resource to address a persistent barrier to family communication about LS.

Barriers and facilitators of cascade testing for Lynch Syndrome in a central Canadian province: a qualitative interview study

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Background In the past decade, many centers have implemented universal screening for Lynch Syndrome (LS) and established multidisciplinary clinics for annual follow-up, generating tremendous potential for cancer prevention. Alerting at-risk individuals to genetic testing and cancer prevention strategies remains reliant on communication within families. There is limited data on barriers and facilitators to cascade testing in Canada, where the universal health care system reduces financial barriers. Using a mixed methods approach, we sought to address a gap in the literature by identifying perceived barriers and facilitators to cascade testing within this patient population. We also developed and assessed an informational resource to facilitate family communication.

Methods We recruited individuals with LS through clinics at a large urban teaching hospital in central Canada. We conducted a survey with 92 respondents (reported separately) and semi-structured interviews with 15 participants to assess the barriers and facilitators to accessing genetic counselling and testing for LS and evaluate the informational resource. Telephone and web-based interviews were audio recorded, transcribed verbatim, and coded inductively by the first author using thematic analysis. The analysis was validated by two additional team members.

Results All participants indicated genetic testing was accessible and the barriers identified related to information sharing. There were three major themes impacting family communication about LS: perceptions of genetic testing, family dynamics, and level of acceptance towards relatives’ autonomy. Individuals who were the first person in their family to have LS identified more challenges informing relatives. A need for increased awareness of LS among primary care providers was identified, as was access to online resources. Participants who were frustrated by their relatives’ decision to decline genetic testing would support direct contact between their healthcare provider and their relative to facilitate communication. The newly developed informational resource was positively appraised by participants, particularly in terms of reading level, comprehension, and sensitivity.

Conclusions There are persistent barriers to family communication about LS, some of which can be mitigated by provision of quality informational resources. An ongoing need for better education of primary care providers and public awareness of LS was illuminated.
Outcomes and attitudes of colorectal cancer risk assessment in a federally qualified health center

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Background Approximately 20% of colorectal cancer (CRC) has a heritable component, with 5% caused by highly penetrant cancer-predisposition syndromes such as Lynch syndrome. Despite incorporation into guidelines, hereditary CRC risk assessment remains underperformed in the primary care setting, with minority and underserved populations disproportionately affected. The aim of this study was to determine feasibility of implementation of a standardized CRC risk assessment tool in a federally qualified health center (FQHC) serving primarily minority and uninsured populations.

Methods Beginning May 2021, a 5-question paper-based CRC risk assessment was administered to patients age 18 and older presenting for primary care visits at a single urban FQHC clinic as part of the check-in process with medical assistants in the exam room. Optional de-identified demographic information was collected, including age, race/ethnicity, gender, and knowledge of family history. Completed tools with positive screen were presented to the provider for genetics or CRC screening referral. Interviews were conducted with patients and providers to for feedback following tool administration.

Results During the first 4 weeks of tool administration, a total of 87 completed tools were returned. Risk assessment was successfully performed in 69 (79.3%) of patients who were able to answer all 5 questions. Demographic information about patients who did or did not complete the tool are shown in Table 1. Twelve (13.8%) patients had a positive CRC risk screen indicating genetic evaluation or early colonoscopy with four confirmed genetics referrals. Results of patient and provider feedback are shown in Fig. 1.

Conclusions CRC risk assessment was successfully performed at a high rate (~80%) among underserved primary care patients at a FQHC. 14% of patients were found to be at risk for familial CRC, similar to rates seen in other populations. Patients and providers felt that tool performance was compatible with clinic workflow and allowed discussion of CRC risk that might not otherwise be performed. Further studies to optimize performance and assess outcomes of risk assessment are needed.
The family history impact on the rate of cancer in pathogenic mutation carriers of MSH6 and PMS2

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PMS2 and MSH6 are moderate penetrant genes for Lynch Syndrome (LS) cancers. Germline mutations (i.e. pathogenic variants) in the aforementioned genes increase cancer risk as they interfere with DNA mismatch repair function (MMR). LS is classically associated with increased risk for colorectal and uterine cancers. The cancer occurrence in these mutation carriers may also be further influenced by family history independent of LS genes. Much of the current body of research on LS examines the condition without subdivision, however, by examining the lower penetrant genes independently from the higher penetrant MSH2 and MLH1, we hope to better inform clinical guidelines on screening for patients with lower penetrant LS mutations. This project conducted a retrospective cohort analysis of data collected from patients presented to Moffitt’s GeneHome Clinic. A total of 96 patients were discovered to carry moderate penetrant germline mutations associated with LS: 56 with PMS2 and 40 with MSH6. A reference group of approximately 1,000 patients tested negative for Lynch Syndrome mutations. Information including cancer type, age of onset, and family history was collected and compared across the reference and LS groups. Differences in categorical variables across compared groups were assessed using a chi-square/Fisher exact test. Results demonstrate varying cancer prevalence between the reference and LS groups; notably in colon (4.3% vs 19.2%), uterine (3.7% vs 32.7%), ovarian (5.6% vs 11.5%). Notably, differences on the counts of cancer and the age of onset are observed between the index case, maternal and paternal side of the family. These are most significantly observed on breast and uterine cancer, where maternal occurrences showed reduced ages of diagnosis compared to the paternal group (55.6 vs 65.8, p = 0.0061 & 50.8 vs 63.8 p = 0.0554). Currently, it is unclear about the factors contributing to such difference. Moderate penetrant LS demonstrate a varying prevalence of cancer types with increased ovarian cancer in addition to the classical LS uterine and colon cancers. Further exploration is needed to elucidate a possible cause for varying ages of diagnosis across maternal and paternal groups in breast and uterine cancer.

Table 1 The pancreas GTS cohort included 411 individuals, 60 of which had a single CFTR mutation. The breast GTS cohort had a total of 480 individuals, 49 of which were CFTR heterozygotes. Excluding poly 5 T variants, the odds of individuals from the pancreas cohort having a single CFTR mutation was greater than the breast cohort (OR = 1.4), which is consistent with a previous study [McWilliams et al., 2010]. Of note, the odds of black individuals in the pancreas cohort having a single CFTR mutation was much higher than the breast cohort (OR 2.6). General population data represents population data from gnomAD

| Study demographics | White, Non-Hispanic Total non-white | Hispanic/Latino | Black/African American | Asian | American Indian or native Alaskan | Native Hawaiian or Pacific Islander | Multi-ethnic | Declined/Unknown | Total |
|--------------------|-------------------------------------|-----------------|-------------------------|-------|-----------------------------------|------------------------------------|-------------|-----------------|-------|
| Pancreas           | 264                                | 136             | 37                      | 14    | 71                                | 2                                  | 3           | 2               | 540   |
| Breast             | 272                                | 164             | 41                      | 22    | 80                                | 3                                  | 5           | 4               | 453   |
| CFTR + (excl. poly 5 T variants) | | | | | | | | | |
| Pancreas           | 39                                 | 18              | 4                       | 7     | 5                                 | 1                                  | 0           | 1               | 57    |
| Breast             | 33                                 | 8               | 2                       | 2     | 3                                 | 0                                  | 0           | 1               | 41    |
| Odds Ratio         | 1.1                                | 1.8             | 2.2                     | 4.3   | 1.9                               | 1.6                                | 1.4         | 1.5             |
| Poly 5 T population (CFTR T/TG) | | | | | | | | | |
| Pancreas           | 15                                 | 3               | 6                       | 4     | 1                                 | 1                                  | 0           | 1               | 37    |
| Breast             | 21                                 | 6               | 1                       | 2     | 2                                 | 0                                  | 0           | 1               | 27    |
| Odds Ratio         | 1.0                                | 3.0             | 3.3                     | 3.7   | 2.3                               | 1.4                                | 1.4         | 1.5             |
| Poly CFTR          | |
| Pancreas           | 15                                 | 15              | 3                       | 6     | 4                                 | 1                                 | 0           | 1               | 37    |
| Breast             | 21                                 | 6               | 1                       | 2     | 2                                 | 0                                  | 0           | 1               | 27    |
| Odds Ratio         | 1.0                                | 3.0             | 3.3                     | 3.7   | 2.3                               | 1.4                                | 1.4         | 1.5             |

Key: ¹p < 0.05; ²p > 0.1; ³p < 0.1* [Note: unable to calculate significance for unmarked odds ratios due to small sample size]

| CFTR Heterozygote General Population | 1/25 | N/A | 1/58 | 1/61 | 1/94 | 1/26 | Unknown | N/A | N/A |
|-------------------------------------|------|-----|------|------|------|------|---------|-----|-----|
| Pancreas                            | 284  | 136 | 37   | 14   | 71   | 2    | 3       | 2   | 540 |
| Breast                              | 272  | 164 | 41   | 22   | 80   | 3    | 5       | 4   | 453 |
| Odds Ratios                         | 1.4  | 1.8 | 2.2  | 4.3  | 1.9  | 1.6  | 1.4     | 1.5 |
| Poly 5 T population (CFTR T/TG)     | 22   | 15  | 3    | 6    | 4    | 1    | 0       | 1   | 37  |
| Odds Ratios                         | 1.0  | 3.0 | 3.3  | 3.7  | 2.3  | 1.4  | 1.4     | 1.5 |

Key: ¹p < 0.05; ²p > 0.1; ³p < 0.1* [Note: unable to calculate significance for unmarked odds ratios due to small sample size]
Carriers of cystic fibrosis from a diverse background are at increased risk of pancreatic cancer

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Background In May of 2018, we implemented a point of care genetic testing model, the genetic testing station (GTS), previously described at CGA.1 UCSF patients with pancreatic adenocarcinoma are eligible for GTS and receive a 133 multi-gene cancer panel, including full sequencing and deletion/duplication analysis of the CFTR gene. Each patient consents to our IRB-approved registry. Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene are known to increase the risk of pancreatic cancer. A previous study, looking at a white-only population, suggested a modest increase in risk of pancreatic cancer for CFTR heterozygotes with an odds ratio (OR) of 1.4.2

We examined the contribution of CFTR variants to pancreas cancer risk in our pancreas GTS patients, a diverse population, to explore whether pancreatic cancer risk in CFTR carriers varies across different racial and ethnic groups.

Methods We reviewed self-reported race and ethnicity data and genetic test results for patients seen at pancreas and breast GTS, which served as our control population, from June 2018 to September 2021. There were 60/411 and 49/480 eligible heterozygous CFTR mutation carriers in the pancreas and breast GTS cohorts, respectively. Individuals with chronic pancreatitis, other pathogenic mutations known to be associated with pancreatic cancer risk, or with more than one CFTR mutation were excluded due to the possibility of subclinical cystic fibrosis.

Results The odds of having a single CFTR pathogenic variant was greater in the pancreas cohort over the breast cohort (OR 1.4), and was statistically significant (p = 0.0233) [Fig. 1], consistent with a previous study1 and confirming that our breast GTS population served as a reasonable control population.

The non-white cohort had higher odds of having a CFTR pathogenic variant than the non-white breast cohort (OR 1.9; p = 0.02743), with black cohort having the highest odds ratio of any racial or ethnic group (OR 2.6). [Table 1].

Conclusions Our data suggest that CFTR mutations serve as a moderate risk factor for pancreas cancer, especially in non-white populations. Given our limited sample size, more attention is warranted in explaining this trend, especially looking at the poly 5 T tract in the black population, who have the highest rate of pancreatic cancer in the United States.3

References
1. Goldberg D, Ko AH, Pedley C, et al. Flipping the model: developing a cutting-edge genetics pipeline to expand access and increase capture of patients at risk for hereditary pancreatic cancer. Poster

Fig. 1 This graph illustrates the percentage of total CFTR heterozygotes observed in our pancreas (13.6%) and breast (9.5%) populations. The pancreas cohort had significantly more CFTR heterozygotes than the breast cohort (p = 0.03005). We also compared the percentages of total CFTR heterozygotes as well as percentages of poly 5 T tract variants (11/TG, 12/TG) between the pancreas and breast cohorts across various ethnicities [Asian, Black, Hispanic, and White (Non-Hispanic)]. There was no significant difference observed between the white breast and pancreas cohorts for total CFTR heterozygotes (p = 0.19215) or poly 5 T tract variants (p = 0.39743). However, there was a significant difference observed between the non-white breast and pancreas cohorts for both total CFTR heterozygotes (p = 0.01923) and poly 5 T tract variants (p = 0.0197). Given the small sample size, we were unable to calculate significance within the non-white ethnicities. The general population column represents population data from gnomAD.
Table 1 The pancreas GTS cohort included 411 individuals, 60 of which had a single CFTR mutation. The breast GTS cohort had a total of 480 individuals, 49 of which were CFTR heterozygotes. Excluding poly 5 T variants, the odds of individuals from the pancreas cohort having a CFTR mutation was greater than the breast cohort (OR = 1.4), which is consistent with a previous study [McWilliams et al., 2010]. Of note, the odds of black individuals in the pancreas cohort having a single CFTR mutation was much higher than the breast cohort (OR 2.6). General population data represents population data from gnomAD

A report of a patient with atypical case of Constitutional Mismatch Repair Deficiency (CMMRD)

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Background Constitutional mismatch repair deficiency (CMMRD) is caused by biallelic mismatch repair pathogenic variants and is characterized by childhood hematologic malignancies, brain tumors, Lynch syndrome related cancers, and dermatologic lesions seen in neurofibromatosis. It is critical to appropriately establish a diagnosis of CMMRD to guide risk counseling, medical management, and clarify risk to other family members. The CMMRD International Consensus Working Group has recently established diagnostic criteria.

Case presentation A 24 year old male from non-consanguineous parents presented with five synchronous colon cancers in the setting of numerous adenomatous polyps. His maternal uncle had rectal cancer at age 50. Genetic testing, revealed a likely pathogenic variant (LPV) c.3227 G>A, and a variant of uncertain significance (VUS) c.2276 T>C, in MSH6. The LPV was paternally inherited and the VUS maternally. The patient subsequently was diagnosed with a low grade glioma, a rectal tubulovillous adenoma with high grade dysplasia, and a pouch cancer. There were no neurofibromatosis dermatologic manifestations. The presenting colon and pouch cancers were microsatellite stable but there was patchy expression of MSH6 in the tumor and normal tissue. Per CMMRD diagnostic criteria, the patient has a likely diagnosis of CMMRD.

Discussion There are limited data available about future cancer risks with atypical CMMRD; more research is needed. Additional and close follow up for patients with CMMRD is needed. In the interim, this patient was advised to undergo full screening for CMMRD associated cancers. Genetic testing recommended for his maternal uncle and brother (Fig. 1, Table 1).

Table 1

| Ethnicity          | White | Non-Hispanic | Total | African | Hispanic | Black/ Latino | Asian | Native American/ or Pacific Islander | Multi-ethnic | Unknown/ Declined | Total |
|--------------------|-------|--------------|-------|---------|----------|--------------|-------|-----------------------------|-------------|-------------------|-------|
| Pancreas           | 284   | 136          | 37    | 2       | 0        | 0            | 0     | 0                           | 0           | 0                 | 420   |
| Breast             | 272   | 161          | 41    | 2       | 0        | 0            | 0     | 0                           | 0           | 0                 | 433   |
| CFTR + (All)       |       |              |       |         |          |              |       |                             |             |                   |       |
| Pancreas           | 30    | 18           | 4     | 5       | 0        | 0            | 0     | 1                           | 0           | 0                 | 57    |
| Breast             | 33    | 8            | 2     | 3       | 0        | 0            | 0     | 0                           | 0           | 0                 | 40    |
| Odds Ratios        | 0.2   | 0.2          | 1.9   | 1.9     | 0.6       | 0.3          | 0.3   | 2                           | 1.2         | 1.4               |       |
| CFTR + (excluding poly 5 T variants) |       |              |       |         |          |              |       |                             |             |                   |       |
| Pancreas           | 17    | 3            | 1     | 1       | 1        | 0            | 0     | 0                           | 0           | 0                 | 20    |
| Breast             | 12    | 2            | 1     | 0       | 0        | 0            | 0     | 0                           | 0           | 0                 | 14    |
| Odds Ratios        | 1.5   |              | 1.1   | 1.1     | 0.8       | 0.6          | 0.6   | 1                           | 0.6         | 1.5               |       |
| Poly 5 T population (11/ TG, 12/TG) |       |              |       |         |          |              |       |                             |             |                   |       |
| Pancreas           | 22    | 15           | 6     | 4       | 1        | 0            | 1     | 0                           | 0           | 0                 | 37    |
| Breast             | 21    | 6            | 1     | 2       | 2        | 0            | 0     | 1                           | 0           | 0                 | 27    |
| Odds Ratios        | 0.7   | 0.6          | 0.3   | 1.1     | 0.2       | 0.6          | 0.6   | 1                           | 0.6         | 1.4               |       |

Key: 1p < 0.05; 2p > 0.1; 3p < 0.1* [Note: unable to calculate significance for unmarked odds ratios due to small sample size]
The percentage of total CFTR heterozygotes observed in the pancreas cohort was greater than the breast cohort, 14.6% and 10.2% respectively [a]. The pancreas cohort had significantly more CFTR heterozygotes than the breast cohort (p = 0.0233). We also compared the percentages of total CFTR heterozygotes as well as percentages of poly 5 T tract variants (11/TG, 12/TG) between the pancreas and breast cohorts across various ethnicities [b]. There was no significant difference observed between the white breast and pancreas cohorts for total CFTR heterozygotes (p = 0.14917) or poly 5 T tract variants (p = 0.31918). However, there was a significant difference observed between the non-white breast and pancreas cohorts for both total CFTR heterozygotes (p = 0.02743) and poly 5 T tract variants (p = 0.04093). Given the small sample size, we were unable to calculate significance within the non-white ethnicities, besides Asian which was not statistically significant. The prevalence of poly 5 T tract variants in the non-white races appeared greater in the pancreas cohort than the breast cohort, marked by the arrows [b], although significance could not be measured due to small sample size. General population data represents population data from gnomAD.

### Table 1 Phenotypic data

| APC alteration | Proband phenotype | Family phenotype |
|---------------|-------------------|------------------|
| c.14C > A (p.Ser5*) | 41-year-old apparently unaffected female | Maternal grandmother ovarian cancer 70 s aunt breast 50 s aunt ovarian 60 s aunt stomach 60 s (unspecified colon polyps) great uncle leukemia 50 s great uncle bone 60 s cousin colon cancer 40 s cousin brain 40 s Paternal grandmother pancreatic 80 s uncle prostate 60 s Nuclear brother small intestine cancer at 41 and colon cancer at 42 – APC ± and has a pathogenic gross deletion in MLH1 Maternal 64 yo apparently unaffected mother APC ± aunt APC ± (one tubular adenoma at 55) aunt ACC grandfather at 86 with reported multiple skin cancers and > 20 unspecified polyps Nuclear brother thyroid 60 s Maternal aunt breast 40 s uncle stomach 70 s grandmother brain 60 s Paternal father prostate 60 s cousin endometrial 40 s half-aunt breast in 40 s and ovarian in 50 s |
| c.14delC (p.Ser5Tyrfs+6) | 39-year-old apparently unaffected male | Nuclear brother small intestine cancer at 41 and colon cancer at 42 – APC ± and has a pathogenic gross deletion in MLH1 Maternal 64 yo apparently unaffected mother APC ± aunt APC ± (one tubular adenoma at 55) aunt ACC grandfather at 86 with reported multiple skin cancers and > 20 unspecified polyps Nuclear brother small intestine cancer at 41 and colon cancer at 42 – APC ± and has a pathogenic gross deletion in MLH1 |
| c.22C > T (p.Gln8*) | 62-year-old female with < 5 unspecified polyps identified over 3–4 colonoscopies | Nuclear brother thyroid 60 s Maternal aunt breast 40 s uncle stomach 70 s grandmother brain 60 s Paternal father prostate 60 s cousin endometrial 40 s half-aunt breast in 40 s and ovarian in 50 s |

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**Fig. 1** The percentage of total CFTR heterozygotes observed in the pancreas cohort was greater than the breast cohort, 14.6% and 10.2% respectively [a]. The pancreas cohort had significantly more CFTR heterozygotes than the breast cohort (p = 0.0233). We also compared the percentages of total CFTR heterozygotes as well as percentages of poly 5 T tract variants (11/TG, 12/TG) between the pancreas and breast cohorts across various ethnicities [b]. There was no significant difference observed between the white breast and pancreas cohorts for total CFTR heterozygotes (p = 0.14917) or poly 5 T tract variants (p = 0.31918). However, there was a significant difference observed between the non-white breast and pancreas cohorts for both total CFTR heterozygotes (p = 0.02743) and poly 5 T tract variants (p = 0.04093). Given the small sample size, we were unable to calculate significance within the non-white ethnicities, besides Asian which was not statistically significant. The prevalence of poly 5 T tract variants in the non-white races appeared greater in the pancreas cohort than the breast cohort, marked by the arrows [b], although significance could not be measured due to small sample size. General population data represents population data from gnomAD.

