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A frame-shift mutation of *PMS2* is a widespread cause of Lynch syndrome

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Abstract

Background—When compared to the other mismatch repair genes involved in Lynch syndrome, the identification of mutations within *PMS2* has been limited (<2% of all identified mutations), yet the immunohistochemical analysis of tumour samples indicates that approximately 5% of Lynch syndrome cases are caused by PMS2. This disparity is primarily due to complications in the study of this gene caused by interference from pseudogene sequences.

Methods—Using a recently developed method for detecting *PMS2* specific mutations, we have screened 99 patients who are likely candidates for *PMS2* mutations based on immunohistochemical analysis.

Results—We have identified a frequently occurring frame-shift mutation (c.736_741del6ins11) in 12 ostensibly unrelated Lynch syndrome patients (20% of patients we have identified with a deleterious mutation in *PMS2*, n = 61). These individuals all display the rare allele (population frequency <0.05) at a single nucleotide polymorphism (SNP) in exon 11, and have been shown to possess a short common haplotype, allowing us to calculate that the mutation arose around 1625 years ago (65 generations; 95% confidence interval 22 to 120).

Conclusion—Ancestral analysis indicates that this mutation is enriched in individuals with British and Swedish ancestry. We estimate that there are >10 000 carriers of this mutation in the USA alone. The identification of both the mutation and the common haplotype in one Swedish

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control sample (n = 225), along with evidence that Lynch syndrome associated cancers are rarer than expected in the probands' families, would suggest that this is a prevalent mutation with reduced penetrance.

An important factor in keeping the integrity of the genome is an ability to detect and repair single nucleotide changes which occur as a result of replication errors. In eukaryotes this process is regulated by a set of evolutionarily conserved genes which belong to the mismatch repair family. There are four key genes involved in this process whose products function as two heterodimers (MLH1 with PMS2 and MSH2 with MSH6).[1] A predisposition to tumorigenesis, known as Lynch syndrome,[2] is associated with mutations in the aforementioned genes.[3] This predisposition is dominantly inherited with a heterogeneous spectrum of tissues being affected (primarily colorectal and endometrial). The development of a cancer, however, is recessive in that, analogous to the mechanism in many tumour suppressor genes, a second hit on the wild type allele is required. The mechanism of the second hit is often unknown, although deletions, chromosomal loss and promoter methylation are often cited.[4–6]

Extensive studies over the last 14 years have identified numerous functional mutations within the mismatch repair genes (http://www.med.mun.ca/mmrvariants). Until recently, the vast majority of Lynch syndrome causing mutations had been identified within either *MLH1* or *MSH2*,[7] which suggested a less significant role for *MSH6* and *PMS2* in the mismatch repair mechanism. It is in our opinion that these results are somewhat misleading in that the majority of studies have avoided looking for *PMS2* mutations due to the presence of extensive, highly homologous, pseudogenes,[8–10] which have made mutation detection by routine methods difficult and error prone. This theory is supported by the fact that tumour studies show that ~5% of Lynch syndrome samples express MLH1, MSH2 and MSH6, but not PMS2 at the protein level,[11] yet the mutation detection rate is currently less than 2% (http://www.med.mun.ca/mmrvariants).

We have recently developed a simple yet effective method of avoiding many of the pseudogene associated problems of *PMS2* screening through the use of long range polymerase chain reaction (PCR).[12] Via this method we have identified a deleterious mutation in ~62% of patients tested (61 out of 99), with 34 different mutations (11 of which occur in more than one family) being identified (authors' unpublished data). Of particular interest from this recent study has been the identification of a frequently occurring insertion/deletion mutation (c.736_741delCCCCCTinsTGT-GTGTGAAG; p.P246CfsX3, referred to herein as indel). Since we first reported this mutation in three Lynch syndrome patients,[12] we have subsequently identified the same mutation in a further nine families.

The following work uses these families to identify a common haplotype, which suggests that the indel is a founder mutation that arose some 1625 years ago.

