HHS Public Access

Author manuscript

Clin Genet. Author manuscript; available in PMC 2015 August 19.

Published in final edited form as:

Clin Genet. 2011 June ; 79(6): 512–522. doi:10.1111/j.1399-0004.2010.01594.x.

Characterization of two Ashkenazi Jewish founder mutations in *MSH6* gene causing Lynch syndrome

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Abstract

Founder mutations are an important cause of Lynch syndrome and facilitate genetic testing in specific ethnic populations. Two putative founder mutations in *MSH6* were analyzed in 2685 colorectal cancer (CRC) cases, 337 endometrial cancer (EnCa) cases and 3310 healthy controls of Ashkenazi Jewish (AJ) descent from population-based and hospital-based case-control studies in Israel, Canada and the USA. The carriers were haplotyped and the age of the mutations was estimated. *MSH6**c.3984_3987dupGTCA was found in 8/2685 CRC cases, 2/337 EnCa cases, and 1/3310 controls, consistent with a high risk of CRC (odds ratio (OR) = 9.9, 95% confidence interval (CI) = 1.2–78.9, p=0.0079) and a very high risk of EnCa (OR = 19.6, 95%CI = 1.8–217.2, p = 0.0006). *MSH6**c.3959_3962delCAAG was identified in 3/2685 CRC cases, 2/337 EnCa cases and no controls. Each mutation was associated with separate conserved haplotypes.

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Disclosure/Conflict of interest: None

*MSH6**c.3984_3987dupGTCA and *MSH6**c.3959_3962delCAAG likely arose around 585 CE and 685 CE respectively. No carriers were identified in Sephardi Jews (450 cases and 490 controls). Truncating mutations *MSH6**c.3984_3987dupGTCA and *MSH6**c.3959_3962delCAAG cause Lynch syndrome and are founder mutations in Ashkenazi Jews. Together with other AJ founder mutations, they contribute substantially to the incidence of CRC and EnCa and are important tools for the early diagnosis and appropriate management of AJ Lynch syndrome patients.

Keywords

MSH6; founder mutation; Lynch syndrome; Ashkenazi Jews

INTRODUCTION

Lynch syndrome is the most common autosomal dominant condition predisposing to colorectal cancer. It is characterized by early onset cancers of the colorectum, endometrium, small bowel, ureter and renal pelvis [1], as well as other malignancies [2]. Lynch syndrome is caused by germline mutations in mismatch-repair (MMR) genes, the common of which occur in *MLH1* [3] and *MSH2* [4], and are less frequently seen in *MSH6* [5, 6] and *PMS2* [7].

The *MSH6* gene is localized at chromosome 2p15–16 and consists of 10 exons encoding a 1360 amino acid protein. The MSH6 protein interacts with the MSH2 protein to form the MutSα heterodimer [5]. The primary function of MutSα is to initiate the repair process by binding to DNA mismatches detected by MSH6. The characteristic role of this heterodimer is to correct single mispaired bases or small insertion or deletion loops [8]. Most reports indicate an attenuated phenotype of *MSH6*-related Lynch syndrome in comparison with mutations of *MLH1* or *MSH2* genes. Although fewer families with *MSH6* mutations fulfill the Amsterdam criteria than families with either *MLH1* or *MSH2* mutations, the characteristic features of *MSH6* mutation families comprise a higher risk for CRC, endometrial, ovarian, upper urinary tract, and stomach cancers [9]. Moreover, families with *MSH6* mutations have a higher incidence of extracolonic cancers in comparison with other Lynch families [10].

Several studies have investigated the frequency of *MSH6* mutations in CRC and endometrial cancers. About 10% of Lynch syndrome colorectal cancers and 0.3% of all colorectal cancers are explained by the mutations in *MSH6* [11, 12]. The prevalence of *MSH6* mutations in endometrial cancer patients who were not selected for family history is about 1.1% [13]. A recent study of 113 *MSH6* families estimated cumulative risks to age 80 years for colorectal cancer to be 44% for men and 20% for women; for endometrial cancer, the risk was 44%. *MSH6* mutation carriers had an eightfold increased incidence of colorectal cancer in comparison with incidence in general population and women with *MSH6* mutations had a 26-fold increased incidence of endometrial cancer [14]. In families with *MSH6* mutations, the onset of CRC and endometrial cancer is significantly delayed

compared to families with *MLH1* or *MSH2* mutations (*MSH6* vs. *MSH2/MLH1* = 55 years vs. 44/41 years for CRC and 55 years vs. 49/48 years for endometrial cancer) [15].

Over 500 unique variants have been identified in *MSH6* gene [16]. Two recently described mutations in *MSH6* exon 9 are truncating mutations identified in several families around the world: the mutation c.3959_3962delCAAG (rs63751151) (p.Ala1320GlufsX6) was first reported in an endometrial cancer patient of unknown ethnicity from the United States, where her MSI-positive tumor with an unmethylated *MLH1* promoter was diagnosed at the age 71 [17] and the mutation c.3984_3987dupGTCA (p.Leu1330ValfsX12), was first reported to occur in an Ashkenazi Jewish (AJ) family with a history of CRC and adenomatous polyps [18] and using slightly different nomenclature was later reported in two families; one from the Netherlands [19] and another family from the United States [20] with recently published Israeli study finding c.3984_3987dupGTCA in 19 members from four AJ families [21].

