

Genetic Analysis of Archived Tumor Specimens for Hereditary Colorectal Cancer Syndromes in the Cajuns of Louisiana, a US Founder Population

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INTRODUCTION: The Louisiana Acadian region (population 1.2 million), home of the Cajuns, has among the highest US colorectal cancer (CRC) rates. Although Cajuns are a known genetic founder population, studies assessing for hereditary CRC have not been performed.

METHODS: A retrospective review of 2 hospital cancer registries was performed to identify young (<55) Cajun CRC patients in Lafayette, Louisiana (the Acadian region population center), diagnosed from 2003 to 2016. Men were studied because of the higher likelihoods of retaining Cajun surnames for ancestry identification compared with women. Immunohistochemistry for mismatch repair proteins associated with the Lynch syndrome (LS) was performed on tumors. Germline sequencing was performed on adjacent normal tissue of these archived formalin-fixed paraffin-embedded surgical resection specimens for pathogenic variants underlying CRC-associated syndromes, including LS, familial adenomatous polyposis, and others.

RESULTS: Of 9 young Cajuns, a germline analysis revealed LS in 2 (*MLH1* frameshift, *MLH1* missense pathogenic variants). Both had immunohistochemistry-deficient MLH1. Two others had the same adenomatous polyposis coli variant of unknown significance (2 algorithms predicting deleterious and probably damaging change), making this a potential familial adenomatous polyposis founder effect candidate.

DISCUSSION: This is the first study assessing for hereditary CRC in a large US regional founder population. This small study did not identify clear Cajun founder pathogenic variants. However, larger studies are warranted, which could also help clarify the clinical significance of the adenomatous polyposis coli variant of unknown significance. This study is important because it demonstrates that a retrospective tumor analysis can be used to ascertain the prevalence of genetic susceptibility in specific populations.

SUPPLEMENTARY MATERIAL accompanies this paper at <http://links.lww.com/CTG/A668>

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INTRODUCTION

The Louisiana Acadian region, Acadiana (population 1.2 million), home of the Cajuns (regional population approximately 700,000), has among the highest US colorectal cancer (CRC) rates, including early-onset disease (1). Because Cajuns are a genetic founder population, we aim to ascertain the prevalence of germline pathogenic variants among those with early-onset CRC by retrospectively analyzing banked CRC surgical resection specimens for Lynch syndrome (LS)-associated mismatch repair deficiency (dMMR) and by analyzing adjacent normal colonic

tissue for pathogenic germline variants underlying LS and other CRC-associated hereditary conditions (1). We also seek to identify potential founder pathogenic variants. LS founder effects have been demonstrated in other populations, and germline variants can explain high malignancy rates in certain areas (1,2). A previous study showed low dMMR testing rates in Acadiana (3).

Previous analyses revealed that Acadiana has disproportionately high CRC rates (1). In parishes with the highest Cajun ancestry rates, CRC incidence in Whites was 13% higher than

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statewide rates ($P < 0.0003$) and 23% higher than US rates ($P < 0.0001$). CRC environmental risk factors, including obesity and smoking, are lower in Acadiana than other areas of Louisiana (1). Furthermore, non-CRC malignancies, including those sharing environmental risk factors with CRC (lung and pancreatic cancer), have not shown disproportionately higher rates than statewide rates in Whites (1). These findings, in the context of the Cajuns being a founder population, support our rationale to assess for genetic variants to explain high CRC rates. Louisiana does not have disproportionately high uterine cancer rates (LS-associated tumor), but because of high hysterectomy prevalence (often for nonmalignant etiologies), corrected rates in Whites in Louisiana are 110.5% higher than previously reported (4).

Retrospectively analyzing archived CRC tissue for germline variants seems to be an infrequently used modality. However, this may be an underappreciated but high-yield tool to assess for germline changes in high-risk populations.

METHODS

A retrospective hospital cancer registry query (2 hospitals in the Acadian population center of Lafayette, Louisiana) identified all CRC patients diagnosed from 2003 to 2016 at these facilities. Subsequent inclusion criteria included White, non-Hispanic men younger than 55 years old at diagnosis, top 100 Cajun surname, residents within the 18 Acadian-parishes region, and available banked CRC resection specimens (1). Men were studied because of the higher likelihoods of retaining Cajun surnames compared with women. For those meeting the inclusion criteria, the patients' primary care providers, oncologists, or surgeons were approached to obtain permission to directly contact patients for informed consent.