PATIENTS AND METHODS

Patients

For the present study only those cases were included (n = 99) in which the tumour did not stain for PMS2 by immunohistochemistry, while it did stain for the MLH1, MSH2 and MSH6 proteins. The National Cancer Institute (NCI) funded Colon Cancer Family Registries provided 35 anonymous samples from four sites: Australasia, Seattle, Mayo Clinic and Ontario. These samples have been accrued to the registry, either through high risk clinic ascertainment or through population based ascertainment. The remaining cases were either from high risk clinics in which patients with an early onset of cancer and/or a family history of cancer predominate (n = 51), or from series in which unselected patients with colorectal cancer (CRC) were screened for mutations by microsatellite instability analysis (n = 13). All patients provided written consent for genetic testing.

Mutation detection

Variants within the *PMS2* locus were detected as described previously,[12] with the following modifications. Exons 6, 7, 8, and 10 were individually amplified directly from genomic DNA, and PCR2 was reduced to a more readily amplifiable product (1618 bp), from which exon 9 can be directly sequenced. Primers differing from the original protocol are displayed in table 1.

Diploid-to-haploid conversion

Haploid converted clones from patient 1 and the sister of patient 3 were created commercially (Mayo Clinic, Rochester, Minnesota, USA; www.mayoclinic.org) using the conversion technology of Yan *et al.*[13]

Allele specific amplifications

Single nucleotide polymorphisms (SNPs) in close proximity to the indel mutation were typed on the mutant allele via long range PCR, using standard protocols and allele specific primers, followed by direct sequencing. Allele discriminating primers were positioned at the site of the indel mutation with the second primers being positioned within exons 5 and 9 (see table 1 for primer sequences).

Haplotype analysis

A combination of three novel (Clen35 (BV725467), Clen36 (BV725468), Clen37 (BV725469)) and three DeCode (D7S481 (Z16478), D7S2201 (G08627), D7S2478 (Z53195)) microsatellite markers (see table 1 for primer sequences) were used to generate genotypes spanning ~1 Mb across the *PMS2* locus. Markers were typed in diploid DNA and haploid clones generated from two mutation carriers. Markers were typed either by direct labelling of a PCR primer or by utilising a labelled M13 primer in conjunction with an M13-tailed, amplicon specific, primer in a three primer PCR. Each 25 μl PCR reaction contained 12.5 μl of HotStarTaq PCR mix (Qiagen), 25 ng of genomic DNA, 10 pmoles of each primer (for the three primer PCR, 2 pmoles of tailed primer and 10 pmoles of FAM labelled M13 primer were used). Reactions were multiplexed when possible and cycled using the

following profile: 96°C for 15 min, 30 cycles (50 cycles were used for the three primer PCR) of 96°C for 30 s, 60°C for 30 s and 72°C for 30 s, and a final extension at 72°C for 10 min. Products were sized using an ABI7000.

Estimating the age of the indel mutation

The DMLE+2.2 software developed by Reeve and Rannala[14] was utilised to estimate the age of the indel mutation. The program, which is freely available from www.dmle.org, uses a Bayesian approach to compare differences in linkage disequilibrium, between the mutation and flanking markers, among DNA samples from mutation carriers and unrelated normal controls. In addition to the genotype data, marker locations, population growth rates, and an estimate for the proportion of disease bearing chromosomes being analysed are used by the software.

RESULTS

The notion that *PMS2* mutations are rarely involved in the onset of Lynch syndrome has been advocated for the last 10 years, and this has been supported in some part by the sparsity of *PMS2* specific mutations recorded in the literature.[15] To address this problem, we developed a relatively simple assay which enabled us to identify *PMS2* specific mutations in a way that minimises the risk of making false diagnoses as a result of interference from pseudogenes.

Among the deleterious mutations we identified during this study was a frequently occurring insertion/deletion mutation (c.736_741del6ins11). This mutation causes an alteration of the PMS2 protein sequence from residue 246 with a termination signal being created at residue 249, some 614 amino acids before the wild type stop codon. We initially identified this mutation in two Swedish patients and an American patient who had a grandparent of Swedish origin. This geographical connection along with the identification of a rare polymorphism (rs2228007), within exon 11, in all three individuals led us to hypothesise that this mutation may have prevailed as a result of a founding event among the population of Sweden. In our subsequent studies we have identified this same mutation in an additional nine probands from various geographical locations throughout North America and Australasia.

