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Prevalence of Germline *PTEN, BMPR1A, SMAD4, STK11,* and *ENG* Mutations in Patients with Moderate-Load Colorectal Polyps

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Abstract

BACKGROUND & AIMS—Gastrointestinal polyposis is a common clinical problem, yet there is no consensus on how to best manage patients with moderate-load polyposis. Identifying genetic features of this disorder could improve management, and especially surveillance, of these patients. We sought to determine the prevalence of hamartomatous polyposis associated mutations in the susceptibility genes *PTEN*, *BMPR1A*, *SMAD4*, *ENG*, and *STK11* in individuals with 5 or more gastrointestinal polyps, including at least 1 hamartomatous or hyperplastic/serrated polyp.

METHODS—We performed a prospective, referral-based study of 603 patients (median age 51 y; range, 2–89 y), enrolled from June 2006 through January 2012. Genomic DNA was extracted from peripheral lymphocytes and analyzed for specific mutations and large rearrangements in *PTEN*, *BMPR1A*, *SMAD4*, and *STK11*, as well as mutations in *ENG*. Recursive partitioning analysis was used to determine cutoffs for continuous variables. The prevalence of mutations was compared using Fischer's exact test. Logistic regression analyses were used to determine univariate and multivariate risk factors.

RESULTS—Of 603 patients, 119 (20%) had a personal history of colorectal cancer and most (461; 76%) had fewer than 30 polyps. Seventy-seven patients (13%) were found to have polyposis-associated mutations, comprising 11 in *ENG* (1.8%), 13 in *PTEN* (2.2%), 13 in *STK11* (2.2%), 20 in *BMPR1A* (3.3%), and 21 in *SMAD4* (3.5%). Univariate clinical predictors for risk of having these mutations included age at presentation less than 40 years (19% vs 10%; P=.008), a polyp burden of 30 or more (19% vs 11%; P=0.014), and male sex (16% vs 10%; P=.03). Patients who had 1 or more ganglioneuromas (29% vs 2%; P<.001) or presented with polyps of 3 or more histologic types (20% vs 2%; P=.003) were more likely to have germline mutations in *PTEN*.

CONCLUSIONS—Age less than 40 years, male sex, and specific polyp histologies are significantly associated with risk of germline mutations in hamartomatous-polyposis associated genes. These associations could guide clinical decision making and further investigations.

Keywords

Hamartomatous polyposis; Juvenile Polyposis Syndrome; Peutz-Jeghers syndrome; Cowden syndrome

Introduction

Each year in the United States, almost 150,000 people will be diagnosed with colorectal cancer (CRC) and close to 50,000 will die from the disease[1]. Most (>95%) CRCs develop from adenomatous polyps[2]. The prevalence of adenomatous polyps increases with age and male gender but is a common finding on screening colonoscopy. Patients with numerous colorectal polyps have an increased risk of CRC and may represent a hereditary polyposis syndrome[3]. These important, potentially heritable polyp conditions are a conundrum, because individuals present with features that overlap one or more of the syndromes, and proper, objective identification is necessary for appropriate clinical management [4]. Patients who meet the gene-testing criteria for known polyposis syndromes are identified through careful evaluation of family history and clinical presentation[5]. However, the reality in the clinic is that often cases either do not fulfill clinical criteria at the time of presentation or meet diagnostic criteria but are mutation negative for the suspected gene(s). At present, there is little consensus or research to help guide clinicians when faced with patients who do not meet established clinical genetic testing criteria but who present with moderate polyp burdens.

The polyposis syndromes are characterized by the dominant type of polyp (whether adenomatous or hamartomatous) present. The hamartomatous syndromes are characterized by an overgrowth of cells native to the area in which they normally occur, i.e., mesenchymal, stromal, endodermal, and ectodermal elements. The represent a significant minority of the inherited gastointestinal cancer predisposition syndromes. It is well established that many of these syndromes carry a substantial risk of developing colon cancer, other gastrointestinal cancers and extra-gastrointestinal malignancies (Table 1).

