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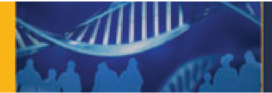

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Original Article

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

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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 United States. 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 observed on separate conserved haplotypes. *MSH6**c.3984_3987dupGTCA and *MSH6**c.3959_3962delCAAG probably 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.

Conflict of interest

Nothing to declare.

Key words: Ashkenazi Jews – founder mutation – Lynch syndrome – *MSH6*

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Lynch syndrome is the most common autosomal dominant condition pre-disposing to colorectal cancer (CRC). 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 CRCs and endometrial cancers (EnCas). About 10% of Lynch syndrome CRCs and 0.3% of all CRCs are explained by the mutations in *MSH6* (11, 12). The prevalence of *MSH6* mutations in EnCa 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 CRC to be 44% for men and 20% for women; for EnCa, the risk was 44%. *MSH6* mutation carriers had an 8-fold increased incidence of CRC in comparison with incidence in general population, and women with *MSH6* mutations had a 26-fold increased incidence of EnCa (14). In families with *MSH6* mutations, the onset of CRC and EnCa 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 EnCa) (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 EnCa 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 CRCs and adenomatous polyps (18), and using slightly different nomenclature was later reported in two families; one from the Netherlands (9) and another family from the United States (19). A recently published Israeli study found c.3984_3987dupGTCA in 19 members of four AJ families (20).

The aim of the present study was to characterize the *MSH6* mutations c.3984_3987dupGTCA (referred below as *MSH6**dup) and c.3959_3962 delCAAG (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: (i) population-based series of cases and controls (Israel); (ii) individuals with a personal and/or family history of CRCs and other Lynch syndrome-associated tumors referred to high-risk clinics (Montreal); (iii) familial gastro-intestinal cancer registry-based cases (Toronto); (iv) hospital-based series of EnCa cases and controls (New York); (v) unaffected control individuals (Montreal and Toronto) and (vi) carriers identified through a CLIA-certified commercial laboratory (Mayo clinic). These datasets are described briefly below.

Population-based MECC case–control study from Israel

DNA samples from 2685 cases and 1591 healthy controls from the large Israeli population-based study of CRC were genotyped at the University of Michigan. The Molecular Epidemiology of

Colorectal Cancer (MECC) study is a population-based case–control study of CRC in northern Israel. The cases include histologically confirmed incident CRC 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.

High-risk clinics

McGill University, 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, Montreal (JGH). Additionally, 22 CRC cases and 22 unaffected individuals from other high-risk CRC 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})].

Familial gastro-intestinal cancer registry

A total of 490 AJ were collected through the Familial Gastro-Intestinal Cancer Registry (FGICR) in Toronto. DNA extracted from their 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.

Hospital series

A total of 337 hospital-based AJ women with EnCa 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.

Volunteering controls

Women's College Hospital, Toronto: a total of 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 to 78 years.

JGH, Montreal: a total of 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 to 93.9 years. The age was unknown for 21 controls.

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 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 S1.

Microsatellite instability analysis and immunohistochemistry

Microsatellite instability assays were performed as described elsewhere (21) 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).

