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Authors
Shin, Brian Y
Chen, Huiping
Rozek, Laura S
et al.

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Diseases of the Colon & Rectum

Low Allele Frequency of *MLH1 D132H* in American Colorectal and Endometrial Cancer Patients

Brian Y. Shin, M.D., M.P.H.,[1] Huiping Chen, Ph.D.,[2] Laura S. Rozek, Ph.D.,[3,4] Leslie Paxton, B.S.,[5] David J. Peel, Ph.D.,[6] Hoda Anton-Culver, Ph.D.,[6] Gad Rennert, M.D.,[7] David G. Mutch, M.D.,[2] Paul J. Goodfellow, M.D.,[2] Stephen B. Gruber, M.D., Ph.D.,[3,4] Steve M. Lipkin, M.D., Ph.D.[1,6]

[1] *Division of Hematology/Oncology, Department of Medicine, University of California Irvine, Irvine, California*
[2] *Department of Surgery and Obstetrics/Gynecology, Washington University School of Medicine, St. Louis, Missouri*
[3] *Department of Medicine, University of Michigan, Ann Arbor, Michigan*
[4] *Department of Epidemiology, University of Michigan, Ann Arbor, Michigan*
[5] *Department of Medicine, University of California Irvine, Irvine, California*
[6] *Division of Epidemiology, Department of Medicine, University of California Irvine, Irvine, California*
[7] *Department of Community Medicine and Epidemiology, Carmel Medical Center and Technion Faculty Medicine, Haifa, Israel*

PURPOSE: Hereditary nonpolyposis colon cancer is caused by mutations in DNA mismatch repair genes, predominantly *MLH1* and *MSH2*. Classic *MLH1* mutations cause an approximately 20-fold increase in colorectal cancer susceptibility. Recently, we identified a hypomorphic allele, *MLH1 D132H*, which impairs, but does not completely eliminate the function *of MLH1* in tumor suppression. *MLH1 D132H* confers an approximately fivefold increase in colorectal cancer susceptibility and was first described in a cohort of Israeli colorectal cancer patients, with an estimated allele frequency of 1.3 percent. Because *MLH1 D132H* has only recently been described, the ethnic distribution of this risk allele is not well understood. This study was undertaken to determine both the frequencies of this risk allele in ethnic groups outside of Israel and whether families harboring this mutation have susceptibility to extracolonic cancers in the hereditary nonpolyposis colon cancer spectrum. METHODS: We genotyped two independent cohorts: 629 population-based colorectal cancer patients ascertained from clinics in Orange, Imperial, and San Diego Counties, and 515 endometrial cancer patients ascertained from gynecologic oncology clinics in the Midwestern United States. RESULTS: *MLH1 D132H* was not detected in either study cohort, which together totaled more than 1,100 American colorectal cancer and endometrial cancer patients. CONCLUSIONS: The *MLH1* D132H risk variant has significantly lower allele frequency in American compared with Israeli cancer patients and, alone, is unlikely to explain significant amounts of American sporadic colorectal cancer or uterine cancer susceptibility. Genetic testing for the *MLH1 D132H* allele exclusively is therefore unlikely to be cost effective for genetic risk assessment in American population-based and clinic-based colorectal cancer and endometrial cancer patients. [Key words: Colorectal cancer; Endometrial cancer; Cancer genetics; *MLH1*]

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Correspondence to: Steve M. Lipkin, M.D., Ph.D., 250 Sprague Hall, ZC 4038, Department of Medicine, UC Irvine, Irvine, California 92697-4038, e-mail: slipkin@uci.edu

