Family History of Colorectal or Esophageal Cancer in Barrett's Esophagus and Potentially Explanatory Genetic Variants

Joel H. Rubenstein, MD, MSc[1,2,3], Anna Tavakkoli, MD, MSc[4], Erika Koeppe, MSc[3], Peter Ulintz, PhD[3], John M. Inadomi, MD[5], Hal Morgenstern, PhD[6,7], Henry Appelman, MD[8], James M. Scheiman, MD[9], Philip Schoenfeld, MD, MSc[10], Val Metko, MD[2] and Elena M. Stoffel, MD, MPH[2,3]

INTRODUCTION: **We aimed to estimate the effects of a family history of colorectal cancer (CRC) or esophageal cancer on the risk of Barrett's esophagus (BE) and identify variants in cancer genes that may explain the association.**

METHODS: **Men scheduled for screening colonoscopy were recruited to undergo upper endoscopy. Cases and noncases were screenees with and without BE, respectively. The effects of family histories on BE were estimated with logistic regression, adjusting for the potential confounders. We additionally recruited men recently diagnosed with BE by clinically indicated endoscopies. Banked germline DNA from cases of BE with ≥2 first-degree relatives (FDRs) with CRC and/or an FDR with esophageal cancer underwent next-generation sequencing using a panel of 275 cancer genes.**

RESULTS: **Of the 822 men screened for CRC who underwent upper endoscopy, 70 were newly diagnosed with BE (8.5%). BE was associated with family histories of esophageal cancer (odds ratio = 2.63; 95% confidence interval = 1.07–6.47) and CRC in ≥2 vs 0 FDRs (odds ratio = 3.73; 95% confidence interval = 0.898–15.4). DNA analysis of subjects with both BE and a family history of cancer identified one or more germline variants of interest in genes associated with cancer predisposition in 10 of 14 subjects, including the same novel variant in *EPHA5* in 2 unrelated individuals.**

DISCUSSION: **We found an increased risk for BE associated with a family history of esophageal cancer or CRC. Although analysis of germline DNA yielded no clinically actionable findings, discovery of the same *EPHA5* variant of uncertain significance in 2 of 14 cases merits additional investigation.**

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/CTG/A242

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INTRODUCTION

Barrett's esophagus (BE) is a precursor to esophageal adenocarcinoma. A familial syndrome of BE and esophageal adenocarcinoma has been described, but a specific genetic variant responsible for the syndrome has not been identified (1,2). Esophageal adenocarcinoma could conceivably be part of other known familial syndromes characterized by adenocarcinomas of other gastrointestinal organs (such as Lynch syndrome, MUTYH-associated polyposis, and familial adenomatous polyposis [FAP]). To the best of our knowledge, there have been few studies examining the associations between BE and familial colorectal cancer (CRC) (3). We hypothesized that a family history of colorectal and/or esophageal cancers is associated with an increased risk for BE and also that a shared germline genetic variant could underlie this association. We aimed to examine whether BE is associated with familial CRC and

[1]Center for Clinical Management Research, Ann Arbor Veterans Affairs Medical Center, Ann Arbor, Michigan, USA; [2]Division of Gastroenterology, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, Michigan, USA; [3]Rogel Cancer Center, University of Michigan, Ann Arbor, Michigan, USA; [4]Division of Gastroenterology, University of Texas Southwestern, Dallas, Texas, USA; [5]Division of Gastroenterology, University of Washington Medical School, Seattle, Washington, USA; [6]Departments of Epidemiology and Environmental Health Sciences, School of Public Health, University of Michigan, Ann Arbor, Michigan, USA; [7]Department of Urology, Medical School, University of Michigan, Ann Arbor, Michigan, USA; [8]Department of Pathology, University of Michigan Medical School, Ann Arbor, Michigan, USA; [9]Division of Gastroenterology, University of Virginia Medical School, Charlottesville, Virginia, USA; [10]Section of Gastroenterology, John A. Dingell Veterans Affairs Medical Center, Detroit, Michigan, USA. **Correspondence:** Joel H. Rubenstein, MD, MSc. E-mail: jhr@umich.edu.
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identify the potential known or novel germline variants that may account for such a familial syndrome.