The aim of the present study was to characterize the *MSH6* mutations c.3984_3987dupGTCA (referred below as *MSH6**dup) and c.3959_3962delCAAG (referred below as *MSH6**del) and estimate the magnitude of the contribution of these mutations to the Lynch syndrome in the AJ population. The frequency of these mutations was evaluated in a large, population-based case-control study in northern Israel and in series of AJ individuals with colorectal, endometrial, or ovarian cancer, ascertained in North America. Furthermore, we sought to establish whether these two mutations are founder mutations and to calculate the age of these mutations in the AJ population.

MATERIALS AND METHODS

Study samples

The present study included individuals of AJ descent from centers in Israel, Canada, and the United States. We studied the frequency of these two mutations in several different types of datasets - a) population-based series of cases and controls (Israel); b) individuals with a personal and/or family history of colorectal cancer and other Lynch syndrome-associated tumors referred to high-risk clinics (Montreal); c) familial gastrointestinal cancer registry-based cases (Toronto); d) hospital-based series of cases and controls (New York); e) unaffected control individuals (Montreal and Toronto) and f) carriers identified through a CLIA-certified commercial laboratory (Mayo clinic). These datasets are described briefly below –

a) Population-based – the MECC case-control study from Israel—DNA samples from 2685 cases and 1591 healthy controls from the large Israeli population-based study of colorectal cancer were genotyped in the University of Michigan. The Molecular Epidemiology of Colorectal Cancer (MECC) study is a population-based case-control study of colorectal cancer in northern Israel. The cases include histologically confirmed incident colorectal cancer patients between 1998 and 2009. Population-based controls were enrolled from the Clalit Health Services database (the largest Israeli health care provider) and matched by year of birth, gender, clinic, and Jewish/Non-Jewish ethnicity.

b) High-risk clinics—Montreal: Individual J2205-2 (Table 1) was found to have the *MSH6**dup mutation after full sequencing of *MSH6*. She was diagnosed with colon and endometrial cancer. The family history met Bethesda guidelines, but did not fulfill Amsterdam criteria. Family members of the index case were analyzed for the mutation and haplotyped at the Jewish General Hospital (JGH). Additionally, 22 CRC cases and 22 unaffected individuals from other high-risk colorectal cancer families referred to the cancer genetics clinic at the JGH were analyzed for the two *MSH6* mutations. All cases were negative for other known AJ founder mutations in CRC genes (*MLH1**c. 394C>G (common name D132H), *MSH2**c.1906G>C (common name A636P), *APC**c.3920T>A (common name I1307K), *BLM**c.2207_2212delATCTGAinsTAGATTC (common name BLM^{Ash}).

c) Familial Gastrointestinal Cancer Registry—Total of 490 AJ were collected through the Familial Gastro-Intestinal Cancer Registry (FGICR) in Toronto, Ontario, Canada and their DNA extracted from blood was tested for *MSH6* exon 9 mutations. This registry constitutes a provincial resource that registers individuals with suspected or verified hereditary cancers and, for the Jewish population, also those who underwent GI cancer screening irrespective of their family history of cancer. Family histories are obtained through a standardized questionnaire.

d) Hospital series—A total of 337 hospital-based AJ women with endometrial cancer and 285 AJ female controls with a mean age of 58 years from Memorial Sloan-Kettering Cancer Center (MSKCC) were tested for both *MSH6* mutations.

e) Volunteering controls—Women's College Hospital, Toronto: 1011 AJ female controls were genotyped for both *MSH6* exon 9 mutations. The mean age of the controls was 48.5 years, ranging from 25 years to 78 years.

Jewish General Hospital, Montreal: 423 unaffected AJ controls (215 females, 201 males, 1 unknown) were tested for both *MSH6* mutations. The mean age of 402 controls was 54.3 years with a range from 18 years to 93.9 years. The age was unknown for 21 controls.

f) Clinical Laboratory series—Two carriers of *MSH6**dup mutation were identified in the Mayo Medical Laboratory mutation database. These carriers were invited to participate in this research through the University of Michigan Cancer Genetics Registry. The patients signed written, informed consent and donated additional blood samples for haplotype analysis.

Genotyping of MSH6 exon 9 and haplotype analysis

Methods for screening of *MSH6* exon 9 for deletions and duplications varied between different centers, but all carriers were re-analyzed and haplotyped at the University of Michigan. DNA fragments containing exon 9 and microsatellite markers were amplified using PCR. PCR product size was determined by fragment length analysis using ABI3730 instrument (Applied Biosystems, Foster City, CA) and compared to the positive control carrying *MSH6**dup and negative control (tested by sequencing) using GeneMarker (State College, PA) software.

Samples with the fragment size different from expected 164 base pairs (bp) were sequenced using the same set of primers from both sides. Single nucleotide polymorphisms (SNPs) were analyzed by direct sequencing. Primers and reaction conditions are described in Supplementary Table 1.