Clinical overview of indel families

The mean age for developing a Lynch syndrome associated cancer among the probands was 52. Their families did not have significant incidences of Lynch syndrome associated cancers, with only one of the families meeting the Amsterdam criteria and six of them meeting the revised Bethesda criteria. When possible, family members, regardless of their cancer status, were screened for the mutation. Data obtained from these family studies (table 2) suggest that this mutation has reduced penetrance. We were able to obtain patient defined ancestral origins for nine of the 12 probands, which indicates a high prevalence of English as well as Swedish ancestry. Two of the three patients without detailed ancestry were from Australia and the third was an African American. Although we cannot be certain, historical records of these populations, especially in the case of Australians, indicate that their

ancestries are significantly more likely to be English rather than Swedish (http://www.censusdata.abs.gov.au).

Mutation analysis and control screening

Complete sequencing of the *PMS2* gene in all probands identified no additional deleterious variants, indicating that the indel mutation is the causative factor for the observed phenotype. Analysis of haploid clones demonstrated that a mutant transcript is generated but no full length protein could be detected (data not shown). We were also unable to detect any truncated protein through the use of an N-terminal antibody which rules out a dominant negative mechanism being responsible for the loss of function of PMS2 (data not shown). In addition to the indel mutation, all 12 probands were shown to have the minor allele variant at codon 1531 (rs2228007; Coriell - A:0.95/G:0.05, n = 96) and this was shown to reside on the same allele as the indel mutation in both haploid clones.

A panel of 399 control DNAs (225 Swedish blood donors, 92 Coriell controls, and 82 African American controls—the latter a gift from Dr Rick Kittles (The University of Chicago)) were screened for the presence of the indel mutation. This control screen showed that a single, anonymous sample from the Swedish blood donor set carried the mutation; it was also shown that the same sample carried the minor allele at rs2228007 (allele frequency was 0.027 in the Swedish control samples).

Haplotype analysis

We sought to identify the extent of any putatively conserved haplotype through the typing of additional SNPs and micro-satellite repeat elements. It should be noted that, as with mutation detection, the identification of unique microsatellite sequences is hampered by extensive homologous sequences. The extent of these sequences is so great that no unique sequence, harbouring a microsatellite repeat, could be identified within 200 kb of the 3′ end of *PMS2* (fig 1). For the haplotype analysis we utilised three database and three novel microsatellite markers which span ~1 Mb across the PMS2 locus. We also assessed a set of seven SNPs, five of which could be typed using allele specific PCR and as such enabled us to decipher a true mutant haplotype, in all samples, for a region spanning from exon 5 to exon 9 of *PMS2*. To get a full length haplotype we typed all markers in haploid cell lines obtained from two individuals (patient 1 and the sister of patient 3, who also carried the mutation). Typing of all markers in the 13 individuals (12 patients and one control sample) with the indel mutation identified a common haplotype of between 104 kb (rs2228007 – D7S481) and 518 kb (SNP-MC1 – Clen35) (fig 2). Although the 3′ end of the haplotype is broken by the absence of an SNP in a single sample (patient 4), which could be put down to mutation, the haplotype is lost by eight more of the 13 individuals a further 60 kb downstream as adjudged by the closest 3′ flanking microsatellite (Clen36).