METHODS

Study design

We performed a secondary analysis of the Newly Diagnosed Barrett's Esophagus Study (NDBES), which has been previously described (Figure 1) (4–7). Briefly, the NDBES enrolled male CRC screenees, aged 50–79 years, presenting for colonoscopy at either the University of Michigan's East Ann Arbor Medical Procedures Center or the Ann Arbor Veterans Affairs Medical Center Endoscopy Suite from February 2008 to December 2011. CRC screenees were recruited to undergo research upper endoscopy regardless of symptoms, thereby identifying newly diagnosed cases of BE without having preselected patients with a gastroesophageal reflux disease (GERD) history. Controls were CRC screenees who were confirmed by upper endoscopy to not have BE. The cross-sectional associations between BE and family histories of CRC or esophageal cancer were estimated by comparing cases of BE and noncases in that study population. In addition, the NDBES concurrently enrolled consecutive cases of patients with newly diagnosed BE at clinically indicated upper endoscopies.

The exclusion criteria for CRC screenees included female sex, age 50 years and younger or 80 years or older, a history of upper endoscopy, BE or esophagectomy at baseline, diagnostic indication for colonoscopy (e.g., bleeding, occult fecal blood, and diarrhea), inflammatory bowel disease, known ascites or esophageal varices, cancer within the previous 5 years with the exception of nonmelanoma skin cancer, significant coagulopathy, inpatient status, or inability to comprehend or cooperate with the study protocol. The exclusion criteria were the same as for the clinically diagnosed cases of BE with the exception that previous upper endoscopies were allowed if the patient was not previously diagnosed with BE.

Data collection included subjects' weight, height, waist circumference, and hip circumference measured in duplicate while wearing hospital gowns or pajamas. Subjects completed questionnaires regarding GERD symptoms, medications, tobacco use, and family history of BE and/or cancer (specifically CRC and esophageal cancer). Subjects were classified as having GERD based on their response to the previously published questionnaire if they typically had symptoms of heartburn or regurgitation at least weekly while not taking acid-reducing medications (4). BE was classified if there was endoscopic suspicion of columnar mucosa proximal to the gastroesophageal junction, and the pathologist reported the presence of specialized intestinal metaplasia. For CRC screenees, the indication for colonoscopy (first screening, repeat screening, and surveillance of polyps) and the largest size and most advanced histology of polyps in each location of the colon were abstracted. Polyps at the splenic flexure or more distal were classified as in the left colon and more proximal polyps in the right colon. Advanced adenomas were classified as those that were ≥10 mm or found to have high-grade dysplasia.

Germline DNA from a subset of subjects underwent next-generation sequencing (NGS) with a multigene panel: subjects with BE with either (i) 1 or more first-degree relatives (FDRs) with esophageal cancer or (ii) 2 or more FDRs with CRC. Germline DNA extracted from banked lymphocytes (buffy coats) and subjected to NGS using a panel of 275 cancer genes (Human Comprehensive Cancer Panel, Qiagen, Germany) (see Table 1, Supplementary Digital Content 1, http://links.lww.com/CTG/A242). DNA library preparation and NGS were performed by the University of Michigan Sequencing Core according to the manufacturer's recommended protocols on the Illumina HiSeq instruments with target read depths of >500×. The average read depth was >2000× using 15–34 million reads per sample. Bioinformatics analysis of NGS data was performed by the UM Bioinformatics Core, with read mapping, variant calling, and annotation performed using the Genome Analysis Toolkit v3.3-2 using the Broad Institute Best Practice guidelines. Reads were aligned to the hg19 human reference genome with BWA v0.7.8, and variants identified using the Broad Unified Genotyper with standard parameters and hard filters. Variants were annotated using Golden Helix VarSeq v1.1.4 (Golden Helix, Bozeman, MT) to draw attention to truncating variants (nonsense, frameshift deletions/insertions, and highly conserved splice site mutations).