**References**

1. Aronson M, et al. Diagnostic criteria for constitutional mismatch repair deficiency (CMMRD): recommendations from the international consensus working group. *J Med Genet* 2021;0:1–10.

**N-terminal truncating variants in APC in patients without overt familial adenomatous polyposis**

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**Background** Individuals with truncating alterations in the APC gene typically present with a classic or attenuated form of familial adenomatous polyposis (FAP/AFAP). The mechanism for pathogenicity of nonsense and frameshift alterations in loss-of-function genes is well established to be premature protein truncation and/or nonsense-mediated decay (NMD); however, N-terminal truncations may be an exception due to incorrect annotation or several rescue mechanisms including use of multiple translational start codons, translation re-initiation, use of unconventional (non-AUG) translational start sites, and/or escape of NMD. As such, review of genotype–phenotype correlations for N-terminal truncations in highly penetrant genes may reveal atypical clinical presentations. Here, we identify a group of APC truncations in which the premature termination codon is within...
Case presentation The clinical data for three patients, tested at a commercial diagnostic laboratory, with unique truncating alterations in APC and whose personal and/or family histories do not exhibit the classic or attenuated colon polyposis/cancer diagnoses are presented (Table 1). Information was ascertained through the test requisition form. Limited family member testing is available for only one case.

Discussion Although the identified alterations are located near or within a highly conserved amino-terminal oligomerization domain that has been established as a critical region for proper dimerization to occur (Fig. 1), there is a lack of published data on truncating alterations impacting only the 5′ region of this domain. Further, there is a lack of truncating alterations detected in patients with a FAP/AFAP phenotype in multiple databases (including HGMD, ClinVar, and LOVD) upstream of p.R24*, a well described pathogenic alteration. We postulate that one mechanism for this phenomenon may be related to the presence of an in-frame methionine at amino acid position 18, that may serve as an alternate translational start. The variants associated with the phenotypes described here fall short of the attenuated phenotype typically described in distal APC truncations. This case series highlights the importance of careful consideration for N-terminal truncating variants and the historic assumption that they unequivocally produce loss-of-function transcripts or polyposis phenotype.

![Fig. 1 APC coding exon 1](image)

Discrepant CDKN2A c.146 T > C variant: a clinical experience

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Background Clinically significant variants (likely pathogenic) in the CDKN2A gene are associated with increased risks of melanoma (MEL) and pancreatic cancer (PCa). The CDKN2A variant c.146 T > C has conflicting interpretations of pathogenicity (variant of uncertain significance vs. likely pathogenic). This study describes patients with CDKN2A variants identified via germline genetic testing (GGT) between April 2010 and March 2020 in a high-volume cancer genetics program.

Methods A retrospective chart review was performed for patient demographics, personal history (phx), family history (fhx), and GGT results. Patients with the c.146 T > C variant were compared to patients with other clinically significant CDKN2A variants using a \( t \) test \( (P < 0.001) \).

Results In the study timeframe, a total of 14,625 patients underwent GGT that included the CDKN2A gene. Overall, 24.8% \( (n = 3629) \) of individuals that underwent CDKN2A testing identified as Hispanic. Fifty-three patients were identified to have the c.146 T > C variant; all of which identified as Hispanic \( (53/3629, 1.5\%) \). One patient with a phx and fhx of CDKN2A-associated cancers carried an additional pathogenic CDKN2A variant and was excluded from further analysis. Excluding individuals with c.146 T > C, 16 (0.1%) patients had a clinically significant CDKN2A variant. The phx and fhx of individuals with the CDKN2A c.146 T > C variant and individuals with other clinically significant CDKN2A variants are outlined in Table 1. The difference in phx of CDKN2A-associated cancers between these groups was statistically significant \( (P = 0.00015) \). There was no statistically significant difference in the fhx of cancers between these groups \( (P = 0.10566) \).

Conclusions The c.146 T > C variant was identified in 1.5% of Hispanic patients who underwent CDKN2A GGT. Compared to other clinically significant CDKN2A variants, the c.146 T > C variant had significantly fewer patients with phx of MEL or PCa. This may be due to the lower general prevalence of MEL amongst Hispanic individuals, the c.146 T > C variant being a low penetrance variant, or the variant is not clinically significant. Further studies are needed to clarify the significance of the CDKN2A c.146 T > C variant to ensure appropriate management.

| Phx PCa and no Fhx MEL or PCa | 2 (3.8) | 0 (0) |
| Phx MEL and no Fhx MEL or PCa | 0 (0) | 2 (12.5) |
| Phx MEL and Fhx MEL and/or PCa | 0 (0) | 4 (25) |
| Fhx MEL and/or PCa | 7 (13.5) | 1 (6.3) |
| No Phx or Fhx MEL or PCa | 43 (82.7) | 9 (56.3) |

The protean phenotype of MSH6 pathogenic variants (PV) in Lynch syndrome (LS) patients (pts)

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Background LS is among the most common hereditary cancer syndromes. PVs in MSH6 are 2–fourfold more common in the population (1/758) than those in MLH1 (1/1946) and MSH2 (1/2841), and are regarded as lower penetrance for CRC due to recent data supporting later age of CRC onset and lower lifetime risk. NCCN 2021 cancer risk estimates for MSH6 recognize only endometrial (EC) and CRC risks in MSH6 + carriers as being much higher than SEER population estimates. Risks of other LS manifestations such as Muir-Torre, ovarian cancer (OC), and rare tumors in LS like sarcoma, have been minimally characterized in MSH6 carriers.

Methods Pedigree data for 44 MSH6 + index pts ascertained since 2009 at FCCC were reviewed. 34% (15/44) index pts were referred to FCCC for cascade testing due to a known MSH6 PV in the family. Of the remaining 29 pts, ascertainment included: 14% w/positive universal LS tumor screening, 21% w/early-onset or synchronous LS cancer, 14% w/multi-gene panel for personal history (PHx) of OC, 10% w/incidental MSH6 + result, and 28% w/PHx and/or family history of LS cancer.

Results Index pts had a mean age of 55.5 years, and 77% were female. 11% (5/44) of MSH6 + index pts were found to have LS at diagnosis of synchronous primary cancers (3 EC/OC, 1 CRC/OC, 1 CRC/EC), and 4/5 of these occurred < 50 yrs. 20% (9/44) index pts reported PHx of > 2 metachronous LS cancers. OC was the presenting cancer in 14% (6/44) female index pts; 2 additional index pts had rarer OC variants (Mullerian duct @ 41, primary peritoneal @ 50). Skin manifestations of LS were documented in 9.1% (4/44) index pts (3 sebaceous, 1 SCC in-situ/Bowen’s disease); 1 other family had documented sebaceous cancers in an FDR (father). Two index pts were found to have LS after developing early-onset BC and contralateral breast cancer. Finally, 7% (3/44) index pts had a diagnosis of sarcoma: 2 were liposarcomas & 1 was DFS. 2 other pts had siblings with childhood sarcomas.

Conclusions Our data, encompassing 44 MSH6 + pts evaluated in our clinic and consecutively ascertained, suggest MSH6 PV carriers develop synchronous primaries (11%), common and rare ovarian cancer histologic types (18%), sarcomas (7%) and skin manifestations/Muir-Torre (9%). While common in the general population and lower penetrance for CRC, MSH6 PV can behave in uncommon ways and may have significant and seemingly rare extra-colonic cancer risks.

Inconclusive genetic test results and a phenotype possibly confounded by prior childhood cancer

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Background In genetic counseling, complex cases exist with nuances that may obscure a definitive diagnosis. We present a case of a possibly mosaic PTEN likely pathogenic variant (PV) with inability to determine variant origin, as well as childhood cancer history that may contribute to the patient’s current clinical presentation.

Case presentation MM is a 50-year-old male referred to Genetics after a colonoscopy detected 22 polyps (14 hyperplastic, 8 adenomatous). He has a history of leukemia treated with full body radiation and bone marrow transplant (BMT) at age 15. His family history includes 4 paternal relatives with colon cancer (CRC). Given a history of BMT, skin fibroblasts were used for germline genetic testing. He was found to have a possibly mosaic PV in the PTEN gene (exon 6 deletion). Physical exam revealed that he did not fulfill the major and minor criteria for Cowden syndrome.

Discussion This case presents various clinical conundrums. First, when possibly mosaic variants are detected on blood/saliva specimens, testing of alternate samples, such as skin, is often pursued to help elucidate the origin of the variant (i.e., constitutional mosaicism, acquired somatic variant). In our patient, a likely mosaic result was
found in skin fibroblasts, and blood/saliva testing would not be appropriate given his history of BMT. As such, there are no other tissue samples for testing via standard clinical pathways as further work up. The patient also has no offspring for transmission testing. Second, germline PTEN PVs are most associated with hamartomatous polyposis, which were not seen in our patient. Thus, his polyposis history and his family history of CRC remain unexplained. A phenomenon of therapy-associated polyposis (TAP) has been identified in patients after childhood and young adulthood cancer treatment (Billier et al., 2020). Given our patient’s history of leukemia, full body radiation, and BMT, TAP is a plausible alternative to hereditary polyposis. Similarly, the PTEN PV could also be an acquired mutation as a result of the cancer history and/or treatment.

**Conclusion**

Despite many advances in germline genetic testing, this case highlights the importance of a phenotypic assessment and consideration of clinical history and exposures in the interpretation of germline genetic test results. Anticipatory guidance should be provided in a pre-test counseling visit that results may be uninformative or indeterminate.

**Interval colorectal cancers in patients with hereditary gastrointestinal syndromes after one year of the SARS-CoV-2 pandemic**

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**Background**

Hereditary colorectal cancer syndromes, including Lynch syndrome, require strict adherence to surveillance protocols. This study evaluated the approach of Italian gastroenterologists to the diagnosis and follow-up of these syndromes and the impact of SARS-CoV-2 on clinical outcomes, including interval cancers.

**Methods**

All members affiliated with the leading gastroenterology Italian societies (*AIGO, SIED,* and *SIGE*) received an online questionnaire through their society newsletter between March 8, 2021, and May 3, 2021.

**Results**

One hundred and twenty-one clinicians from 96 Italian hospitals answered the questionnaire (males: 73, 60.3%; average clinical experience: 20.13 ± 11.69 years). 61.2% performed immunochemistry (IHC) staining presents challenges in interpretation, the standard protocol using mismatch repair (MMR) immunohistochemistry (IHC) staining presents challenges in interpretation, particularly in the setting of normal germline results in patients with abnormal staining. Recent implementation of paired germline/tumor analysis allows for better characterization of previously unexplained findings. We present the experience of the universal screening program at Beaumont Health (BH), including recent utilization of a paired approach.

**Methods**

MMR IHC results for CRC resection specimens from August 2012 through June 2021 were included. Absence of MLH1/MSI2 prompted MLH1 promoter hypermethylation analysis with reflex to BRAF V600E mutation testing. Patients with abnormal results were referred for cancer genetics evaluation. Results of MMR staining, germline testing, and paired analysis are reported.

**Results**

Pathology specimens from 2,537 CRC resections underwent MMR IHC staining, and 537 were abnormal (21%). Most cases of absent MLH1/MSI2 were explained by hypermethylation or BRAF analysis (n = 356, 89% of MLH1/MSI2). Of the remaining cases with MMR deficiency (dMMR) (n = 181), the most common was absence of MSI2/MSI6 (n = 69), then MLH1/MSI2 (n = 43, Table 1). Germline testing performed on 88 individuals revealed 50 cases of LS [MLH1 (n = 9), MSI2 (n = 25), MSI6 (n = 7), PMS2 (n = 8), EPCAM (n = 1)]. A significant proportion of cases (n = 38, 43%) had unexplained dMMR. Since implementation of an updated
protocol, 18 patients underwent paired testing. Of those, 8 had somatic findings explaining their dMMR, 6 had LS, and 4 (22%) remained unexplained.

**Conclusions** Recent advances in oncology underscore the importance of MMR analysis, with ongoing challenges to implementation and interpretation of results. We identified a large number of dMMR tumors (21% of all CRC resections) and LS patients (2%), with significant impact on management. Recent implementation of paired germline/tumor testing has improved the testing algorithm, resulting in more accurate interpretation, and a significant decrease in unexplained dMMR. This updated approach allows for more precise identification of LS, resulting in more optimal risk-based screening, early detection, and personalized therapies.

| IHC Result | N  |
|------------|----|
| Loss of MLH1 and MSH6 | 60 |
| Loss of MLH1 and PMS2 (No Methylation/BRAF) | 43 |
| Loss of PMS2 | 35 |
| Loss of MSH6 | 35 |
| Loss of MLH1, PMS2, MSH2, and MSH6 | 7 |
| Loss of MSH2 | 3 |
| Loss of MLH1, PMS2, MSH2, and MLH6 | 5 |
| Other | 5 |

**Impact of a single-institution hereditary cancer screening program on uptake of genetic testing and counseling for pathology-identified high-risk patients**

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**Background** Hereditary nonpolyposis colorectal cancer (Lynch Syndrome (LS)) is the most common hereditary colorectal cancer syndrome, conferring an 80–90% lifetime risk for colorectal cancer, among other cancer types [1]. To prevent and detect cancer and decrease mortality, early identification of those with LS is critical [2]. Immunohistochemistry (IHC) testing is now considered the gold standard screening modality for Lynch Syndrome regardless of clinical and family history [3]. Despite this recommendation, referrals for germline testing in the case of abnormal IHC results is often complicated and can lead to missed opportunities. Thus, our institution implemented the Hereditary Cancer Screening and Risk Reduction Program (HCSRRP) to facilitate counseling and genetic testing for patients identified as high-risk for familial cancer syndromes. The purpose of this study is to describe and assess the impact of the HCSRRP on uptake of genetic counseling and testing by pathology-identified patients at high-risk for LS.

**Methods** Adult patients identified as high-risk for LS as defined by abnormal IHC stains for MLH1, PMS2, MSH2, and MSH6 in any tissue specimen were included. The pre-intervention cohort constituted patients identified by pathology between January 2013- August 2014 and post-intervention between January 2017 and March 2020. Data were abstracted from electronic medical records, the Department of Pathology’s clinical database, and the HCSRRP visit logs. Statistical analysis was performed using SAS.

**Results** 42 patients were identified in the pre-intervention cohort and 151 patients were identified in the post-intervention cohort. Before implementation of the program, 6.3% (N = 2) of pathology-identified high-risk patients were seen by a genetic specialist (Fig. 1). After implementation, 35% of patients had at least one visit with the HCSRRP for genetic counseling, and 54% of patients underwent genetic testing. Age and cancer type were significantly associated with uptake of genetic testing, with patients younger than 65 years old and those with gastrointestinal or gynecologic cancer having higher testing rates (p = 0.01543, p = 0.0082 respectively).

**Conclusions** Implementation of a designated hereditary cancer program had a significant impact on the proportion of patients identified as high-risk for Lynch Syndrome who visited a genetics specialist and underwent genetic testing.

**Fig. 1 Uptake of genetic testing and counseling before and after implementation of the HCSRRP**

**Increased risk of gastric and pancreatic adenocarcinoma associated to hereditary breast and ovarian cancer syndrome**

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**Background** Hereditary breast and ovarian cancer syndrome (HBOC) is associated with germline pathogenic variants (GPV) mainly in BRCA1, BRCA2 and PALB2 genes. In addition to breast and ovarian cancer, it is also associated with an increased risk of other tumours such as gastric(GC) and pancreatic(PC) adenocarcinoma. The aim of the study is to describe the prevalence of GC and PC in these families.

**Methods** National multicenter study (7 Spanish centers) with retrospective inclusion of families with a GPV in BRCA1, BRCA2 or PALB2 genes, diagnosed after genetic counseling of an individual with cancer and fulfilling clinical criteria of a hereditary syndrome (defined as index case). Personal and family medical history was reported.

**Results** A total of 192 families were included: 78 families had a GPV in BRCA1 (40.6%), 89 in BRCA2 (46.3%) and 25 in PALB2 (13%).

Regarding the index case, 177 (92.1%) were women, and the median age at diagnosis of the tumor was 46 years. In 131/192 (68.2%) cases, the tumor in the index case was breast cancer, 45(23.4%) ovarian, 7(3.6%) prostate, 4(2%) pancreas, 2(1.1%) stomach, 2(1.1%) colon and 1(0.5%) endometrium. Thus, in 6(3.1%) of the families the diagnosis was made after presenting PC or GC, and 3(1.6%) of the
Early age of onset and broad cancer spectrum persist in MSH6 and PMS2-associated Lynch syndrome

Ying L. Liu MD MPH1,5, Karen A. Cadoo MD2, Anna Maio BS1, MSH6
in Early age of onset and broad cancer spectrum persist
BRCA2 gene was first-degree relatives of the index case. The predominantly affected
GC, with a median age at diagnosis of 62, of which 10(40%) were
median age at diagnosis of 70, and 16(68%) cases were first-degree
Of the 192 families with HBOC, 25(13%) had a history of PC, with a
personal history of PC or GC.

cases diagnosed with breast, ovarian or prostate cancer had a previous
personal history of PC or GC.
Of the 192 families with HBOC, 25(13%) had a history of PC, with a
median age at diagnosis of 70, and 16(68%) cases were first-degree
relatives of the index case. The predominantly affected
GC, with a median age at diagnosis of 62, of which 10(40%) were
first-degree relatives of the index case. The predominantly affected
gene was BRCA2 (29/50,58%).

Conclusions Up to 3% of families with HBOC were diagnosed as a
result of a case of GC or PC. In 25% of the families, there was a
history of GC or PC, mostly associated with germline pathogenic
variant in BRCA2.

Although more data is needed, our study supports the increased risk of
GC and PC in individuals with HBOC, and screening for GC and PC
in these families should be considered.

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Table 1 Cancer Spectrum in Lynch Syndrome Patients with P/LP MSH6 and PMS2 Variants

| Cancer          | Overall (N = 160) | MMRD/ MSI-H (N = 77) | Overall (N = 101) | MMRD/ MSI-H (N = 44) |
|-----------------|-------------------|----------------------|-------------------|----------------------|
| Colorectal (CRC)|                   |                      |                   |                      |
|                 | 47 (29%)          | 33 (43%)             | 31 (31%)          | 23 (52%)             |
| Endometrial (EC)| 41 (26%)          | 25 (32%)             | 12 (12%)          | 10 (23%)             |
| Breast          | 13 (8%)           | 1 (1%)               | 13 (13%)          | 0                    |
| Prostate        | 11 (7%)           | 1 (1%)               | 3 (3%)            | 1 (2%)               |
| Urothelial (UC) | 5 (3%)            | 4 (5%)               | 2 (2%)            | 1 (2%)               |
| Pancreas/Biliary| 5 (3%)            | 3 (4%)               | 7 (7%)            | 1 (2%)               |
| Sarcoma         | 2 (1%)            | 0                    | 0                 | 0                    |
| Gastric/ Esophageal (GEJ)| |                      |                      |                      |
| Esophageal      | 5 (3%)            | 3 (4%)               | 1 (1%)            | 0                    |
| Lymphoma        | 3 (2%)            | 0                    | 0                 | 0                    |
| Melanoma        | 4 (3%)            | 0                    | 0                 | 0                    |
| CNS/Brain       | 1 (1%)            | 1 (1%)               | 4 (4%)            | 0                    |
| Small Bowel (SB)| 1 (1%)            | 1 (1%)               | 5 (5%)            | 5 (11%)              |
| Skin, Non-      | 6 (4%)            | 3 (4%)               | 5 (5%)            | 0                    |
| melanoma        |                    |                      |                   |                      |
| Kidney          | 2 (1%)            | 0                    | 1 (1%)            | 0                    |
| Thyroid         | 2 (1%)            | 0                    | 0                 | 0                    |
| Testicular/Germ Cell |          |                      |                   |                      |
| Carcinoma of Unknown Primary | |                      |                   |                      |
| Lung            | 2 (1%)            | 0                    | 0                 | 0                    |
| Other           | 4 (3%)            | 0                    | 3 (3%)            | 0                    |

Conclusions Patients with MSH6/PMS2 P/LP variants remain at risk for a broad-spectrum of cancers and very early-onset CRC, with 16% of MMRD/MSI-H CRC presenting prior to upper threshold of initiation of colonoscopic screening per NCCN.

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Table depicts cancer types and distribution for MSH6 and PMS2 P/LP variant carriers, both overall and those that were MMR-D/MSI-H.

**Risk-stratified FIT for urgent colonoscopy in Lynch Syndrome: a clinical service throughout the COVID-19 pandemic**

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**Background** Lynch syndrome (LS) is an inherited disorder characterised by pathogenic variants within mismatch repair (MMR) genes and results in an increased risk of colorectal cancer. We report preliminary results from a national clinical service implemented during the COVID-19 pandemic which used FIT as a novel intervention in this patient population to prioritise colonoscopy in the highest risk patients while endoscopy services were limited.