Estimating the age of the mutation

It has been shown that the rate of recombination and population frequency of flanking marker alleles can be used to estimate the age of a conserved haplotype. In this study we utilised a well established method that considers the linkage disequilibrium across all markers in a single analysis, and is implemented in the DMLE+2.2 software. Marker

locations were obtained from the human genome reference sequence, and converted to map distances using a conversion factor of 1.67 cM/Mb which was obtained from a comparison of Centimorgan and megabase values for the 7p22 region (based on deCODE mapping data[18]). We used a population growth rate of 1.05 fold per generation based on European estimates.[19] To calculate the proportion of disease bearing chromosomes being studied we extrapolated *PMS2* specific data for both the USA and Sweden, from the literature and our own unpublished data (USA: 6% lifetime risk of CRC (American Cancer Society; http://www.cancer.org/downloads/STT/CAFF2006PWSecured.pdf), 2.8% of CRC are Lynch syndrome (authors' unpublished data)[20]; Sweden: 5% lifetime risk of CRC, 1.2% of CRC are Lynch syndrome[21]), to calculate the number of Lynch syndrome cases. Using these figures and our own data for the incidence of the indel we estimated that there would be ~10 874 cases of this mutation in the USA and ~181 cases in Sweden, based on the population sizes of the 2000 censuses. The DMLE+2.2 software predicted an age of ~1625 years (65 generations; 95% confidence interval 22 to 120) (fig 3), which would suggest that this indel mutation arose sometime during the first millennium.

DISCUSSION

We show here that many *PMS2* mutations occur, but the determination of the true proportion of all cases of Lynch syndrome that are due to *PMS2* mutations must await further, larger, population based studies; nevertheless, our estimate for the present number of carriers of the indel mutation (>10 000 in the USA alone) does implicate *PMS2* as a potentially important gene to consider in clinical practice. The clinical and family history data that we were able to assemble and critically evaluate (table 2) do not allow us to assess precisely the penetrance of this mutation. An apparent lack of disease segregation among the affected families (there was no Lynch syndrome associated cancer in either parent for eight of the 12 probands) is a strong indication that this mutation, along with many other truncating mutations within *PMS2* (authors' unpublished data), has reduced penetrance in comparison to the other mismatch repair genes. Lynch syndrome has been defined as a heritable, high penetrance, predisposition to cancer. If some mutations, such as the one described here, carry a lower penetrance, they may be seen not to fall under this definition of Lynch syndrome. Once the penetrance of *PMS2* mutations can be more precisely defined, their role in Lynch syndrome will become better established and modifications to the counselling and clinical management of carriers of these mutations may be called for.

A mutation that is seen in many ostensibly unrelated individuals can be either recurrent—that is, it arises repeatedly de novo—or it represents an ancestral event that occurs in the population. In this case the highly conserved haplotype that occurs in all patients virtually excludes a recurrent event. The alternative would be that the associated haplotype somehow predisposes to this particular mutation, a highly unlikely assumption. It follows that even though we were not able to investigate DNA from most parents of the probands, it is likely that in each case one of them carried the mutation.

Our calculations, based on a conserved haplotype of ~520 kb (~0.85 cM), estimated that this mutation originated around 1625 years ago. Although these kinds of estimates tend to have broad margins of error, it is consistent with the ages predicted for similarly sized

haplotypes.[2223] Many of the earlier studies along these lines have studied disease in distinct populations such as the Ashkenazi Jews, Icelanders and Finns,[2324] and so they have been able to support their age estimates with historical records of population movements. With our data we are unable to make such reassuring comparisons due to the patient cohort being heterogeneous, with several having limited or no information regarding their ancestral background. Without further data it would be unwise to make firm conclusions as to the geographical origins of this indel mutation; however, our current data would indicate that this mutation originated at a time when there was significant interaction between people of Scandinavian ancestry and those of British ancestry, which could explain our findings that the mutation occurs primarily in people of English and Swedish descent.

The identification of the indel in a control sample (Swedish blood donor) highlights further that this mutation is of significant prevalence; although our clinical findings would indicate that this mutation, like several other reported mutations within *PMS2*,[2526] has a reduced penetrance, it is in our opinion that this indel could be responsible for a significant proportion of cases of Lynch syndrome that are associated with an aberration in *PMS2*. It is also worth considering the long term impact of this kind of *PMS2* mutation on the affected populations. Over time, if the number of heterozygous carriers increases in the population, we would expect to see a rise in the number of homozygous mutation carriers for *PMS2* which presents as a severe childhood cancer syndrome typified by Turcot's syndrome.[27–29]

Unlike many other mutations in *PMS2*, this alteration is located within a region not complicated by pseudogenes; it can therefore be easily screened for by several methods including, but not limited to, direct sequencing, multiplex ligation dependent probe amplification (MRC Holland), and allele size screening, either on an ABI7000 utilising a fluorescently labelled primer or by high resolution agarose electrophoresis.

