Current clinical criteria for Lynch syndrome are not sensitive enough to identify *MSH6* mutation carriers

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ABSTRACT

Background Reported prevalence, penetrance and expression of deleterious mutations in the mismatch repair (MMR) genes, *MLH1*, *MSH2*, *MSH6* and *PMS2*, may reflect differences in the clinical criteria used to select families for DNA testing. The authors have previously reported that clinical criteria are not sensitive enough to identify MMR mutation carriers among incident colorectal cancer cases.

Objective To describe the sensitivity of the criteria when applied to families with a demonstrated MMR mutation.

Methods Families with an aggregation of colorectal cancers were examined for deleterious MMR mutations according to the Mallorca guidelines. All families with a detected MMR mutation as of November 2009 were reclassified according to the Amsterdam and Bethesda criteria.

Results Sixty-nine different DNA variants were identified in a total of 129 families. The original Amsterdam clinical criteria were met by 38%, 12%, 78% and 25% of families with mutations in *MSH2*, *MSH6*, *MLH1* and *PMS2*, respectively. Corresponding numbers for the revised Amsterdam criteria were 62%, 48%, 87% and 38%. Similarly, each of the four clinical Bethesda criteria had low sensitivity for identifying *MSH6* or *PMS2* mutations.

Conclusion Amsterdam criteria and each of the Bethesda criteria were inadequate for identifying *MSH6* mutation-carrying kindreds. *MSH6* mutations may be more common than currently assumed, and the penetrance/expression of *MSH6* mutations, as derived from families meeting current clinical criteria, may be misleading. To increase detection rate of MMR mutation carriers, all cancers in the Lynch syndrome tumour spectrum should be subjected to immunohistochemical analysis and/or analysis for microsatellite instability.

INTRODUCTION

The concept of hereditary non-polyposis colorectal cancer (HNPCC) was developed to denote families with inherited colorectal cancer (CRC). The Amsterdam (AMSI) criteria identified families with CRC.[1] As extracolonic cancers, especially endometrial cancer, were shown to be part of the inherited syndrome,[2 3] the revised Amsterdam criteria (AMSII) were introduced.[4] The Bethesda guidelines included the tumour marker microsatellite instability (MSI),[5] and the revised Bethesda criteria (BII) specified all cancers known at the time to be associated with the syndrome.[6] Prostate cancer has recently been shown to possibly be part of the syndrome.[7] Germline mutations in the mismatch repair (MMR) genes, *MLH1*, *MSH2*, *MSH6* and *PMS2*, have been identified to cause HNPCC (reviewed by Lynch and Lynch[8]). However, it has become clear that not all families fulfilling the clinical criteria have an identifiable deleterious mutation (hereafter called 'mutation') in one of these genes. In addition, because MMR mutations confer an increased risk of several types of cancer in addition to CRC, it has been suggested that the term Lynch syndrome should replace HNPCC in families where a mutation has been detected.[9] This definition of Lynch syndrome will be used in the present report. Families fulfilling the AMSII criteria without a demonstrable MMR mutation may be denoted HNPCC. Families with an aggregation of CRC and not corresponding to Lynch syndrome or HNPCC may be referred to as familial CRC.[9]

Tumours caused by mutations in *MLH1*, *MSH2*, *MSH6* and *PMS2* show a high degree of MSI. It has been shown by immunohistochemical analysis (IHC) that the gene product from the mutated gene is absent in tumour tissue (reviewed by Vasen *et al*[10]). IHC and MSI analysis have high sensitivity in detecting carriers of MMR mutations.[11 12] It is now customary to examine tumours in families that fulfil clinical criteria by IHC/MSI analysis, and select those families with abnormal results for analysis of constitutional DNA.[10] As a consequence, families not meeting the clinical criteria will not be subjected to mutation analyses.

Varying prevalence of mutations in the MMR genes has been reported. Some variations are obviously caused by geographically local and frequent founder mutations.[13–16] It is, however, reasonable to assume that the criteria used to select families for testing may also have influenced the results.

Over the last two decades, Norwegian cancer genetic clinics have recruited families with an aggregation of cancers of any type. Thousands of cancer kindreds have been examined for hereditary cancer syndromes. Reports from this are listed on http://www.inherited-cancer.com. Upon referral, the families were classified using preset wide-ranging criteria, and IHC/MSI analyses were performed not only on the families that met the clinical criteria for HNPCC and familial CRC. We here report the sensitivities of the AMSI, AMSII and BII criteria when applied to families that were shown by genetic testing to have an MMR mutation. As Norwegian legislation

dictates that genetic testing is restricted to clinical departments, and as all genetic departments collaborated in this report, we here present a complete report of all clinical genetic activity in a defined population up to November 2009.