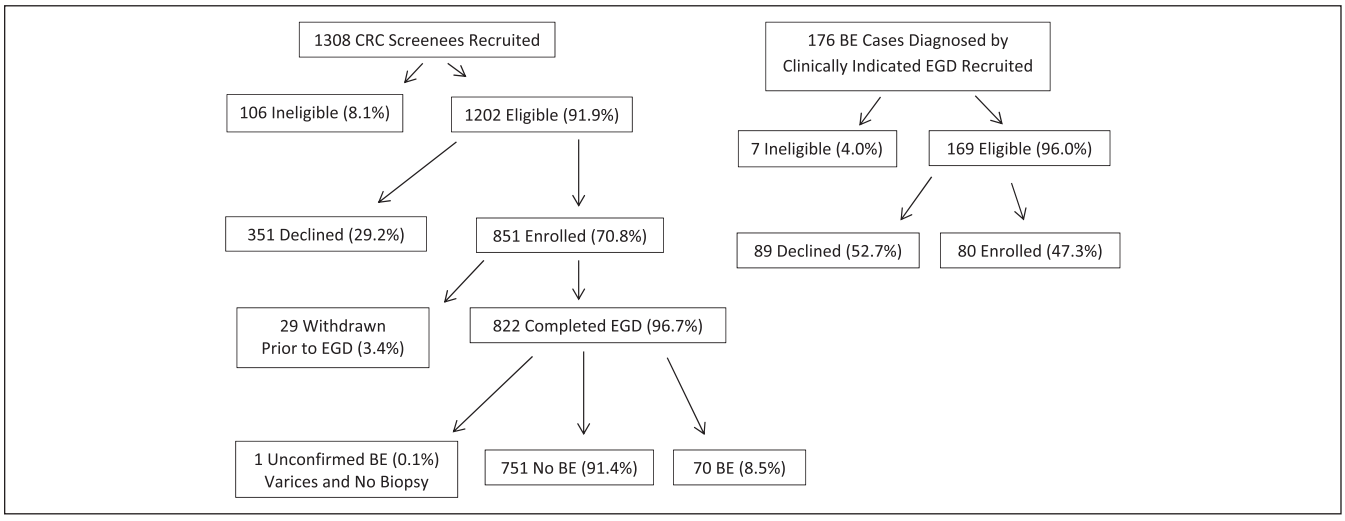

Figure 1. Flowchart of cohort. Effects of family histories were estimated among the CRC screenees. Analysis of germline DNA was performed on relevant cases of BE selected from CRC screenees and clinically diagnosed cases of BE. BE, Barrett's esophagus; CRC, colorectal cancer; EGD, esophagogastroduodenoscopy.

RefSeq v105v2 gene models were used for annotation. The study was approved by the Institutional Review Boards of the University of Michigan (HUM00013564) and the Ann Arbor Veterans Affairs Medical Center (2008-116).

Analysis

Data were manually entered into Microsoft Access (Microsoft, Bellevue, WA) and then imported into SAS 9.4 (SAS Institute, Cary, NC). Using the cross-sectional cohort of CRC screenees, we fitted logistic regression models to estimate the magnitude of association (odds ratio [OR] and 95% confidence interval [CI]) between findings of BE (outcome) and colorectal adenomas or family histories of esophageal cancer and CRC. We hypothesized that there is a shared germline genetic variant for a subset of cases of CRC and BE. A family history does not directly cause disease in an individual. Rather, a family history is an outcome of genes, environment, and number of family members. Therefore, an association of a family history of CRC or esophageal cancer with the risk of BE would be confounded by the germline genetic variant (because it causes both the family history and the development of BE) or shared environmental factors among family members. The logistic regression models were therefore adjusted for the potential confounders, including age and waist-to-hip circumference ratio (each treated as continuous variables), cigarette use (current, former, or never), GERD status, and indication for colonoscopy. If these are the only confounders of the associations between a family history and BE, and there are no other sources of bias, then the observed crude associations would attenuate to the null after adjusting for these factors. If there is an appreciable residual association after adjustment, it suggests that there are other confounders (such as an unspecified germline genetic variant) on the risk of BE.