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	EnCa (66)	No	N/A	MSH6(−)
McGill	1	2	Female	44	c.3984_3987dupGTCA	CRC (40), EnCa (43)	No	MSS	N/A
McGill	2	1	Female	87	c.3984_3987dupGTCA	Metachronous CRC (64, 65), SCC (86), BCC (87)	No	N/A	N/A
McGill	2	2	Female	54	c.3984_3987dupGTCA	CRC (50), EnCa (50)	No	N/A	MSH6(−)
MECC_case	3	1	Male	70	c.3984_3987dupGTCA	CRC (70)	No	MSS	N/A
MECC_case	4	1	Female	86	c.3984_3987dupGTCA	EnCa (47), ovarian cancer (66), CRC (86)	No	MSI-High (mono)	N/A
MECC_case	5	1	Female	79	c.3984_3987dupGTCA	CRC (79)	No	MSI-High (mono)	N/A
MECC_case	6	1	Male	55	c.3984_3987dupGTCA	CRC (55)	No	MSI-High	N/A
MECC_case	7	1	Female	73	c.3984_3987dupGTCA	EnCa (58), cecal cancer (73)	Amsterdam II	N/A	MSH6(−)
MECC_case	8	1	Female	52	c.3984_3987dupGTCA	CRC (52)	N/A	N/A	N/A
MECC_case	9	1	Male	56	c.3984_3987dupGTCA	CRC (56)	No	MSI-High (mono)	N/A
MECC_control	10	1	Female	71	c.3984_3987dupGTCA	EnCa (61), cecal cancer (76)	No	N/A	N/A
Toronto	11	1	Female	85	c.3984_3987dupGTCA	Breast cancer (75), CRC (81)	No	N/A	N/A
Toronto	11	2	Female	54	c.3984_3987dupGTCA	CRC (50), EnCa (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	EnCa (57)	No	N/A	MSH6(+)
MSKCC	20	1	Female	68	c.3984_3987dupGTCA	EnCa (68)	No	N/A	MSH6(−)
MECC_case	13	1	Male	69	c.3959_3962delCAAG	CRC (69)	No	MSS	N/A
MECC_case	14	1	Male	82	c.3959_3962delCAAG	CRC (60), CRC (82)	No	N/A	N/A
MECC_case	15	1	Female	84	c.3959_3962delCAAG	CRC (84)	N/A	N/A	N/A
Toronto	16	1	Male	73	c.3959_3962delCAAG	CRC (70)	No	MSI-High	MSH6(−)
Toronto	17	1	Male	68	c.3959_3962delCAAG	CRC (62)	No	MSI-High	N/A
Toronto	18	1	Male	72	c.3959_3962delCAAG	Rhabdomyosarcoma (39), CRC (64), cancer of pancreas (71)	Amsterdam II	MSI-High	N/A
MSKCC	21	1	Female	43	c.3959_3962delCAAG	EnCa (43)	No	N/A	N/A
MSKCC	22	1	Female	49	c.3959_3962delCAAG	EnCa (49)	No	N/A	N/A

CRC, colorectal cancer; BCC, basal cell carcinoma; EnCa, endometrial cancer; IHC, immunohistochemistry; MECC, Molecular Epidemiology of Colorectal Cancer; Mono, MSI in mononucleotide microsatellite markers only; MSKCC, Memorial Sloan-Kettering Cancer Center; MSH6(−), MSH6 protein not present on immunohistochemical staining with an anti-MSH6 antibody; MSH6 (+), MSH6 protein present on immunohistochemical staining with an anti-MSH6 antibody; MSI-High−a high level of microsatellite instability is present; MSS, microsatellite stable; N/A, not available; SA, sebaceous carcinoma; SCC, squamous cell carcinoma.

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

The association between *MSH6* mutations and risk of CRC and EnCa was estimated using Fisher's exact test at SAS 9.1 (The SAS Institute, Cary, NC). Haplotype reconstruction was performed using PHASE v.2.1.1 (22, 23). 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) (24). 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 northeastern European region of 11,000 in the year 600 and 5 million in the year 1900. Considering a lifetime risk of CRC of 6.85%, a worldwide population of Ashkenazi Jews of 13 million (25), 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: 2672 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 CRC cases, EnCa 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. As 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 CRC

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 EnCa 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 (26), we included this carrier to both case and control groups to calculate the odds ratio (OR). To evaluate the risk of CRC 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 ($n = 1011$), MSKCC, New York ($n = 285$) and JGH, 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 JGH, Montreal, one of the 22 (4.5%) CRC affected probands from high-risk CRC 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 EnCa from MSKCC, two carriers (0.6%) of *MSH6**dup and two carriers (0.6%) of *MSH6**del mutations were

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
CRC					
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
EnCa[a]					
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

CRC, Colorectal cancer; EnCa, endometrial cancer.
[a]EnCa risk was estimated from MSKCC hospital-based series.