These hamartomatous syndromes occur at approximately 1/10th the frequency of the adenomatous sundromes and account for < 1% of colorectal cancer[4, 6–8]. Despite the uncommoness of the disease, proper identification has major clinical significance for the affected individual as well as for at-risk families. In an attempt to better understand how we ought to clinically approach patients with moderate polyp burdens, we previously explored the feasibility of molecularly classifying patients with clinically unclassifiable hamartomatous polyposis or with hyperplastic/mixed polyposis with a pilot study[9]. We found that ~20% of 49 such individuals carried germline mutations in *PTEN* (susceptibility gene for *PTEN* Hamartoma Tumor Syndrome [PHTS]; NCBI Entrez Gene ID 5728), *BMPR1A* (1 of 2 susceptibility genes for juvenile polyposis syndrome [JPS]; NCBI Entrez Gene ID 12166), *SMAD4* (2nd susceptibility gene for JPS; NCBI Entrez Gene ID 4089), *ENG* (susceptibility gene for hereditary hemorrhagic telangiectasia; NCBI Entrez Gene ID 2022), or *STK11/LKB1* (susceptibility gene for Peutz-Jeghers syndrome [PJS]; NCBI Entrez Gene ID 6794). Despite the relatively small sample size in our pilot, it would appear that germline mutations or deletions of *PTEN* and *BMPR1A* were over-represented in polyp presentations. We also reported that gastrointestinal polyps were common amongst patients with germline *PTEN* mutation. In a separate study of 127 pathogenic *PTEN* mutation positive patients, 69 had undergone 1 or more endoscopic evaluations, of which 64 (93%) had polyps, often with a mixed polyp histology[10]. This study suggests that the previous paradigm that only hamartomatous polyps are seen in PHTS may not be true.

Based on the above studies, we hypothesized that germline mutations in hamartomatous polyposis related genes *PTEN*, *BMPR1A*, *SMAD4*, *STK11* and *ENG* accounts for subsets of cases of unexplained, modest-burden gastrointestinal polyp presentations typically seen in patients. In this study, we sought to determine the prevalence of germline mutations in these genes in a prospectively accrued series of >500 individuals with ≥5 cumulative lifetime gastrointestinal polyps, at least one of which must be hamartomatous or hyperplastic/ serrated.

Methods

Study Design

Prospective, referral-based study of 603 patients from the Cleveland Clinic (n = 77) and 148 outside institutions (n=526), conducted from June, 2006, until January, 2012. Individuals, irrespective of age and family history, who met the minimal criteria of ≥5 cumulative lifetime gastrointestinal polyps, one or more lesion(s) being hamartomatous or hyperplastic/ serrated could be referred. A total of 603 eligible patients were accrued with polyp histology documented by report, of which a random 148 (25%) had central pathology re-review (by X.L./L.Y./J.W.). Medical records were requested to document polyp and cancer history. Pedigrees obtained by a genetic counselor/physician were also reviewed. Where eligibility criteria data are not complete, eg, no medical documentation of polyp numbers, the patient was excluded from the study.

All subjects had their polyp phenotypes extracted from available records. Histology slides for polyps from each subject were requested and blindly read by our study gastrointestinal

pathologists (X.L./L.Y./J.W.). Juvenile polyps show a normal epithelium with a dense stroma, an inflammatory infiltrate, and a smooth surface with dilated, mucus-filled cystic glands in the lamina propria. Muscle fibers and the proliferative characteristics of adenomas are typically not seen in juvenile polyps[11]. In contrast, Peutz-Jegher polyps show extensive smooth muscle arborization throughout the polyp. In the absence of these distinguishing factors, hamartomatous polyps were labeled as unspecified hamartomatous polyps. Central pathology review showed that 53% (78/148) had their original histological diagnoses amended but this did not significantly lead to changes in the clinical syndromic diagnoses. Based on available clinical data, polyp data including pathological diagnosis, where available, patients were classified under known clinical polyposis syndromes if they met clinical diagnostic criteria for JPS, PJS, PHTS, familial adenomatous polyposis (FAP), attenuated familial adenomatous polyposis (AFAP), *MUTYH*-associated polyposis (MAP), serrated polyposis syndrome (SPS), and hereditary non-polyposis colorectal cancer syndrome (HNPCC)[5, 12–14] (Table 1). This study was approved by the Cleveland Clinic Institution Review Board (#8458).