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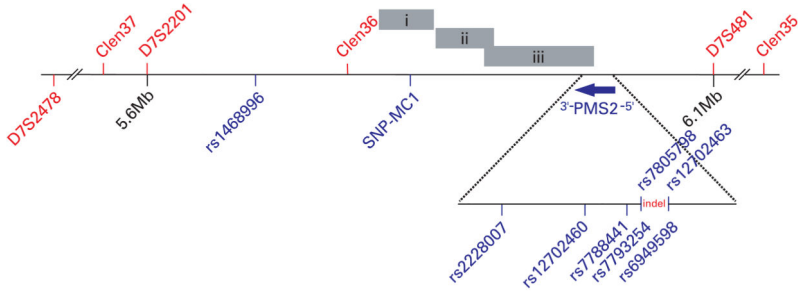
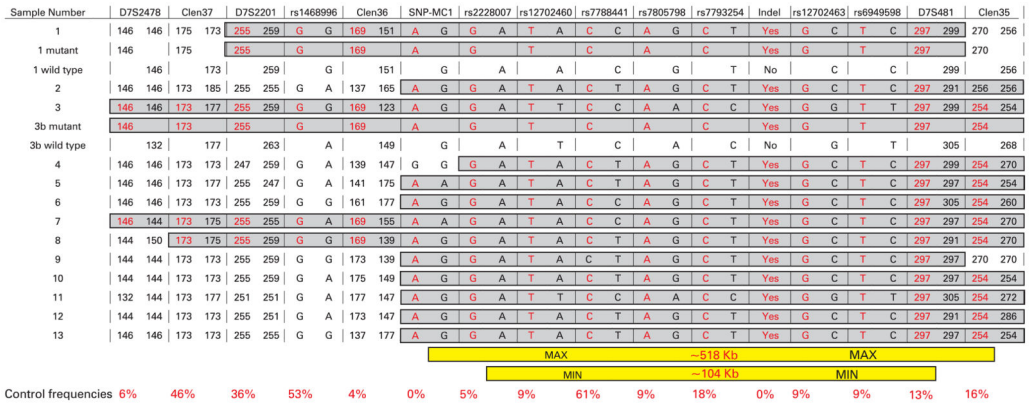


Figure 1.

Genomic region flanking *PMS2*. Microsatellite and single nucleotide polymorphism (SNP) markers used for the haplotype analysis are shown in red and blue fonts, respectively. Chromosomal fragments which show high levels of homology to the 3′ end of *PMS2* are represented by grey bars; (i) chr 7: 5,126,099…5,174,845 bp; (ii) chr 7: 97,435,392…97,486,400 bp; (iii) chr 7: 6,741,350…6,839,016 bp. Physical locations are based on NCBI's reference build (ver36.2).