Microsatellite Instability analysis and Immunohistochemistry

Microsatellite instability assays were performed as described elsewhere [22] using microdissected DNA from paraffin-embedded tissue blocks. Five markers (BAT25, BAT26, D2S123, D5S346, and D17S250, often called the Bethesda panel) were PCR amplified using radioactively labeled primers. The patterns of the microsatellite markers in normal tissue and tumor were compared to find changes in marker length. Samples were categorized according to established criteria [7]: MSI-High (two or more markers are unstable), MSI-Low (one marker is unstable), microsatellite stable (MSS) (no markers being unstable).

Tumor samples from *MSH6* exon 9 mutation carriers were retrieved and tested for the expression of MLH1, MSH2, MSH6 and PMS2 proteins. Immunohistochemistry (IHC) was performed on 4-μm sections of formalin-fixed and paraffin-embedded tissues. Sections were dried, de-paraffinized and re-hydrated. Slides were stained with monoclonal antibodies. The protein expression in normal lymphocytes and colonocytes adjacent to the tumor served as an internal positive control.

Statistical Analysis

Association between *MSH6* mutations and risk of colorectal and endometrial cancers was estimated using Fisher exact test at SAS 9.1 (The SAS Institute, Cary, NC). Haplotype reconstruction was performed using PHASE v.2.1.1 [23, 24]. Uncertain genotype phases in haplotype estimates were excluded from the analysis. The age of the mutation was estimated using a Bayesian MCMC linkage disequilibrium mapping approach (DMLE+ software) [25]. Genetic distance was estimated at 1 Mb corresponding to 1 cM. We assumed population growth rate 1.125-fold per generation, considering the population of Jewish people in a north-eastern European region of 11,000 in the year 600 and 5 million in the year 1900. Considering a lifetime risk of colorectal cancer of 6.85%, a worldwide population of Ashkenazi Jews of 13 million [26], and the mutation prevalence among CRC cases of 0.3% (duplication) and 0.1% (deletion) estimated from the MECC study, we calculated the number of disease chromosomes in AJ population: 2,672 for duplication and 891 for deletion. Accordingly, the proportion of mutation carrying chromosomes sampled was 0.00299 (8/2672) and 0.00337 (3/891) for duplication and deletion respectively.

RESULTS

Identification and screening for MSH6 exon 9 indel mutations

An AJ family found to carry *MSH6**dup was evaluated at McGill University for Lynch Syndrome. Based on this case and prior reports of this mutation in the literature, we hypothesized that this variant might represent a founder mutation identifiable among Ashkenazi Jews. This led to a comprehensive search among investigators with large series of AJ colorectal cancer cases, endometrial cancer cases, and controls. One further family

from Montreal, one family from Toronto, eight families from northern Israel, and two families from MSKCC were found to carry the same mutation. One family ascertained through Mayo Medical Labs with *MSH6**dup mutation was also ascertained. Since the mutation screening relied on fragment size analysis of PCR products, a second mutation within the same region was also recognized corresponding to a truncating mutation, *MSH6**del in three families from northern Israel, three families from Toronto, and two families from MSKCC (Table 1).

Frequency of MSH6 exon 9 dup-del mutations in Ashkenazi Jews and risk of colorectal cancer

We evaluated the frequency of *MSH6**dup and *MSH6**del mutations in the MECC study with the addition of control AJ individuals from multiple centers in Israel, Canada, and the United States. *MSH6**dup was found in 8 of 2685 (0.3%) cases and 1 of 3310 (0.03%) controls. The only control carrying *MSH6**dup had an endometrial cancer at age 61 and developed cecal cancer at age 76, several years after being ascertained as a control without CRC. Considering this fact and epidemiologic principles [27], we included this carrier to both case and control groups to calculate the odds ratio (OR). To evaluate the risk of colorectal cancer associated with these two *MSH6* mutations, we used MECC cases (n = 2685) and controls (n = 1591) as well as large series of healthy Ashkenazi Jews from a study carried out at Women's College Research Institute, Toronto (n =1011), Memorial Sloan-Kettering Cancer Center, New York (n = 285) and Jewish General Hospital, Montreal (n = 423). The carriers of *MSH6* had a very high risk of CRC (OR = 9.9, 95% confidence interval (CI) = 1.2–78.6, p = 0.0079) (Table 2). The *MSH6**del was less common, identified in 3 of 2685 (0.11%) cases and no controls. No carriers of either mutation were identified in 450 Sephardi Jewish cases or 490 Sephardi Jewish controls from Israel.

MSH6*dup and MSH6*del mutations in patients and controls from hospital-based series—At the Jewish General Hospital, Montreal, one of the 22 (4.5%) CRC affected probands from high-risk colorectal cancer families carried *MSH6**dup, and there were no *MSH6**del mutations found.

One carrier of *MSH6**dup and three carriers of *MSH6**del were identified in 490 AJ patients from Toronto registry. After testing family members of the identified carriers, two carriers of *MSH6**dup (family 11) and four *MSH6**del carriers (families 16 and 17) were detected (Figs. 1 and 2). In 337 hospital-based cases of AJ patients with endometrial cancer from MSKCC, two carriers (0.6%) of *MSH6**dup and two carriers (0.6%) of *MSH6**del mutations were identified. Roughly estimated risk of endometrial cancer in AJ *MSH6**dup mutation carriers is high (OR = 19.6, 95%CI = 1.8–217.2, p = 0.0006) (Table 2).