DNA Extraction and Molecular Genetic Analyses

Germline genomic DNA was extracted from peripheral lymphocytes (protocols at www.lerner.ccf.org/gmi/gmb). Mutation analysis of the entire coding sequence, the exon-intron boundaries and the flanking sequences of *PTEN*, *BMPR1A*, *SMAD4*, *STK11*, and *ENG* was carried out on coded samples in a blinded fashion with a combination of denaturing gradient gel electrophoresis, high-resolution melting curve analysis (Idaho Technology, Salt Lake City, Utah)[15] and confirmed with direct Sanger sequencing (ABI 3730xl, Life Technologies, Carlsbad, California)[16–20]. Deletion analysis with the multiplex ligation-dependent probe amplification assay[21] was performed for *PTEN, SMAD4, BMPR1A* and *STK11* (MRC-Holland, Amsterdam, Holland). All suspected deletion/duplications were confirmed with quantitative polymerase chain reaction. All patients underwent re-sequencing of the *PTEN* promoter region as previously described[22]. Resulting sequence was analyzed using Mutation Surveyor (Softgenics, State College, Pennsylvania) in comparison with the reference sequences of human *PTEN* [NM_000314.4], *BMPR1A* [NM_004329.2], *SMAD4* [NM_005359.5], *STK11* [NM_000455.4], and *ENG* [NM_000118.2]. The primers used for all genes are available in Supplemental Table 1.

Classification of Variants

Critical to this study, is distinguishing disease-causing mutation carriers from those who are mutation negative from our analysis. Non-synonymous, frameshift, splice-site, nonsense mutations as well as large deletions, whole gene deletion or any variant known to be disease-causing are assigned to the category, ‘mutation positive (Mut+)’. All intronic and synonymous mutations were classified as variants of unknown significance (VUS) and considered as mutation negative. Prediction databases were used to assist missense mutation annotations[22–25]. To be conservative, all cases without proof of functionality or that were predicted to be non-pathogenic in 2 of 3 prediction databases were considered as VUS and, for the purposes, of this study were considered as mutation negative. All variants were discussed in a monthly protocol meeting. Unless the specific *PTEN* promoter mutations have been shown to affect PTEN function[22] and are associated with Cowden Syndrome phenotypes[26, 27], to be conservative, we consider them here as mutation negative.

Statistical Considerations

The primary outcome was the prevalence of pathogenic germline mutations in the five genes tested. Covariates of interest included the number and age at diagnoses of 5th colorectal polyp, gender, the presence of CRC, family history of gastrointestinal polyps or CRC,

number of polyps, specific polyp histology subtypes as well as the presence of clinical polyposis syndromes. Patients meeting diagnostic criteria for more than one clinical polyposis syndrome were included in the separate analyses of the individual syndrome met. Univariable recursive partitioning analysis (RPA) was used to identify optimal cutpoints in continuous variables that best predict the presence of any mutation. RPA indicated that the best cutpoint is <55 vs. ≥55 for age; 5–31 vs. ≥32 for total number of polyps; 0 vs. ≥1 for hamartomas and ganglioneuromas; 0 vs. 1–17 vs. ≥18 juvenile polyps. No cutpoints were identified for adenomas. The primary aim of identifying genetic predisposition is to impact management and inform on surveillance strategies, i.e. who may need earlier colonoscopies. We, therefore, chose to use age ≥ 40 vs < 40 as our cutoff for age instead of the RPA optimal of 55 years because the 40 cutpoint is both statistically significant and clinically significant. For adenomatous polyposis, a cutoff of ≥20 has been found to be clinically useful in predicting risk for *APC* and *MUTYH* mutations[28]. A cutoff of ≥20 in our study did not significantly predict mutations in the 5 genes tested. Based on our RPA results, although a cutoff of ≥32 was identified to be the best cutoff point, we elected to use a more clinically useful cutoff of ≥30 in our subsequent analysis.

For each variable, the number and percentage of patients with mutations was calculated and compared between groups using Fisher's exact test. Risk factors for any mutation were assessed using logistic regression analysis. Stepwise logistic regression analysis with a variable entry criterion of P≤0.10 and a variable retention criterion of P≤0.05 was used to identify multivariable risk factors. Logistic regression results are summarized as the odds ratio (OR), 95% confidence interval (CI) for the OR, and *P*-value. Data were analyzed using SAS® software (SAS Institute, Inc., Cary, NC, USA). All statistical tests were two-sided, and $P<0.05$ was used to indicate statistical significance.