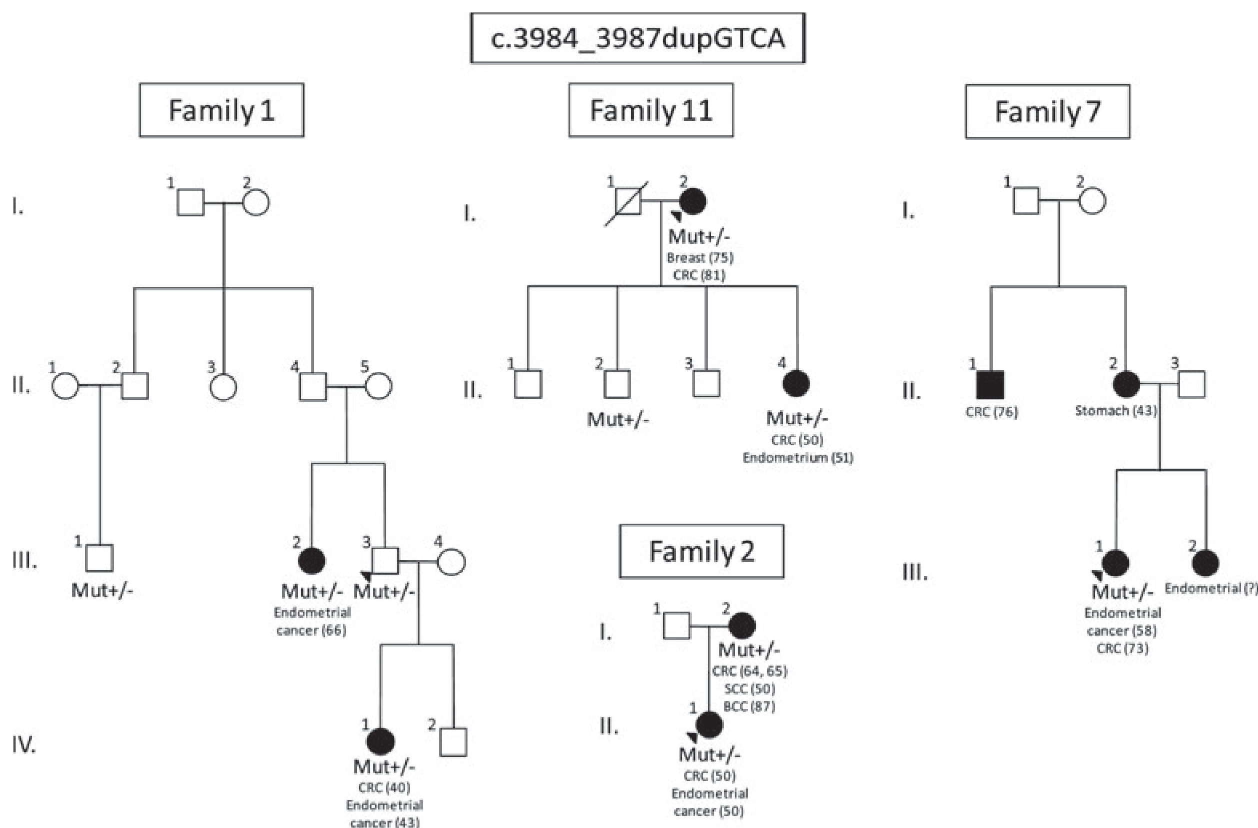

Fig. 1. Representative families carrying c.3984_3987dupGTCA. Mutation carriers are indicated by '+/−'. None of the families meet Amsterdam criteria, but history of family cancers is suggestive of mismatch-repair mutations. Probands are indicated with arrowheads.