**Methods** Regional genetic and endoscopy services across England were invited to participate. Patient eligibility was determined by 1) Diagnosis of LS 2) Planned colonoscopy between 1 March 2020 and 31 March 2021. Requests for FIT testing from participating NHS Trusts were sent to the NHS Bowel Cancer Screening South of England Hub. The Hub sent patients a FIT kit (OC-Sensor™ (Eiken, Japan)), FIT instructions, a questionnaire, and a pre-paid return envelope. Faecal haemoglobin (f-Hb) results were returned for clinical action. LS patients were risk-stratified for colonoscopy based upon the following f-Hb thresholds: (1) f-Hb ≥10 µg Hb/g faeces: triaged for colonoscopy via two-week wait (2WW) pathway, (2) f-Hb ≥10 µg Hb/g faeces: colonoscopy in 6–12 weeks, where availability permits.

**Results** Seventeen centres across England participated in the clinical service from 9 June 2020 to 31 Mar 2021. A patient uptake rate of 67% was observed (394/588 invites). Of the 394 participating patients, 16% (n = 65) had f-Hb ≥10 µg Hb/g faeces and met criteria for urgent colonoscopy triage via the 2WW pathway. In a subgroup analysis of 18 patients from St Mark’s Hospital with elevated FIT results ≥10 f-Hb, 15 patients proceeded to priority colonoscopy. Of the 15 prioritised LS patients in this subgroup, 7 (47%) had detectable adenomas which were subsequently resected for further examination by pathology.

**Conclusions** The utility of FIT during the pandemic has demonstrated clinical value for LS patients requiring routine surveillance. Further longitudinal investigation into the specificity and sensitivity of FIT in LS patients is warranted.

**Penetrance of colorectal, endometrial and other cancers among lynch syndrome families identified through universal tumor screening**

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**Background** Studies have demonstrated MLH1, MSH2, MSH6, and PMS2 pathogenic variants are associated with differing cancer risks, suggesting clinical management for Lynch syndrome (LS) should be stratified by MMR gene. However, many prior analyses have incorporated high-risk clinic-based families, potentially inflating penetrance estimates. This study aimed to estimate LS penetrance with an entirely population-based cohort to reduce ascertainment bias.

**Methods** Data were ascertained from three population-based universal tumor screening programs conducted in Ohio between 1999 and 2021. 3,182 relatives (553 positive, 2,629 untested) from 203 LS families were included in the analyses. Probands (n = 226) and mutation-negative relatives were excluded from the analyses. The Kaplan–Meier statistic was used to estimate the age-dependent cumulative risk of six cancer types. The number of colorectal, endometrial, and ovarian cancers that could have been prevented by following National Comprehensive Center Network (NCCN) LS management guidelines was calculated.

**Results** For all four MMR genes, the risk of developing colorectal, endometrial, and ovarian cancer was increased compared to the general population. The lifetime risks for colorectal cancer ranged from 9.2–36.5%, and endometrial cancer ranged from 4.1–17.7% (see Fig. 1). PMS2 was associated with the lowest risk for endometrial cancer (4.1%) and no increased risk for upper gastrointestinal cancer. Only MSH2 was associated with elevated prostate cancer risk (13.1%). Only MLH1 was associated with elevated breast cancer risk (12.3%). If all individuals with LS followed the NCCN Guidelines (v.1.2020), greater than 90% of CRCs could have been prevented. However, only (80.8%) of endometrial and (60%) of ovarian cancers would have been prevented if all the women with LS had a hysterecortomy and/or bilateral salpingo-oophorectomy by age 40.

**Conclusions** Our risk estimates were comparable to prior studies, including those that utilized clinic-based ascertainment with complex analyses correcting for ascertainment bias. This finding suggests that these analyses accurately correct for clinic-based ascertainment, reducing the likelihood of inflated penetrance estimates. Our data support the continued use of gene-specific guidelines and current colorectal cancer screening recommendations. However, it is possible that gynecologic management guidelines may need to start at an earlier age, given the large proportion of early-onset endometrial and ovarian cancers.
Fig. 1 Age-dependent cumulative risks for colorectal, endometrial, ovarian, breast, prostate, and upper gastrointestinal cancer by MMR gene

Rates of gynecologic cancer and endometrial precursor lesion detection via endometrial biopsy among women with Lynch syndrome

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Background Women with Lynch syndrome (LS) have elevated lifetime risk of endometrial (EC) and ovarian cancers (OC). NCCN guidelines recommend patients (pts) > age 30 consider screening endometrial biopsy (sEMB) every 1–2 years prior to risk-reducing hysterectomy/bilateral salpingo-oophorectomy (RR-Hys/BSO). We sought to characterize rates of EC/OC, EC precursor lesions, and EMB results among women with LS.

Methods Clinical and pathology records of pts consented to IRB-approved protocols of tumor/norom sequencing or prospective registry of LS pts from 2/2005–4/2021 were reviewed to identify female LS pts > age 30 diagnosed with EC/OC and those that underwent Hys/BSO for cancer treatment or RR-Hys/BSO. sEMB was defined as that done in asymptomatic pts, diagnostic EMB (dEMB) in symptomatic pts. Clinical variables were correlated by germline MMR PV-status using non-parametric tests.

Results Of 293 female LS pts > age 30, 163(56%) had EC/OC, inclusive of 124(76%) EC, 21(13%) OC, and 18(11%) synchronous/metachronous EC/OC. Among overall EC pts (142), 24(17%), 63(44%), 42(30%) and 13(9%) had an MLH1, MSH2/EPCAM, MSH6 or PMS2 PV, respectively. Median age at diagnosis was 49[MLH1:46, MSH2/EPCAM: 47, MSH6: 55, PMS2: 54 (p < 0.001)]. Among overall OC pts(39), 5(13%), 17(44%), 8(21%), and 9(23%) had an MLH1, MSH2/EPCAM, MSH6 or PMS2 PV, respectively. Median age at diagnosis was 46[MLH1:43, MSH2/EPCAM:41, MSH6:52.5, PMS2:63(p = 0.002)]. Of EC pts, 113(80%) had Hys with pathology available for review. 49(43%) had EMB within one year, of which 47(96%) were dEMB in symptomatic pts yielding 98% detection rate of EC/CAH. 93 asymptomatic pts underwent RR-Hys/BSO of which 78(84%) had pathology available for review. 49(43%) had EMB within one year, of which 47(96%) were dEMB in symptomatic pts yielding 98% detection rate of EC/CAH. 93 asymptomatic pts underwent RR-Hys/BSO of which 78(84%) had pathology available for review. 27(35%) of such pts had abnormal pathology on RR-Hys/BSO, including 9%(7/78) EC, 12%(9/78) CAH, 10%(8/78) hyperplasia/EH, and 9%(7/78) other. Among such pts, 37%(10/27) had sEMB within 1 year [mean interval 3.6 months prior to surgery (range 1–11 months)], with 90% having normal results, including 2 pts with overt EC found on RR-Hys/BSO.

Conclusions While lower penetrance, MSH6 and PMS2 PV carriers remain at risk for gyn cancers. Though dEMB detected EC/CAH in 98% of cases, sEMB lacked accuracy. Enhanced EC screening is needed in high-risk populations.

Unusual location of a juvenile polyp in juvenile polyposis syndrome – a case report

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Background  Juvenile Polyposis Syndrome (JPS) is a rare hereditary hamartomatous polyposis syndrome due to a pathogenic variant (PV) in SMAD4 or BMPR1A. It is associated with a risk of colorectal and gastric polyposis and cancer, and rarely small bowel polyps. Guidelines suggest endoscopic surveillance in JPS begins at the age of 15 years old with upper endoscopy and colonoscopy and repeated no less frequently than every 3 years pending findings. No recommendations are made for small intestine surveillance as the evidence of small bowel involvement in not well established.

Case presentation  We report a 29 year old female patient with a personal and paternal family history of JPS and hereditary hemorrhagic telangiectasia. The patient was diagnosed with a pathogenic variant in SMAD4 at the age of 10 years after presenting with rectal bleeding. She underwent a restorative proctocolectomy with ileal pouch-anal anastomosis at age 15 for colorectal polyposis. She since then has been having upper endoscopy and pouchoscopy every 2–3 years. In July 2021 pouchoscopy was normal. Upper endoscopy demonstrated numerous sessile polyps up to 10 mm in size at the cardia removed with a hot snare. The rest of the stomach was normal. The duodenum was normal except for an abnormal appearance of the duodenal papilla with juvenile polyp histology. The patient has no symptoms attributed to the duodenal papilla involvement and will undergo repeat EGD in one year. We believe this a rare finding and warrants further research.

Clinical characteristics of Lynch syndrome patients with multiple and extracolonic cancers

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Background  Phenotypic presentation of Lynch syndrome (LS) can vary greatly. Reasons for the heterogeneity in spectrum of cancer remain largely unexplained. We sought to explore factors associated with the development of extracolonic and multiple cancers and whether specific medical comorbidities, specifically history of autoimmune disease, might be associated with severity of cancer phenotype.

Methods  We queried the University of Michigan Cancer Genetics Clinic database for individuals with clinically confirmed LS seen between 2002–2021. Cancer diagnoses, genetic variant, sex, age, and medical history, including history of autoimmune disease (e.g., rheumatoid arthritis, inflammatory bowel disease) or use of immunosuppressive medications, were abstracted via retrospective chart review. Chi-square and t-tests were used to compare characteristics of individuals with multiple primaries to those with one or no cancer diagnoses, and to look for trends associated with development of extracolonic cancers.

Results  494 individuals were included (MLH1 n = 110, MSH2 n = 193, MSH6 n = 116, PMS2 n = 70, EPCAM n = 5). 279 (56.5%) had at least one cancer diagnosis and 132 (26.7%) had a history of multiple primary cancers (average of 2.7, range 2–7). Extracolonic cancers, including renal, urothelial, and pancreatic cancers, were observed more than colorectal cancers after age 50 (56% vs 44% of

Table 1  Characteristics of MSH6 and PMS2 carriers with at least one in-house surveillance colonoscopy prior to age 50

| Variable                        | Total (N = 113) | MSH6 (N = 76) | PMS2 (N = 37) | P (MSH6 vs. PMS2) |
|---------------------------------|----------------|---------------|---------------|-------------------|
| Female sex                      |                |               |               |                   |
| 69 (61%)                        | 49 (64%)       | 20 (54%)      | 0.31          |
| Race/Ethnicity                  |                |               |               |                   |
| NH White                        | 104 (92%)      | 73 (96%)      | 31 (84%)      | 0.05              |
| Black                           | 4 (4%)         | 1 (1%)        | 3 (8%)        |                   |
| Hispanic                        | 1 (1%)         | 0             | 1 (3%)        |                   |
| Asian/Pacific Islander          | 3 (3%)         | 1 (1%)        | 2 (5%)        |                   |
| Unknown                         | 1 (1%)         | 1 (1%)        | 0             |                   |
| AJ ancestry                     | 23 (20%)       | 21 (28%)      | 2 (5%)        | < 0.01            |
| Smoking                         |                |               |               |                   |
| Never                           | 79 (70%)       | 51 (67%)      | 28 (76%)      |                   |
| Ever                            | 29 (26%)       | 20 (26%)      | 9 (24%)       |                   |
| Unknown                         | 5 (4%)         | 5 (7%)        | 0             |                   |
| Personal history of any cancer  | 50 (44%)       | 36 (47%)      | 14 (38%)      | 0.42              |
| Personal history of colon cancer| 21 (19%)       | 16 (21%)      | 5 (14%)       | 0.44              |
| Total number of colonoscopies   | 314            | 202           | 112           | 0.33              |
| Number of colonoscopies per patient (median, IQR) | 2 (1–4) | 2 (1–3) | 3 (1–4) | 0.32 |
| Age at first colonoscopy (median, IQR) | 39 (32–44) | 39 (33–44) | 38 (32–44) | 0.75 |

NH—Non-Hispanic; AJ—Ashkenazi Jewish; IQR—Interquartile range

Table 2  MSH6 and PMS2 carriers with colonic neoplasia identified

| Colonoscopy before age 50 | Total (N = 113) | MSH6 (N = 76) | PMS2 (N = 37) | P (MSH6 vs. PMS2) |
|---------------------------|----------------|---------------|---------------|-------------------|
| Any neoplastic lesion     | 46 (41%)       | 32 (42%)      | 14 (38%)      | 0.69              |
| Any advanced adenoma/     | 7 (6%)         | 5 (7%)        | 2 (5%)        | 0.99              |
| advanced serrated lesion  |               |               |               |                   |
| identified before 50      |               |               |               |                   |
| Any CRC identified before | 4 (4%)         | 3 (4%)        | 1 (3%)        | 0.99              |

Table 2  MSH6 and PMS2 carriers with colonic neoplasia identified

| Colonoscopy before age 30 | Total (N = 20) | MSH6 (N = 16) | PMS2 (N = 4) | P (MSH6 vs. PMS2) |
|---------------------------|----------------|---------------|--------------|-------------------|
| Any neoplastic lesion     | 14 (43%)       | 11 (69%)      | 3 (75%)      | 1.00              |
| Any advanced adenoma/     | 2 (6%)         | 2 (12%)       | 0 (0%)       | 1.00              |
| advanced serrated lesion  |               |               |              |                   |
| identified before 50      |               |               |              |                   |
| Any CRC identified before | 1 (5%)         | 1 (6%)        | 0 (0%)       | 1.00              |

NH—Non-Hispanic; AJ—Ashkenazi Jewish; IQR—Interquartile range

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cancers after age 50 compared to 38% vs 62% prior to age 50, p = 0.001). Overall, the proportion of individuals developing incident colorectal and endometrial cancers decreased with age. The incidence of second primary cancers increased among individuals in their 5th decade of life. In total, 25 patients had a history of autoimmune disease and these were more likely to have a second primary cancer diagnosis (OR 2.3).

Conclusions These findings support the success of screening and prevention efforts at reducing colorectal and endometrial cancers, thus lengthening the lifespans of LS patients. As a result, the incidence of extracolonic LS cancers among older individuals is increasing. Our data also suggest that immune status may play a role in the development of LS cancers (Fig. 1).

Recurrent frameshift neoantigen vaccine in a Lynch syndrome mouse model: first evidence of a tumor-preventive effect

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Table 1  Study population demographics

| N (%) | Total study population | With PV/ LPV | Without PV/ LPV | P value |
|-------|------------------------|--------------|-----------------|---------|
| N = 1380 |                        |              |                 |         |
| Age at diagnosis, mean ± stand dev | 65.7 ± 11.2 | 65.2 ± 12.2 | 65.7 ± 11.0 | 0.58    |
| Gender |                         |              |                 |         |
| female | 694 (50.4) | 95 (48.5) | 599 (50.6) | 0.58    |
| male   | 686 (49.7) | 101 (51.5) | 585 (49.4) |         |
| Race   |                         |              |                 | 0.02    |
| Asian  | 103 (7.5) | 11 (5.6) | 92 (7.8) |         |
| Black  | 84 (6.1) | 7 (3.6) | 77 (6.5) |         |
| Mixed  | 44 (3.2) | 2 (1.0) | 42 (3.6) |         |
| Other  | 71 (5.1) | 6 (3.1) | 65 (5.5) |         |
| White  | 1051 (76.1) | 170 (86.7) | 881 (74.4) |         |
| Unknown | 27 (2.0) | 0 | 27 (2.3) |         |
| Ethnicity |                  |              |                 | 0.06    |
| Non-Hispanic | 1237 (89.6) | 185 (94.4) | 1052 (88.9) |         |
| Hispanic | 99 (7.2) | 8 (4.1) | 91 (7.7) |         |
| Unknown | 44 (3.2) | 3 (1.5) | 41 (3.5) |         |
| Ashkenazi Jewish | 1042 (75.5) | 155 (79.1) | 887 (74.9) | 0.07    |
| No | 183 (13.3) | 16 (8.2) | 167 (14.1) |         |
| Yes | 155 (11.2) | 25 (12.7) | 130 (11.0) |         |
| Smoking status at PDAC diagnosis |              |              |                 | 0.39    |
| Current | 120 (8.7) | 19 (9.7) | 101 (8.5) |         |
| Never | 665 (48.2) | 103 (52.5) | 562 (47.5) |         |
| Past | 466 (33.8) | 56 (28.6) | 410 (34.6) |         |
| Unknown | 129 (9.3) | 18 (9.2) | 111 (9.4) |         |
| Personal history of other cancer* |              |              | < 0.0001 |         |
| No | 1127 (81.7) | 128 (65.3) | 999 (84.4) |         |
| Yes | 252 (18.3) | 68 (34.7) | 184 (15.5) |         |
| Unknown | 1 (0.0) | 0 (0.0) | 1 (0.1) |         |

*Excludes basal cell and squamous cell carcinomas of the skin

Yield of surveillance colonoscopy in young patients with MSH6 and PMS2-associated Lynch syndrome

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Background Lynch syndrome is the most common hereditary colorectal cancer (CRC) predisposition syndrome. MLH1/MSH2/ EPCAM likely pathogenic/pathogenic variant (PV)-carriers have the highest CRC risk with colonoscopy recommended to start between age 20–25. CRC risk amongst MSH6 and PMS2 PV-carriers is lower with recent guidelines advocating for later colonoscopy initiation between age 30–35. However, there are limited data on colonoscopy findings in young MSH6/PMS2 PV-carriers that could help inform the ideal age for colonoscopy initiation.

Methods Data was collected from a multi-institutional cohort of MSH6/PMS2 PV-carriers who had an in-house surveillance colonoscopy performed prior to age 50. Neoplastic lesions were defined as a colonic adenocarcinoma, adenoma, or non-hyperplastic serrated lesion. Continuous and categorical variables were compared using non-parametric tests.

Results Of 386 total MSH6/PMS2 PV-carriers, 113(29%; 76 MSH6; 37 PMS2) had an in-house surveillance colonoscopy performed prior to age 50. Neoplastic lesions were defined as a colonic adenocarcinoma, adenoma, or non-hyperplastic serrated lesion. Continuous and categorical variables were compared using non-parametric tests.

Conclusions Amongst a multi-institutional cohort of MSH6/PMS2 carriers who underwent in-house colonoscopy before age 50, colonic neoplasia was found at similar rates in both MSH6 and PMS2 PV-carriers. While CRC was not identified on screening before age 30,
there was still colonic neoplasia in both MSH6 and PMS2 PV-carriers before age 30, highlighting that the age of surveillance colonoscopy initiation in MSH6/PMS2 PV-carriers needs further study (Tables 1, 2).

### Does family history of pancreatic cancer in pathogenic variant carriers identify patients who are diagnosed with pancreatic cancer: Results of a multi-site collaboration

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**Background** Previously reported single institution data on family history of pancreatic adenocarcinoma (PDAC) showed that most individuals with a germline pathogenic or likely pathogenic variant (PV/LPV) in a PDAC susceptibility gene who were diagnosed with PDAC would not have met current recommendations for PDAC surveillance established by the National Comprehensive Cancer Network, the American College of Gastroenterology, or International Cancer of the Pancreas Screening Consortium. These recommendations rely on the assumption that PV/LPV carriers with family history of PDAC are at greater risk for developing PDAC as compared to carriers without a family history. This study is a multi-site collaboration to validate the previous findings.

**Methods** Individuals with PDAC who had a germline PV/LPV in ATM, BRCA1, BRCA2, EPCAM, MLH1, MSH2, MSH6, PALB2, or PMS2 were assessed for family history of PDAC in first-degree relatives (FDR) or second-degree relatives (SDR). A comparison group of individuals with PDAC who had no germline PV/LPV identified through multi-gene panel testing was also assessed. Chi-square and t-tests were used to determine statistical significance.

**Results** Nine institutions compiled a cohort of 196 individuals with PDAC who had a germline PV/LPV in one of the aforementioned genes. See Table 1 for demographics. Fifty (25.5%) had an FDR and/or SDR affected by PDAC and 146 (74.5%) had no family history of PDAC. The cohort was significantly more likely to have a PDAC-affected FDR or SDR than individuals with PDAC who had no germline PV/LPV (p = 0.004). Significance was also reached for affected FDR alone (p = 0.003), but not for affected SDR alone (p = 0.344). See Table 2.

**Conclusions** This multi-site study confirms that most individuals with PDAC and a PV/LPV in ATM, BRCA1, BRCA2, EPCAM, MLH1, MSH2, MSH6, PALB2, or PMS2 would not meet current pancreatic cancer surveillance recommendations because they do not have family history of PDAC. Family history, particularly an affected FDR, enriches the cohort but alone is insufficient in identifying the majority of high-risk individuals who are at risk for developing PDAC.