Both affected twins from Mayo clinic were diagnosed with sebaceous adenoma, squamous cell carcinoma, and basal cell carcinoma, but did not have any Lynch syndrome associated cancers. In one of the brothers, IHC analysis of sebaceous adenoma showed impaired expression of MSH6 protein. Testing for mutations in exon 9 in *MSH6* showed *MSH6**dup in both brothers.

Clinicopathological features of MSH6 founder mutation carriers

Among all carriers the mean age at diagnosis of colorectal cancer was 66 years (median 67 years), and the mean age at diagnosis of endometrial cancer was 54 years (median 52 years). Microsatellite instability and immunohistochemistry staining against mismatch repair proteins were performed in carriers with available tumor tissue (Table 1). MSI status of the tumors was determined for 37% of carriers (10/27) and IHC was available for three colorectal cancers, three endometrial tumors, and 1 sebaceous adenoma. In our data, 33% (3/10) of carriers had MSS tumors and 67% (7/10) had MSI-High tumors. It is noteworthy that 3 out of 7 MSI-High tumors were unstable in mononucleotide markers only. In six out of seven tumors with IHC, the MSH6 protein was not expressed, while other mismatch repair proteins were intact (MSH2, MLH1, PMS2).

Characteristics of MSH6 mutation-positive families

A total of 19 *MSH6**dup carriers and 12 *MSH6**del carriers from 22 unrelated families were identified (Table 3, Figs. 1 and 2). Among all carriers, 18 *MSH6**dup carriers and 8 *MSH6**del carriers were affected with colorectal or endometrial cancers (Table 1). None of the families met Amsterdam criteria I and only two families met Amsterdam criteria II (families 7 and 18). Although families did not fulfill Amsterdam criteria, the family history of cancer is suggestive of mutations in the mismatch-repair genes. In family 11, both mother (I.2) and daughter (II.4) had colorectal cancer, while the daughter was diagnosed at the age of 50 followed by the diagnosis of endometrial cancer in a year. In families 16 and 17, the patients (I.1 and I.1) had two synchronous colorectal tumors; the proband in the family 7 and the patient I.2 from family 2 were diagnosed with metachronous colorectal cancer. It is noteworthy that three MSH6*dup carriers had squamous cell carcinoma (SCC) and basal cell carcinoma (BCC).

Haplotype analysis

To characterize the haplotypes associated with *MSH6* exon 9 deletion and duplication we analyzed four microsatellite markers (AtnACAGn, CAn, D2S1352, D2S123) and four SNPs (rs1800932, rs1800935, c.1146+14A>T, rs3136367) in all identified carriers (Table 3). The markers cover about 3.4 Mb including *MSH6* sequence. The marker AtnACAGn is approximately 144 kb upstream of *MSH6*, followed by SNPs in the coding region of *MSH6*, two polymorphic microsatellite loci (CAn, D2S1352), with the last marker D2S123 located 32 Mb away from the 3'-UTR of the *MSH6*. The physical order of the markers is tel - AtnACAGn - rs1800932 - rs1800935 - c.1146+14A>T - rs3136367 - CAn - D2S1352 - D2S123 – cen. Haplotype analysis demonstrated separate conserved haplotypes for each mutation. The haplotype tel-163-G-C-T-G-*MSH6**dup-179-113-225-cen was observed with *MSH6* exon 9 dup in 16/19 carriers. Three *MSH6**dup carriers had recombination events involving the markers at the ends of the haplotype (AtnACAGn, D2S1352, D2S123). The *MSH6**del was associated with the haplotype tel-151-A-T-T-G-*MSH6**del-175-110-210-cen found in 8/12 carriers. Four *MSH6**del carriers had recombination events involving the marker D2S1352.

Age of MSH6 exon 9 indel mutations

We estimated the age of *MSH6**dup and *MSH6**del using an established method of combined markers that utilizes rate of recombination and population frequency of flanking marker alleles [25]. Using the growth rate of Ashkenazi population of 0.125 per generation, the estimated age of *MSH6**dup is about 57 (45–83) generations and the age of *MSH6**del is about 53 (40–76) generations. Considering that one generation approximately equals to 25 years, the age of the mutations are 1425 (1125–2075) and 1325 (1000–1900) years for *MSH6**dup and *MSH6**del respectively. In other words, the duplication mutation likely arose around 585 CE and the deletion mutation probably originated in 685 CE.

DISCUSSION

In the present study we characterized two *MSH6* truncating founder mutations c.3984_3987dupGTCA and c.3959_3962delCAAG (rs63751151) that cause Lynch syndrome in Ashkenazi Jews. Both *MSH6**dup and *MSH6**del create premature stop codons and likely result in nonfunctional proteins. We found 19 *MSH6**dup carriers from 14 unrelated AJ families who all share the same haplotype associated with c.3984_3987dupGTCA, while all 12 *MSH6**del carriers from 8 unrelated families share a different haplotype associated with c.3959_3962delCAAG. These results confirm that both *MSH6**dup and *MSH6**del are founder mutations in the AJ population.