Germline DNA NGS multigene panel results were filtered to focus on those nonsynonymous single-nucleotide polymorphisms or indels that occur in <5% of the general population, are found in >30% of reads, and are not identified as benign or likely benign in the National Center for Biotechnology Information ClinVar database. Variants were considered clinically actionable if they were classified as likely pathogenic or pathogenic in ClinVar. The remaining variants of uncertain significance (VUS) were considered of interest if they were predicted to be deleterious by both the Sorting Intolerant from Tolerant (SIFT) and Polymorphism Phenotyping (PolyPhen2) *in silico* prediction models. Functional prediction voting annotations for each variant were provided by the dbNSFP functional prediction and scores database, v.3.0, curated 2015-10-29, representing votes by the tools SIFT, PolyPhen2, MutationTaster, MutationAssessor, FATHMM, and FATHMM MKL. Variant allele frequencies were based on ExAC v.0.3 and gnomAD Exomes v.2.0.1 v2 curated by BROAD 2017-05-09.

RESULTS

A total of 851 CRC screenees enrolled in the study (consent rate = 70.7%), and 822 (96.7%) completed upper endoscopy (mean age 58.7 years). Four hundred forty-five (54.1%) were undergoing their first colonoscopy, 123 (15.0%) were undergoing repeat colon cancer screening after previous normal colonoscopy, and 254 (30.9%) had a history of colon polyps. Among the CRC screenees, BE was found in 70 (8.5%), and one additional subject had endoscopic findings suspicious for BE, but no biopsies were obtained because of coexistent esophageal varices. The median length by the Prague criteria was C0M2 (interquartile range = C0M1–C1M3), and dysplasia or adenocarcinoma was present in 5 (7.1%). Descriptive characteristics of the potential confounding factors are displayed in Table 1.

Among the CRC screenees, BE was associated with colorectal adenomas, particularly left-sided adenomas, adjusting for the potential confounders of age, abdominal obesity, cigarette use, indication for colonoscopy, and GERD (OR = 1.93; 95% CI = 1.05–3.56) (Table 2). This association was stronger for advanced adenomas than for nonadvanced adenomas (Table 2).

Among the CRC screenees, a family history was missing in 25 subjects (3.0%); 38 (4.6%) reported any family history of esophageal cancer, including 21 with at least one FDR with esophageal cancer (2.6%) and 2 with at least 2 FDRs (0.25%). Eleven CRC screenees (1.3%) reported any family history of BE, but 8 of them also reported a family history of esophageal cancer, suggesting that patients are generally unaware of a family history of BE in the absence of cancer. A family history of one FDR with CRC was reported in 109 (13.3%) and at least 2 FDRs in 11 (1.3%). Among the 70 CRC screenees diagnosed with BE, there were 3 subjects (4.3%) with at least one FDR with esophageal cancer and 3 (4.3%) with CRC in 2 or more FDRs. By comparison, among 80 individuals with BE diagnosed by clinically indicated endoscopy, 6 (7.5%) had an FDR with esophageal cancer and 2 (2.5%) had at least 2 FDRs with CRC.

BE among the screening colonoscopy patients was associated with any family history of esophageal cancer, adjusting for age, abdominal obesity, smoking, GERD, and indication for colonoscopy (OR = 2.63; 95% CI = 1.07–6.47) (Table 3). However, the estimated association of BE with a history of an FDR with esophageal cancer was very imprecise (not shown) because of the small number of cases having FDR with esophageal cancer. A distant family history of CRC or only one FDR with CRC was minimally associated with BE; however, a family history of at least 2 FDRs with CRC was positively associated with BE, although still imprecisely estimated (unadjusted OR = 4.13; 95% CI = 1.06–16.1; adjusted OR = 3.73; 95% CI = 0.898–15.4) (Table 3). Further adjusting for the use of proton pump inhibitor medications yielded similar results.

Having found these associations of a family history with BE, we selected cases of BE with an FDR with esophageal cancer or at least 2 FDRs with CRC among the CRC screenees or clinically diagnosed cases of BE for genetic analysis. Eighty subjects with BE diagnosed by clinically indicated endoscopy were enrolled within 1 month of the initial diagnosis (45% consent rate). Among those clinically diagnosed cases of BE, 6 (7.5%) had an FDR with esophageal cancer and 2 (2.5%) had at least 2 FDRs with CRC. Among the 70 CRC screenees with BE, there were 3 subjects (4.3%) with each of those family histories.