**Table 2 Family history of PDAC in PV/LPV carriers with PDAC compared to individuals with PDAC and no PV/LPV**

| Total study population | With PV/LPV | Without PV/LPV | p-value |
|------------------------|-------------|----------------|---------|
| PDAC affected FDR      | N = 1380    | N = 196        | N = 1184 | 0.003   |
| No                     | 1208 (87.5) | 159 (81.1)     | 1049     |         |
| Yes                    | 172 (12.5)  | 37 (18.9)      | 135 (11.4)| |
| PDAC affected SDR only*| N = 1208    | N = 159        | N = 1049 | 0.344   |
| No                     | 1130 (93.5) | 146 (91.8)     | 984 (93.8)|         |
| Yes                    | 78 (6.5)    | 13 (8.2)       | 65 (6.2) |         |
| PDAC affected FDR and/or SDR | N = 1380 | N = 196 | N = 1184 | 0.004 |
| No                     | 1130 (81.9)| 146 (74.5)     | 984 (83.1)|         |
| Yes                    | 250 (18.1) | 50 (25.5)      | 200 (16.9)|         |

*Individuals with PDAC affected FDR were excluded from total

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**Allergic and immune mediated factors as drivers of serrated polyposis syndrome**

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Serrated Polyposis Syndrome (SPS) presents with multiple sessile serrated lesions (SSL) and confers high risk for colorectal cancer. Etiology of SPS remains unknown. Genetic differences do not seem to cause most SPS cases. Eosinophilic esophagitis (EoE) is an inflammatory condition of the esophagus that causes chronic dysphagia, probably driven by immune responses from food-specific antigens or other allergens. Anecdotally, we have encountered many SPS patients with seasonal or other allergies, and, interestingly, sporadic SSL have frequently been observed in EoE patients. The purpose of this study was to analyze RNA-sequencing (RNA-Seq) results of SSL from SPS patients and esophageal tissues from EoE patients, and assess whether certain immune or allergy-related pathways overlap between SPS SSL colon and EoE esophagus.

**Methods** RNA-Seq data from a) 10 SPS patients’ right-colon SSL, b) 9 right-colon adenomatous polyps (AP) from non-SPS patients, c) 10 normal right-colon from colonoscopy patients never identified with a colon polyp (control colon), d) 14 EoE patients’ esophagus, and e) 14 non-EoE esophagus (control esophagus) were aligned to GRCh38 from Ensembl version 102. Differentially expressed genes were identified in SPS-SSL vs. control colon, AP vs. control colon, and EoE esophagus vs. control esophagus using a 5% false discovery rate (FDR) with DESeq2. Enriched pathways were analyzed using a 10% FDR with Gene Set Enrichment Analysis (GSEA).

**Results** There are 1807 significant genes shared between SPS-SSL and EoE, compared to only 7 between AP and EoE (Fig. 1). 57 protein-coding genes were significant at FDR < 0.0001 in both SPS-SSL colon and EoE esophagus. Hallmark gene sets overlapping in SPS-SSL and EoE included positively enriched Apical Junction, and negatively enriched Oxidative Phosphorylation and Myc Targets V1 (Fig. 2A). Similarly, KEGG pathways included positively enriched Chemokine Signaling and Jak Stat Signaling (Fig. 2B).

**Conclusion** Differentially expressed genes and enriched pathways that overlap in SPS-SSL (but not AP) and EoE suggest allergic and/or immune-based etiologies as drivers of SPS development. Further studies of genes, pathways and clinical correlations in these and other cohorts may assist prevention and treatment of SPS and EoE.

**Endoscopic submucosal dissection (ESD) of a large gastric white mucosal patch in a patient with FAP**

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**Background** We have recently described the increasing incidence of gastric cancer in patients with familial adenomatous polyposis (FAP) in the US. White mucosal patches (WMP) within an area of proximal gastric polyposis may be seen in some patients with FAP and when present are associated with other high risk gastric histology including gastric cancer both within and outside the WMP. WMPs are often flat, large, and in the fundus or upper gastric body. Due to their positioning in the stomach, flatness, and large size they may be difficult to resect completely using traditional endoscopic techniques. Here we demonstrate a case where we used endoscopic submucosal dissection (ESD) to resect a very large WMP.

**Case presentation** A 64 year old female with FAP presented for ongoing EGD polyposis surveillance. Previous EGD revealed more than 100, 2–9 mm gastric polyps in the cardia, fundus and gastric body and Spigelman stage I duodenal polyposis. Additionally, an 80 mm flat WMP (Fig. 1) was noted in the proximal gastric body along the greater curvature of the stomach. Multiple biopsies of the white patch revealed gastric adenoma with low-grade dysplasia. Due to the high risk nature of this lesion, we decided to pursue en-bloc resection using ESD for complete resection of the neoplasm. The WMP was approached in retroflexion and a ProdiGI traction wire (ERD-TW35, Medtronic, Dublin, Ireland) was used for traction. Complete en-bloc R0 ESD resection was achieved. Post resection histology showed the foveolar-type gastric adenoma with low grade dysplasia.

**Fig. 1** xxx

**Fig. 2** xxx
dysplasia. The patient went home after the procedure and was doing well on follow up and had no adverse events. Given the gastric polyposis and high risk histology, surveillance will be continued no less frequently than yearly.

**Discussion** This report describes a novel application of ESD in patients with FAP and WMP. Certain endoscopic features including a carpeting of proximal polyps, polyoid mounds, polyps > 1 cm, and WMPs are associated with gastric cancer in FAP. High risk histology such as pyloric gland adenoma, tubular adenoma, fundic gland polyp with high grade dysplasia is more commonly seen within and outside of the WMP. ESD allows for complete resection and more accurate histopathological assessment of margins and presence of high-risk histology.

**Outcomes of push enteroscopy for upper GI tract screening in Lynch syndrome**

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**Background** Lynch syndrome (LS) carriers have an increased risk for small bowel cancers and they are more common in the proximal small bowel. Guidelines previously endorsed extended visualization of the proximal small bowel at the time of upper endoscopy, but this has fallen out of favor without clear reasons. Our aim was to assess the incidence of neoplasia identified during screening with push enteroscopy in LS.

**Methods** A retrospective review of patients followed in the Hereditary and High-Risk GI Clinic for LS from 8.2014 through 12.2020 was approved by the IRB. Per our clinical protocol, push enteroscopy utilizing a pediatric colonoscope was the standard procedure for upper tract screening during the study period. Inclusion criteria included age ≥18 years, pathogenic/likely pathogenic variant in a mismatch repair gene and at least one enteroscopy performed for screening. Exclusion criteria included multiple hereditary cancer syndromes or previous small bowel neoplasia.

**Results** There were 129 patients that met study criteria. Demographic and clinical details are included in Table 1. A total of 172 enteroscopies were performed during the study with an average of 1.3 per patient (range 1–3). Small bowel neoplasia was identified in 3 procedures (1.7%), with 2 adenomatous polyps removed from the distal duodenum/proximal jejunum and 1 duodenal cancer located in the duodenal bulb (Table 2). No procedural complications were noted. The 3 patients with small bowel neoplasia were women age 45—59 with MSH2 or MLH1 variants. Two had a history of endometrial cancer and none had a history of colon cancer. They did not have a family history of small bowel cancer. The distal duodenal and proximal jejunal adenomatous polyps were found on initial screening enteroscopy in patients that had received previous standard upper endoscopy within 3 years.

**Conclusions** Push enteroscopy identified distal duodenal and proximal jejunal neoplastic lesions at a rate at least similar to gastric and proximal duodenal neoplasia and these lesions would not have been identified with standard upper endoscopic screening. Given this increased yield, we favor the use of push enteroscopy if upper endoscopic screening is performed in LS (Figs. 1, 2).

**Table 1** Characteristics of Lynch syndrome patients receiving screening enteroscopy

| Characteristic                                      | n = 129 |
|----------------------------------------------------|---------|
| Age (median [IQR]), years                          | 48 [39–56] |
| Women                                              | 86 (66.6%) |
| Body mass index (median [IQR])                     | 28.3 [24.0–35.2] |
| Race                                               |         |
| Caucasian                                          | 123 (95.3%) |
| African American                                   | 1 (0.8%) |
| Hispanic                                           | 1 (0.8%) |
| Asian                                              | 4 (3.1%) |
| Mutation type                                      |         |
| MLH1                                               | 27 (21.0%) |
| MSH2                                               | 44 (34.1%) |
| MSH6                                               | 39 (30.2%) |
| PMS2                                               | 18 (14.0%) |
| EPCAM                                              | 1 (0.8%) |
| Aspirin use (daily)                                | 69 (53.5%) |
| Family history small bowel cancer                  |         |
| First degree relative                              | 7 (5.4%) |
| Second degree relative                             | 4 (3.1%) |
| Number of enteroscopies (mean [range])             | 1.3 [1–3] |

**Table 2** Results of screening enteroscopy in Lynch syndrome

| Neoplasia Type                   | n = 172 |
|---------------------------------|---------|
| Distal duodenal/jejunal adenoma | 2       | 1.2%   |
| Distal duodenal/jejunal cancer   | 0       | 0%     |
| Proximal duodenal adenoma       | 0       | 0%     |
| Proximal duodenal cancer        | 1       | 0.6%   |
| Gastric adenoma                 | 0       | 0%     |
| Gastric cancer                  | 1       | 0.6%   |
Diagnoses of Lynch Syndrome in pediatric cancer patients undergoing paired tumor-germline sequencing

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Background Lynch syndrome (LS) is an adult-onset hereditary cancer syndrome, meaning generally there is no expected increased risk for childhood cancers. Rarely, children in families with LS present with cancer but little is known about the potential contribution of LS to these malignancies. Paired tumor-germline sequencing can inform our knowledge of a possible role of LS when childhood malignancies occur in these families.

Methods Results from patients ages 0–25 years enrolled in the pediatric arm of one institution’s paired tumor-germline sequencing study over eight years were analyzed for frequency of germline pathogenic variants (PVs) associated with LS. Tumor loss-of-heterozygosity (LOH), immunohistochemistry (IHC) staining, and/or tumor hypermutation were used to determine whether LS may be causative of the cancers.

Results Of 698 participants, three (0.4%) had a germline pathogenic variant in a gene related to LS. All three had tumor findings consistent with LS-associated cancers; a 15-year-old (MLH1 PV) with a hypermutated astrocytoma with absent MLH1/PMS2 staining on IHC, a 17-year-old (MSH6 PV) with a hypermutated glioblastoma with absent MSH6 staining on IHC, and a 22-year-old (MSH6 PV) with a hypermutated astrocytoma with MSH6 LOH. None reported family history of brain cancers in a three-generation pedigree. LS-associated cancers and/or screening in younger individuals with LS. Additional work in this area could further inform a possible role for germline genetic testing for LS in pediatric patients with astrocytoma and glioblastoma given that LS-related germline PVs were identified in more than 10% of patients in this population with these cancer types (Table 1).

Discussion While LS-related pediatric cancer diagnoses are extremely uncommon and brain tumors are a relatively rare manifestation of LS, this work suggests that when cancers develop in children with LS this may be a common site. Brain cancer screening is currently not part of standard medical management for individuals with LS. These findings raise the question of the appropriate age for predictive testing and indicate a possible role for education on signs of neurological cancers and/or screening in younger individuals with LS. Additional work in this area could further inform a possible role for germline genetic testing for LS in pediatric patients with astrocytoma and glioblastoma given that LS-related germline PVs were identified in more than 10% of patients in this population with these cancer types (Table 1).

Table 1 Barriers to genetic testing

| Barriers | Mean Endorsement score (sum % of 4 s and 5 s) | Correlation to readiness *p < 0.05 |
|----------|---------------------------------------------|----------------------------------|
| I need more information about the how the testing process works | 3.74 70.1 | 0.160 |
| Knowing that I carry an altered gene would cause me to worry more about other family members who could be carriers | 3.78 71.1 | 0.114 |
| I don't know how genetic testing has to offer | 3.64 62.3 | 0.247* |
| If I were found to carry an altered gene, I would worry about passing the gene to future generations | 3.13 50.4 | 0.339* |
| I don’t know how genetic testing benefits me | 3.15 47.4 | -0.139 |
| I am concerned about my family's reaction to my genetic testing results | 1.72 11.9 | -0.120 |
| If I were found to carry an altered gene, I would feel hopeless | 1.85 16.3 | -0.093 |
| Getting genetic testing is not consistent with my religious or spiritual beliefs | 1.19 0.7 | -0.091 |
| If I were found to carry an altered gene, I would feel ashamed | 1.21 8.1 | -0.089 |
| I would worry about how these results affect my employment | 1.67 8.0 | -0.085 |
| I would feel anxious while waiting for my results | 2.83 43.0 | -0.062 |
| I worry that my health insurance would not cover the cost of genetic testing | 3.42 51.1 | -0.061 |
| Knowing that I carry an altered gene would cause me to feel less healthy than other people | 2.22 17.8 | -0.049 |
| If I were found to carry an altered gene, I would feel singled out | 1.59 5.9 | -0.047 |
| If I were found to carry an altered gene, it would cause others to view me negatively | 1.59 5.1 | -0.0071 |
| I would feel guilty if one of my relatives had an altered gene and I did not | 1.70 9.6 | -0.005 |
| I don't know how to get genetic testing | 3.58 62.0 | -0.00 |
| If I were found to carry an altered gene, I would feel guilty if my family member developed cancer | 2.50 28.9 | 0.009 |
| If I were found to carry an altered gene, I would feel anxious | 2.74 45.9 | 0.037 |
| If I were found to carry an altered gene, I would feel scared | 2.80 46.7 | 0.090 |
| Genetic testing would not provide me with any means of preventing cancer | 2.87 29.0 | -0.396* |
Table 1 continued

| Barriers                                                                 | Mean endorsement score (sum % of 4s and 5s) | Correlation to readiness |
|-------------------------------------------------------------------------|---------------------------------------------|--------------------------|
| I’m not sure if the test is accurate                                    | 2.47                                       | -0.357*                  |
| I believe that if someone is diagnosed with cancer there is no purpose for genetic testing at that point | 1.74                                       | -0.352*                  |
| Genetic testing would not help me deal with my fears and uncertainty about having my cancer come back or getting a new one | 2.93                                       | -0.285*                  |
| I have too much going on with my current cancer diagnosis to think about genetic testing right now | 1.85                                       | -0.260*                  |
| I would worry about how genetic testing would affect my health insurance | 2.71                                       | -0.229*                  |
| If I were found to carry an altered gene, I worry it would be considered a pre-existing condition for health insurance | 3.01                                       | -0.227*                  |
| If I were found to carry an altered gene, I would worry about who would have access to my test results | 2.10                                       | -0.206*                  |
| If I were found to carry an altered gene, I worry it would affect my life insurance policy | 2.64                                       | -0.186*                  |
| Getting genetic testing would be too expensive for me                   | 2.98                                       | -0.178*                  |
| If I were found to carry an altered gene, I would feel angry            | 1.89                                       | -0.162                   |
| Knowing my genetic status wouldn’t impact my cancer screening/follow-up monitoring schedule | 3.35                                       | -0.146                   |
| I am concerned about my partner’s reaction to my genetic testing results | 1.51                                       | -0.144                   |
| Knowing that I carry an altered gene would cause me to worry more about other family members who could be carriers | 3.78                                       | 0.114                    |
| I need more information about the how the testing process works         | 3.74                                       | 0.160                    |
| I need to get more information about what genetic testing has to offer  | 3.64                                       | 0.247*                   |
| If I were found to carry an altered gene, I would worry about passing the gene to future generations | 3.13                                       | 0.339*                   |
Utility of adenomatous polyp testing for detection of constitutional mosaic \(APC\) mutations in unexplained polyposis patients

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Background A subset of patients with colorectal adenomatous polyposis have familial adenomatous polyposis (FAP) caused by pathogenic germline \(APC\) variants. In polyposis patients without an identified pathogenic \(APC\) variant, constitutional mosaic \(APC\) (cm\(APC\)) mutations are known to occur. Mosaic \(APC\) mutations can be difficult to detect in peripheral blood or saliva, and new methods are needed to improve detection.

Methods We developed the 3-sample ColoSeq Polyposis next-generation sequencing (NGS) test to improve the detection of cm\(APC\) mutations in patients with unexplained adenomatous polyposis. The test simultaneously evaluates neoplastic tissue from at least two adenomas (formalin-fixed paraffin-embedded, FFPE) and a germline sample, either peripheral blood, saliva or normal FFPE tissue. Using a custom NGS capture and bioinformatics, data from multiple samples from 28 patients were analyzed to identify shared \(APC\) mutations between adenomas and assess the presence of any shared mutation in the germline sample. Data were correlated with the age at initial adenomatous polyp(s) detection and the estimated number of adenomatous polyps.

Results We identified cm\(APC\) mutations in 18/28 cases (64%). Half (9/18) of patients with mosaic \(APC\) had the mutation detected only in polyps, with mosaic mutations detected at low allelic fractions in the germline sample of the remaining cases (9/18). In 10/28 cases (36%), independent \(APC\) mutations were detected in each of the tested polyps, making a diagnosis of FAP less likely. Compared to patients with apparently sporadic polyposis, patients with cm\(APC\) mutations were younger at diagnosis (Wilcoxon rank sum test, one-tailed, \(W = 24, p = 0.0008\)) with median age 49 years (range 13 to 61 years) compared to 62 years (range 30 to 72 years) (Fig. 1). Patients with cm\(APC\) mutations had more polyps (Wilcoxon rank sum test, one-tailed, \(W = 125, p = 0.048\)), with a median of 41 polyps (range 10 to 300) compared to 25 (range 10 to 63) in patients with sporadic polyposis (Fig. 2).

Conclusion Testing multiple adenomas with a paired germline sample using the 3-sample ColoSeq Polyposis technique effectively identifies constitutionally mosaic \(APC\) mutations in patients with unexplained polyposis, particularly among patients under age 60 with greater than 30 adenomas.

Table 1 Characteristics of patients with relevant findings in surgical specimens

| Patient | Histological subtype | Stadium | sex | Age (y) | AIT | Previous colectomy |
|---------|----------------------|---------|-----|---------|-----|-------------------|
| 1       | Multifocal papillary | pT2 mN1a (1/10) RX | w   | 31      | n   | n                 |
| 2       | Cribiform-morular papillary | pT1 NO M0 L0 V0 R0 | m   | 23      | n   | y                 |
| 3       | Papillary | pT1a (m), pNx, R0, L0, V0 | w   | 19      | n   | n                 |
| 4       | Papillary | pT1b L0 V0 R0 | w   | 48      | y   | y                 |
| 5       | Papillary | pT1a R0 V0 L0 | m   | 49      | n   | y                 |
| 6       | Papillary | pT3a(m) pN0 L0 V0 R0 | w   | 21      | n   | n                 |

Routine screening for lynch syndrome: data from the lynch syndrome screening network membership data, 2011–2020

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Background Lynch syndrome (LS) accounts for approximately 2–3% of colorectal (CRC) and endometrial (EC) cancers and is the most common inherited cause of these malignancies. Evidence-based recommendations support testing for LS on all newly diagnosed CRCs and ECs. The Lynch Syndrome Screening Network (LSSN) has promoted institutional implementation of universal tumor screening
for LS (UTS-LS) and collaborative data collection over the past decade.

Methods
Since LSSN was launched in 2011, interested institutions have joined by completing annual membership applications that included information regarding their existing UTS-LS protocols, and the number and type of LS cancers screened since 2008. Application and renewal information received between 2011 and 2020 was analyzed to determine trends in membership and tumor screening.

Results
In 2011, 91 institutions joined LSSN. This number dropped in 2013 to 80 institutions and to 72 institutions in 2018–2020. In 2020, 85% of member institutions reported routine screening for LS on all or a subset of newly diagnosed CRCs. Of these institutions, 97% screen all cases, an increase from 56% in 2012 and 81% in 2013, while the remainder limit by age or other selection criteria. In 2013, 21% of member institutions reported screening all ECs, which increased to 53% in 2020. For both CRCs and ECs in 2013, immunohistochemistry was the most common initial screen (79% and 87%, respectively), which increased to 92% and 95% in 2020. In 2012, 44% of reporting institutions indicated that their genetics department is responsible for reviewing all screens and initiating follow-up on abnormal screen results for CRCs. This changed slightly to 47% by 2020. There was a clear increase in the number of cancers screened over time with over 42,000 cancers screened from 2008 to 2018 (Fig. 1). Over half of the institutions with existing or soon-to-be implemented protocols indicated their willingness to provide additional patient-level data.

Conclusions
LSSN is an important resource to support, track, and promote UTS-LS through collaborative data collection. This membership data demonstrates an increase in the rates of UTS-LS among LSSN member institutions and suggests there is significant interest in collaborative data collection among institutions involved in cancer genetics.

### Risk of malignant and benign thyroid disease in patients with familial adenomatous polyposis – a prospective surveillance study

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Introduction
Patients with familial adenomatous polyposis (FAP) are at high risk to develop colorectal, ampullary or duodenal cancer. FAP is furthermore associated with a substantial lifetime risk for thyroid cancer (TC) and benign thyroid disease. Screening and surveillance guidelines differ substantially between countries. The aim of our study was to assess the prevalence of TC and benign thyroid disease and possible surveillance intervals in a large European center.