Results

Of 603 research participants, 360 (60%) were women (Table 2). Median age of patients at time of identification of their 5th polyp was 51 years (range 2–89 years). From 5 to 302 (median 13) polyps/patient were detected, with a median of 3 colonoscopies (range 1–19). Personal history of CRC was noted in 20% of patients (119/603), with median age at diagnosis 53 years (21–80). Patients with a personal history of CRC and an underlying germline genetic alteration were younger compared to those without a germline mutation at the time of their cancer diagnosis (median 48, range 32–65). Patients self-reported the presence of CRC (325/603; 54%) and polyps (295/603; 49%) in at least one family member in a 3-generational pedigree (Table 2). 31% (186/603) of patients had a first-degree relative with CRC. 440 (73%) patients did not meet criteria for any clinical polyposis or colorectal cancer syndrome.

Frequency of and Patient Demographics Associated with Germline Mutations in PTEN, BMPR1A, SMAD4, STK11 and ENG

A total of 77 pathogenic germline mutations were detected in 603 (12.8%) patients, comprising 11 (1.8%) in *ENG*, 13 (2.2%) *PTEN*, 13 (2.2%) *STK11*, 20 (3.3%) *BMPR1A* and 21 (3.5%) *SMAD4* (Table 3). One patient had 2 *SMAD4* mutations and 1 *BMPR1A* mutation (Supplemental Table 2). Clinical features associated with a higher likelihood of an underlying germline mutation includes age at 5th polyp presentation <40 years (19% vs 10%; $P=0.008$), polyp number ≥ 30 (19% vs 11%; $P=0.014$) and male gender (16% vs 10%; $P=0.03$). Interestingly, family history of colonic polyps and a personal history of CRC were not helpful in predicting who may harbor a mutation in these genes (Table 3). Importantly, germline mutations in one of these 5 genes were more common amongst patients who had

no family history of CRC than those who had a positive family history for CRC (18% vs 8%; $P<0.001$).

While the numbers were small, there were gene-specific patterns of note: among the 77 mutation-positive patients, *SMAD4* mutations were more commonly seen in patients with unexplained polyps if they were <40 years of age (7% vs 2%; $P=0.009$) and in patients who had no family history of CRC (6% vs 2%; $P=0.006$) and in patients with a positive family history of gastrointestinal polyps (5% vs 2%; $P=0.013$). Patients were more likely to have *PTEN* mutations if they did not have a family history of CRC (5% vs 0%; $P<0.001$). *STK11* mutations were more common in those <40 years (4% vs 1%; $P=0.047$) and less frequently seen in females presenting with unexplained polyps (1% vs 4%; $P=0.044$). There were no significant associations with *ENG* mutations. However, of the 11 participants with *ENG* mutations, only 1 had juvenile/inflammatory polyps, all others had hyperplastic/serrated polyps.

Polyp Histology Associated with Mutations in Specific Genes

Because polyp histology is a major clinical diagnostic criterion for most polyposis syndromes, we analyzed if the predominant polyp histology was associated with mutations in any specific gene (Table 4). Patients were enrolled if they met the criteria of ≥5 cumulative lifetime gastrointestinal polyps, at least one of which must be hamartomatous or hyperplastic/serrated. RPA demonstrated that patients whose polyps included ≥1 unspecified hamartomatous polyps were more likely to have any germline mutation (32% vs 11%; $P<0.001$) and specifically, *STK11* mutations (11% vs 1%; $P=0.001$) compared to those who did not. An increasing number of juvenile polyps was associated with *SMAD4* ($P<0.001$) and *BMPR1A* ($P< 0.001$) mutations. Of 14 patients who presented with at least one ganglioneuroma, 6 (43%) had pathogenic germline mutations, 4 of whom harbored germline *PTEN* mutations ($P< 0.001$). A mixed polyposis presentation (≥3 different histological subtypes of adenoma, hamartoma, lipoma, ganglioneuroma, juvenile, inflammatory polyps) was associated with an increased prevalence of underlying germline mutation (53% vs 12%; $P<0.001$) and specifically of *PTEN* (20% vs 2%; $P<0.001$) [Table 4].

Mutation-Frequencies in Research Participants Meeting Clinical Criteria for Polyposis Syndromes versus Those Not Meeting Criteria

We found that a significant number (46/440; 11%) of patients who did not meet clinical criteria for heritable polyposis or colorectal cancer syndromes harbored an underlying germline mutation. Not surprisingly, patients meeting criteria for known hamartomatous polyposis syndromes were more likely to test positive for an underlying germline mutation. Of the 69 patients who met clinical criteria for JPS, 22 (32%) had germline mutations: 13 in *SMAD4* and 9 in *BMPR1A*. Patients meeting criteria for PJS were more likely to have an underlying mutation in *STK11* (35% vs 1%; $P<0.001$).