Germline DNA from a total of 14 cases of BE underwent NGS using the panel of 275 cancer genes. After filtering and annotation with *in silico* prediction models, we focused our attention on 12 germline variants found in 10 of these 14 subjects (Table 4). None of the 12 germline variants were classified as pathogenic or likely pathogenic in ClinVar, but they were predicted to be deleterious by both the SIFT and PolyPhen models. The same missense VUS in *EPHA5* (c.242A>C with minor allele frequency 0.04789) was identified in 2 unrelated subjects, one with a family history of CRC in 2 brothers and the other with a family history of both esophageal cancer and CRC diagnosed in his mother (subjects D and E in Table 4).

Table 1. Descriptive characteristics of subjects

	CRC screenees		BE diagnosed by clinically indicated endoscopy (n = 80)
	No BE (n = 751)	BE (n = 70)	
Age (yr)[a]	58.5 (6.7)	61.0 (6.5)	61.4 (7.1)
BMI (kg/m^2)[a]	29.9 (5.6)	30.3 (4.2)	31.0 (5.6)
WHR[a]	1.001 (0.056)	1.020 (0.053)	1.021 (0.054)
GERD ≥weekly (%)[b]	131 (18)	24 (34)	65 (81)
Current smoker (%)[b]	158 (22)	20 (29)	22 (28)
Former smoker (%)[b]	338 (47)	36 (53)	43 (54)
Never smoker (%)[b]	224 (31)	12 (18)	15 (19)
White race (%)[b]	640 (90)	57 (88)	76 (95)
Colonoscopy indication			
First screening (%)[b]	410 (55)	35 (50)	NA
Surveillance of polyps (%)[b]	230 (31)	23 (33)	NA
Repeat screening (%)[b]	111 (15)	12 (17)	NA

BE, Barrett's esophagus; BMI, body mass index; CRC, colorectal cancer; GERD, gastroesophageal reflux disease; NA, not applicable; WHR, waist-to-hip ratio.
[a]Data expressed as mean (SD).
[b]Data expressed as number (proportion). Proportions are among subjects with available data.

DISCUSSION

We found that BE is associated with a family history of esophageal cancer (1 or more FDR) and/or CRC (2 or more FDR). Although we did not identify any clinically actionable pathogenic germline variants to explain this association, multigene panel testing did identify 12 VUS that deserve further study based on *in silico* prediction. In particular, we found the identical germline variant in *EPHA5* in 2 cases of BE with family histories of esophageal cancer or at least 2 FDRs with CRC.

A familial syndrome of BE and esophageal adenocarcinoma has been previously described (1,2). The familial aggregation could be the result of shared environmental risk factors (such as diet, obesity, and smoking) or an underlying genetic basis. To that end, we explored whether BE is associated with a family history of esophageal cancer, adjusting for the potential confounders. We also explored an association with a family history of CRC because there are a number of syndromes with adenocarcinomas throughout the gastrointestinal tract and CRC is the most common gastrointestinal cancer. We found no evidence of a strong association with a distant family history or a single FDR with CRC, but we did find a strong association with at least 2 FDRs with CRC. We also confirmed the previously described increased occurrence of BE in individuals with a family history of esophageal cancer, with a more precise although weaker association than previously reported (OR = 2.63; 95% CI = 1.07–6.47 in the current study compared with OR = 12.2; 95% CI = 3.34–44.8 in the study by Chak et al.) (1). Because of the small number of cases of BE with the rare family history of at least 2 FDRs with CRC, our estimate of the magnitude of that adjusted association was imprecise, i.e., with a very wide CI (OR = 3.73; 95% CI = 0.898–15.4). Thus, this finding needs to be replicated in larger studies.