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Mutations in the DNA mismatch repair gene *MLH1* are a common cause of hereditary nonpolyposis colon cancer (HNPCC).[1] Recently, a hypomorphic *MLH1* allele has been identified that impairs,

but does not completely eliminate, the roles of *MLH1* in tumor suppression (*MLH1* D132H).[2] Unlike classic *MLH1* mutations, which cause an ~20-fold increase in colorectal cancer (CRC),[1] *MLH1 D132H* confers an ~fivefold increase in CRC risk.[2] This risk allele was first described in Israelis, including members of Ashkenazi Jewish, non-Ashkenazi Jewish, Muslim and Christian Arab, and Druze Christian population subgroups, with an estimated allele frequency in CRC patients approximating 1.3 percent.[2] This mutation also was unusual because it was associated with CRCs that lack microsatellite instability (MSI), a phenotype closely associated with classic *MLH1* mutations in HNPCC.[2] Because *MLH1 D132H* has only recently been described, the ethnic and cancer susceptibility characteristics of families with identified *MLH1 D132H* mutations are not well understood. In particular, it is important to address what are the frequencies of this risk allele in ethnic groups outside of Israel, and whether families harboring this mutation have susceptibility to other cancers, particularly extracolonic cancers in the HNPCC spectrum. Endometrial cancer is the most common gynecologic malignancy in the United States and is the most frequent extracolonic cancer in HNPCC.[1] Epigenetic silencing of *MLH1* (evidenced by methylation of the MLH1 promoter) is the most frequent cause of microsatellite instability in sporadic endometrial and colorectal cancers.[3,4] Although nearly 20 percent of a large series of endometrial cancers studied had epigenetic inactivation of *MLH1*,[4] the majority of sporadic colorectal and endometrial cancers do not exhibit the MSI phenotype and the role that the *MLH1 D132H* variant plays in these North American patients is unknown.

To understand more precisely the contribution of *MLH1 D132H* to sporadic CRC and endometrial cancer in the United States, we genotyped two independent cohorts: 629 population-based CRC patients ascertained from clinics in Orange, Imperial, and San Diego Counties,[5] and 515 endometrial cancer patients ascertained from gynecologic oncology clinics in the midwestern United States. In both cohorts of CRC and endometrial cancer patients, together comprising more than 1,100 Americans, we did not detect *MLH1 D132H*. Altogether, these data suggest that *MLH1 D132H* has significantly lower allele frequency in American compared with Israeli cancer patients.

METHODS

A total of 1,134 CRC patients were ascertained through the population-based registries of the Cancer Surveillance Program of Orange County/San Diego Imperial Organization for Cancer Control. These subjects were diagnosed between 1994 and 1996. Detailed information about the population sampling has been previously described.[5] Positive family history was defined as having at least one first-degree relative with CRC, or no first-degree relative with CRC, and at least two maternal or two paternal second-degree relatives with CRC, or at least three maternal or three paternal third-degree relatives. DNA was extracted from blood samples taken from participants. A total of 629 patients had DNA samples available. For endometrial cancer patients, 515 patients were ascertained through Washington University Gynecology Oncology Clinics in Missouri.[4]

MLH1 D132H analyses of CRC patient samples were performed in the laboratory of Dr. Steve Lipkin. Allelic discrimination of the CRC sample set was optimized using a custom TaqMan-based SNP Genotyping assay and the ABI Prism 7900 HT Sequence Detection System (Applied Biosystems, Foster City, CA). Each 5-µl reaction included 2.5 µl of TaqMan Universal Master Mix® (Roche Molecular Systems Inc., Branchburg, NJ), 0.125 µl of assay reagents for Single Nucleotide Polymorphism (SNP) genotyping from the Applera Assays-by-Design (Applied Biosystems) consisting of a 40× mix of unlabeled PCR primers and TaqMan MGB probes (FAM and VIC dye labeled), in addition to 2 ng of genomic DNA diluted to 2.375 µl in dH_2O. Primer sequences were as follows: Forward 5′-GTATCTATCTCTCTACTGGATATTAATTTGTTATATTTTCTCATTAG-3′; Reverse 5′-TTGCCAGCACATGGTTTAGGA-3′; VIC (Reporter 1 Sequence) TTCAGTTTTCCATCTGAGTAA; and FAM (Reporter 2 Sequence) TCAGTTTTCCATGTGAGTAA. Positive control genomic DNA samples containing heterozygous *MLH1 D132H* alleles were used in every assay. PCR was performed at denaturing step of 92°C for 15 seconds and annealing step of 60°C for 60 seconds, for 40 cycles, after an initial hold of 95°C for 10 minutes.