Risk Factors For Mutations In Any Of The 5 Genes

Using univariate logistic regression analysis, we found nine clinical variables (clinical characteristics and histology subtypes) which significantly predicted for the presence of germline mutation (Table 5). Age < 40, male gender, polyp burden ≥ 30, absent family history of CRC, presence of any unspecified hamartomas, ganlioneuromas or mixed histology. Stepwise logistic regression revealed that 5 variables were associated with risk for any mutation: male gender, any hamartoma, juvenile polyps and mixed histology, along with the absence of a family history of CRC (Table 5).

Discussion

This paper focuses on the prevalence of hamartomatous polyposis-related genes in patients with moderate burdens of varied histology colorectal polyps, predominantly hamartomatous and hyperplastic/serrated. Because the entry criteria for this study was any individual with ≥5 polyps, of which at least one must be either hamartomatous or hyperplastic/serrated, the majority of our patients have <30 polyps (76%). The study was designed as such, as this most closely reflects the spectrum of patients presenting to clinics whom are etiologically puzzling resulting in unclear clinical management.

Our findings of 13% germline mutation in a large cohort (77/603) accrued from a wide cross-section of institutions is significant as it implies that clinicians would need to be vigilant for the possibility of an underlying gene mutation, even in these patients with very modest polyp burdens. It is noteworthy that the majority of patients who had an underlying germline mutation do not have positive family histories of CRC. While research participants had 20% prevalent CRC, this was not particularly associated with presence of germline mutations in the five genes tested. This suggests that neither family history nor personal history of CRC are not good predictors of underlying germline mutations in genes germane to hamartomatous polyps, in contrast to adenomatosis-related polyposis[28]. While this finding was surprising, there are possible explanations that may in part account for this observation including the contribution from de novo mutations[29, 30]. Specifically with *PTEN* mutations, we now understand that the frequency of de novo *PTEN* mutation could range between 11% to >40%. It is for this reason that absence of PHTS features within a family history should not preclude consideration of this diagnosis for patients with relevant personal history[31]. The cumulative lifetime risk for CRC individually for the genes in our study is thought to be lower than with those associated with MAP, AFAP or FAP, potentially accounting for this lack of association with both family and personal history of CRC[27, 32, 33].

While the prevalence of mutations in individual genes tested was low, gene-specific correlations, which could shed light on how to approach patients with polyps, were noted. Patients <40 years were more likely to have germline mutation compared to those ≥40 years (19% vs 10%; P=0.008). Age was also predictive of specific-gene involvement. For example, patients with *SMAD4* and *STK11* mutations tended to be younger. The predominant polyp histological subtype was informative in predicting germline mutation involvement. High-risk presentations included increasing numbers of juvenile polyps or the presence of ganglioneuromas (≥1), both of which were significantly more likely to be associated with harboring specific underlying mutations. For example, germline *PTEN* mutations were associated with ganglioneuromas (Ngeow and Eng, unpublished). Presentations with any ganglioneuromatous polyp(s) or mixed-polyposis should alert clinicians to the possibility of an underlying *PTEN* mutation and trigger a detailed assessment for other clinical features associated with PHTS[26] including mucocutaneous lesions, macrocephaly and history of breast, thyroid, endometrial and renal carcinomas, which would require more extensive surveillance. Although the genetic differential diagnosis of ganglioneuromas in the gut also includes multiple endocrine neoplasia type 2B and type 1 neurofibromatosis, these syndromes typically have submucosal ganglioneuromas/matosis that are not polypoid[34]. As previously reported, *PTEN* mutation carriers having an increased propensity for multiple histological subtypes[10], which was again observed here.

In our pilot study, two out of 14 subjects with early-onset JPS had *ENG* mutations[9]. Eleven patients in our cohort had *ENG* mutations but amongst 69 JPS patients, none harbored a mutation in *ENG*. This as well as similar findings in another study of *SMAD4*/*BMPR1A*-negative JPS patients[35] suggest that *ENG* may not be a JPS susceptibility gene. It is possible

that in the absence of co-segregation and functional studies, missense variants such as those seen in *ENG* could be over-interpreted by software prediction models and will need to be interpreted with caution. Further research is needed to determine what role, if any, *ENG* plays in polyposis syndromes.