Immunohistochemistry analysis (Mayo Clinic Laboratories) was of LS-associated dMMR proteins (MLH1, MSH2, MSH6, and PMS2) and performed on formalin-fixed paraffin-embedded CRC tumors from surgical resection specimens. For MLH1 loss, *BRAF* mutation and MLH1 promoter methylation analyses were conducted to assess for promoter methylation sporadic CRC.

DNA for germline sequencing was extracted from formalin-fixed paraffin-embedded blocks of adjacent normal tissue of the surgical resection specimens. Analyses (see Supplemental Methods, Supplementary Digital Content 1, <http://links.lww.com/CTG/A668>) were conducted at the Washington University McDonnell Genome Institute (10 hereditary CRC-associated genes: *MLH1*, *MSH2*, *MSH6*, *PMS2*, *epithelial cellular adhesion molecule* [*EPCAM*], *breast cancer* [*BRCA1*], *BRCA2*, *adenomatous polyposis coli* [*APC*], *MutY glycosylase homologue*, and *ataxia telangiectasia mutated* [*ATM*]).

Analyses were conducted from September 2016 to February 2017. Archived surgical specimen years from those consented ranged from 2009 to 2015. Consent was obtained from family members if patients were deceased.

RESULTS

Of all patients with CRC identified, 62 met the inclusion criteria. We received permission to directly contact all 62 patients for consent. Consent was obtained in 9 (14.5%). Others were unable to be consented for multiple reasons including the patients being deceased and cancers being diagnosed years earlier, with changes in contact information.

Immunohistochemistry was completed in 7 patients (Table 1). All 9 patients underwent germline testing. Two of 9 (22%) had 2 different germline *MLH1* variants. Both had dMMR consistent with LS. Patient 3 had an *MLH1* frameshift pathogenic variant (c.1195del/p.Arg399fs). Patient 6 had an *MLH1* missense pathogenic variant (c.116G > A/p.Cys39Tyr). One of these patients reported outside commercial testing showing an *MLH1* pathogenic variant confirming LS.

Four others had variants of unknown significance (VUS) including *BRCA2*, *APC*, *ATM*, and *EPCAM*. The *EPCAM* VUS was not a large 3' deletion (not considered CRC-associated). The same exact *APC*, *BRCA-2*, and *ATM* VUSs were seen in 2 distinct patients (patient 4 and 8). For patient 4, no colonoscopy reports before CRC diagnosis were available to assess for a history of polyps removed (which could potentially indicate a polyposis phenotype if present). No other lesions/adenomas were seen on the surgical resection specimen; however, only 19 cm of colon was resected. For patient 8, no previous colonoscopy reports were available, and no other lesions/adenomas were seen on the surgical resection specimen; however, only 22 cm of colon was resected. This patient did report 1 first-degree relative with both CRC and small bowel cancer (which can be commonly associated with *APC* variants). The *APC* variant (c.3205A > G/p.Arg1069Gly) has been reported 6 times in ClinVar (ID: 142240). The *BRCA-2* (c.9772G > A/p.Glu3258Lys) and *ATM* (c.2650C > G/p.Pro884Ala) VUSs have not been reported.

DISCUSSION

To our knowledge, this is the first study assessing hereditary CRC in a large US regional founder population. One Ohio study among early-onset CRC patients demonstrated that 10.7% had dMMR, with 83.3% of these having LS confirmed by germline testing (5). We found 28.6% (2/7) of Cajun early-onset patients had dMMR, and 100% (2/2) of those had 2 different *MLH1* pathogenic variants. We also found 2 patients with the same *APC* VUS. This small study did not identify Cajun founder pathogenic variants to help explain the higher regional CRC incidence. However, larger studies are warranted, which could also help clarify the clinical significance of the *APC* VUS. This study is important because it demonstrates retrospective tumor analysis can be used to ascertain the prevalence of genetic susceptibility in specific populations.