For the endometrial cancer cohort, *MLH1 D132H* analyses were optimized and performed in the laboratory of Dr. Paul Goodfellow, using the Pyrosequencing technique. Briefly, 20-µl PCR reactions were performed in 96-well plates, each well containing 11 µl of AmpliTaq Gold® PCR Master Mix (Applied Biosystems), 0.5-µl each of 4 µM forward and reverse primers, and 20 ng of genomic DNA. Primer sequences were as follows: PCR Forward 5′-TCTCTTTTCCCCTTGGGATT-3′; PCR Reverse 5′-TCCC-

ATGTACCATTCTTACCG-3′; Pyrosequencing primer 5′-GGGCTTTCAGTTTTCC-3′. Positive control genomic DNA (*MLH1 D132H* heterozygote) was run to ensure quality control for each 96-well plate reaction run. PCR was performed with a denaturing step of 95°C for 30 seconds, an annealing step of 60°C for 30 seconds, and an extension step of 72°C for 30 seconds for 49 cycles after an initial hot start.

RESULTS

Clinical characteristics of the CRC cohort from Southern California and the endometrial cancer (EC) patients from Missouri are described in Tables 1 and 2. For CRC patients, the mean age of diagnosis was 59 years. The vast majority (87.6 percent) of this American CRC subject cohort consisted of non-Hispanic whites. Slightly < three percent (2.7 percent) were of Jewish ethnicity.[5,6] Approximately one-third (30.7 percent) of cohort has a positive family history of colon or rectal cancer. Almost one-half (48.1 percent) of them were found to have early localized state of disease (Stage 0–2) without involvement of adjacent organs or lymph nodes, but only 7.1 percent of patients had Stage IV disease. Close to two-thirds (65 percent) of the tumors were located in the sigmoid colon or rectum. Complete typing of MSI status for each individual in the cohort is not available at present. From the total of 629 DNA samples analyzed, alleles were determined for 621 patients (98.7 percent). Positive control DNA samples in duplicate from *MLH1 D132H* heterozygotes were used in each TaqMan assay that was run. In the 621 CRC cases from the Southern California CRC cohort who were assayable, 0 of 621 *MLH1* D132H carriers (0 percent) were detected.

For the endometrial cancer patients, the mean age of diagnosis was 64.7 years (Table 2). The vast majority of this cohort were non-Hispanic whites and only ~1.8 percent of the endometrial cancer patient population was of Ashkenazi Jewish ethnicity. Approximately 15 percent of endometrial cancer probands have a first-degree or second-degree relative with colorectal or endometrial cancer. Approximately 80 percent of patients ascertained had endometrioid cancer. Twenty-nine and one-half percent of EC patients have tumors that are MSI-H, and 3 percent MSI-L, similar to our previously described clinic-based population.[3] Of the endometrial tumors that were MSI-H, 71.7 percent lacked *MLH1* expression because of epigenetic silencing of *MLH1*, the most common cause of MSI-H status in this cancer.[3,7] From the total of 527 DNA samples analyzed, alleles were determined for 515 patients (97.7 percent). Positive control DNA samples in duplicate from *MLH1* D132H heterozygotes were used in each pyrosequencing assay that