For adenomatous polyposis syndromes such as FAP, increasing polyp burden is known to be a good clinical predictor for underlying germline mutation in *APC*[28]. It is less clear, if polyp burden in non-adenomatous polyposis behaves similarly. We saw that polyp burden (≥30) was more likely to be associated with the presence of a germline mutation in one of the 5 genes (OR 1.93; P=0.012), but on multivariate analysis, polyp burden was no longer predictive. Indeed, our data show that polyp histology remains the key determinant for mutation status contributing towards 3 of the 5 risk factors identified from multivariate analysis, thus reaffirming the importance of re-review of polyp histology during routine clinical practice. Male patients were also significantly more likely to harbor a germline mutation but only marginally so (OR 1.76, 95% CI 1.05–2.96) and should be interpreted with caution. The absence of a family history of CRC was on multivariate analysis found to be significantly associated with germline mutations. While we have explored possible reasons for this in our discussion above including rate and age of transformation to CRC and *de novo* mutation frequency, it is possible that recall bias may inflate the true impact of family history. Patients with family history of CRC may have been identified for genetic testing and may not have been included in this study as a result.

It is not uncommon for patients to present with polyp burdens that are shy of diagnostic criteria or with varied polyp histologies, an undefined group with no consensus on how best to manage these cases. Our study was designed as a referral-based study in an attempt to recapitulate the cases. This results is both a strength as well as a weakness in study design. Prior gene testing to exclude *APC* or other adenomatous polyposis-related genes was not necessary for enrollment in our study, and it is possible that this may result in ascertainment bias. It is possible that the lack of clinical testing for patients meeting clinical syndromic criteria could have inflated the prevalence of mutations seen. It is of note however that the prevalence of an underlying germline mutation in patients who do not meet any clinical criteria was still elevated (46/440; 11%). There is really no elegant way in which we could have assessed if clinicians were referring only cases of heightened suspicion for an underlying polyposis syndrome. This is potentially problematic and our data should be interpreted in light of this.

The approach to gastrointestinal polyps, especially modest-burden and comprising non-adenomatous polyps, is something which continues to trouble clinicians given the lack of clinical guidance. To the best of our knowledge, this is the only study of its kind looking to comprehensively elucidate prevalence of germline mutations in these 5 genes in a cohort with moderate colorectal polyp burdens. Regrettably, due to the sample size, the ORs were small and the true clinical impact will require further validation. Our study shows that patients with moderate burden polyposis with at least one hamatormatous or hyperplastic/serrated polyposis have a significant prevalence of germline mutations in hamartomatous-polyposis-related genes. Certain clinical settings increase this possibility: in patients who present under 40 years of age, males and those who present with juvenile polyposis, ganglioneuromatous polyposis or an admixture (≥3) of multiple histological subtypes are at increased risk for specific genetic mutations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations Used

AFAP	attenuated familial adenomatous polyposis
CRC	colorectal cancer
CI	confidence interval
FAP	familial adenomatous polyposis
HNPCC	hereditary non-polyposis colorectal cancer syndrome
JPS	juvenile polyposis syndrome
Mut+	mutation positive
MAP	*MUTYH*-associated polyposis
OR	odds ratio
PJS	Peutz Jeghers syndrome
RPA	recursive partitioning analysis
PHTS	*PTEN* hamartoma tumor syndrome
SPS	serrated polyposis syndrome
VUS	variants of unknown significance

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Table 1

Incidence And Cancer Risks For Known Polyposis Syndromes

Syndrome	MIM No.*	Gene(s)	Population prevalence	Cancer Risks	Diagnostic Criteria Reference
Familial adenomatous polyposis	175100	*APC*	1/5000	Colorectal, duodenal, papillary thyroid, pancreatic, hepatoblastoma, CNS tumors, desmoid tumors	Park et al[36]
Attentuated familial adenomatous polyposis	175100	*APC*	unknown	Colorectal, stomach, thyroid, desmoid tumors (rare)	Nielson et al [37]
MUTYH-associated polyposis	608456	*MUTYH*	1/5000	Coloectal tumors, other?	Sieber et al[38]
Serrated polyposis syndrome		NA	1/100 000	Colorectal tumors, other?	Burt et al[39]
Juvenile polyposis syndrome	175050/174900	*SMAD4/BMPR1A*	1/100 000	Colorectal, gastric, duodenal, pancreatic tumors	Jass et al[40]
Peutz-Jeghers syndrome	175200	*STK11*	1/30 000–1/100 000	Colorectal, small intestine, stomach, breast, pancreatic, sex cord tumors	Giardiello et al[41]
Cowden syndrome	158350	*PTEN*	1/200 000	Breast, thyroid, uterine, melanoma, renal cell tumors, colon	Eng et al[13]
Hereditary non-polyposis colorectal cancer	120435	*MLH1/MSH2/MSH6/PMS2/EPCAM*	1/440	Colon, uterine, stomach, ovary, urinary tract, small bowel, brain/central nervous system, sebaceous neoplasms	Vasen et al[12]