Methods
We identified consecutive patients with FAP at a single center from 2010 till September 2020. Inclusion criteria were a known pathogenic variant in the \( \text{APC} \) gene and age \( \geq 18 \) years. All patients were enrolled in our prospective hereditary cancer registry and signed an informed consent. Thyroid surveillance consisted of ultrasound and endocrinologic testing for thyroid disease. We collected demographic, ultrasound, laboratory and histopathological results in case of biopsy or surgery.

Results
A total number of 230 patients with FAP were part of our prospective registry. We had to exclude 15 patients (\( \leq 18 \) y), 28 patients refused to undergo thyroid examination. In 187 patients (100 female; mean age 39 (± 15 y); 187 (100%) pathogenic variant in the \( \text{APC} \) gene) at least one thyroid examination was performed at our center. Endocrinologic testing for thyroid disease revealed abnormal results in 92/187 (49%) with hypothyreodism diagnosed in 32/92 (35%). In 85/186 patients (46%) thyroid nodules were detected with a significant female predominance (55/99 vs 31/87; \( p = 0.01 \)). In 16/187 patients (mean age 41 (± 13y) thyroid surgery was performed revealing thyroid cancers in seven patients, adenoma in one patient and C cell hyperplasia in additional one (Table 1). No patient reported any symptoms prior surgery.

Autoimmune thyreoiditis (AIT) was diagnosed in 28/187 (15%) patients with a female predominance (21/99 vs 7/87; \( p = 0.012 \)), including one patient with thyroid cancer.

In 101 patients, a surveillance examination was done with an interval between 1–3 years. In patients with a normal index examination, no cancer or large thyroid nodule (\( > 5 \) mm) was observed.

Conclusions
Patients with FAP are at increased risk for developing benign and malignant diseases of the thyroid gland irrespective to gender. Our data indicate that if the index examination is inconspicuous, the examination interval may be extended.
Evaluation of upper gastrointestinal tract surveillance in individuals with Lynch syndrome. An international, multicenter registry. EARLY-Study

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Background Lynch syndrome (LS) is the most common hereditary colorectal cancer (CRC) syndrome and accounts for 3% of all CRCs. This autosomal dominant disorder is caused by germline mutations in DNA mismatch repair (MMR) genes (MLH1, MSH2, MSH6, PMS2 and EPCAM). LS includes a variety of extracolonic malignancies such as gastric carcinoma (life-time risk 13%) or small bowel cancer (life-time risk 8%). Whereas several national or European guidelines propose no screening, others propose a baseline esophagogastroduodenoscopy (EGD) and testing for Helicobacter pylori. Also risk factors for upper GI cancer remain unclear. All studies so far were either retrospective or monocentric, which leads to a selection bias.

Methods Patients with a proven (likely-) pathogenic germline variant in the DNA mismatch repair genes (MLH1, MSH2, MSH6, PMS2 or EPCAM) are included in a surveillance program. All detected lesions are assessed for MSI/dMMR and adjacent MMRd crypts.

Results At least one esophagogastroduodenoscopy with mucosal biopsy was performed in 129 proven carriers of a pathogenic variant since 2008. Two new cases of gastric cancer, three new cases of duodenal cancer and one ampullary cancer were observed (age 50–71 years) during routine endoscopic surveillance. The detection of (pre-) neoplastic upper gastrointestinal lesions was often associated with the occurrence of synchronous LS-associated cancer (7/11 patients, 64%; Table 1). One MMRd crypt focus adjacent to a duodenal carcinoma was detected (Image 1). Cancer was detected in patients with and without prior endoscopy (time since last endoscopy 0–48 months).

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 primary endpoint is to compare the 10-year cumulative incidence of upper GI tract neoplasms (gastric cancer and duodenal cancer) between surveillance strategy and symptom-triggered endoscopic examinations in an international setting. Further, we want to identify factors associated with a higher risk to develop upper GI cancer. We intend to provide the basis for systematically assessing the relation of immune surveillance with upper GI cancer risk. To address these important questions, we plan to conduct the study as a joint project of the European Hereditary Tumor Group (EHTG) and The Collaborative Group of The Americas on Inherited Gastrointestinal Cancer (CGA-IGC).

Ampullary adenomas in patients with familial adenomatous polyposis (FAP): Biopsy, management, risk assessment

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Introduction After colectomy has been performed in patients with familial adenomatous polyposis (FAP), ampullary and duodenal cancer are the leading causes of death. Risk stratification of duodenal polyposis and cancer risk is based on Spigelman stage. It is not defined, whether the papilla is part of the Spigelman staging system.
Methods We identified consecutive patients with FAP at a single center from January 2012 till September 2020 with at least one upper GI endoscopy. Patients with a foregut surgery (Whipple procedure or pancreas-sparing duodenectomy) were excluded. We collected demographic, endoscopic and histology data and calculated Spigelman stage with and without papilla results.

Results A total number of 213 patients with FAP were part of our prospective registry. We had to exclude 19 patients due to prior upper GI surgery. In 193 patients (46%) an ampullary adenoma was detected, in 75/89 cases mircoscopically suspected and confirmed by histopathology, in 14/89 cases only detected via routine biopsy.

The inclusion of the histopathological result into the Spigelman score led to an upgrade in 16 cases (7%), with consideration of prophylactic duodenectomy for 2 patients (1%). No adverse events effects were observed following ampullary biopsy. The size of the ampullary adenoma led to an upgrade in 25 patients (28%) and consideration of prophylactic duodenectomy in 4/89 patients (4%).

Fourteen patients underwent a papillectomy at our department. After papillectomy, 6 cases of pancreatitis (42%) and four bleedings (28%; endoscopically managed) occurred. Follow-up data were available for all patients. During follow-up, 6 out of 14 (43%) resection sites demonstrated recurrence. In all six patients, additional EMR and radiofrequency ablation were performed. In 4/6 (67%) patients, a pancreatitis was observed.

Conclusions Biopsy of the ampulla is safe and feasible in FAP patients. Histology and size of the papilla can change Spigelman stage and therefore affect patient management. Endoscopic papillectomies showed substantial adverse events, especially interventional treatment for recurrence. Benefits and risks should carefully be weighted (Figs. 1, 2).

Age of onset of surveillance colonoscopy for MSH6 mutation carriers

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Background Current guidelines by NCCN, the BSG and ESGE stratify the age of onset of surveillance colonoscopy according to the gene associated with Lynch syndrome (LS). They suggest starting colonoscopic surveillance from age 25 years for carriers of a pathogenic variant in MLH1 and MSH2 and 35 years for carriers with a pathogenic variant in MSH6 and PMS2. Previous studies demonstrated pathogenic MSH6 variants to be associated with the occurrence of CRC at later age compared to MLH1/MSH2 variant with a low risk of CRC before the age of 30 years. However, data on cancer development in MSH6 carriers are sparse.

Methods: We analyzed data of our prospective register of the “German Consortium for Familial Intestinal Cancer”, which currently comprises 2108 patients with LS, including 255 carriers (127 males; 50%) of a pathogenic variant in MSH6.

Fig. 1 Endoscopic surveillance

Fig. 2 Ampullary assessment
Results CRCs were observed in 128/255 (50%) MSH6 carriers with a mean age at diagnosis of 47.5 years (range 19–78 years) (Fig. 1). CRCs occurred more often in the left (75 cases; 59%) than in the right hemicolon (53 cases; 41%), with males being more frequently affected than females (55% vs 45%). Of note, in 19/128 (15%) patients, CRCs were detected before the age of 35 years. Most of the MSH6 carriers with CRC (113/128; 88%) were index patients. A total of 139 MSH6 carriers underwent 749 (range 1–28; mean 5.4) surveillance colonoscopies with a cumulative prospective observation time of 785 person-years. Adenoma detection rate (ADR) by either index or follow-up colonoscopy was 14% overall. Below the age of 35, ADR in index colonoscopies was already 13% and thus just as high as in the age group of 60–69 years (Fig. 2). This might imply that adenoma formation in MSH6 carriers is already beginning at a young age. Recently published data from Germany, the Netherlands, and Finland showed that adenoma or advanced adenoma incidence was similar or even higher in MSH6 carriers compared to MLH1 carriers.

Conclusion Taken together, we show that CRCs and adenomas were already apparent in a substantial proportion of MSH6 carriers before the age of 35 years. The high number of index cases might lead to an ascertainment bias. However, our data clearly indicate early cancer development in a relevant subgroup of MSH6 carriers. Thus, we believe that colonoscopic surveillance should start before the age of 35 years in these patients.

Clinical characteristics of colorectal cancer cases (CRC) in MSH6 carriers. Age distribution, gender and localization of CRC is shown.

Adenoma detection rate (ADR) in MSH6 carriers. Adenoma detection in index and follow-up colonoscopies in different age segments are shown.

Overlap in patients meeting NCCN testing criteria for Lynch Syndrome and hereditary breast and ovarian cancer

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Background LS and HBOC can present with phenotypic overlap. Despite this, NCCN has unique testing criteria for these conditions. Prior studies analyzed genetic testing requisition forms (TRF) to identify patients who meet NCCN criteria for genetic testing for LS and/or HBOC. By analyzing self-reported history rather than TRF, data is likely to be more complete, providing a more robust analysis.

Methods Participants recruited from two clinical sites at Michigan Medicine (Breast and Ovarian Cancer Risk Evaluation Clinic (BOCREC), University Health Services (UHS)) from 9/1/20 through 6/30/21 were asked to complete an online personal and family health history module at their convenience, on any internet-connected device. The module was written at a 4th grade reading level to reduce literacy concerns. Answers were analyzed against NCCN guidelines to determine if participants met testing criteria for LS and/or HBOC. All BOCREC participants were asked to complete the module after referral. UHS patients were invited to complete the module if they had a personal and/or family history of cancer. Not all patients invited to participate did so.

Results A total of 723 histories were analyzed, of which 66.11% of patients met at least one testing criterion and 9.82% of patients met both (LS and HBOC) testing criteria. The UHS clinic, which is not designed to identify high-risk patients, had 42.65% of patients meeting at least one criterion, including 11.76% meeting LS criterion and 7.35% meeting both testing criteria. BOCREC, which specializes in seeing patients at increased risk of HBOC, had 10.69% of patients also meeting LS criterion and 10.08% of patients meeting both criteria.

Conclusions This study emphasizes the importance of collecting personal health history and multi-generational (3–7 generations) pedigree in all populations, including low-risk populations, to generate appropriate referrals to genetic counseling and/or determine
appropriate genetic testing. While our study found lower rates of individuals meeting NCCN testing criteria than prior studies looking at TRFs, this is not surprising, as the study analyzed all individuals, as opposed to those at the highest risk undergoing testing.

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Disclosure: I, Bailey Hulswit, disclose that I am a part-time employee of InHerET Inc, receiving salary.

**Creation of a cloud-based database to facilitate multi-institutional collaborative research on universal screening for Lynch syndrome: a partnership between Kintalk@UCSF, LSSN and CGA**

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**Background** Lynch syndrome (LS) is a hereditary cancer syndrome that accounts for the majority of hereditary colorectal and endometrial cancers and approximately 3% of all colorectal cancers. Universal tumor screening has been shown to greatly increase the identification of individuals at risk for Lynch syndrome, and has been implemented at many institutions, but benefit from implementation relies on timely referral and genetic evaluation. To better understand obstacles to diagnosis of Lynch syndrome, our team applied for and was awarded $20 K from the Collaborative Fund of CGA-IGC to create a multi-center cloud-based database for de-identified universal tumor screening records for institutional members of the Lynch Syndrome Screening Network (LSSN).

**Methods** We contracted with a developer to create the database, using database elements developed by the LSSN Database Committee, and housed temporarily on a staging server. In order to add patient records, a graphic user interface to manually input records was created, as well as a bulk upload option by CSV file. A blank CSV template file was created to facilitate other institutions with long-standing databases to map their data for bulk upload. Both methods of adding records were tested for data fidelity by uploading and downloading dummy records from the staging server and any errors were corrected. To ensure that data-sharing requests go through the LSSN, institute accounts cannot view records added by other institute accounts. Once the database was confirmed to work correctly, it was transferred to a live server. This was then tested again to ensure login and account creation, and manual and bulk upload and download, were error-free.

**Conclusion** We have successfully created a cloud-based collaborative database with capability to both manually add and view de-identified records through a graphic user interface, and add or download records in bulk by CSV file. This database was created in collaboration with the LSSN and will be utilized by their participating member institutions to generate a large pool of multi-center universal screening records, allowing for large multi-center studies. Next steps include the finalizing of data use agreements and facilitation of IRB considerations with LSSN member institutions.

**Methods** Our analysis included all cases of endometrial or colon tumors that were found to have abnormal IHC or MSI through our LS universal tumor screening protocol, separated into two cohorts: Jan 1, 2019 to June 1, 2020 (16 months) and June 2, 2020 to June 30, 2021 (13 months). We captured primary language, race, and ethnicity for all patients with abnormal LS screening, and evaluated whether they were referred appropriately to cancer genetics for follow-up. Cases sent to UCSF for outside pathology review only, diagnosed with LS previously, or had MLH1 promoter methylation analysis ordered after 11/30/2020 were excluded.

**Results** 114 cases were included in our analysis, 49 of which met criteria for referral for genetic counseling to have germline genetic testing; the remaining were determined to have abnormal IHC/MSI due to MLH1 promoter hypermethylation. A higher proportion of individuals who self-identified as African American were not referred (missed referral) compared to those who self-identified as Caucasian (58 [62.5%] versus 11/31 [35.5%]). While the overall proportion of missed referrals decreased after June 1, 2020, there was no decrease among individuals who self-identified as African American. There were no differences in referral patterns based on primary language.

**Conclusion** While our overall numbers for these two time periods are small, the heightened awareness of DEI issues and anti-racism initiatives did not seem to impact the disproportionate number of individuals self-identifying as African American who were not referred to genetic counseling following abnormal universal tumor screening for LS. We will continue to analyze this data through the lens of DEI to more fully understand the barriers leading to this disparity, with the hopes of implementing systemic institutional changes that translate to equitable care for our African American patients.

**Universal tumor screening for Lynch syndrome through the lens of diversity, equity, and inclusion**

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**Background** The murder of George Floyd highlighted the ongoing impacts of racism across all aspects of life in America. In response UCSF developed mandatory diversity, equity, and inclusion (DEI) training for providers and staff. We set out to determine if the heightened awareness of DEI issues at UCSF impacted referral patterns after universal tumor screening for Lynch syndrome (LS).

**Methods** Our analysis included all cases of endometrial or colon tumors that were found to have abnormal IHC or MSI through our LS universal tumor screening protocol, separated into two cohorts: Jan 1, 2019 to June 1, 2020 (16 months) and June 2, 2020 to June 30, 2021 (13 months). We captured primary language, race, and ethnicity for all patients with abnormal LS screening, and evaluated whether they were referred appropriately to cancer genetics for follow-up. Cases sent to UCSF for outside pathology review only, diagnosed with LS previously, or had MLH1 promoter methylation analysis ordered after June 30, 2021 were excluded.

**Results** 114 cases were included in our analysis, 49 of which met criteria for referral for genetic counseling to have germline genetic testing; the remaining were determined to have abnormal IHC/MSI due to MLH1 promoter hypermethylation. A higher proportion of individuals who self-identified as African American were not referred (missed referral) compared to those who self-identified as Caucasian (58 [62.5%] versus 11/31 [35.5%]). While the overall proportion of missed referrals decreased after June 1, 2020, there was no decrease among individuals who self-identified as African American. There were no differences in referral patterns based on primary language.

**Conclusion** While our overall numbers for these two time periods are small, the heightened awareness of DEI issues and anti-racism initiatives did not seem to impact the disproportionate number of individuals self-identifying as African American who were not referred to genetic counseling following abnormal universal tumor screening for LS. We will continue to analyze this data through the lens of DEI to more fully understand the barriers leading to this disparity, with the hopes of implementing systemic institutional changes that translate to equitable care for our African American patients.
Timely and correct diagnosis of constitutional MLH1 methylation at first presentation of cancer

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Constitutional MLH1 methylation, or “epimutation”, is a rare cause for a cancer phenotype reminiscent of Lynch syndrome, most frequently colorectal cancer (CRC) and endometrial cancer (EC). Characterized by methylation of one allele of the MLH1 CpG island promoter with allelic loss of expression throughout normal tissues, this molecular mechanism predisposes to early-onset mismatch repair deficient (MMR-d) cancers displaying immunohistochemical loss of MLH1/PMS2 expression, microsatellite instability, and MLH1 hypermethylation. While some familial cases of constitutional MLH1 methylation have been linked to germline non-coding variants (e.g. the c.-27C > A; c.85G > T haplotype and promoter deletions), most cases arise de novo and test negative in cancer gene panel testing. With the presence of MLH1 methylation in the tumors and absence of germline mutations, patients with constitutional MLH1 methylation may be mistaken for more common sporadic MLH1-methylated cancers in universal testing schemes of CRC and EC for MMR-d and Lynch syndrome, or undergo a molecular diagnostic odyssey before the cause of their cancer is diagnosed. Given carriers of constitutional MLH1 methylation are at high risk for metachronous cancers, it is important to diagnose them at first presentation. The selection algorithm with the highest positive detection rate is presence of MLH1 methylation in the tumor of a patient with first cancer presentation < 60 years of age. While CLIA testing for MLH1 methylation in whole blood samples does identify carriers of this defect, this has led to one case with a false-positive test result, most likely due to the detection of MLH1 methylation within circulating tumor DNA (ctDNA) in the blood plasma originating from an undiagnosed tumor at the time of the blood draw. It is therefore important to perform CLIA testing on leukocyte nuclei, not whole blood. In confirmed carriers of constitutional MLH1 methylation, it is important to test first degree relatives, and/or undertake extended sequencing around the MLH1 gene in the proband to identify potential linked germline non-coding variants. The Hitchins Laboratory has NCI/NIH R01 funding to undertake an extended molecular “work-up” of cases with either confirmed MLH1 methylation, or early-onset MLH1 methylated tumors, which includes allelic methylation testing and extended sequencing around MLH1. I am seeking patient samples for inclusion on a collaborative basis.

Characterization of gastrointestinal neoplasms in the NCI Li-Fraumeni syndrome study

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Background Li-Fraumeni syndrome (LFS) is a variably penetrant cancer predisposition syndrome typically caused by heterozygous germline pathogenic variants in TP53. Increased risk of colorectal adenocarcinoma (CRC) and other gastrointestinal (GI) cancers in LFS has led to screening recommendations of colonoscopy and endoscopy starting at age 25 years. However, understanding of the spectrum of GI neoplasms in LFS is limited.

Methods This study evaluated 510 individuals with germline TP53 pathogenic or likely pathogenic (P/LP) variants. High-grade dysplasia (HGD) was included with the respective site malignancy. LFS-associated cancer incidences were compared with the Surveillance, Epidemiology, and End Results (SEER) 1975–2017 registry. Cumulative incidences were calculated using Aalen Johansen fitter.

Results GI malignancies were diagnosed in 55 (10%) individuals. CRC/HGD occurred in 26 (5.1%) individuals at a median age of 41 years (range 19–69). Twelve of the 26 CRC/HGD were identified prior to age 40, including two before age 25 years. Esophageal and gastric cancer/HGD were diagnosed in four (0.8%) and 11 (2.2%) individuals respectively, at median ages of 49 (range 34–62) and 54 (range 32–70) years. Other lower GI tract neoplasms included nonepithelial malignancies in five (1.0%) and benign polyps in 73 (14.3%) individuals. Other upper GI tract neoplasms consisted of small intestine lymphoma in one (0.2%) and benign polyps in 11 (2.2%) individuals. Solid GI organ findings included pancreatic cancer in nine (1.8%), liver carcinoma in three (0.6%), liver sarcomas in three (0.6%), and benign liver tumors in four (0.8%) individuals. We observed increased incidence and earlier median age at diagnosis for all GI cancers in LFS compared with the general population; standardized incidence ratios showed 15-fold (9.6–21.5) and 31-fold (14.3–59.5) incidence of CRC/HGD and gastric cancer/HGD, respectively. Cumulative incidences of CRC/HGD, gastric cancer/ HGD, and pancreatic cancer were 18.5% (9.6–33.6%), 11.6% (4.6–27.7%), and 7.3% (3.5–15.1%), respectively, by age 70.

Conclusions Our study highlights the increased burden of GI malignancies in individuals with P/LP germline TP53 variants compared with the general population and reinforces the importance of existing high-risk surveillance for GI cancers. Further studies are needed to evaluate the impact of cancer screening in this group and further tailor these recommendations.

Bone morphogenic protein receptor 2 (BMPR2) as a potential germline driver in Juvenile Polyposis Syndrome (JPS)

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Background Juvenile Polyposis Syndrome (JPS) is a cancer predisposition syndrome characterized in some cases by germline mutations in BMPR1A or SMAD4. However, in 40–60% of patients the germline driver is unknown. Whole exome sequencing (WES) in a cohort of JPS patients identified bone morphogenic protein receptor 2 (BMPR2) as a candidate driver in one patient with high polypl burden. We established patient-derived, three-dimensional colon organoids
(colonoids) from this individual for comparative analysis of proliferation and downstream pathway activation.