We then explored with NGS whether germline variants in any of 275 genes known to be involved in any cancer could explain the association of a family history of CRC with BE. Among individuals with BE and at least 2 FDRs with CRC, we did not find any

Table 2. Associations of BE with colorectal adenomas among colorectal cancer screenees

	No. of BE/No. of no BE	Crude OR (95% CI)	Adjusted[a] OR (95% CI)
No adenoma	31/451	1 (reference)	1 (reference)
Nonadvanced adenoma	27/227	1.73 (1.01–2.97)	1.59 (0.900–2.82)
Advanced adenoma	10/65	2.24 (1.05–4.78)	1.79 (0.802–4.01)
No adenoma	31/451	1 (reference)	1 (reference)
Any right adenoma	25/215	1.69 (0.975–2.94)	1.37 (0.759–2.47)
No adenoma	31/451	1 (reference)	1 (reference)
Any left adenoma	22/152	2.11 (1.18–3.75)	1.93 (1.05–3.56)

BE, Barrett's esophagus; CI, confidence interval; OR, odds ratio.
[a]Adjustments are made for age (continuous), waist-to-hip ratio (continuous), cigarette use (never, former, or current), gastroesophageal reflux disease frequency (≥weekly vs <weekly or never), and indication for colonoscopy (first screening, repeat screening, or surveillance of polyps).

Table 3. Associations of BE with family history among CRC screenees

	No. of BE/No. of no BE	Crude OR (95% CI)	Adjusted[a] OR (95% CI)
No family history of EC	61/697	1 (reference)	1 (reference)
Any family history of EC	7/31	2.58 (1.09–6.11)	2.63 (1.07–6.47)
No family history of CRC	51/562	1 (reference)	1 (reference)
Any family history of CRC	17/166	1.13 (0.635–2.01)	1.08 (0.584–1.98)
Distant family history of CRC	4/59	0.747 (0.261–2.14)	0.895 (0.306–2.62)
1 FDR with CRC	10/99	1.11 (0.547–2.27)	0.935 (0.433–2.02)
≥2 FDRs with CRC	3/8	4.13 (1.06–16.1)	3.73 (0.898–15.4)

BE, Barrett's esophagus; CI, confidence interval; CRC, colorectal cancer; EC, esophageal cancer; FDR, first-degree relative; OR, odds ratio.
[a]Adjustments are made for age (continuous), waist-to-hip ratio (continuous), cigarette use (never, former, or current), gastroesophageal reflux disease frequency (≥weekly vs <weekly or never), and indication for colonoscopy (first screening, repeat screening, or surveillance of polyps).

variants that are known to be pathogenic but did find 5 VUS that are predicted by *in silico* methods to potentially disrupt protein function. These findings should be interpreted with caution, however, and future studies are needed to replicate these findings. One such gene was *EXO1*, which encodes exonuclease 1 and interacts with *MSH2*, one of the DNA mismatch repair genes responsible for Lynch syndrome, which has an increased risk of CRC and other gastrointestinal cancers (8). The same patient also had a germline variant in *DNMT3A*, which encodes DNA (cytosine-5)-methyltransferase 3A, and a variant in that gene has been associated with an increased risk of CRC (9,10). To the best of our knowledge, neither *EXO1* nor *DNMT3A* has been associated with BE or esophageal adenocarcinoma. Another patient had a variant in *PTCH1* that was predicted to be pathogenic. *PTCH1* encodes a tumor suppressor that is a receptor for sonic hedgehog. Variants in *PTCH1* are associated with Gorlin (basal cell nevus) syndrome; however, these have also been found to be associated with microsatellite-unstable CRC, and hypermethylation of the *PTCH1* promoter has been associated with aberrant crypt foci (11,12). Other variants in *PTCH1* have been associated with esophageal squamous cell carcinoma, but *PTCH1* is upregulated in BE (13–15).

Among individuals with BE and an FDR with esophageal cancer, we also found 8 suspicious germline variants, including a VUS in *MSH6*, a DNA mismatch repair gene known to be associated with Lynch syndrome. A variant in *APC* was found in a subject whose brother had both esophageal cancer and CRC. FAP is caused by inherited inactivating variants in *APC*, leading to CRC at early ages, and associated with extracolonic malignancies, including gastric, duodenal, and biliary (16). Attenuated forms of FAP can lead to CRC at somewhat later ages. A number of studies have implicated mutation or inactivation of *APC* by hypermethylation as an uncommon pathway for neoplastic progression of BE (3,17–26). One study suggested that individuals with FAP may be at an elevated risk for BE compared with age-matched controls undergoing endoscopy for clinical indications (3).