Sample Number	D7S2478		Clen37		D7S2201		rs1468996		Clen36		SNP-MC1		rs2228007		rs12702460		rs7788441		rs7805798		rs7793254		Indel	rs12702463		rs6949598		D7S481		Clen35	
1	146	146	175	173	255	259	G	G	169	151	A	G	G	A	T	A	C	C	A	G	C	T	Yes	G	C	T	C	297	299	270	256
1 mutant	146		175		255		G		169		A		G		T		C		A		C		Yes	G		T		297		270	
1 wild type		146		173		259		G		151		G		A		A		C		G		T	No		C		C		299		256
2	146	146	173	185	255	255	G	A	137	165	A	G	G	A	T	A	C	T	A	G	C	T	Yes	G	C	T	C	297	291	256	256
3	146	146	173	177	255	259	G	G	169	123	A	G	G	A	T	T	C	C	A	A	C	C	Yes	G	G	T	T	297	299	254	254
3b mutant	146		173		255		G		169		A		G		T		C		A		C		Yes	G		T		297		254	
3b wild type		132		177		263		A		149		G		A		T		C		A		C	No		G		T		305		268
4	146	146	173	173	247	259	G	A	139	147	G	G	G	A	T	A	C	T	A	G	C	T	Yes	G	C	T	C	297	299	254	270
5	146	146	173	177	255	247	G	A	141	175	A	A	G	A	T	A	C	T	A	G	C	T	Yes	G	C	T	C	297	297	254	254
6	146	146	173	173	255	259	G	G	161	177	A	G	G	A	T	A	C	C	A	G	C	T	Yes	G	C	T	C	297	305	254	260
7	146	144	173	175	255	255	G	A	169	155	A	A	G	A	T	A	C	C	A	G	C	T	Yes	G	C	T	C	297	297	254	270
8	144	150	173	175	255	259	G	G	169	139	A	G	G	A	T	A	C	T	A	G	C	T	Yes	G	C	T	C	297	291	254	270
9	144	144	173	173	255	259	G	G	173	139	A	G	G	A	T	A	C	T	A	G	C	T	Yes	G	C	T	C	297	297	270	270
10	144	144	173	173	255	259	G	A	175	149	A	G	G	A	T	A	C	T	A	G	C	T	Yes	G	C	T	C	297	297	254	254
11	132	144	173	177	251	251	G	A	177	147	A	G	G	A	T	T	C	C	A	A	C	C	Yes	G	G	T	T	297	305	254	272
12	144	144	173	173	255	251	G	A	173	147	A	G	G	A	T	A	C	T	A	G	C	T	Yes	G	C	T	C	297	291	254	286
13	146	146	173	173	255	255	G	G	137	177	A	G	G	A	T	A	C	T	A	G	C	T	Yes	G	C	T	C	297	297	254	254
											MAX										~518 Kb			MAX						
													MIN								~104 Kb			MIN						
Control frequencies	6%		46%		36%		53%		4%		0%		5%		9%		61%		9%		18%		0%	9%		9%		13%		16%	

Figure 2.

Genotype data spanning the *PMS2* locus of 17 DNAs. The 17 DNAs comprise 12 patients, one blood donor control (13), and four haploid clones. Wild type and mutation bearing haploid clones were typed for both patient 1 and the sister of patient 3 (3b). Disease associated genotypes are highlighted in grey, with the disease allele depicted in red. The size of the minimum and maximum conserved haplotypes along with the frequency of the disease associated allele among a panel of control DNAs are shown at the bottom.