identified. Roughly estimated, the risk of EnCa 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 (SCC), and basal cell carcinoma (BCC), 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 CRC was 66 years (median 67 years), and the mean age at diagnosis of EnCa was 54 years (median 52 years). Microsatellite instability and IHC staining against mismatch-repair (MMR) 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 CRCs, three

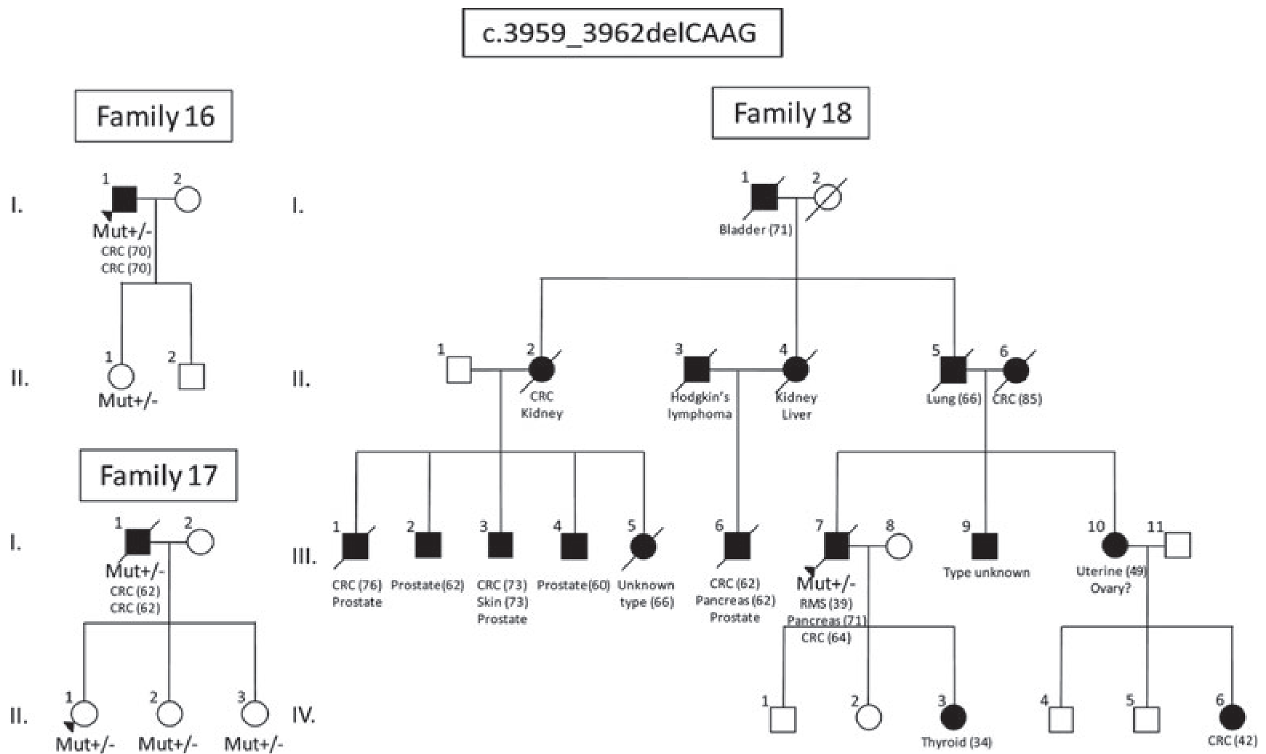

Fig. 2. Representative families carrying c.3959_3962delCAAG. Mutation carriers are indicated by '+/−'. None of the families meet Amsterdam criteria, but the history of family cancers is suggestive of mismatch-repair mutations. RMS, Rhabdomyosarcoma. Probands are indicated by arrowheads.

endometrial tumors, and one 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 three out of seven 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 MMR 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 CRC or EnCa (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 MMR genes. In family 11, both mother (I.2) and daughter (II.4) had CRC, while the daughter was diagnosed at the age of 50 followed by the diagnosis of EnCa 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 CRC. It is noteworthy that three MSH6*dup carriers had SCC and 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 (rs1800 932, 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 showed 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,