Perhaps most intriguing, the identical rare germline VUS in *EPHA5* was found in 2 of 14 unrelated individuals with BE—one subject had 2 brothers with CRC and another subject reported that his mother had both esophageal cancer and CRC. *EPHA5* encodes the tyrosine kinase ephrin type-A receptor 5. CRC tumors tend to have hypermethylation in the promoter region of *EPHA5* or decreased expression of the protein (27,28), and decreased expression is associated with stage at presentation and prognosis of CRC (28,29). In cell lines of CRC, expression of the protein inhibited epidermal growth factor receptor, which is also an important marker of neoplasia and prognosis in esophageal adenocarcinoma (28). *EPHA5* appears to be differentially expressed in gastric adenocarcinoma, with somatic methylation observed in >50% of tumors (30,31). Although this rare *EPHA5* missense variant is predicted to affect protein function by *in silico* models, it has not been reported in ClinVar, and thus, there is no additional information to assess its clinical significance. We are not aware of any studies examining the role of *EPHA5* in BE or esophageal adenocarcinoma. We also found that BE is associated with the presence of colorectal adenomas.

A number of previous studies have suggested an association between BE and colorectal neoplasms. A meta-analysis published in 2013 of those studies concluded that BE was associated with colorectal neoplasms with a summary OR of 1.96 (95% CI 1.56–2.46) (32). Only 2 of the 11 studies adjusted for factors aside from age and sex. Since then, there have been at least 2 other case–control studies finding unadjusted associations between BE and colorectal neoplasms with magnitudes similar to what we found (33,34). However, de Jonge et al. (35) found that the increased risk of CRC was limited to within the first year after the first diagnosis of BE, suggesting that the association with CRC is due largely to diagnostic bias (e.g., a patient is diagnosed with BE and CRC in short succession of each other after presenting for upper and lower endoscopy for iron deficiency anemia). Our study minimized the risk of diagnostic bias in that men presenting for colonoscopy for screening or surveillance were recruited to undergo upper endoscopy for research purposes regardless of symptoms, and the consent rate was quite high. In addition, we adjusted for a number of potential confounders, including age, tobacco use, abdominal obesity, GERD symptoms, and the indication for colonoscopy, and yet, we still found that men with colorectal adenomas (particularly left-sided adenomas) were almost twice as likely to be diagnosed with BE. The association could be due to unmeasured confounders, such as dietary or nutritional factors, or an underlying genetic etiology shared by both conditions. Either way, this association may be useful for identifying patients who are at risk for esophageal adenocarcinoma.

Table 4. Suspicious germline variants of uncertain significance identified in men with BE and either a first-degree relative with esophageal cancer or at least 2 first-degree relatives with CRC

Subject	Gene	Variant	Functional prediction voting	Alt allele frequency gnomAD/ExAC	Family history	Case population
A	*EXO1*	NM_006027.4:c.836A>G (NC_000001.10: g.242023898A>G)	5 of 6 predicted as damaging	0.02416/0.024	Father, brother, and paternal uncle with CRC	CRC screenee
	DNMT3A	NM_022552.4:c.89A>C (NC_000002.11: g.25523096T>G)	5 of 6 predicted as damaging	0.00331/0.00292		
B	*PTCH1*	NM_000264.3:c.2485G>A (NC_000009.11: g.98229473C>T)	6 of 6 predicted as damaging	0.00039/0.00031	Mother and father with CRC	Clinically diagnosed BE
C	*MYC*	NM_002467.4:c.77A>G (NC_000008.10: g.128750540A>G)	4 of 6 predicted as damaging	0.02311/0.022	Mother and sister with CRC	CRC screenee
D	*EPHA5*	NM_001281765.2:c.242A>C (NC_000004.11: g.66509085T>G)	4 of 6 predicted as damaging	0.04789/0.049	Two brothers with CRC	Clinically diagnosed BE
E	*EPHA5*	NM_001281765.2:c.242A>C (NC_000004.11: g.66509085T>G)	4 of 6 predicted as damaging	0.04789/0.049	Mother with both EC and CRC	Clinically diagnosed BE
F	*MSH6*	NM_000179.2:c.3557G>A (NC_000002.11: g.48032757G>A)	6 of 6 predicted as damaging	0.000016355/ 0.00002471	Sister with EC	CRC screenee
G	*TET2*	NM_001127208.2:c.5333A>G (NC_000004.11: g.106197000A>G)	4 of 6 predicted as damaging	0.03662/0.045	Mother with EC	Clinically diagnosed BE
H	*BCL6*	NM_001706.4:c.1375C>T (NC_000003.11: g.187446313G>A)	4 of 6 predicted as damaging	0.00109/0.00105	Father with EC and mother with BE	Clinically diagnosed BE
	BCR	NM_004327.3:c.455C>T (NC_000022.10: g.23523602C>T)	4 of 6 predicted as damaging	0.00287/0.00305		
I	*DDR2*	NM_006182.2:c.187C>G (NC_000001.10: g.162724415C>G)	6 of 6 predicted as damaging	0.00005699/ 0.00006589	Sister with EC	CRC screenee
	DOT1L	NM_032482.2:c.4327G>T (NC_000019.9:g.2226847G>T)	3 of 6 predicted as damaging	0.03819/0.036		
J	*APC*	NM_000038.5:c.7574G>A (NC_000005.9: g.112178865G>A)	6 of 6 predicted as damaging	0.0001425/ 0.0001812	Brother with EC and CRC	CRC screenee