*From Online Mendelian Inheritance in Man[42]

Table 2

Patient Demographics (N=603)

Clinical Characteristics	N= 603
Median age at presentation of 5th polyp (years)	51 (2–89)
Gender	
Female	360 (59.7)
Male	243 (40.3)
Median number of polyps	13 (5–302)
Median number of scopes	3 (1–19)
Personal history of CRC	119 (19.7)
Median age of onset of CRC	53 (21–80)
Family history of CRC	
Any in 3 generation pedigree	325 (53.8)
First Degree Relative	186 (30.8)
FHx of polyps	295 (48.9)
Clinical criteria met	
JPS	69 (11.4)
PJS	20 (3.3)
MAP	39 (6.5)
HNPCC	10 (1.7)
FAP	2 (0.3)
AFAP	43 (7.1)
SPS	45 (7.5)
NONE	440 (73.0)

Abbreviations: CRC, colorectal cancer; FHx, family history; JPS, Juvenile Polyposis Syndrome; PJS, Peutz-Jeghers Syndrome; MAP, MUTYH Adenomatous Polyposis; HNPCC, Hereditary Non-Polyposis Colorectal Cancer; FAP, Familial Adenomatous Polyposis; AFAP, Attenuated Familial Adenomatous Polyposis; SPS, Serrated Polyposis Syndrome

Table 3

Univariate Risk Factors (Clinical Characteristics) for Germline Mutations in *ENG*, *PTEN*, *STK11*, *BMPR1A* and *SMAD4*

		***ENG* (11; 1.8%)**		***PTEN* (13; 2.2%)**		***STK11* (13; 2.2%)**		***BMPR1A* (20; 3.3%)**		***SMAD4* (21; 3.5%)**		**Any Gene (77; 12.8%)**	
Variable	**N**	**#**	**(%)**	**#**	**(%)**	**#**	**(%)**	**#**	**(%)**	**#**	**(%)**	**#**	**(%)**
Clinical Characteristics													
Age, years													
<40	155	3	(1.9)	3	(1.9)	7	(4.5)	6	(3.9)	11	(7.1)	30	(19.4)
≥40	448	8	(1.8)	10	(2.2)	6	(1.3)	14	(3.1)	10	(2.2)	47	(10.5)
P-value		1.0		1.0		**0.047**		0.61		**0.009**		**0.008**	
Gender													
F	360	5	(1.4)	7	(1.9)	4	(1.1)	11	(3.1)	10	(2.8)	37	(10.3)
M	243	6	(2.5)	6	(2.5)	9	(3.7)	9	(3.7)	11	(4.5)	40	(16.5)
P-value		0.36		0.78		**0.044**		0.65		0.26		**0.034**	
Number of polyps													
5–29	461	10	(2.2)	8	(1.7)	9	(2.0)	9	(2.0)	14	(3.0)	50	(10.8)
≥30	142	1	(0.7)	5	(3.5)	4	(2.8)	11	(7.7)	7	(4.9)	27	(19.0)
P-value		0.47		0.20		0.52		**0.002**		0.30		**0.014**	
Family history of colonic polyps													
No	308	5	(1.6)	8	(2.6)	7	(2.3)	7	(2.3)	5	(1.6)	32	(10.4)
Yes	295	6	(2.0)	5	(1.7)	6	(2.0)	13	(4.4)	16	(5.4)	45	(15.3)
P-value		0.77		0.58		1.0		0.17		**0.013**		0.09	
Personal history of CRC													
No	484	7	(1.4)	11	(2.3)	12	(2.5)	16	(3.3)	20	(4.1)	65	(13.4)
Yes	119	4	(3.4)	2	(1.7)	1	(0.8)	4	(3.4)	1	(0.8)	12	(10.1)
P-value		0.24		1.0		0.48		1.0		0.10		0.36	
Family history of CRC													
No	278	5	(1.8)	13	(4.7)	8	(2.9)	10	(3.6)	16	(5.8)	51	(18.3)
Yes	325	6	(1.8)	0	(0.0)	5	(1.5)	10	(3.1)	5	(1.5)	26	(8.0)
P-value		1.0		**<0.001**		0.28		0.82		**0.006**		**<0.001**	