PATIENTS AND METHODS

Patients, registries and ethics

The initial material included all families investigated for inherited CRC in Norway until November 2009. Wide-ranging selection criteria were used to identify the families. Besides including all kindreds meeting the AMSII or BII criteria, we explored all families with four CRC cases irrespective of age and including skipped generations or with an aggregation of any cancer associated with Lynch syndrome. All activities were conducted as part of the healthcare system, all information was included in the patient files, all genetic testing was conducted according to national legislation, including genetic counselling before and after genetic testing, and all genetic testing was performed with written informed consent from the participants. All relevant diagnoses in the families were validated in the medical files or cancer registry after consent from relatives or descendants if the subject was dead. No research registry that included names was produced; only summarised data were taken from medical files for compilation of the present report. All information described has been disclosed to the patients/families, and family members were offered appropriate healthcare according to the Mallorca guidelines.[9]

MSI/IHC examinations

Upon referral and inclusion according to the wide-ranging criteria, families were subjected to examination for Lynch syndrome as described in the Mallorca guidelines, with IHC/MSI analysis of at least two affected family members if available,[9] continuing to full mutation analysis of the relevant gene(s) of the patient (or obligate carrier in the family or offspring if dead) if an abnormal IHC result was obtained. A family was scored as having an abnormal IHC result if one or more tumour(s) showed lack of staining for the gene product of one or more of the MMR genes. Full mutation analysis of all MMR genes was performed if IHC was normal but the tumours were MSI (MSI-high). In some selected families, mutation analyses were also performed in the absence of MSI/abnormal IHC.

Molecular methods

MMR mutation analyses included heteroduplex identification followed by DNA sequencing of the actual MMR gene(s). Analysis of gross deletions and duplications was performed by multiplex ligation-dependent probe amplification assay (MLPA; SALSA P003 *MLH1/MSH2*, P008 *MSH6/PMS2* and P072-*MSH6*; MRC-Holland, http://www.mrc-holland.com). Results for *PMS2* exon 13–15 probes were disregarded because many related sequences are present in the genome and the probes provided very variable results. Sequencing analyses were performed on an ABI Genetic Analyzer model 3100 or 3130 (Applied Biosystems, Carlsbad, CA, USA), and DNA sequences were computed using SeqScape v2.5 software (Applied Biosystems). Primer and sequence details are available on request. In some cases of putative splice effects, cDNA analyses of *MLH1, MSH2, MSH6* and *PMS2* were performed. The molecular analyses were performed according to standard procedures and manufacturers' instructions. Methods used varied over time and between the different laboratories involved. It was beyond our means to reanalyse the whole series so that one method was applied to all cases for the present report.

Classification of DNA variants

Reference sequences used were as follows (GeneBank http://www.ncbi.nlm.nih.gov/genbank): *MLH1*, NT_022517 (transcript: NM_000249.2); *MSH2*, NT_022184 (transcript: NM_000251.1); *MSH6*, NT_0221844 (transcript: NM_000179.1); *PMS2*, AC005995.3 (transcript: BC093921.1). Detected DNA variants were checked against published mutations in the following websites: http://www.insight-group.org (LOVD: Leiden Open Variation Database), https://portal.biobase-international.com/hgmd/pro/start.php (Human Gene Mutation Database), Pub Med and http://www.med.mun.ca/MMRvariants.[17] Mutations causing direct stop/nonsense, frameshifts, splice defects and large insertions/deletions were considered deleterious. Missense mutations or small in-frame deletions were subjected to segregation analysis when possible.[18] If a review of the international databases or segregation analyses strongly suggested the variant to be deleterious, the mutations were scored accordingly. The reasons for scoring of each mutation are given in table 1. All other DNA variants were considered part of normal variation or the information available on the variant and family was insufficient for conclusive scoring. These variants were excluded from the report.