The case-control settings of the MECC study allowed us to estimate frequency and CRC related risk of *MSH6**dup and *MSH6**del in Ashkenazi and Sephardi Jews. *MSH6**dup was found in 0.3% and *MSH6**del in 0.1% of Ashkenazi Jews with CRC, and was not observed in 940 Sephardi Jewish cases and controls. We identified one *MSH6**dup carrier among the MECC controls, who developed colorectal cancer after being ascertained as a control. This carrier was included in the analysis both as a control and a case. Our data indicate that carriers of *MSH6**dup have an almost 10 times higher risk to develop colorectal cancer than do non-carriers (OR = 9.9, 95% CI = 1.2–78.9, p = 0.0079). The frequency of Lynch syndrome among unselected CRC cases is ~ 3% – 5%, which corresponds to 81 to 134 cases of Lynch syndrome in the 2685 MECC CRC cases. Therefore, although the combined frequency of *MSH6**dup and *MSH6**del mutations is only 0.4% in CRC patients of AJ origin, the frequency of these mutations in AJ patients with Lynch syndrome is likely to be 8–14% (11 of 134 to 81 cases). Endometrial cancer risk, roughly estimated from the hospital-based series, was about 20 times higher in the carriers of *MSH6**dup than in non-carriers (OR = 19.6, 95% CI = 1.8–217.2, p = 0.0006). We used the only identified AJ control carrying *MSH6**dup from the MECC study for approximate calculation of the risk of endometrial cancer, although this patent had an endometrial cancer before being ascertained as a control. Despite the wide confidence intervals, the point estimates of the risk of colorectal and endometrial cancer in our study are, however, very comparable to the risk demonstrated in the recent analysis of 113 families with *MSH6* mutations [14]. This study found an eight-fold increase in risk of colorectal cancer and 26-fold increase in risk of endometrial cancer. In addition, the fact that we only observed one carrier in 3310 controls, and this person later developed a Lynch-related cancer is supportive of our conjecture that these are highly penetrant alleles.

In our data the mean age at diagnosis of colorectal cancer among carriers of *MSH6* exon 9 mutations was 66 years, 20 years older than in carriers of *MSH2* or *MLH1* mutations (44 years) [28]. The mean age at diagnosis of endometrial cancer was 54 years, comparable with previous studies [29]. This later age of onset may explain the fact that only two families meet Amsterdam criteria in our data.

About half of the MSI-High tumors (3/7) were instable in mononucleotide markers only (BAT25, BAT26, beta-catenin) in line with previous observations that mononucleotide microsatellite markers are more frequently affected in tumors harboring *MSH6* germline mutations [30].

Recombination events allowed estimation of the age of the mutations. *MSH6**dup arose ~1425 years ago in the 6th century CE, and *MSH6**del arose ~1325 years ago in the 7th century CE, both at the time of formation of the AJ ethnicity in Western Europe. These mutations are much older than other previously described Ashkenazi founder mutations in MMR genes, such as *MSH2* c.1906G>C, that probably arose in 15th–18th century CE, but more recent than *BRCA2* c.6174delT (3rd century BCE) and *APC* p.I1307K (7th century BCE) [31]. The estimated age of the founder mutations can in part explain their frequency in Ashkenazi Jews, more recent mutations (*MSH6**dup, *MSH6**del) have lower than 0.03% frequency in general population, while the older mutations (*BRCA2* c.6174delT and *APC* p.I1307K) have much higher frequency of 1.5% and 6% respectively [32, 33].

Only a few AJ founder mutations leading to colorectal and endometrial cancers have been described until now. In addition to the *MSH6* exon 9 deletion and duplication characterized here, previous studies report another mismatch-repair gene mutation, *MSH2**c.1906G>C (common name A636P), associated with increased risk of both colorectal [34] and endometrial cancers [34, 35], as well as mutations in *APC**c.3920T>A (common name I1307K) [33] and *BLM**c.2207_2212delATCTGAinsTAGATTC (common name *BLM^{Ash}*) [36] associated with increased risk of colorectal cancer only. The reported frequency of *APC**I1307K, *BLM*^{Ash} and *MSH2**A636P among AJ colorectal cancer patients is 12% [37], 1%–3% [36], and 1% [34] respectively. The frequency of *MSH2**A636P in AJ endometrial cancer patients is about 1% [34]. Altogether AJ founder mutations (*APC**I1307K, *BLM*^{Ash}, *MSH2**A636P, *MSH6**dup, and *MSH6**del) can be involved in approximately 14%–16% of all colorectal cancer cases and 2.2% of all endometrial cancer cases in Ashkenazi Jews. The frequency of these mutations in AJ patients with family history of colorectal and/or endometrial cancers can be significantly higher. A panel designed to detect known AJ founder mutations consisted of *APC**I1307K, *BLM*^{Ash}, *MSH2**A636P, *MSH6**dup, and *MSH6**del could have value as a first-line screen in all AJ colorectal and/or endometrial cancer cases, irrespective of family history, IHC or MSI status.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank Philip H. Gordon for this helpful assistance with this study.