Table 3. Haplotype analysis of the carriers of *MSH6**c.3984_3987dupGTCA and *MSH6**c.3959_3962delCAAG mutations

Center/Study	Family	Patient	AtnACAGn	ex2_ rs1800932	ex3_ rs1800935	IVS5+14A>T	int8_ rs3136367	*MSH6* ex9 indel	CAn	D2S1352	D2S123
			2:47866183-47866350[a]	2:48018081	2:48023115	2:48030838	2:48033551	2:48033748-48033776[b]	2:48131630-48131817	2:50833714-50833825	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*

MECC, Molecular Epidemiology of Colorectal Cancer; MSKCC, Memorial Sloan-Kettering Cancer Center.

[a]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 shown in italics.

[b]Chromosomal location includes both *MSH6**del and *MSH6**dup.

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 (24). 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 probably 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_3987dup GTCA and c.3959_3962delCAAG (rs63751151) that cause Lynch syndrome in Ashkenazi Jews. Both *MSH6**dup and *MSH6**del create premature stop codons and probably result in non-functional 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 CRC 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 CRC 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 81–134 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 probably 8–14% (11 of 81–134 cases). EnCa 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 EnCa, although this patient had an EnCa before being ascertained as a control. Despite the wide CIs, the point estimates of the risk of CRC and EnCa in our study are, however, very comparable to the risk showed in the recent analysis of 113 families with *MSH6* mutations (14). This study found an 8-fold increase in risk of CRC and 26-fold increase in risk of EnCa. 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 CRC among carriers of *MSH6* exon 9 mutations was 66 years, 20 years older than in carriers of *MSH2* or *MLH1* mutations (44 years) (10). The mean age at diagnosis of EnCa was 54 years, comparable with previous studies (9). 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 (27).

Recombination events allowed estimation of the age of the mutations. *MSH6**dup arose ∼1425 years ago in the sixth century CE, and *MSH6**del arose ∼1325 years ago in the seventh 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.5946delT (traditionally known as 6174delT, 3rd century BCE) and *APC* p.I1307K (7th century BCE) (28). 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.5946delT and *APC* p.I1307K) have much higher frequency of 1.5% and 6%, respectively (29, 30).

Only a few AJ founder mutations leading to CRC and EnCa have been described until now. In addition to the *MSH6* exon 9 deletion and duplication characterized here, previous studies report another MMR gene mutation, *MSH2**c.1906G>C (common name A636P), associated with increased risk of both CRC (31) and EnCa (31, 32), as well as mutations in *APC**c.3920T>A (common name I1307K) (30) and *BLM**c.2207_2212delATCTGA insTAGATTC (common name BLM^{Ash}) (33) associated with increased risk of CRC only. The reported frequency of *APC**I1307K, BLM^{Ash} and *MSH2**A636P among AJ CRC patients is 12% (34), 1–3% (33), and 1% (31), respectively. The frequency of *MSH2**A636P in AJ EnCa patients is also about 1% (31). Altogether AJ founder mutations (*APC**I1307K, BLM^{Ash}, *MSH2**A636P, *MSH6**dup, and *MSH6**del) are associated with approximately 14–16% of all CRC cases and 2.2% of all EnCa cases in Ashkenazi Jews. The frequency of these mutations in AJ patients with family history of CRC and/or EnCa can be significantly higher. A panel designed to detect known AJ founder mutations consisting of *APC**I1307K, BLM^{Ash}, *MSH2**A636P, *MSH6**dup, and *MSH6**del could have value as a first-line screen in all AJ CRC and/or EnCa cases, irrespective of family history, IHC or MSI status.

Supporting Information

The following Supporting information is available for this article:

Table S1. Primers and reaction conditions used for genotyping and sequencing.

Additional Supporting information may be found in the online version of this article.

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