**Methods** Through whole exome sequencing, an individual with high polyp burden and negative genetic testing for variants in *SMAD4* and *BMPRIA* was identified to have a potentially pathogenic germline variant in *BMPR2*. Under an IRB-approved protocol, patient-derived, three-dimensional colonoids were established from adjacent colon and polyp tissue of this individual, as well as an individual with a *BMPRIA* deletion. Proliferation and metabolic activity of each line were measured by Ki67 staining, EdU labelling, and cell titer glo (CTG) assay alongside age- and sex-matched controls. Relative protein expression of known drivers and downstream effectors in colonoids was examined by Western Blot.

**Results** Distinct phenotypic differences are evident between colonoids from an individual with a *BMPRIA* deletion and both the colon and polyp *BMPR2* variant colonoids, such as increased crypt budding and Ki67 staining. CTG assay data indicate higher metabolic activity in *BMPR2* variant colonoids in comparison to normal controls (*p* = 0.0104). Through Western blot analysis, both *BMPR2* colon and polyp colonoids showed decreased *SMAD4* expression and phosphorylation. These data demonstrate that both known and candidate genotypes are consistent with a hyperproliferative phenotype. In vitro data support *BMPRIA* as a candidate germline driver of the JPS phenotype, and additional study is ongoing regarding downstream pathway regulation in colonoids with a *BMPR2* variant.

**Conclusions** These demonstrate that clear phenotypic differences exist between colonoid lines established from normal controls, individuals with known JPS predisposition genes, and a novel candidate driver of disease, *BMPR2*. Proliferation and protein expression data suggest that both known and candidate genotypes are consistent with a hyperproliferative phenotype. These data indicate higher metabolic activity in *BMPR2* variant colonoids in comparison to normal controls. These data demonstrate that both known and candidate genotypes are consistent with a hyperproliferative phenotype. In vitro data support *BMPRIA* as a candidate germline driver of the JPS phenotype, and additional study is ongoing regarding downstream pathway regulation in colonoids with a *BMPR2* variant.

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**Management of a transgender male with Lynch syndrome**

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**Background** There is currently limited data and no guidelines for the management of transgender individuals with inherited cancer risk. We present a case of a young transgender male with newly diagnosed Lynch syndrome (LS) and the complex care considerations of his future management.

**Case Presentation** EN is an unaffected 18-year-old transgender male (assigned female at birth) referred to Genetics for cascade testing, as his father was known to carry a MSH2 PV. Regarding his transition, he took puberty blockers from age 11 and testosterone since age 16. He completed gender-reaffirming top surgery at age 15 but had not thought deeply about plans for gender-reaffirming bottom surgery. His family history of cancer is significant for 2 paternal aunts had colon cancer (CRC) reportedly diagnosed as early as age 20, synchronous uterine and ovarian cancer in an aunt diagnosed in her 40s, and sebaceous adenomas in his father. He consented to a 77-gene germline genetic testing panel and was found to carry the familial MSH2 PV only. He was recommended to return to Genetics for a physical exam and to coordinate his first colonoscopy screening.

**Discussion** This case brings several biopsychosocial considerations to light, namely that there is little data and no formalized recommendations for cancer screening of trans individuals with LS. As such, we are following the current NCCN guidelines while thoughtfully attempting to tailor this patient’s care plan to account for his gender-related healthcare. We are discussing the timing and impact of a risk-reducing hysterectomy/salpingo-oophorectomy, which intrigued him because this procedure would have been considered elective in the

| Table 1 Demographic, clinical, and endoscopic features of Barrett’s esophagus in Lynch syndrome |
|---------------------------------------------------------------|
| **LS with** | **LS without** | **P-value** | **Odds Ratio** |
| **BE** | **BE** | (95% confidence interval) |
| Male | 13 | 106 | 106 | 3.00 (1.21–7.48) |
| MMR PV | | | | |
| **MSH2** | 8(38.1) | 109 (36.1) | > 0.99 | 1.09 (0.44–2.71) |
| **MLH1** | 4 (19) | 85 (28.1) | 0.52 | 0.60 (0.20–1.84) |
| **MSH6** | 6(28.6) | 67 (22.2) | 0.59 | 1.40 (0.52–3.76) |
| **PMS2** | 3 (14.3) | 41 (13.6) | > 0.99 | 1.06 (0.30–3.76) |
| Age at LS diagnosis | 54.3 [45.3, 59.5] | 47.9 [35.3, 56.2] | 0.04 |
| Age at 1st LS related EGD | 55.3 [49.2, 59.5] | 49.1 [38.0, 58.1] | 0.05 |
| Age at last LS EGD | 59.5 [55.9, 63.8] | 50.7 [40.3, 60.3] | 0.009 |
| Age at BE diagnosis | 56.0 [47.0, 59.0] | NA | NA |
| Hiatal hernia present | 19 (90.5) | 97 (32.1) | < 0.001 |
| Hiatal hernia size ≥ 3 cm | 0 (0) | 10 (3.3) | > 0.99 | NA |
| *H. pylori* positive | 0 (0) | 10 (3.3) | > 0.99 | NA |
| Personal Hx of Ca | 18 (85.7) | 198 (65.6) | 0.10 |
| Personal Hx of Ca 3.15 (0.91–10.95) | 0.003 | 11.19 (2.41–52.1) |
| Family Hx gastric or esophageal Ca | 2 (9.5) | 10 (3.3) | 0.18 | 3.07 (0.63–15.03) |
| History of smoking | 59.1 (18.5) | 108 (35.8) | 0.68 | 1.35 (0.55–3.30) |
| History of BMI > 30 kg/m² | 11 (52.4) | 122 (40.4) | 0.40 |
| NSAID/Aspirin use | 9 (26.3) | 3 (3.1) | 5 | 0.001 |
| PPI use | 12 (42.9) | 133 (44) | > 0.99 | 0.95 (0.39–2.33) |
| NSAID/Aspirin use | 9 (26.3) | 3 (3.1) | 5 | 0.001 |
| PPI use | 12 (42.9) | 133 (44) | > 0.99 | 0.95 (0.39–2.33) |
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| PPI use | 12 (42.9) | 133 (44) | > 0.99 | 0.95 (0.39–2.33) |
Germline mutations In MSH2, RAD51D and ATM gene in patients with GIST (gastrointestinal stromal tumor) and second epithelial tumors

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Background Adult GISTs are frequently sporadic, while rarely GISTs are linked to Carney triad and Carney-Stratakis Syndrome and NF1. GISTs with second primary tumours are reported in 4-33% of patients in literature and genetic counselling is suggested to explore an underlying germline mutations pathway.

Methods In our Academic Hospital Centre (EURACAN member) in Florence, Italy, we are following patients with GIST and multiple primary tumors with genetic counseling (72/185) and germline analysis of the following genetic panel is performed as clinically indicated: BRCA1, BRCA2, MUTYH, MLH1, MSH2, MSH6, CDH1, ATM, TP53, PTEN, CHEK2, PALB2, BARD1, BRIP1, BLM, RAD51C, RAD51D, XRCC2, PMS2, MRE11A, RAD50, NBN, FAM175A, EPKAM, TSK1, MEN1 by sequencing analysis with Illumina MiSeq by kit multiplicom BRCA Hereditary cancer Magrini plus, and bioinformatic analysis by software SOFHIADD (Sophia genetics) for point genetic alterations of BRCA1 NM_007294.3, BRCA2 NM_000059.3, MUTYH NM_0000249, MSH2 NM_0000251, MSH6 NM_000179, CDH1 NM_00444360, ATM NM_000051, TP53 NM_0000546, PTEN NM_000314, CHEK2 NM_00105735, PALB2 NM_024675, BARD1 NM_000465, BRIP1 NM_032043, BLM, NM_000057, RAD51C NM_002876, RAD51D NM_001142571, XRCC2 NM_005431, PMS2 NM_0000535, MRE11A NM_005590, RAD50 NM_006732, NBN NM_002485, FAM175A NM_139076, EPCAM NM_002354, STK1 NM_000455, MEN1 NM_000244 and MLPA (Multiplex Ligation-dependent Probe Amplification) test analysis for patients with kit P087-BRCA1,P045-BRCA2(2),(2), P248-MLH1-MSH2, P003-MLH1/MSH2, P072-MSH6-MUTYH (MRC-Holland).

Results In 4 patients germline mutations have been observed: 1 patient showed the c.1192dup, p.(Ala398Glyfs*19) pathogenetic variant in exon 7 MSH2 gene confirmed by Sanger Sequencing, 1 patient showed c.565-?_1130?del pathogenic variant consisting in 3–4–5–6 exons deletion of MSH2 gene, confirmed by MLPA analysis, in 1 patient the following ATM alteration has been identified in heterozygosis: c.5319 + 2 T > C, p.(?) and in 1 patient with synchronous GIST and serous ovarian cancer, c.694C > T, p.(Arg239Trp) pathogenic variant and c.715C > T, p.(Arg239Trp) variant of uncertain significance in RAD51D gene have been observed.

Conclusion Our analysis suggests that GIST diagnosis could be tumour-related to multiple hereditary tumour syndromes as Lynch Syndrome, RAD51D and Ataxia-telangiectasia syndrome, the latter being linked in heterozygosis to tumour susceptibility to the breast in female.

Table 1 continued

| Current Study/2021 | United States/Cohort | BE | N | LS with out BE | P-value | Odds Ratio (95% confidence interval) |
|-------------------|----------------------|----|---|---------------|---------|-----------------------------------|
| Kumar/2020/United States/| Cohort | 295 | LS related | Surveillance |
| Eusebi/2021/North America/Meta-analysis | 26,521 | Patients with GERD | 7.2% | 13.9%/1.2% |
| BE: Barrett’s esophagus, MMR = mismatch repair, PV = pathogenic variant, LS = Lynch syndrome, EGD = esophagogastroduodenoscopy, H. pylori = helicobacter pylori, BMI = body mass index, NSAID = nonsteroidal anti-inflammatory drug, PPI = proton pump inhibitor, GERD = gastroesophageal reflux disease, NA = not applicable, ND = not described; Hx = history, Ca = cancer |
Comparison of colorectal cancer patients with Lynch syndrome, double somatic mutations, and MLH1 promoter hypermethylation

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Background Colorectal cancer (CRC) demonstrating MLH1 promoter hypermethylation (MLH1-hm) is generally accepted as sporadic; however, less information is available regarding outcomes and familial risk for patients with double somatic (DS) mutations. Here we describe paired tumor/germline testing results based on clinical risk factors and compare clinical characteristics of DS CRC patients to those with Lynch syndrome (LS) and MLH1-hm.

Methods 647 CRC patients with classic IHC loss patterns [MLH1-/PMS2- (n = 360), MSH2-/MSH6- (n = 145), MSH6- (n = 61), PMS2- (n = 81)] underwent paired testing at a commercial laboratory. Patients were excluded for: prior germline LS and/or MLH1-hm testing (n = 189); concurrent DS and MLH1-hm (n = 2); DS discordant with IHC (n = 3). Patients were grouped based on clinical characteristics aligning with NCCN LS testing criteria: CRC ≤ 50y (n = 75); CRC ≤ 50y with additional risk factors (?RF) (e.g. family history, multiple LS-associated cancers) (n = 42); CRC > 50y + RF (n = 108); no risk factors (CRC > 50y) (n = 228).

Results Regardless of IHC pattern, LS is the most likely result for patients diagnosed ≤ 50y + RF and the least likely for patients with no additional risk factors. For those diagnosed < 50y or > 50y + RF, LS germline mutations were identified in a majority of patients with MSH2-/MSH6- or MSH6- and a minority of patients with MLH1-/PMS2- or PMS2- (Fig. 1). DS patients have an earlier mean age of onset for first LS-associated cancer (58.85y) than MLH1-hm patients (66.03y), and a later age of onset than LS (52.67y) (p < 0.001) based on ANOVA testing. Logistic regression analysis showed that when comparing DS to LS, a similar proportion have CRC < 50y as their only risk factor (p = 0.117), while significantly fewer DS have CRC < 50y + RF (p < 0.001). MLH1-hm are more likely than DS (p = 0.003) or LS (p < 0.001) to have no risk factors and are less likely to present with CRC < 50y compared to DS (p = 0.015) (Fig. 2).

Conclusions Results from this analysis revealed a clinical gradient correlated with origin of mismatch repair deficiency, in which DS lies between LS and MLH1-hm with respect to age of onset. Paired testing outcomes are correlated with clinical presentation; thus, this data can help inform testing and management decisions for CRC patients.

Prevalence and risk factors of Barrett’s esophagus in patients with Lynch Syndrome

Table 2 Characteristics of Universal Testing Group. Demographic and clinical characteristics are provided for long-term survivors (LTS) and short-term survivors (STS) in the universal testing group. Age at PDAC diagnosis is reported in years as the mean ± SD. Sex, age, ethnicity, PDAC stage at the time of diagnosis, and genetic testing results are reported as n (%)

| Characteristics | LTS (n = 20) | STS (n = 166) |
|----------------|-------------|---------------|
| Age at dx       | 65.7 ± 8.8  | 67.6 ± 11.1   |
| Sex             |             |               |
| Male            | 10 (50.0%)  | 93 (56.0%)    |
| Female          | 10 (50.0%)  | 73 (44.0%)    |
| Race            |             |               |

Fig. 1 xxx

Fig. 2 xxx
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**Background** Lynch syndrome (LS) predisposes to upper gastrointestinal (UGI) cancer. A female patient with a germline MSH2 DNA mismatch repair (MMR) pathogenic variant (PV) and Barrett’s esophagus (BE) followed in our LS-related upper gastrointestinal endoscopy (EGD) surveillance program developed a BE-related esophageal adenocarcinoma (EAC) with loss of MSH2 expression on immunohistochemistry. Since BE predisposes to EAC and LS predisposes to UGI cancer, we assessed the prevalence of BE, BE-related dysplasia, and BE-related EAC and factors associated with BE in a cohort of patients with LS.

**Methods** An IRB approved, hereditary database of patients undergoing asymptomatic LS-related EGD surveillance was reviewed. BE was diagnosed by presence of intestinal metaplasia in salmon colored mucosa > 1 cm above the gastroesophageal junction. Demographic, clinical, and endoscopic factors in LS patients with and without BE were compared. Unadjusted univariate logistic regression analysis assessed factors associated with BE.

**Results** 323 patients with > 1 EGDs were included. 21 patients (6.5%) were diagnosed with BE. Of patients with BE, BE-associated dysplasia was observed in 2 patients (9.5%), one (4.8%) of whom developed BE-related EAC. Factors associated with BE included male gender, older age at LS diagnosis and at 1st and last EGD, and presence of a hiatal hernia, especially hernia size > 3 cm (Table 1). No association between MMR PV and BE was observed.

**Conclusions** In our cohort, BE occurred 2.9 greater than observed in the Kumar study, the only other LS related EGD surveillance program reporting BE (Table 1). Neither ours nor Kumar’s study reported the frequency of gastroesophageal reflux (GERD), the greatest risk factor for BE. The BE prevalence and frequency of BE-related dysplasia in our study is similar to a meta-analysis by Eusebi of adults in North America with GERD symptoms (Table 1). Although we had only 1 BE-related EAC, this is 3× more common than reported in the Eusebi study. This may represent a synergistic risk between BE-related dysplasia and LS mutagenesis although we found no association between BE and a specific MMR PV. Larger studies are needed to determine if the risk of BE and BE-associated neoplasia is higher in patients with LS, including those with and without GERD.

### Table 1 PDAC risk perception by demographics, personal and family history

| n | PDAC risk perception over average risk (fold change) | p-value |
|---|---------------------------------|---------|
| Age | < 50 | 52 | 3.61 | 0.68 |
| | > 50 | 98 | 3.29 | |
| Sex | M | 38 | 3.57 | 0.68 |
| | F | 109 | 3.11 | |
| Race | White | 129 | 3.65 | 0.60 |
| | Black | 9 | 1.19 | |
| | Asian | 5 | 2.02 | |
| | Other | 6 | 1.75 | |
| Highest education/ HS | less | 16 | 1.55 | 0.22 |
| | undergrad | 56 | 4.25 | |
| | post-grad | 75 | 3.21 | |
| Pathogenic variant | Y | 116 | 3.31 | 0.23 |
| Gene | BRCA1 | 22 | 4.44 | 0.26 |
| | BRCA2 | 49 | 3.52 | |
| | Lynch | 25 | 2.35 | |
| | other | 14 | 2.97 | |
| Cancer history | Y | 72 | 3.08 | 0.50 |
| | N | 75 | 3.72 | 0.02 |
| PDAC FDR | Y | 41 | 5.14 | |
| | N | 109 | 2.76 | |

PDAC, pancreatic ductal adenocarcinoma; M, male; F, female; HS, high school; Y, yes; N, no; FDR, first degree relative.
Universal insurance coverage of germline testing in patients with colorectal cancer: uptake and potential impact on patient care

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Background Colorectal cancer (CRC) affects about 104,000 patients (pts) annually in the U.S., and up to one-third of cases are estimated to be genetic and/or familial. In 2020, a large U.S. insurer established Medical Policy allowing for and reimbursing germline genetic testing (GGT) for all CRC pts. We report overall uptake of GGT in CRC pts under this coverage policy with actionable findings and management implications for pts tested.

Methods Two independent de-identified datasets were reviewed, including administrative claims data of commercially insured (COM) and Medicare Advantage (MA) enrollees from a large national health plan in the U.S., which included CRC pts, based on ICD10 code, who were continuously enrolled (CE) in the health plan from 1/2019–12/2020. GGT was based on CPT codes during 2020. A second de-identified dataset of CRC pts whose GGT was performed by a large genetic testing laboratory and was billed to the insurer under the Medical Policy in 2020 was also reviewed.

Results Of the >14 million CE enrollees, 39,382 were newly diagnosed CRC pts in 2020; 16,539 COM and 22,843 MA pts. Overall, 5.6% (2,196) of the cohort received GGT (8.1% of COM pts compared to 3.8% of MA pts). Those that received GGT were younger than the overall cohort of CRC pts. From the GGT dataset, 787 pts diagnosed CRC pts in 2020; 16,539 COM and 22,843 MA pts. Overall, 5.6% (2,196) of the cohort received GGT (8.1% of COM pts compared to 3.8% of MA pts). Those that received GGT were younger than the overall cohort of CRC pts. From the GGT dataset, 787 pts had test results available for review. 142 (18%) pts had 152 pathogenic/likely pathogenic germline variants (PGVs) in 30 genes, including: MSH2, MLH1, PMS2, MSH6, CHEK2, APC, BRCA2, ATM, MUTYH (biallelic). Overall, 133 of the 142 (93%) had PGVs in genes with precision therapy, clinical trial and/or published management guidelines. In a subset of pts (n = 674) with ethnicity data, Asian, Black/African-American and Hispanic pts showed lower uptake of GGT relative to Caucasians (Table 1).

Conclusions Despite Medical Policy supporting GGT for all pts with CRC, <10% of eligible pts received testing. If all CRC pts enrolled in the health plan in 2020 had received GGT, potentially ~6,300 additional pts with PGVs conferring potential eligibility for precision therapy (PD-1/PD-L1 inhibitors), clinical treatment trials (PARP inhibitors) and/or specific management recommendations including counseling and cascade testing in relatives, could have been identified, but were missed. Additional research is needed to identify obstacles to systematic implementation of this Medical Policy and to improve access to underrepresented populations.

Table 1 CRC patients with germline genetic testing (VUS—variant of uncertain significance)

| Self-reported ancestry/ethnicity | Patients | P/LP | Negative | VUS |
|---------------------------------|---------|------|----------|-----|
|                                 | N, %    | N, % | N, %     | N, %|
| Asian                           | 28, 4%  | 6, 21%| 11, 39%  | 11, 39%|
| African-American                | 65, 9%  | 15, 23%| 26, 40%  | 24, 36%|
| Hispanic                        | 60, 8%  | 6, 10%| 28, 46%  | 26, 43%|
| Caucasian                       | 520, 77%| 98, 19%| 241, 46% | 181, 34%|
| Total                           | 673     | 125,18%| 306, 45%| 242, 35%|

Are long-term pancreatic cancer survivors more likely to have pathogenic variants in PDAC-susceptibility genes?

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Background Long-term survival for individuals with pancreatic ductal adenocarcinoma (PDAC) is rare. Anecdotally, we observed that long-term survivors referred to our clinic for genetic testing seemed more likely to have pathogenic variants (PV) identified in PDAC-susceptibility genes. This study aims to determine if our observation was borne out by data or simply due to an ascertainment bias.

Methods We queried our pancreatic cancer database (PAGER) to identify individuals with PDAC who underwent genetic testing (n = 393) and selected individuals diagnosed at least 5 years ago. The PV prevalence rate of individuals with greater than 5-year survival (n = 34) was compared to individuals with less than 5-year survival (n = 155).