BE, Barrett's esophagus; CRC, colorectal cancer; EC, esophageal cancer.

Our study was limited by the few number of cases with the rare family history of at least 2 FDRs with CRC. In addition, our questionnaires only queried family histories of esophageal cancer and CRC and did not include other cancer sites and any verification of the family history beyond subject self-report. The questionnaire did not distinguish between adenocarcinoma and squamous cell carcinoma of the esophagus in family members. There may have been unmeasured confounders that are responsible for the observed associations, such as environmental toxins. We only sequenced germline DNA for 14 subjects with BE, and given that our sequencing technology was neither comprehensive nor optimized for the detection of large deletions and duplications, it is possible that clinically actionable germline variants could have been missed. Finally, because of cost considerations, we did not sequence DNA from controls but instead report allele frequencies from population reference databases. Our study also had important strengths, including a design that decreases the potential for diagnostic bias and the ability to adjust for several important potential confounders.

In conclusion, we found that colorectal adenomas and a family history of at least 2 FDRs with CRC or with a FDR with esophageal cancer are associated with BE among men. Although sequencing germline DNA from affected individuals did not identify any variants

known to be pathogenic, we did identify a number of germline VUS that might explain the familial associations, including *EPHA5*. Future studies are needed to confirm these findings. Such a family history may be important to consider in selecting men for screening for esophageal adenocarcinoma.

CONFLICTS OF INTEREST

Guarantor of the article: Joel H. Rubenstein, MD, MSc.

Specific author contributions: J.H.R. conceived and designed the study, acquired the data, analyzed and interpreted the data, drafted the manuscript, had full access to all the data in the study, and takes responsibility for the integrity of the data and the accuracy of the data analysis. A.T., J.M.I., and H.M. interpreted the data and critically revised the manuscript. H.A., J.M.S., and P.S. acquired the data, interpreted the data, and critically revised the manuscript. V.M. acquired the data and critically revised the manuscript. E.K. and P.U. acquired and analyzed sequencing data. E.M.S. conceived and designed the study, acquired the data, analyzed and interpreted the data, and critically revised the manuscript. All authors approved the final manuscript.

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Study Highlights

WHAT IS KNOWN

- ✓ BE has been associated with colorectal adenomas.
- ✓ There is a familial syndrome of esophageal adenocarcinoma.
- ✓ Familial syndromes of adenocarcinomas of the gastrointestinal tract can affect multiple organs.

WHAT IS NEW HERE

- ✓ BE was associated with family histories of esophageal cancer or at least 2 FDRs with CRC.

TRANSLATIONAL IMPACT

- ✓ Germline DNA analysis did not identify any clinically actionable germline variants.
- ✓ However, 10 cases carried a variant predicted to be damaging by in silico models.
- ✓ The same rare VUS in *EPHA5* was found in 2 cases of BE.

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