Table 1.
Clinical Characteristics of the Colorectal Cancer Patient Cohort

Southern California Colorectal Cancer Cohort	
Age at diagnosis (yr)	
<45	64 (10.3)
45–49	66 (10.6)
50–54	91 (14.6)
55–59	121 (19.4)
60–64	175 (28)
65+	107 (17.1)
Race/ethnicity	
Non-Hispanic whites	543 (87.6)
African American	9 (1.4)
Hispanic	28 (4.5)
Asian/Pacific islander	39 (6.3)
Other	1 (0.2)
Family history of colon cancer	
Yes	191 (30.7)
No	432 (69.3)
SEER summary stage	
In situ	43 (6.9)
Localized	257 (41.2)
Regional, direct extension only	112 (17.9)
Regional, lymph nodes only	62 (9.9)
Regional, direct extension and lymph nodes	98 (15.7)
Distant metastases	44 (7.1)
Unstageable	8 (1.3)
Grade of differentiation	
Well differentiated	87 (13.9)
Moderately differentiated	379 (60.8)
Poorly differentiated	67 (10.7)
Unknown	91 (14.6)
Size of tumor (mm)	
<28	105 (16.8)
28–39	90 (14.4)
40–60	142 (22.8)
60+	120 (19.2)
Unknown	167 (26.8)
Site of tumor	
Cecum	70 (11.2)
Appendix	6 (1)
Ascending	47 (7.5)
Hepatic flexure	21 (3.4)
Transverse	31 (5)
Splenic flexure	17 (2.7)
Descending	32 (5.1)
Pelvic/sigmoid/sigmoid flexure	176 (28.2)
Overlapping lesions of colon	5 (0.8)
Not otherwise specified	6 (1)
Rectosigmoid/rectum	80 (12.8)
Rectum, not otherwise specified	133 (21.3)

Data are numbers with percentages in parentheses.

Table 2.
Clinical Characteristics of the Endometrial Cancer Patient Cohort

Cases analyzed for the *MLH1 D132H* variant	515
Mean age (range)	64.7 (26–99)
Age at diagnosis (yr)	
<45	23 (4.5)
45–49	35 (6.8)
50–54	46 (8.9)
55–59	73 (14.2)
60–64	80 (15.5)
65+	258 (50.1)
Histology	
Endometrioid	407 (79)
Others	108 (21)
Tumor differentiation/grade	
Well differentiated	229 (44.5)
Moderately differentiated	148 (28.7)
Poorly differentiated	64 (12.4)
Not applicable	74 (14.4)
Stage	
IA	93 (18)
IB	166 (32.2)
IC	65 (12.6)
IIA	18 (3.5)
IIB	17 (3.3)
IIIA	21 (4.1)
IIIB	3 (0.6)
IIIC	57 (11)
IVA	3 (0.6)
IVB	19 (3.7)
Unknown	53 (10.4)
Race	
White	424 (82.3)
African American	82 (16)
Other	4 (0.7)
Unknown	5 (1)
MSI	
MSI-H	152 (29.5)
MSI-L	15 (3)
MSS	348 (67.5)
MLH1 Methylation in MSI-H	
Yes	109 (71.7)
No	41 (27)
Unknown	2 (1.3)

MSI = microsatellite instability.

Data are numbers with percentages in parentheses unless otherwise indicated.

was run. In the 515 EC cases from the Missouri EC cohort who were assayable, 0 of 515 *MLH1* D132H carriers (0 percent) were detected.

DISCUSSION

We analyzed the allele frequencies of *MLH1 D132H* in two cohorts of American cancer patients with HNPCC-related cancers (colorectal and endometrial cancers). Together, these cohorts comprise more than 1,100 patients, most of who were white or mixed European ancestry. In a previous study of population-based Israeli CRC patients, we detected *MLH1 D132H* among Ashkenazi Jewish, non-Ashkenazi Jewish, Muslim and Christian Arab, and Druze Christian patients with an estimated allele frequency in CRC patients approximating 1.3 percent.[2] If the allele frequency in the American population were similar, we would expect to find approximately 8 carriers in this 621 American CRC patients, and 7 carriers in the 515 endometrial cancer patients, respectively. However, the *MLH1 D132H* variant allele was not detected in either of these cohorts. The reasons for this difference in allele frequency could be attributable to differences in either the ethnic makeup, or to a lesser degree, to differences in the clinical characteristics of these two cohorts.