An independent cohort of individuals (n = 186) were ascertained in a consecutive manner through participation in a universal genetic testing study was used to represent our baseline prevalence rates in unsel ected cases. Since that study was completed less than 5 years ago, we defined long-term survival as more than 4 years for this group. Prevalence was compared with Chi2 tests.

Results In our PAGER cohort, 10 of 34 (29.4%) 5-year survivors had a PV in a PDAC-susceptibility gene, whereas 30 of 155 (19.4%) individuals who lived less than 5 years after diagnosis had a PV (p = 0.19). See Table 1 for PAGER cohort characteristics. In our universal testing cohort, 1 of 20 (5.0%) 4-year survivors had a PV in a PDAC-susceptibility gene, whereas 16 of 166 (9.6%) less than 4-year survivors had a PV (p = 0.50). See Table 2 for the universal testing cohort characteristics.

Conclusions Our data suggests that the higher PV prevalence rate seen in our long-term PDAC survivors is the result of ascertainment bias since these individuals are more likely to have had genetic testing before the advent of universal genetic testing for PDAC, when the requirement for personal or family history of other cancers inflated the positive rate. Further research is needed to define factors that impact survival of PV carriers with PDAC, including stage at diagnosis (which could be influenced by high-risk surveillance) and use of targeted therapies.

Table 1 Characteristics of PAGER Cohort. Demographic and clinical characteristics are provided for the long-term survivors (LTS) and short-term survivors (STS) of the PAGER cohort. Age at PDAC diagnosis is reported in years as the mean ± SD. Sex, race, ethnicity, PDAC stage at the time of diagnosis, and genetic testing results are reported as n (%)

| Characteristic                  | LTS (n = 34) | STS (n = 155) |
|--------------------------------|--------------|---------------|
| Age at dx                       | 60.9 ± 11.6  | 68.7 ± 11.4   |
| Sex                            |              |               |
| Male                           | 19 (55.9%)   | 79 (51.0%)    |
| Female                         | 15 (44.1%)   | 76 (49.0%)    |
| Race                           |              |               |
| White                          | 33 (97.1%)   | 147 (94.8%)   |
| Black/African American         | 1 (2.9%)     | 8 (5.2%)      |
Table 1

| Ethnicity       | LTS (n = 34) | STS (n = 155) |
|-----------------|--------------|---------------|
| Non-Hispanic    | 34 (100.0%)  | 154 (99.4%)   |
| Hispanic        | 0 (0.0%)     | 1 (0.6%)      |
| PDAC Stage      |              |               |
| I/II            | 30 (88.2%)   | 81 (52.3%)    |
| III             | 4 (11.8%)    | 22 (14.2%)    |
| IV              | 0 (0.0%)     | 52 (33.5%)    |
| Genetic Testing |              |               |
| Positive for PV in PDAC Gene | 10 (29.4%) | 30 (19.4%) |
| APC             | 0 (0.0%)     | 1 (3.3%)      |
| ATM             | 5 (50.0%)    | 6 (20.0%)     |
| BRCA1           | 2 (20.0%)    | 3 (10.0%)     |
| BRCA2           | 3 (30.0%)    | 11 (36.7%)    |
| CDKN2A          | 0 (0.0%)     | 1 (3.3%)      |
| MSH2            | 0 (0.0%)     | 1 (3.3%)      |
| MSH6            | 0 (0.0%)     | 2 (6.7%)      |
| PALB2           | 0 (0.0%)     | 3 (10.0%)     |
| TP53            | 0 (0.0%)     | 2 (6.7%)      |
| Negative for PV in PDAC Gene | 24 (70.6%) | 125 (80.6%) |
| Negative/VUS Result | 22 (91.7%) | 120 (96.0%) |
| PV in non-PDAC Gene | 2 (8.3%) | 5 (4.0%) |

Pancreatic ductal adenocarcinoma risk perception and surveillance in at-risk individuals

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**Background**
Surveillance for pancreatic ductal adenocarcinoma (PDAC) is recommended for at-risk individuals due to pathogenic variants (PV) in PDAC-associated genes and/or PDAC family history. Surveillance uptake and adherence could depend on perception of PDAC risk and cancer worry. The study aim was to understand factors associated with PDAC risk perception and surveillance in at-risk individuals.

**Methods**
An IRB-approved RedCap survey was emailed to individuals with PV in PDAC-associated genes and/or PDAC family history from an academic center registry. Questions included demographics, personal and family cancer history, PDAC risk perception, cancer worry and surveillance. Responses through June 30, 2021 were included. Univariate comparisons were performed using chi-square tests, t-tests, and one-way ANOVA.

**Results**
The overall response rate was 29% (150/526). Respondents were 73% female, 86% White, 50% with post-graduate degree, and median age of 55 years (IQR 42.3–64). Personal history of cancer was reported in 50%; 27% with family history of a first degree relative (FDR) with PDAC. The average perceived risk was 3.43-fold above respondent’s reported average general population risk. 91% of respondents report PDAC risk to be slightly higher, higher, or much higher than an average person’s risk. Respondents with an affected FDR perceived their risk to be significantly higher than individuals without history (p = 0.02) (Table 1). Age, sex, race, education level, PV status, and personal cancer history were not predictors of perceived PDAC risk. A third of respondents reported undergoing PDAC surveillance. Of those who did not, 88% would undergo surveillance if offered. No demographic factors differed by surveillance (Table 2). Respondents with an affected FDR were significantly more likely to report surveillance than those without this history (p < 0.0001). PDAC risk perception and PDAC cancer worry were not associated with a significantly different rate of PDAC surveillance (p = 0.20 and p = 0.35, respectively).

**Conclusions**
An affected FDR is predictive of higher PDAC risk perception and surveillance; this should be incorporated when counseling at-risk individuals. Additional factors might also contribute to risk perception and surveillance practices but more data are needed, especially among minority populations and individuals with rare genetic syndromes with an elevated PDAC risk.

Table 2

| Surveillance | No surveillance | p-value |
|--------------|-----------------|---------|
| Age          |                 |         |
| < 50         | 14              | 35      | 0.40 |
| > = 50       | 33              | 60      |      |
| Sex          |                 |         |
| M            | 15              | 22      | 0.31 |
| F            | 32              | 70      |      |
| Race         |                 |         |
| White        | 43              | 80      | 0.23 |
| Non-White    | 4               | 15      |      |
| Highest education |       |         |
| HS or less   | 7               | 8       | 0.26 |
| undergrad    | 18              | 36      |      |
| post-grad    | 22              | 48      |      |
| Pathogenic variant |         |         |
| Y            | 33              | 80      | 0.06 |
| N            | 10              | 10      |      |
| Gene         |                 |         |
| BRCA1        | 4               | 18      | 0.07 |
| BRCA2        | 19              | 28      |      |
| Lynch        | 4               | 21      |      |
| other        | 6               | 7       |      |
| Cancer history |               |         |
| Y            | 20              | 49      | 0.31 |
| N            | 27              | 46      |      |
| PDAC FDR     |                 |         |
| Y            | 25              | 16      | < 0.0001 |
| N            | 22              | 79      |      |
| PDAC risk perception |         |         |
| < 3.43-fold  | 26              | 63      | 0.20 |
| > = 3.43-fold| 21              | 32      |      |
| Cancer worry |                 |         |
| Worried      | 11              | 16      | 0.35 |
| Not worried  | 36              | 79      |      |

PDAC, pancreatic ductal adenocarcinoma; M, male; F, female; HS, high school; Y, yes; N, no; FDR, first degree relative
Table 1 Co-existing MLH1 promoter hypermethylation (PHM) and germline MMR mutation cases

| Co-existing MLH1 Promotor Hypermethylation (PHM) and Germline MMR mutations cases | Age | Family History | Origin | Loss of expression by IHC | BRAF | Germline Variants | Ref |
|---|---|---|---|---|---|---|---|
| PHM + MLH1 mutation, CRC | 37 | Father; CRC at 43, died at 47 | + | MLH1 and PMS2 | + | MLH1: c.678G > T | 1 |
| Brother*, adenomatosus and hyperplastic colonic polypos at 40 | | | | | | | |
| 59 | 4 'Affected' family members | Transverse colon cancer | + | MLH1 and PMS2 | - | MLH1: c.378 T > C, p. (Ser247Pro) | 2 |
| N/A | A | Proximal CRC | + | N/A | N/A | N/A | |

**MLH1:** genomic deletion of exons 3-5 (c.208-2_453 + 76del)

| N/A | A | 2 ind. with proximal CRC | + | N/A | N/A | N/A | |

| N/A | A | Proximal CRC | + | N/A | N/A | MLH1: c.1376G > C | 4 |
| [Exon 17 skipping] |

**PHM + MLH1 mutations, CRC**

| N/A | A | Prominin CRC | + | N/A | N/A | MLH1: c.1376G > C | 4 |
| [Exon 18 skipping] |

| 40 | Amsterdam criteria | Carcinoma in situ (CRC adenoma) | + | MSI-II or loss of MLH1 | - | MLH1: c.405insA | 4 |

| 44 | Amsterdam criteria | CRC | + | N/A | N/A | MLH1: c.1376G > C | 4 |

| 53 | 1 relative with EC | CRC | + | N/A | N/A | MLH1: c.1376G > C | 4 |

**PHM + MSH2, EC**

| 49 | 1 relative; CRC at 52 | Grade 2 Endometrial adenocarcinoma (Endometrium) | + | MLH1 and PMS2 | N/A | Large deletion in exon 5 of MLH1 | 5 |

| N/A | A | Distal CRC | + | N/A | N/A | N/A | |

**PHM + non-MLH1 mutations**

| N/A | A | N/A | N/A | N/A | N/A | N/A | |

**MSH2:** c.342 + 3A > T

| N/A | A | CRC | + | MSH2 and MSH6 | N/A | N/A | |

| 71 | Mother; died of OC at 49 | ***Perforated cecal cancer at 63 | + | MLH1, MSH2, PMS2 and MSH6 | - | MSH2: c.379G > C | 6 |
| Son**: CRC at 42 | 2 daughters; BRCA at 42 and 46 | 1 daughter; died of OC at 38 | + | N/A | N/A | N/A | |

| 75 | Father; died of CRC at 58 | ****Right-sided colorectal adenocarcinoma | + | MLH1, PMS2 and MSH6 | - | MSH2: c.3699delAGAA, p. (Lys1233Asnfs) | 7 |
| 2 brothers; CRCs in 60 s, 1 died at 63 | | | + | MLH1, PMS2 and MSH6 | N/A | N/A | |

| 63 | N/A | Carcinoma in situ (CR adenoma) | + | MSH6 | - | MSH2: c.3699delAGAA, p. (Lys1233Asnfs) | 7 |

| 70 | Bethesda criteria | ****CRC | + | MSH6 | N/A | MSH6: c.3514dup, p. (Arg1172Lys) | 9 |

SC: stomach cancer, CRC: colorectal cancer; EC: endometrial cancer, OC: ovarian cancer, BRCA: breast cancer

In bold: germline variants that have been classified as pathogenic

N/A: information was unavailable, or test was not done

* Tumour also had a MSH6 somatic mutation – c.3674C > T, p. (Thr1225Met)

** Carried the same pathogenic variant as proband

*** Personal history of ureteral cancer at 69

**** Personal history of ovarian cancer at 56 and urothelial carcinoma at 74

***** Metachronous urothelial cancer at 70
African American pancreatic ductal adenocarcinoma patients significantly less likely to be recommended and undergo genetic testing

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Background In 4/2019, the National Comprehensive Cancer Network (NCCN) guidelines recommended all patients with pancreatic ductal adenocarcinoma (PDAC) consider germline genetic testing for potential targeted therapies and identification of at-risk family members. Differences in genetic testing and recommendation among diverse PDAC patients have not been previously reported. The aim of this study was to compare genetic testing practices between African Americans (AA) and non-Hispanic Whites (NHW) PDAC patients.

Methods A retrospective chart review of PDAC patients seen at the University of Chicago or affiliated satellites between 4/2019 and 12/2020 was performed. Rates of genetic testing and recommendation documented in the medical record were compared between AA and NHW patients using chi-square tests.

Results A total of 580 PDAC patients (63% NHW, 20% AA) were included. Average age at diagnosis was 67 years; 52% were male. In total, 216 (37%) patients underwent genetic testing. Of those tested, 47 (22%) had a pathogenic/likely pathogenic variant (P/LPV) identified. Of those with P/LPVs, 25 (12%) were in genes with an increased risk of PDAC (ATM, BRCA1/2, CDKN2A, Lynch, and TP53) (Fig. 1). AA patients had significantly lower rates of genetic testing compared to NHW patients (19% vs. 42%, p < 0.0001) (Fig. 2A). P/LPVs were found in 2 (8%) AA patients and 37 (24%) NHW patients (p = 0.10). AA patients had significantly lower rates of documented recommendation for genetic testing (34% vs. 56%, p < 0.0001) (Fig. 2B). When recommendation for genetic testing was documented, there was no statistically significant difference in rates of testing between AA and NHW patients (62% vs. 75%, p = 0.08) (Fig. 2C).

Conclusions Genetic testing in PDAC patients is unacceptably low especially among AA patients. The disparity in testing is likely related to lack of provider recommendation more than patient uptake. Lack of testing leads to missed opportunities for potential targeted therapies and improved outcomes as well as identification of at-risk family members who could potentially benefit from screening. Strategies to improve genetic testing in PDAC patients, especially AA patients, are needed to ensure equitable benefits for patients and their family members.

Prevalence of 5 cancer types in families with a pathogenic or likely pathogenic variant in the ATM gene

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Background Pathogenic (P) and likely pathogenic (LP) variants in ATM are associated with increased risk of pancreatic and female breast cancer. Some data also suggest an increased risk of ovarian, colorectal, prostate, gastric, and possibly male breast cancer. More data are needed to evaluate these associations, which could broaden testing indications and affect cancer surveillance and treatment. Here, we report the frequency of 5 cancer types in families with a P/LP ATM variant.

Methods We reviewed data for 328 consecutive individuals tested for ATM variants who had findings of P, LP, or variant of uncertain significance. Personal and family (to 3rd-degree relatives) cancer histories were assessed for individuals with a P/LP finding. We noted whether individuals underwent targeted or comprehensive panel testing. Segregation was also assessed.

Results P or LP variants in ATM were observed in 59 of 328 individuals. Clinical information was available for 51; 2 individuals were related: 1 was counted in the study cohort and 1 was counted as a family member. For the 50 families, a cancer type of interest was reported in at least 1 family member: 18 (36%) colorectal, 9 (18%) ovarian, 8 (16%) gastric, 10 (20%) prostate, and 2 (4%) male breast. ATM variants were identified by targeted tests for 15 (30%) of the 50 carriers and panel tests for 35 (70%). Segregation was suspected or confirmed for 9 of 18 (50%) of the families with colorectal cancer.

Conclusions The data presented adds support to an association between P/LP ATM variants and these 5 cancer types. However, further research is still needed for confirmation. The high prevalence of colorectal cancer in this cohort was noteworthy. Given the rarity of

Fig. 1 Genes with pathogenic/likely pathogenic variants identified in 47 PDAC patients

Fig. 2 Rates of (A) genetic testing, (B) documented provider recommendation and (C) genetic testing when recommended in AA and NHW PDAC patients. *p < 0.0001, **p = 0.08
male breast cancer, the identification of 2 individuals (4%) with a family history of the cancer may be of interest. If any associations are confirmed and the phenotype broadened, ATM testing may be indicated for more patients. This could increase the number of carriers identified and prompt consideration of additional cancer surveillance, prophylactic surgery, or other risk-reducing options to minimize cancer burden.

Not as rare as you might think: Lynch syndrome in persons with cancers showing MLH1 promoter hypermethylation

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Background Lynch Syndrome (LS) is a hereditary disorder caused by germline pathogenic variants in mismatch repair genes (MMR – MLH1, MSH2, MSH6 and PMS2) that predispose carriers to colorectal cancer (CRC) and other cancers. Screening for LS is recommended for all newly-diagnosed CRC patients. If somatic testing reveals microsatellite instability (MSI) and/or deficient MMR (dMMR) via immunohistochemistry (IHC), the tumor samples are further tested for MLH1 promoter hypermethylation (PHM) and/or a BRAF V600E mutation – two strong indicators of non-hereditary cancer. In many settings, when one or both of these indicators is/are present, germline testing is not offered. However, cases with concomitant germline MMR mutations and MLH1 PHM and/or BRAF V600E have recently emerged in the literature – questioning the robustness of these tests to definitely rule out LS. We sought to identify the subset of LS patients who would have been ‘missed’ using the current screening methods.

Methods We conducted a literature search using PubMed to find articles describing cases with concurrent germline MMR mutations and MLH1 PHM.

Results 21 cases were found; 19 colorectal and 2 endometrial cancer patients. Their ages spanned from 37 to 75 and most had a positive family history. 16 patients had germline mutations in MLH1, 4 in MSH6 and 1 in MSH2. BRAF V600E was absent in 5 out of 6 cases with available testing results (present in 1 MLH1 carrier). Importantly, 5 out of 10 patients were > 50y, the Amsterdam and Bethesda criteria age cut-off.

Conclusions Co-occurring MLH1 PHM and MMR germline mutation cases are rare but may have been underestimated. However, the absence of BRAF mutation in most of the cases where testing was done, shows that it is likely a stronger indicator of LS. Nonetheless, our data suggest that decisions on when to not offer germline testing to persons with MLH1 PHM and/or BRAF V600E mutation need to be carefully considered. We suggest that patients with dMMR/MSI tumours who are +55y at time of diagnosis and/or have +1 relative(s) with a LS-related cancer diagnosed at +55y, should systematically have their germline tested—perhaps without needing to investigate their MLH1 methylation and BRAF status initially (Table 1).

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Characterization of esophagogastric cancers in pathogenic and likely pathogenic germline ATM variant carriers

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Background ATM is a cancer predisposition gene that has been associated with breast, pancreatic, and other cancers. Although recent studies suggest a possible association between ATM and gastric cancer, the risk and associated tumor pathologies have not been well described. We sought to characterize the prevalence as well as tumor and clinical characteristics of esophagogastric (EG) cancers in a cohort of cancer patients found to harbor ATM germline pathogenic/likely pathogenic (PLP) variants via cancer-agnostic paired tumor/normal testing.

Methods Retrospective review of cancer patients consented to an IRB-approved matched tumor/normal next-generation sequencing (NGS) protocol (MSK-IMPACT) was performed to identify patients with germline PLP variants in the ATM gene. Clinical records for
ATM + patients with EG cancers were reviewed, and descriptive statistics were performed. 

**Results** Among 22,260 patients, 1.1% (n = 238) carried a P/LP variant in ATM. 8.4% (20/238) of ATM + patients had an EG tumor that underwent NGS profiling, of which 45% (9/20) were gastric, 50% (10/20) gastroesophageal junction (GEJ), and 5% (1/20) esophageal adenocarcinoma. 40% (8/20) of tumors were diffuse or had signet ring cell features. Loss of heterozygosity (LOH) at ATM was observed in 88% (15/17) of tumors assessed. The median age-of-onset of EG cancer was 58, with 45% (9/20) of patients presenting ≤ age 50. The mean age-of-onset in gastric cancer patients was 43.7 years compared to 59.4 in the GEJ and esophageal cancer patients (p < 0.01). 35% (7/20) of patients had a family history of EG cancer. Notably, one patient and his daughter, both diagnosed with diffuse gastric cancer, were ATM +. 60% (12/20) of patients reported a family history of an ATM-associated malignancy (breast, pancreas cancer). 5% (1/20) of ATM + patients had a personal history of an ATM-associated malignancy (pancreas cancer). None of the patients had a co-occurring P/LP variant in a known gastric cancer predisposition gene. 

**Conclusions** These findings suggest that ATM may play a role in the development of EG cancers, including diffuse gastric cancer. Additional research is necessary to determine whether consideration of ATM germline testing in all EG cancer patients and routine upper endoscopic surveillance for P/LP germline ATM variant carriers is warranted.

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**Towards developing a model to ascertain functionality of germline MSH3 variants**

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**Background** Patients with multiple colorectal adenomas with germline mutations in the MSH3 gene have been reported in two families (now defined as MSH3-associated polyposis). Somatic frameshift mutations of APC of two nucleotides or longer were detected in the adenomas, suggesting loss of MSH3 may be causal. MSH3 germline analyses may not yield definitive pathogenic variants to diagnose MSH3-associated polyposis.

**Methods** We report two patients with CRC carrying potentially pathologic germline MSH3 variants. We generated MSH3 knockout cell lines as a tool to evaluate variants for functionality.