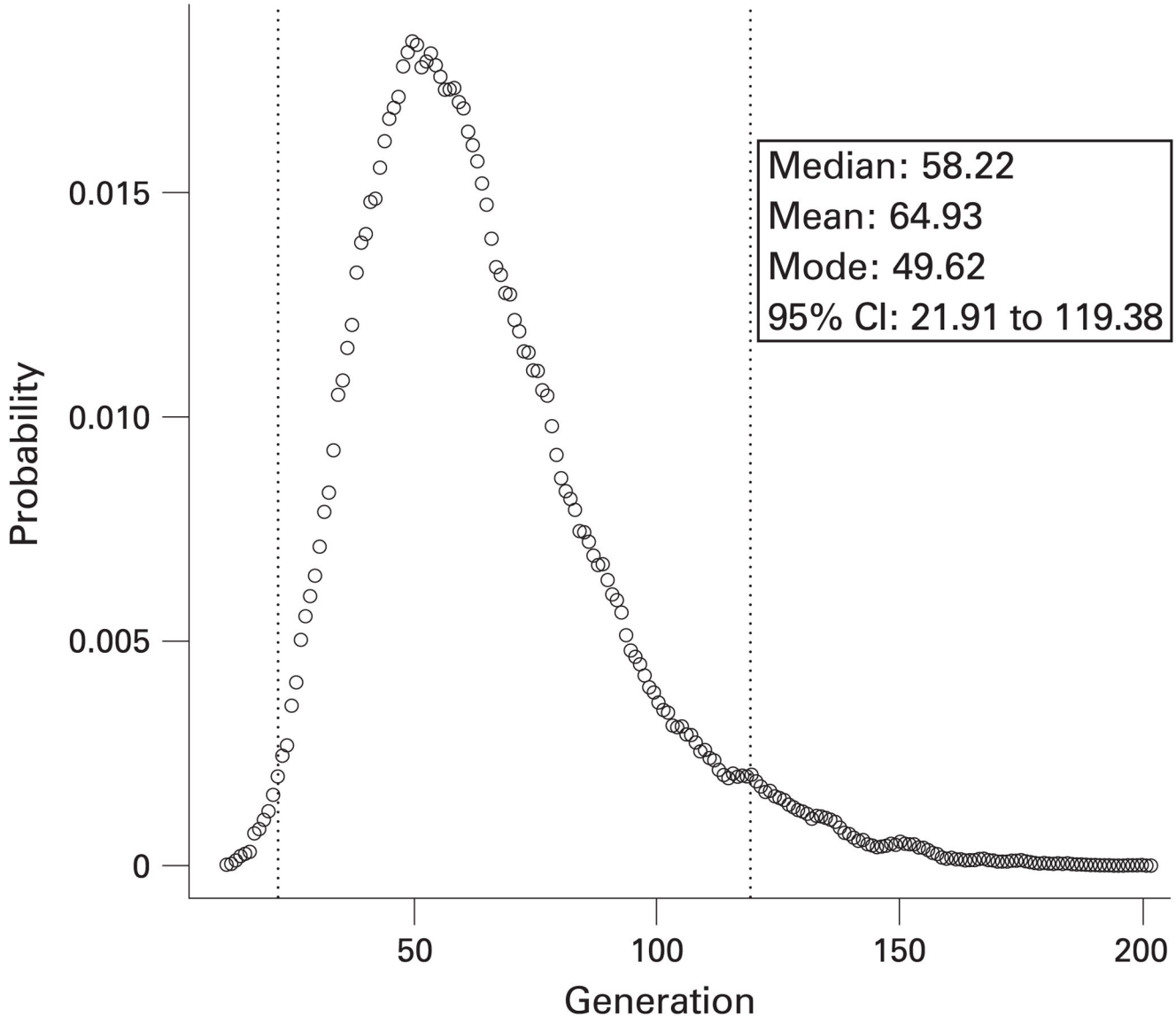


Figure 3.

Age estimates for the haplotype associated with the indel mutation. The posterior probability density of the age (in generations), as estimated by the software DMLE+2.2, is shown when a population growth rate of 1.05-fold per generation (25 years) is assumed. The dotted lines show the 95% confidence intervals for the calculation.

Table 1

Oligonucleotides used for sequencing, allele specific polymerase chain reaction (PCR) and genotyping