The most striking difference between these cohorts is the low frequency of Jewish patients in the Southern California CRC Cohort analyzed (2.7 percent)[5,6] and the Missouri Endometrial Cancer cohort (1.8 percent) compared with the previous Molecular Epidemiology of Colorectal Cancer (MECC) study (>80 percent) of population-based Israeli CRC.[2] In the MECC population, Jewish patients comprised 85 percent (Ashkenazi 71 percent, non-Ashkenazi 29 percent).[7,8] Additionally, distinct clinical characteristics of the two cohorts may account for this allele frequency differences. Approximately 80 percent of the Southern California CRC cohort was diagnosed at aged 50 years or older compared with 94 percent of Israeli population from the MECC Study, and >90 percent of the general population of the United States.[6] Because the average age at which *MLH1* D132H carriers developed CRC was 69 compared with 59 in this cohort, the overall younger average age of the American cohort compared with the MECC study population also may contribute to this difference. Only 7.1 percent of this cohort has Stage IV disease compared with 21.9 percent in the general United States population. A considerably higher proportion of familial cases in this Southern California sample (30.7 percent) was noted, because patients with familial risk of CRC makeup approximately 20 percent of all CRC.[8]

Although these clinical factors are unlikely to account for the majority of the observed difference in allele frequencies, they perhaps indicate different mechanisms of carcinogenesis in this younger, earlier-staged CRC cohort. For the endometrial cancer patients, it may be notable that there were no endometrial cancers described in the probands or first-

degree and second-degree relatives of the 21 Israeli families carrying *MLH1 D132H*.[2] Indeed, at this time there is no direct evidence suggesting increased endometrial cancer susceptibility in *MLH1 D132H* carriers. We conclude that *MLH1 D132H* has a very low frequency in American sporadic CRC and endometrial cancer patients, mostly of mixed European ancestry. Future studies will be required to assess the frequencies of this allele in other ethnic groups.

Molecular diagnostic mutation analysis of *MLH1* and *MSH2* can cost as much as $2,500 per patient.[1] Because of this high cost, individual alleles can be analyzed in specific patient groups to minimize patient expense (such as the panel of Ashkenazi founder mutations for *BRCA1* and *BRCA2*, which reduces patient cost almost tenfold compared with sequencing of the coding region). Our work suggests that genetic testing exclusively for the *MLH1 D132H* allele is unlikely to be cost effective for genetic risk assessment in American population-based and clinic-based CRC and endometrial cancer patients.

CONCLUSIONS

The *MLH1* D132H risk variant has significantly lower allele frequency in American compared with Israeli cancer patients and is unlikely to explain significant amounts of American sporadic CRC and uterine cancer susceptibility. Targeted genetic testing for *MLH1 D132H* in isolation is unlikely to prove cost-effective.

REFERENCES

1. Lynch HT, Riley BD, Weissman SM, *et al*. Hereditary nonpolyposis colorectal carcinoma (HNPCC) and HNPCC-like families: problems in diagnosis, surveillance, and management. Cancer 2004;100:53–64.
2. Lipkin SM, Rozek LS, Rennert G, *et al*. The MLH1 D132H variant is associated with susceptibility to sporadic colorectal cancer. Nat Genet 2004;36:694–9.
3. Cunningham JM, Kim CY, Christensen ER, *et al*. The frequency of hereditary defective mismatch repair in a prospective series of unselected colorectal carcinomas. Am J Hum Genet 2001;69:780–90.
4. Goodfellow PJ, Buttin BM, Herzog TJ, *et al*. Prevalence of defective DNA mismatch repair and MSH6 mutation in an unselected series of endometrial cancers. Proc Natl Acad Sci USA 2003;100:5908–13.
5. Peel DJ, Ziogas A, Fox EA, *et al*. Characterization of hereditary nonpolyposis colorectal cancer families from a population-based series of cases. J Natl Cancer Inst 2000;92:1517–22.
6. Kolodner RD, Tytell JD, Schmeits JL, *et al*. Germ-line MSH6 mutations in colorectal cancer families. Cancer Res 1999;59:5068–74.
7. Niell BL, Long JC, Rennert G, Gruber SB. Genetic anthropology of the colorectal cancer-susceptibility allele APC I1307K: evidence of genetic drift within the Ashkenazim. Am J Hum Genet 2003;73:1250–60.
8. Niell BL, Rennert G, Bonner JD, Almog R, Tomsho LP, Gruber SB. BRCA1 and BRCA2 founder mutations and the risk of colorectal cancer. J Natl Cancer Inst 2004;96:15–21.