**Results: Case 1** 45yo male with stage IV adenocarcinoma in the descending colon showed somatic mutations in KRAS (c.35G > A, p. Gly12Asp) but not in NRAS or BRAF; and PCR-based MSI test was negative. IHC showed presence of PMS2/MSH6. Two mutations within MSH3 were detected in his tumors: c.2436-1G > A (splice acceptor) and c.3265 > T (p. Lys1089*, translational stop signal). Because instability in dinucleotide or longer microsatellites are hallmarks of MSH3-deficiency, tumor DNA was tested for microsatellite instability, with instability detected in 2/5 dinucleotide and 6/7 tetranucleotide but not in 2 mononucleotide markers, suggesting that the tumor is completely deficient of MSH3 function. **Case 2** 42yo female with CRC demonstrating MSI-H and IHC loss of MSH2/MSH6. Germline demonstrates 2 variants of MSH3: c.3032A > G; p. Tyr1011Cys and c.670C > T; p. Arg224Trp. IHC for MSH3 was present in normal tissue. Because of the presence of MSI-H, direct functional loss of MSH3 by microsatellite analysis could not be undertaken. **Cell lines** Both alleles of the MSH3 locus of the immortalized normal human colon cell line HCEC-1CT were knocked out by CRISPR/Cas9, and 5 MSH3-deficient cell lines were established. Cell lines exhibit dinucleotide or greater instability and lack homology-directed double strand break repair. These cell lines can now be used to evaluate biological effects of MSH3 variants by re-introducing patient-specific MSH3 cDNAs containing the variant sequence.

**Conclusions** MSH3-knockout human cell lines could prove to be useful for determining the effects of MSH3 variants, and can be more definitive over microsatellite evaluation. This model may also be useful for studying MSH3-associated adenoma formation.

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**Adolescent diffuse gastric cancer and Li Fraumeni Syndrome: a case report**

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**Background** Gastric cancer risk is elevated in several hereditary cancer predisposition syndromes, including Hereditary Diffuse Gastric Cancer syndrome (up to 83% risk), Peutz Jeghers syndrome (up to 29% risk) and Lynch syndrome (up to 9% risk). The gastric cancer risk associated with Li Fraumeni syndrome (LFS) is estimated to be less than 5% lifetime risk with a mean age of diagnosis of 43 years. When diagnosed, it has been seen in association with both intestinal and diffuse gastric cancers (DGC).

**Case presentation** A 16-year-old African American female presented to pediatric gastroenterology after several months of early satiety and 40-pound weight loss. Abdominal MRI noted diffuse bowel wall thickening of 2.5 cm and multiple masses of the left upper quadrant and pancreatic body; subsequent biopsy confirmed stage IV poorly cohesive diffuse gastric cancer. The patient has been treated with 6 cycles of FOLFOX plus nivolumab. Germline testing included analysis with a 77 multi-gene panel from a clinical laboratory; a TP53 pathogenic variant (c.379 T > C; p.S127P) was identified. This is a rare, missense dominant negative mutation that has not previously been reported in individuals with LFS.

**Discussion** This case represents the youngest known diagnosis of gastric cancer, let alone DGC, in a patient with LFS. Current National Comprehensive Cancer Network guidelines (v2.2021) for LFS recommend colonoscopy and endoscopy at age 25. This case highlights a diagnosis that occurred well before the recommended age of screening initiation, as well as a diagnosis that may have been missed at an early stage as endoscopic surveillance for DGC is highly challenging. Further, it has been previously shown that dominant negative missense mutations reduce the transcriptional activity of wild-type p53 protein and are significantly associated with earlier age of cancer onset in childhood and adolescence. This case serves to strengthen the phenotype of LFS, particularly for those with dominant negative mutations, as a highly penetrant syndrome associated with early ages of cancer diagnosis that can affect a vast variety of tissue types. Clinicians should include LFS in the differential for patients presenting with early-onset DGC.
Evaluation of colonoscopy screening intervals in a large series of patients with Lynch Syndrome from Toronto

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**Background** Lynch Syndrome (LS) is the most common hereditary colorectal cancer (CRC) syndrome and accounts for 2–5% of all CRCs. General screening guidelines for LS families recommend colonoscopy every 1 to 2 years, beginning between the ages of 20–25 years for MLH1/MSH2 and 30–35 years for MSH6/PMS2. Recent research findings have questioned the benefit of shorter colonoscopy screening intervals in reducing CRC incidence or improving overall survival. Our aim in this study is to assess the detection of CRC and adenomas in LS patients undergoing colonoscopy screening at varied intervals.

**Methods** A total of 440 patients with LS from 274 different families were identified through the Familial Gastrointestinal Cancer Registry in the Zane Cohen Centre at Mount Sinai hospital, Toronto (Table 1). We assessed how the detection of adenomas can mediate the association between colonoscopy screening intervals and CRC. The number of adenomas detected at each screening visit was modeled as a function of the screening interval. Then, the predicted number of adenomas corresponding to the different screening intervals was used as a time-dependent variable in a survival model for CRC.

**Results** 46 CRCs were found during follow-up and 47.5% of LS patients had at least one adenoma detected either at the first colonoscopy or during follow-up. The mean number of adenomas detected decreases by 1.6 (p = 0.04), 2.5 (p < 10–3) and 4.8 (p < 10–3) for a screening interval between 1–2 years, 2–3 years, > 3 years vs. + 1 year, respectively, after 10 years follow-up. In turn, a large number of adenomas was associated with a decreased risk of CRC over time with a corresponding hazard ratio of 0.80 [95% CI = 0.66–0.97] (p = 0.02). The cumulative risk of CRC in males and females for different screening intervals (for individuals with adenomas detected) and for individuals without adenomas is represented in Fig. 1.

**Conclusions** Shorter intervals between colonoscopies are efficient at detecting and removing adenomas, the precursor of CRC, which in turn reduce CRC risk. Yet, a large proportion of LS patients had no adenomas detected in our study and remained at high risk of CRC.

Referrals to genetic counseling due to abnormal tumor screening for Lynch syndrome: impact of champions, maintenance and outreach

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Background Evidence-based guidelines exist for identifying colorectal cancer patients at risk of having Lynch syndrome (LS) through tumor screening; however, referral rates to genetic counseling after an abnormal tumor screen vary by institution. Gaining an understanding of genetic counselor experiences is needed to increase successful implementation and maintenance of colorectal tumor screening programs.

Methods An online survey was administered to genetic counselors in the United States to explore their experiences with colorectal tumor screening programs. Participants were recruited from the Collaborative Group of the Americas on Inherited Gastrointestinal Cancer, the American Board of Genetic Counseling, the National Society for Genetic Counselors, and the Lynch Syndrome Screening Network. Participants were asked a series of survey questions regarding referral rates, champions for implementation and maintenance, and ownership of the tumor screening program. Descriptive statistics are reported alongside results of Fisher’s Exact Test and Chi-Square test.

Results Of the 78 respondents, around half could provide exact (10.7%) or estimated (37.3%) numbers of patients referred from positive tumor screening. The other half of respondents did not know (13.3%) and could not estimate (38.7%) the number of patients referred due to positive tumor screening. Nearly half (47.4%) of participants knew of a champion for implementation and 32% knew of a champion for maintenance (Figs. 1, 2). Less than half of participants (43.6%) knew which specialty had ownership of the tumor screening program. In general, participants who were unsure of champions for implementation and maintenance, or ownership of the program were more likely to not know when the tumor screening program was implemented (p = 0.001) and were unable to provide referral rate estimates (p = 0.001).

Conclusions It is concerning that many genetic counselors who participated did not know when, who, or what specialty championed the implementation and maintenance or had ownership of the tumor screening program. In general, participants who were unsure of champions for implementation and maintenance, or ownership of the program were more likely to not know when the tumor screening program was implemented (p = < 0.001) and were unable to provide referral rate estimates (p = < 0.001).

Expanding the phenotype: case report of PMS2-associated early onset colorectal cancer

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Background Lynch syndrome (LS) is caused by heterozygous pathogenic variants in the mismatch repair genes (MLH1, MSH2, MSH6, PMS2, EPCAM) and confers increased risks of primarily colorectal cancer (CRC) and endometrial cancer. Of the five LS genes, PMS2 is considered the least penetrant with a lifetime colorectal cancer risk ranging up to 20% and an average age of CRC onset between ages 61–66 years. In comparison, the higher penetrant MLH1 gene is associated with up to a 61% lifetime risk of CRC with average age of onset at 44. There were no diagnoses of CRC under age 50 within 407 pathogenic PMS2 carriers from the Prospective Lynch Syndrome Database, leading to this group’s conclusion that PMS2 does not increase the risk of colon cancer before the age of 50. Accordingly, whereas colonoscopy initiation is recommended between ages 20–25 for the higher penetrant MLH1 and MSH2 genes, current NCCN Guidelines (v1.2021) recommend initiating colonoscopy between ages 30–35 for pathogenic PMS2 carriers.

Case details The patient is a 26-year-old male diagnosed with stage III adenocarcinoma of the transverse colon diagnosed at age 25. Immunohistochemistry staining revealed isolated loss of PMS2 gene product. He was treated with right hemicolectomy and adjuvant chemotherapy. Germline testing revealed a pathogenic deletion (p. EX14_15 del) in the PMS2 gene. Subsequent cascade testing determined that the variant was maternally inherited.

Discussion This case represents one of the youngest known diagnoses of CRC within an individual due to PMS2-associated LS. This report supplements a French cohort that identified seven PMS2 carriers with CRC diagnosed under age 30 with the youngest age of onset at 21 years. This additional data of a 25-year-old male with late-stage CRC contributes to an expanding phenotype of PMS2-associated LS. The variability of disease onset should be accounted for when establishing medical management guidelines and considering the risks and benefits of CRC surveillance.
Incidence of (adenomatous) polyps and colorectal cancer in patients with PMS2-associated Lynch syndrome undergoing surveillance: a prospective cohort analysis

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Background Of the four mismatch repair (MMR) genes, PMS2 variant carriers have the lowest colorectal cancer risk. We collected a prospective cohort to estimate the prevalence of adenomas. As a next step we would like to assess the proportion of adenomas with PMS2-deficiency.

Methods Our current cohort consists of 171 PMS2 variant carriers. We recorded the occurrence and characteristics of incident adenomas and CRC. After receiving consent to request clinical data, we obtained information through PALGA, the Dutch nationwide network and registry of histo- and cytopathology, and by requesting colonoscopy reports at gastroenterology departments. We had a pilot cohort of twenty polyps available for immunohistochemical staining of the PMS2 protein.

Results During a total of 675 colonoscopies (1044 observation years, median surveillance interval 2 years), 435 polyps were removed, of which 237 (54.5%) were adenomatous. Forty-one (16.9%) of those polyps were immunohistochemically stained showed loss of PMS2 expression, suggesting late involvement of PMS2 deficiency in the pathway to cancer. One incident CRC was reported.

Discussion In this large cohort of PMS2 variant carriers, only one incident CRC were observed. This tumor was preceded by a colonoscopy with insufficient bowel preparation. Further analyses are required to investigate the timing of PMS2 deficiency in the development of colorectal cancer, for which we are actively searching for collaborators.

From counseling to registering: Opportunities unveiled in a five-year experience of registering familial cancers in Iran

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Background Not all developing countries have established familial cancer registries, a task which requires vision and concerted efforts. Here, we propose that with certain crucial elements in place, clinical cancer genetic counseling settings could undertake the basic tasks of small intra-institutional registries and thus generate the potential for larger, more effective ones.

Methods This commentary is based upon our five-year multi-central experience on familial cancer genetic counseling service and registry.

Results To be able to function as a basic registry, we had to add mechanisms for registering and surveillance downstream to genetic counseling and diagnosing. Thus, organizational and workflow designs had to be modified to reflect the additional tasks such as arranging, facilitating and documenting diagnoses and screenings. Among the participating personnel, the coordinator was a key element and the hinge of services of our basic registry. We found the coordinator’s dedication and tenacity vital to registry efficiency. Nevertheless, participation of clinicians was an equally important factor in our workflow.

In our experience, in order to have access to necessary medical services, counseling settings are best to be located in hospitals with capacity to meet their medical investigation demands. Furthermore, surgery ward was our preferred location. This choice brought a number of advantages including access, sufficient transit time, oriented staff and steady logistical provision. Also, in most cases, a close relative accompanied the patient and this provided us with better information acquiring, family testing and follow-up planning. Also notably, surgeons were available for possible consideration of management options.

Finally, our participation in multidisciplinary tumor board (MDT) tumor boards made informative sessions with clinicians of various fields possible where we adapted the familial/hereditary cancer guidelines to our local capacities. Likewise, we tried to convey the importance of hereditary/familial approach, a notion we found effective in complementing surgical management with a dimension of precision medicine. We also found that MDT sessions are proper places to select familial/hereditary cases.

Conclusions The path from cancer genetic counseling settings to a basic functional registry may differ in each country. Nonetheless, from our standpoint, one could be certain that opportunities greatly outweigh the challenges.

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Evaluating Genetic Testing Barriers to Inform Tailored Messaging

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Background Genetic testing for hereditary cancer syndromes can provide lifesaving information, allowing for targeted cancer
screening, prevention, and treatment. However, many barriers to testing exist. We assessed patient barriers to inform content of a patient-facing tailored messaging tool aimed at increasing uptake of genetic testing.

**Methods** A novel online survey was sent to academic medical center patients ≥18 years old who met criteria for genetic evaluation based on their cancer type and diagnosis at age. Untested participants were asked how true 33 barriers to genetic testing were to them using a 5-point Likert scale (1 = not true at all, 5 = very true). Barrier items encompassed several domains including worry/fear, lack of education, cost, impact of testing, psychosocial distress, genetic discrimination, stigma, religious beliefs, and logistics. Mean value (M) and endorsement score (ES, total percentage of 4’s and 5’s) were calculated for each item (Table 1). A readiness score was calculated based on mean response to questions of confidence, importance, and readiness to get genetic testing in the next year (10-point scale). Items with negative correlation to readiness score were counted as true barriers.

**Results** 138 participants were included in this analysis; the majority were white (86%) and 55% described themselves as male. Items with the highest mean value and ES included worry about family members (M=3.78, ES=71.1%), lack of information on the testing process (M=3.74, ES=70.1%), and lack of information about the value of testing (M=3.64, ES=62.3%). These three items, and four additional items were positively correlated with readiness. 25 items had negative correlation with readiness. Items with the strongest negative correlation were related to genetic testing knowledge including limited utility for cancer prevention (−0.396, p<0.05), concern with test accuracy (−0.357, p<0.05), and belief that there is no purpose in testing (−0.352, p<0.05).

**Conclusions** Some items commonly reported as barriers with high mean and ES were positively correlated with readiness, suggesting they may be facilitators rather than barriers to testing. Many items with strong negative correlations to readiness were related to knowledge about genetic testing, which may be an effective target for intervention through educational content and tailored counter-messaging. In addition, awareness of barriers to genetic testing is key for healthcare providers to help better facilitate genetic testing discussions with patients.

| Table 1. Barriers to Genetic Testing |
|-------------------------------------|
| Item | M | ES | p |
| I don’t know how genetic testing benefits me. | 3.15 | 47.4 | -0.139 |
| I am concerned about my family’s reaction to my genetic testing results. | 1.72 | 11.9 | -0.120 |
| I am not sure what they would do with the results. | 1.85 | 16.3 | -0.093 |
| Getting genetic testing is not consistent with my religious or spiritual beliefs. | 1.39 | 7.0 | -0.091 |
| I worry about how these results affect my employment. | 2.12 | 4.0 | -0.089 |
| I would feel anxious while waiting for my results. | 1.98 | 3.5 | -0.080 |
| I worry that my health insurance would not cover the cost of genetic testing. | 2.42 | 7.1 | -0.061 |
| Knowing that I carry an altered gene would cause me to feel less healthy than other people. | 2.22 | 17.8 | -0.049 |
| I would feel guilty if one of my relatives had an altered gene and I did not. | 1.59 | 5.9 | -0.047 |
| I would feel guilty if one of my relatives had an altered gene and I did not. | 1.70 | 6.0 | -0.005 |
| I don’t know how to get genetic testing. | 1.75 | 6.2 | 0.00 |
| I would feel guilty if my family member developed cancer. | 2.50 | 28.9 | 0.009 |
| I worry that I would have to tell all of my relatives about my test results. | 2.74 | 45.9 | 0.037 |
| I worry that I would have to tell all of my relatives about my test results. | 2.80 | 46.7 | 0.090 |

| Item | M | ES | p |
| Knowing that I carry an altered gene would cause me to worry more about other family members who could be carriers. | 3.78 | 71.1 | 0.114 |
| I need more information about the how the testing process works. | 3.74 | 71.1 | 0.160 |
| I need to get more information about what genetic testing has to offer. | 3.64 | 62.3 | 0.247 |
| If I were found to carry an altered gene, I would want to pass the gene to future generations. | 3.13 | 50.4 | 0.338 |

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Factors predictive of endoscopic detection of signet ring cell foci in CDH1 patients

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Background Individuals with a pathogenic or likely pathogenic (PLP) CDH1 variant are advised to undergo a risk-reducing total gastrectomy (TG) given elevated risk for hereditary diffuse gastric cancer (HDGC). Annual endoscopic surveillance, an alternative to surgery, may result in detection of occult foci of signet ring (SRC), a hallmark of early HDGC. We aim to identify factors predictive of detecting SRC foci on biopsy during esophagogastroduodenoscopy (EGD) in patients with CDH1 mutation and to identify the time to detect SRC foci on endoscopic biopsies.

Methods All patients with a PLP CDH1 mutation were evaluated for the presence/absence of SRC foci on EGD performed from 2013 to 2020. Demographic, endoscopic, and pathology covariates were recorded. Logistic regression analysis identified factors predictive of SRC foci detection on EGD. Time to detection of SRC on surveillance was estimated using Kaplan-Meier method.

Results 227 patients with PLP CDH1 mutations were identified. 205 (90.3%) patients underwent > 1 surveillance EGD. SRC foci were identified in 134 (59%) patients on surveillance EGD; 93 patients (41%) did not have any SRC foci identified. Most patients were Caucasian (95.2%), females (67%), with a mean age of 44.7 ± 14.7 years at time of CDH1 diagnosis. On univariate analysis, younger age at time of CDH1 diagnosis (OR 0.98, 95% CI 0.96 - 1.00) and elevated body mass index (BMI) (OR 1.05, 95% CI 1.01 - 1.10) were associated with increased risk for the presence of SRC foci on biopsy (P=0.03 for both). Younger age and elevated BMI remained predictive for SRC on multivariate analysis (OR 0.97, 95% CI 0.96 - 0.99; OR 1.07, 95% CI 1.01 - 1.11, respectively, P = 0.01 for both) (Figure 1). Median time for identification of SRC foci on EGD surveillance was 41 months (Figure 2).

Conclusion From the time of first surveillance endoscopy, our results demonstrate that it takes a median of 41 months on active surveillance before successful detection of SRC foci on endoscopic biopsies. While age and BMI may influence detection, our findings highlight the occult nature of this disease and the viability of surveillance as an alternative to TG.
The immune profile of normal colonic mucosa as a possible tumor risk modifier in Lynch syndrome

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Abstracts

The immune profile of normal colonic mucosa as a possible tumor risk modifier in Lynch syndrome

The immune milieu of Lynch syndrome (LS), the most common inherited colorectal cancer (CRC) syndrome, is characterized by immune evasion and a pronounced immune infiltration. In contrast to the immune milieu of LS CRC, the immune status of normal colorectal mucosa in LS is not well characterized. In this study, we analyzed the immune infiltrate in tumor-distant normal colorectal mucosa from LS CRC patients, sporadic MSI and MSS CRC patients, and cancer-free LS carriers.

Methods 215 and 234 FFPE normal colonic mucosa tissue sections, 140 and 124 adenomas, and 24 and 22 LS CRCs, were immunohistochemically stained and quantified for CD3-positive and FOXP3-positive T cells, respectively. In addition, CD3-positive T cells were quantified in an independent cohort of 97 FFPE normal rectal mucosa tissue sections from LS carriers enrolled in the CAPP2 clinical trial. Gene expression profiling using the NanoString nCounter platform covering 770 immune-relevant genes was performed in a subset of samples.

Results Significantly elevated CD3-positive and FOXP3-positive T cell densities were observed in the normal mucosa of LS individuals compared to non-MSI controls. Distinct immune profiles in the colonic mucosa of LS carriers with and without tumor manifestation were revealed by the gene expression and cluster analysis. The mucosa of cancer-free LS carriers was particularly characterized by an overrepresentation of CD45-positive, exhausted CD8-positive, NK, Mast and B cell populations. In comparison to normal mucosa of both LS cancer patients and cancer-free LS carriers, LS tumor tissue showed overrepresentation of regulatory T cells and neutrophils. A long-term follow-up of LS carriers within the CAPP2 trial showed a correlation between mucosal T cell infiltrate and time to subsequent tumor occurrence.

Conclusions Our observations suggest that LS carriers present with an altered mucosal immune environment even in the absence of manifest cancer and support the hypothesis that the immune profile in the colorectal mucosa may be a temporary or permanent tumor risk modifier in LS carriers.