Primer name	Sequence	Temp °C	Primer name	Sequence	Temp °C
Exon 7 seq	GCTCTCAGGATAAAATGTTC	55	Clen37 For	GCATTTGGAGCAAGATTTCCCTACT	60
Exon 6 seq	CCCGCTATAATCACTAGAGC	55	Clen37 Rev-tailed	† GCCAACACCTCTAATTAGCTCTGAA	60
ex9 LR For	TTGCTTGTAATCTGCCAGATGTGGT	65	D7S2201For	GGTTCACACCTGAAATCCCAACACT	60
ex9 LR Rev	ATCTACTTTCTCCCTTGGTTGACAT	65	D7S2201Rev-FAM	TCTGTACCCAATGTAGAGCAGGACA	60
indel wt for	ATTCCTTTTGTTCAGCTGCCCCCTA	65	D7S2478 For-tailed	† CAGATTCCATATGCAATCCCCATCA	60
indel mut for	TTTTGTTCAGCTGTGTGTGTGAAGA	65	D7S2478 Rev	CGTGCTCCGCCATTTCTGTATACTT	60
indel wt rev	TCACACACGGAGTCACTAGGGGGCA	65	rs12702460seq	AGAAGTTCAACCACATCTGGCAGAT	60
indel mut rev	CACGGAGTCACTCTTCACACACACA	65	rs12702463seq	GCGTTGAAGCAATTCTCCTACCTTA	60
exon 5 AS For	CGAAGGTTGGAACTCGACTGATGTT	65	rs6949598seq	AGATTCAAGCAATTCTCCTGCCTCA	60
exon 9 AS Rev	TGGTGTCGATTATACATGTGGTAGA	65	rs7793254seq	TTCTTGAGACGGAGTCTTGCTTTGT	60
Clen36 For	AAGGCTGGGTCCAGTAGTGGGA	60	rs7788441seq	TTTCCAGAAGAACACCACCTTCACA	60
Clen36 Rev-tailed	* CATCTTGCTTCCTTAAGGTCTGTCA	60	rs7805798seq	TTTCCAGAAGAACACCACCTTCACA	60
D7S481 For	TAGCGTCTAGTCAGCTACCGTATTA	60	SNP-MC1† For/Seq	GTTTATGGCTACACTCCTGTCTAGT	60
D7S481 Rev-tailed	* CAAAATAGCTAGACACCACCCCACT	60	SNP-MC1† Rev	CCCTTTGTACTCTCCCTCACTGAGA	60
Clen35 For	AATTAGCTGGGCGTGGTACCAGGCA	60	rs1468996 For/Seq	AGAAATACAGTTCTTAGTTGGTGGA	60
Clen35 Rev-tailed	* CCTGTGGGAAGAACGAAGTGTTTCT	60	rs1468996 Rev	TTCTCTGTAGCTGCGTAGCTTGTGT	60
FAM labelled M13	TGTAAAACGACGGCCAGT	60			

*Primers have a common tail sequence 5′-tgtaaaacgacggccagt-3′.

†52 bp upstream of rs1974766.

Table 2

Clinical attributes of the 12 patients who tested positive for the indel

Patient	Proband location	Sex/age at diagnosis	Site of cancer	Criteria met‡	No. of affected* parents	No. of affected* siblings	Microsatellite status unstable/No. typed	de novo status	Ancestral information†
1	USA	♂ 49 years	Rectum	Bethesda	0	0(1)	6/9	Unknown	pat—Ger; mat—Swe/Nor
2	Canada	♀ 44 years	Ascending	Bethesda	0	1(14)	2/2	Unknown	pat—Eng; mat—Eng/USA
3	USA	♂ 46 years	Splenic flexure	Bethesda	1	0(9)	5/5	Mother was an obligate carrier	African American
4	Sweden	♂ 54 years	Ascending	None	1	1(1)	6/6	Father carried the mutation	Swedish
5	Sweden	♀ 51 years	Descending	None	0	0(2)	2/5	Unknown	Swedish
6	Canada	♀ 74 years	Ascending	Bethesda	1	1(2)	5/5	Sister carries the mutation	English
7	USA	♂ 57 years	Caecum	None	0	0(3)	8/10	Unknown	pat—Irish/English; mat— n/a
8	USA	♂ 58 years	Caecum	None	0	0(2)	9/10	Unknown	Swedish
9	USA	♀ 51 years	Caecum	None	0	0(5)	8/10	Unknown	pat—Swe/Ger; mat—Eng
10	Canada	♂ 29 years	Rectum	Bethesda	0	0(1)	5/6	Unknown	English/Canadian
11	Australia	♀ 67 years	Stomach	Amsterdam II	1	2(8)	3/4	Brother carries the mutation	Australian
12	Australia	♀ 48 years	Caecum	Bethesda	0	0(2)	8/9	Sister carries the mutation	Australian

*Affected with any of the following types of cancer: colorectal, endometrial, stomach, ovarian, pancreas, ureter and renal pelvis, biliary tract, brain, small bowel, sebaceous adenoma and keratoacanthoma.

†pat, paternal side; mat, maternal side; Ger, German; Swe, Swedish; Nor, Norwegian; Eng, English; Can, Canadian; n/a, data not available.

‡Based on guidelines by Umar *et al* and Vasen *et al.*[1617]