Grant support: This work was supported in part by the Jewish General Hospital Weekend to End Women's Cancers. WDF holds a Fonds de la Recherche en Santé du Québec (FRSQ) national scientist award and MT holds a FRSQ clinician-scientist award. SBG is supported by NCI 1R01CA81488 and University of Michigan Comprehensive Cancer Center Core Support grant (NIH 5P30CA46592).

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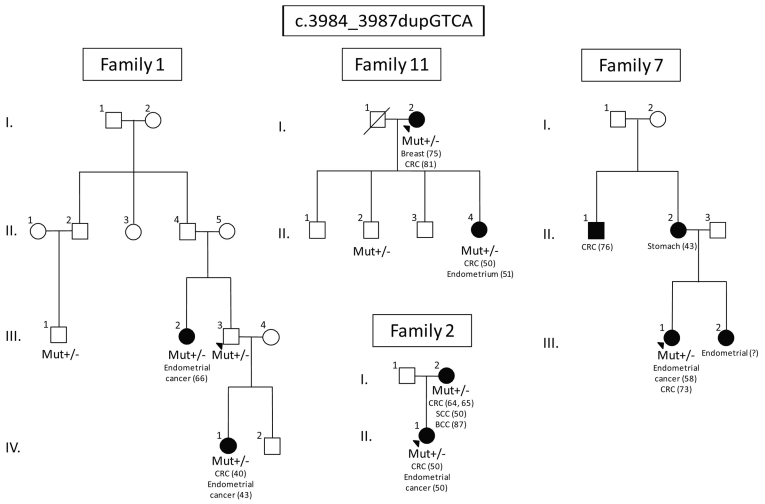

Figure 1.

Representative families carrying c.3984_3987dupGTCA. Mutation carriers are indicated by "+/−". None of the families meet Amsterdam criteria, but history of family cancers is suggestive of mismatch-repair mutations.

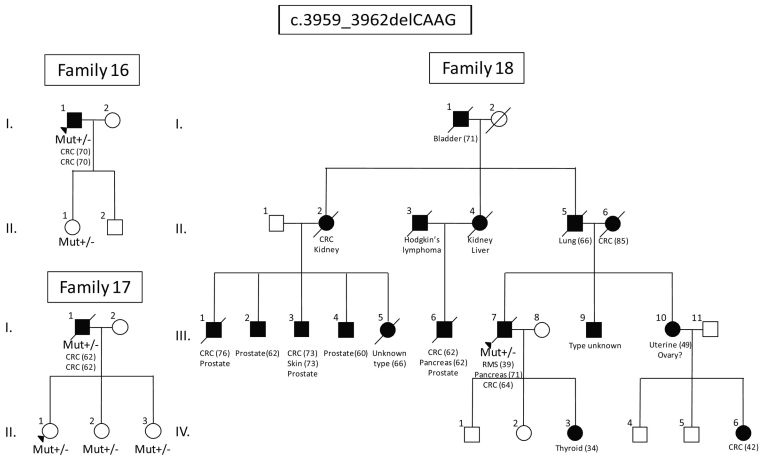

Figure 2.

Representative families carrying c.3959_3962delCAAG. RMS – Rhabdomyosarcoma. Mutation carriers are indicated by "+/−". None of the families meet Amsterdam criteria, but history of family cancers is suggestive of mismatch-repair mutations.

Table 1

Clinicopathological characteristics of affected Ashkenazi Jewish carriers of the MSH6* c.3984_3987dupGTCA and MSH6* c.3959_3962delCAAG mutations identified in the participating centers