Table 4

Univariate Risk Factors (Polyp Histology) for Germline Mutations in *ENG, PTEN, STK11, BMPR1A* and *SMAD4*

		ENG (11; 1.8%)		*PTEN* (13; 2.2%)		*STK11* (13; 2.2%)		*BMPR1A* (20; 3.3%)		*SMAD4* (21; 3.5%)		Any Gene (77; 12.8%)	
Variable	**N**	**#**	**(%)**	**#**	**(%)**	**#**	**(%)**	**#**	**(%)**	**#**	**(%)**	**#**	**(%)**
Polyp Histology													
Hamartomas													
0	559	10	(1.8)	11	(2.0)	8	(1.4)	16	(2.9)	19	(3.4)	63	(11.3)
≥1	44	1	(2.3)	2	(4.5)	5	(11.4)	4	(9.1)	2	(4.5)	14	(31.8)
P-value		0.57		0.24		**0.001**		0.05		0.66		**<0.001**	
Juvenile polyps													
0	521	10	(1.9)	13	(2.5)	12	(2.3)	10	(1.9)	9	(1.7)	53	(10.2)
1–17	71	1	(1.4)	0	(0.0)	1	(1.4)	5	(7.0)	10	(14.1)	17	(23.9)
≥18	11	0	(0.0)	0	(0.00	0	(0.0)	5	(45.5)	2	(18.2)	7	(63.6)
P-value		1.0		0.52		1.0		**<0.001**		**<0.001**		**<0.001**	
Ganglioneuromas													
0	589	10	(1.7)	9	(1.5)	13	(2.2)	19	(3.2)	21	(3.6)	71	(12.1)
≥1	14	1	(7.1)	4	(28.6)	0	(0.0)	1	(7.1)	0	(0.0)	6	(42.9)
P-value		0.23		**<0.001**		1.0		0.38		1.0		**0.005**	
Adenomas													
0–4	423	10	(2.4)	10	(2.4)	11	(2.6)	15	(3.5)	16	(3.8)	62	(14.7)
≥5	180	1	(0.6)	3	(1.7)	2	(1.1)	5	(2.8)	5	(2.8)	15	(8.3)
P-value		0.19		0.76		0.36		0.81		0.63		**0.033**	
Mixed histology													
No	588	10	(1.7)	10	(1.7)	12	(2.0)	18	(3.1)	20	(3.4)	69	(11.7)
Yes	15	1	(6.7)	3	(20.0)	1	(6.7)	2	(13.3)	1	(6.7)	8	(53.3)
P-value		0.24		**0.003**		0.28		0.08		0.42		**<0.001**	

Table 5

Univariate and Multivariate Logistic Regression Analysis of Risk Factors for Any Mutation in *ENG*, *PTEN*, *STK11*, *BMPR1A* and *SMAD4*

Variable	Univariable			Multivariable		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Age, years						
≥ 40/< 40	0.49	0.30–0.80	**0.005**			
Gender						
Male/Female	1.72	1.06–2.78	**0.027**	1.76	1.05–2.96	**0.032**
Number of polyps						
≥30/5–29	1.93	1.16–3.22	**0.012**			
Family history of colonic polyps						
Yes/No	1.55	0.96–2.52	0.08			
Personal history of CRC						
Yes/No	0.72	0.38–1.39	0.33			
Family history of CRC						
Yes/No	0.39	0.23–0.64	**<0.001**	0.44	0.26–0.76	**0.003**
Unspecified Hamartomas						
≥1/0	3.68	1.85–7.30	**<0.001**	3.05	1.39–6.67	**0.005**
Juvenile polyps						
1–17/0	2.78	1.50–5.14	**0.001**	2.33	1.22–4.47	**0.010**
≥18/0	15.45	4.38–54.53	**<0.001**	14.37	3.83–53.94	**<0.001**
Ganglioneuromas						
≥1/0	5.47	1.84–16.23	**0.002**			
Adenomas						
≥5/0–4	0.53	0.29–0.96	**0.036**			
Mixed Histology						
Yes/No	8.59	3.02–24.44	**<0.001**	4.29	1.36–13.51	**0.013**