The *MLH1* pathogenic variants have been previously reported in the genomic database, ClinVar (<http://www.ncbi.nlm.nih.gov/clinvar/>). The *MLH1* variant in patient 3 (c.1195del/p.Arg399fs) was reported in 2 studies conducted in an Ohio population (6). The *MLH1* variant in patient 6 (c.116G > A/p.Cys39Tyr) was reported in studies conducted in Scotland, Australia, Germany, and the US (MD Anderson) (7). Given that Cajuns are descended from the Acadians of northeast Canada (Nova Scotia), it is noteworthy there are no ClinVar reports of these variants in Canada. However, according to 2016 census data, after mass migration out of the province in the 1700s, only 2.6% of the total Nova Scotia population reports Acadian ancestry (23,700 of 923,598) (8).

Two patients having the same VUSs in *BRCA2*, *APC*, and *ATM* implies that they might be closely related (but not first or second degree relatives, given differing family histories). Because the genes are on different chromosomes, these cannot all 3 represent founder mutations. The *BRCA2* and *ATM* variants have not been reported in ClinVar.

Table 1. Immunohistochemistry and germline sequencing results

Patient	Age at diagnosis	Family history of cancer			Patient tumor location	MLH1	MSH2	MSH6	PMS2	BRAF mutation	MLH1 promotor methylation	IHC consistent with Lynch	DNA sequencing
		CRC in first degree relative	CRC in second degree relative	LS associated cancer ^b (non-CRC) in relative									
1	46–50 ^c	No	Yes, 2 family members	No	Left distal descending colon	N/A	N/A	N/A	N/A	N/A	N/A	N/A	No mutation/no VUS
2	46–50 ^c	No	No	No	Rectum into sigmoid colon	N/A	N/A	N/A	N/A	N/A	N/A	N/A	No mutation/no VUS
3	31–35 ^c	Yes, 2 family members	Yes, 1 family member	Yes	Rectum	Loss	Present	Present	Loss	No mutation	No methylation	Yes	Frameshift MLH1 rs63750855 ^e (c.1195del/p.Arg399fs)
4	51–55 ^c	No	No	No	Sigmoid colon	Present	Present	Present	Present	N/A	N/A	No	VUS APC/ATM ^f /BRCA2 ^g APC rs375408871 ^e (c.3205A>G/p.Arg1069Gly)
5	51–55 ^c	No	No	No	Rectum into sigmoid colon	Present	Present	Present	Present	N/A	N/A	No	VUS EPCAM
6	21–25 ^c	No	Yes, 2 family members	No	right colon	Loss	Present	Present	Loss	No mutation	No methylation	Yes	Missense MLH1 ^d rs63751701 ^e (c.116G>A/p.Cys39Tyr)
7	46–50 ^c	No	Yes, 1 family member	No	Rectum	Present	Present	Present	Equivocal	N/A	N/A	Possible	No mutation/no VUS
8	36–40 ^c	Yes, 1 family member	No	Yes	Right colon	Present	Present	Present	Present	N/A	N/A	No	VUS APC/ATM ^f /BRCA2 ^g APC rs375408871 ^e (c.3205A>G/p.Arg1069Gly)
9	41–45 ^c	No	Yes, 1 family member	Yes	Right colon extending into terminal ileum	Present	Present	Present	Present	N/A	N/A	No	Insertion-low read support MSH6 ^a , VUS EPCAM

APC, adenomatous polyposis coli gene; ATM, ataxia telangiectasia mutated gene; BRCA, breast cancer gene; CRC, colorectal cancer; EPCAM, epithelial cellular adhesion molecule gene; IHC, immunohistochemistry; LS, Lynch syndrome; RS, reference SNP cluster; SNP, single nucleotide polymorphisms; VUS, variant of unknown significance.

^aLow read support in patient 9 indicates that an insertion was seen, but the number of sequence reads was low. The read support is not associated with the phenotype as much as it is to the level of confidence in the variant call.

^bNon-CRC LS-associated cancers as per the revised Bethesda Criteria (13).

^cAge range is reported to preserve patient anonymity.