Center/Study	Family	Patient	Sex	Age at ascertainment	MSH6 ex9 indel	Clinical Features	Amsterdam Criteria	MSI	IHC
McGill	1	1	female	68	c.3984_3987dupGTCA	Endometrial cancer (66)	No	N/A	MSH6(−)
McGill	1	2	female	44	c.3984_3987dupGTCA	Colorectal cancer (40), Endometrial cancer (43)	No	MSS	N/A
McGill	2	1	female	87	c.3984_3987dupGTCA	Metachronous Colorectal cancer (64, 65), SCC (86), BCC (87)	No	N/A	N/A
McGill	2	2	female	54	c.3984_3987dupGTCA	Colorectal cancer (50), Endometrial cancer (50)	No	N/A	MSH6(−)
MECC_case	3	1	male	70	c.3984_3987dupGTCA	Colorectal cancer (70)	No	MSS	N/A
MECC_case	4	1	female	86	c.3984_3987dupGTCA	Endometrial cancer (47), Ovarian cancer (66), Colorectal cancer (86)	No	MSI-High (mono)	N/A
MECC_case	5	1	female	79	c.3984_3987dupGTCA	Colorectal cancer (79)	No	MSI-High (mono)	N/A
MECC_case	6	1	male	55	c.3984_3987dupGTCA	Colorectal cancer (55)	No	MSI-High	N/A
MECC_case	7	1	female	73	c.3984_3987dupGTCA	Endometrial cancer (58), Cecal cancer (73)	Amsterdam II	N/A	MSH6(−)
MECC_case	8	1	female	52	c.3984_3987dupGTCA	Colorectal cancer (52)	N/A	N/A	N/A
MECC_case	9	1	male	56	c.3984_3987dupGTCA	Colorectal cancer (56)	No	MSI-High (mono)	N/A
MECC_control	10	1	female	71	c.3984_3987dupGTCA	Endometrial cancer (61), Cecal cancer (76)	No	N/A	N/A
Toronto	11	1	female	85	c.3984_3987dupGTCA	Breast cancer (75), Colorectal cancer (81)	No	N/A	N/A
Toronto	11	2	female	54	c.3984_3987dupGTCA	Colorectal cancer (50), Endometrial cancer (51)	No	N/A	N/A
Mayo	12	1	male	75	c.3984_3987dupGTCA	SA (71), SCC (71), BCC (71)	No	N/A	MSH6(−)
Mayo	12	2	male	75	c.3984_3987dupGTCA	SCC (71), SA (71), BCC (75), SA (75)	No	N/A	N/A
MSKCC	19	1	female	57	c.3984_3987dupGTCA	Endometrial cancer (57)	No	N/A	MSH6(+)
MSKCC	20	1	female	68	c.3984_3987dupGTCA	Endometrial cancer (68)	No	N/A	MSH6(−)
MECC_case	13	1	male	69	c.3959_3962delCAAG	Colorectal cancer (69)	No	MSS	N/A
MECC_case	14	1	male	82	c.3959_3962delCAAG	Colorectal cancer (60), Colorectal cancer (82)	No	N/A	N/A
MECC_case	15	1	female	84	c.3959_3962delCAAG	Colorectal cancer (84)	N/A	N/A	N/A
Toronto	16	1	male	73	c.3959_3962delCAAG	Colorectal cancer (70)	No	MSI-High	MSH6(−)

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Center/Study	Family	Patient	Sex	Age at ascertainment	MSH6 ex9 indel	Clinical Features	Amsterdam Criteria	MSI	IHC
Toronto	17	1	male	68	c.3959_3962delCAAG	Colorectal cancer (62)	No	MSI-High	N/A
Toronto	18	1	male	72	c.3959_3962delCAAG	Rhabdomyosarcoma (39), Colorectal cancer (64), Cancer of pancreas (71)	Amsterdam II	MSI-High	N/A
MSKCC	21	1	female	43	c.3959_3962delCAAG	Endometrial cancer (43)	No	N/A	N/A
MSKCC	22	1	female	49	c.3959_3962delCAAG	Endometrial cancer (49)	No	N/A	N/A

*CRC – colorectal cancer; SCC – squamous cell carcinoma; BCC – basal cell carcinoma; SA – sebaceous carcinoma; N/A- not available; Mono – MSI in mononucleotide microsatellite markers only.

Table 2

Risk of colorectal and endometrial cancers among MSH6 exon 9 indel mutation carriers in an Ashkenazi Jewish population

Mutation	Cases	Controls	OR	95%CI	p-value
Colorectal cancer					
c.3984_3987dupGTCA	8/2685 (0.3%)	1/3310 (0.03%)	9.9	1.2–78.9	0.0079
c.3962_3965delCAAG	3/2685 (0.1%)	0/3310	∞	∞	0.0546
MSH6 exon 9 dup or del	11/2685 (0.4%)	1/3310 (0.03%)	13.6	1.8–105.1	0.0011
Endometrial cancer[*]					
c.3984_3987dupGTCA	2/337 (0.6%)	1/3310 (0.03%)	19.6	1.8–217.2	0.0006
c.3962_3965delCAAG	2/337 (0.6%)	0/3310	∞	∞	<0.0001
MSH6 exon 9 dup or del	4/337 (1.2%)	1/3310 (0.03%)	39.3	4.4–352.5	<0.0001

[*] Endometrial cancer risk was estimated from MSKCC hospital-based series.

Table 3

Haplotype analysis of the carriers of MSH6* c.3984_3987dupGTCA and MSH6* c.3959_3962delCAAG mutations

Center/Study	Family	Patient	AtnACAGn 2:47866183-47866350*	ex2_rs1800932 2:48018081	ex3_rs1800935 2:48023115	IVS5+14A>T 2:48030838	int8_rs3136367 2:48033551	MSH6 ex9 indel 2:48033748-48033776**	CAn 2:48131630-48131817	D2S1352 2:50833714-50833825	D2S123 2:51288437-51288647
McGill	1	1	**163**/155	**G**/A	**C**/T	**T**/T	**G**/G	**c.3984_3987dupGTCA**	**179**/165	**113**/110	**225**/225
McGill	1	2	**163**/163	**G**/A	**C**/T	**T**/A	**G**/G	**c.3984_3987dupGTCA**	**179**/171	**113**/113	**225**/227
McGill	1	3	**163**/155	**G**/A	**C**/T	**T**/T	**G**/G	**c.3984_3987dupGTCA**	**179**/165	110/116	225/208
McGill	2	1	**163**/163	**G**/A	**C**/T	**T**/T	**G**/G	**c.3984_3987dupGTCA**	**179**/175	**113**/125	**225**/212
McGill	2	2	**163**/165	**G**/A	**C**/T	**T**/A	**G**/G	**c.3984_3987dupGTCA**	**179**/171	**113**/113	**225**/227
MECC_case	3	1	**163**/175	**G**/A	**C**/T	**T**/A	**G**/C	**c.3984_3987dupGTCA**	**179**/173	**113**/110	**225**/210
MECC_case	4	1	**163**/155	**G**/A	**C**/T	**T**/A	**G**/G	**c.3984_3987dupGTCA**	**179**/171	110/110	210/214
MECC_case	5	1	**163**/157	**G**/A	**C**/T	**T**/A	**G**/G	**c.3984_3987dupGTCA**	**179**/171	**113**/113	**225**/210
MECC_case	6	1	**163**/155	**G**/A	**C**/T	**T**/T	**G**/G	**c.3984_3987dupGTCA**	**179**/165	**113**/116	**225**/225
MECC_case	7	1	**163**/157	**G**/A	**C**/T	**T**/A	**G**/C	**c.3984_3987dupGTCA**	**179**/175	**113**/110	**225**/214
MECC_case	8	1	**163**/175	**G**/A	**C**/T	**T**/A	**G**/C	**c.3984_3987dupGTCA**	**179**/173	**113**/113	**225**/210
MECC_case	9	1	**163**/177	**G**/A	**C**/C	**T**/A	**G**/G	**c.3984_3987dupGTCA**	**179**/165	**113**/116	**225**/225
CAF_5544	9	2	**163**/177	**G**/A	**C**/C	**T**/A	**G**/G	**c.3984_3987dupGTCA**	**179**/165	**113**/116	**225**/225
MECC_control	10	1	**163**/163	**G**/G	**C**/C	**T**/T	**G**/G	**c.3984_3987dupGTCA**	**179**/171	**113**/116	**225**/210
Toronto	11	1	157/175	**G**/A	**C**/T	**T**/A	**G**/G	**c.3984_3987dupGTCA**	**179**/171	**113**/110	**225**/210
Mayo	12	1	**163**/161	**G**/A	**C**/T	**T**/T	**G**/G	**c.3984_3987dupGTCA**	**179**/165	**113**/113	**225**/210
Mayo	12	2	**163**/161	**G**/A	**C**/T	**T**/T	**G**/G	**c.3984_3987dupGTCA**	**179**/165	**113**/113	**225**/210
MSKCC	19	1	**163**/155	**G**/A	**C**/T	**T**/A	-	**c.3984_3987dupGTCA**	**179**/171	**113**/113	**225**/210
MSKCC	20	1	**163**/163	**G**/A	**C**/T	**T**/A	-	**c.3984_3987dupGTCA**	**179**/171	**113**/116	**225**/225
MECC_case	13	1	**151**/155	**A**/A	**T**/T	**T**/A	**G**/G	**c.3959_3962delCAAG**	**175**/171	**110**/116	**210**/225
MECC_case	14	1	**151**/161	**A**/A	**T**/T	**T**/A	**G**/C	**c.3959_3962delCAAG**	**175**/171	**110**/116	**210**/208
MECC_case	15	1	**151**/169	**A**/G	**T**/C	**T**/T	**G**/G	**c.3959_3962delCAAG**	**175**/181	**110**/116	**210**/210
Toronto	16	1	**151**/163	**A**/A	**T**/T	**T**/A	**G**/G	**c.3959_3962delCAAG**	**175**/171	**110**/113	**210**/214
Toronto	16	2	**151**/155	**A**/A	**T**/T	**T**/A	**G**/G	**c.3959_3962delCAAG**	**175**/171	**110**/113	**210**/214
Toronto	17	1	**151**/159	**A**/A	**T**/T	**T**/A	**G**/C	**c.3959_3962delCAAG**	**175**/171	**110**/122	**210**/210
Toronto	17	2	**151**/163	**A**/A	**T**/T	**T**/A	-	**c.3959_3962delCAAG**	**175**/171	113/122	210/226
Toronto	17	3	**151**/159	**A**/A	**T**/T	**T**/A	**G**/C	**c.3959_3962delCAAG**	**175**/171	**110**/122	**210**/210
Toronto	17	4	**151**/163	**A**/A	**T**/T	**T**/A	**G**/C	**c.3959_3962delCAAG**	**175**/177	116/122	210/212
Toronto	18	1	**151**/163	**A**/G	**T**/T	**T**/T	**G**/G	**c.3959_3962delCAAG**	**175**/185	113/122	210/210
MSKCC	21	1	**151**/151	**A**/A	**T**/T	**T**/T	-	**c.3959_3962delCAAG**	**175**/175	**110**/116	**210**/210
MSKCC	22	1	**151**/157	**A**/G	**T**/C	**T**/T	-	**c.3959_3962delCAAG**	**175**/179	116/116	210/225

* Chromosomal location is taken from ENST00000234420 Ensembl transcript of MSH6 (Genome assembly GRCh37). Both affected and unaffected carriers were used for haplotype analysis. The common haplotypes associated with *MSH6*dup and *MSH6*del mutations are highlighted by an outlined box.

** Chromosomal location includes both *MSH6*del and *MSH6*dup.