^dNote that although this variant is reported as missense in ClinVar, MLH1 has several splice forms, and the annotation software picked the most severe change (in a short isoform where change introduces a stop codon).

^eThe RS number is an accession number used to refer to specific SNPs. It stands for Reference SNP cluster. The RS number can be used to access information in ClinVar.

^fc.2650C>G/p.Pro884Ala.

^gc.9772G>A/p.Glu3258Lys.

The APC variant (c.3205A > G/p.Arg1069Gly) has been reported 6 times as a VUS in ClinVar (10). It has not been reported in the scientific literature in those with familial adenomatous polyposis (FAP). This sequence change involves the replacement of arginine with glycine at codon 1069. The physicochemical difference between arginine and glycine is moderate. Algorithms predicting protein structure/function change with this missense variation do not agree on potential effects; however, 2 algorithms suggest that it is “deleterious” and “probably damaging” (9). Given its presence in 2 different young Cajun patients with CRC potentially indicates a founder effect variant. Although these patients did not have known polyposis, a limitation is that the study was not designed to obtain previous colonoscopy data. However, patient 8 reported a first-degree relative with small bowel cancer, which can be associated with APC variants. Furthermore, some APC variants are associated with attenuated FAP (fewer polyps compared with traditional FAP). However, larger confirmatory studies are required to assess for this and other genes that may be associated with founder effects. Testing in other populations sharing Cajun ancestry, including Acadians of northern Maine (Aroostook county), which also has disproportionately high CRC incidence, will be important (1).

A retrospective germline analysis of adjacent normal tissue in resection specimens has been infrequently used for CRCs and has not been the predominant modality to analyze high-risk populations, as in our study (10). This may be an underappreciated, yet high-yield, tool to obtain previously unavailable genetic information. This could be in the context of research settings of high-risk populations and/or in families to obtain previously unavailable genetic information in deceased relatives to guide testing of surviving relatives. In other cancers (ovarian and breast cancer), studies validating archived tumor specimens for germline analysis have been performed (11,12).

Study limitations include a relatively low consent response and small study population. The analysis did not include copy number variations. Furthermore, testing was performed on a research basis without result confirmation with standard testing protocols, except in one who reported *MLH1* commercial testing. Finally, to more reliably capture Cajun surnames, only men were analyzed. However, because most hereditary CRC syndromes are autosomal dominant (including LS and FAP), underlying variants would be expected to be present in similar frequency in women.

In conclusion, in this small Cajun CRC cohort, we identified 2 patients with 2 different *MLH1* pathogenic variants and 2 others with the same APC VUS. We did not identify a clearly pathogenic founder variant. However, the presence of the same APC VUS in different patients could potentially indicate a founder effect variant, but larger studies are needed to assess phenotypic-genotypic correlations. This study demonstrates retrospective tumor analysis can be used to ascertain the prevalence of genetic susceptibility in specific populations.

CONFLICTS OF INTEREST

Guarantor of the article: Jordan J. Karlitz, MD.

Author contributions: J.J.K.: study concept, study design, primary writing of the manuscript, data analysis, and literature review. A.P.-S. and S.R.: data acquisition, data analysis, and critical revision of manuscript. K.S.: data acquisition and data analysis.

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Potential competing interests: J.J.K.: Advisor for Exact Sciences, former consultant and Speaker's Bureau for Myriad Genetics, and equity position in Gastro Girl (virtual care/telehealth company). All other authors have no reported conflicts of interest.

Access to data statement: J.J.K. and A.P.-S. had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study Highlights

WHAT IS KNOWN

- ✓ The Cajuns of Louisiana, a founder population, have among the highest colorectal cancer (CRC) incidence rates in the US.
- ✓ Studies assessing for hereditary CRC have not been performed in this population.
- ✓ Retrospective germline sequencing of the normal colonic tissue margin of archived surgical resection specimens has been infrequently used to retrospectively assess patients with CRC.

WHAT IS NEW HERE

- ✓ Of 9 young Cajun patients with CRC, an analysis of archived surgical resection specimens revealed Lynch syndrome in 2.
- ✓ Two others had the same *adenomatous polyposis coli* genetic variants of unknown significance, making this a potential founder effect variant.

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