Clinical classification

All families in which an MMR mutation (ie, with confirmed Lynch syndrome) had been detected were reclassified according to clinical criteria with the information obtained as of November 2009. Thus the classification does not reflect the starting point with the information at hand at referral, but rather the information obtained after having expanded all Lynch syndrome families and verified all relevant diagnoses for all family members in the medical files or cancer registry. The families were classified according to the AMSI, AMSII or BII criteria. Furthermore, the scoring for BII criteria was specified according to the subgroups given in Umar *et al*[6]: BII_1 (CRC<50 years), BII_2 (synchronic/metachronic cancers), BII_4 (two affected relatives, one <50 years) and/or BII_5 (relatives with HNPCC-associated tumours). For precise definitions of groups as applied, see Umar *et al*.[6] BII_3 includes MSI, which was a selection criterion for DNA analysis, and was not used to categorise mutation-carrying kindreds revealed this way. Also, MSI is a laboratory finding and not a clinical criterion. The combined BII criteria were possibly too close to our inclusion criteria for the total cohort studied, and scoring for the combined BII criteria could not be considered a result.

RESULTS

Sixty-nine different mutations were identified in a total of 129 families. Of these, 31 (45%) were detected in *MSH2*, 19 (27%) in *MSH6*, 15 (22%) in *MLH1*, and four (6%) in *PMS2*. Sixty-five (50%) of the families had a mutation in *MSH2*, 33 (26%) in *MSH6*, 23 (18%) in *MLH1*, and eight (6%) in *PMS2*. The total numbers of mutation carriers were 514, of whom 248 (48%), 146 (28%), 98 (19%) and 22 (4%) had a mutation in *MSH2*, *MSH6*, *MLH1* and *PMS2*, respectively.

Frameshift mutations (n=24) and splice defects (n=18) were the most common aberrations. Other types of mutations were nonsense mutations creating new stop codons (n=13), large genomic (exon) deletions (n=8), in-frame deletions of three nucleotides (n=3) and missense mutations (n=3). There were no indications that the nature of mutations differed between the different genes, and no further statistical analyses based on the nature of the mutation were undertaken.

Table 1 Deleterious mismatch repair (MMR) mutations in the Norwegian population[22 31]

Type of mutation/ gene	Mutation	Effect of mutation (verified or predicted)	Family No	No of mut+	IHC (missing protein)	Inclusion criteria: Amsterd.‡‡	Inclusion criteria: BII §§	Ref.
Frameshift								
MLH1e1	c.39_40dupGA	p.Thr14ArgfsX3	H1855 (D4354)	6	MLH1/PMS2	I & II	1, 2, 4 & 5	LOVD
			T343	1	MLH1/PMS2	I & II	1, 2, 4 & 5	
			D20	1	MLH1/PMS2	I & II	1, 2, 4 & 5	
MLH1e5	c.413delC	p.Pro138LeufsX21	U82517	1	MLH1/PMS2	I & II	1, 2, 4 & 5	¶¶
MLH1e10	c.866_867delAC	p.His289ProfsX16	H836	2	MLH1/PMS2	I & II	1, 2, 4 & 5	LOVD
MLH1e13	c.1411_1414delAAGA	p.Lys471AspfsX19	H892*	3	MLH1/PMS2& MSH2/MSH6	II	1& 5	LOVD
MLH1e16	c.1771dupG	p.Asp591GlyfsX1	U97760	3	MLH1&PMS2	I & II	1, 2, 4 & 5	¶¶
MSH2e2	c.229_230delAG	p.Ser77CysfsX3	H3323	1	MSH2/MSH6	0	1	LOVD
MSH2e4	c.675_678delAGAA	p.Thr225ThrfsX19	D2679	1	MSH2/MSH6	II	1, 4 & 5	¶¶
MSH2e6	c.969_970delTC	p. Gln324ValfsX8	U85816	2	MSH2/MSH6	I & II	1, 2, 4 & 5	¶¶
			D2033	1	MSH2/MSH6	I & II	1, 2, 4 & 5	
MSH2e7	c.1204delC	p.Gln402LysfsX10	H677	1	MSH2/MSH6	II	1, 4 & 5	LOVD
MSH2e10	c.1594dupG	p.Val532GlyfsX3	D139	5	MSH2/MSH6	I & II	1, 2, 4 & 5	LOVD
MSH2e11	c.1705_1706delGA	p.Glu569IlefsX1	D2938	4	MSH2/MSH6	II	1, 2, 4 & 5	LOVD
MSH2e13	c.2120_2122delGCA insCGGGCTAAGAAGTG	p.Cys707SerfsX2	D1570	5	MSH2/MSH6	I & II	1, 2, 4 & 5	¶¶
MSH6e4	c.900dupG	p.Lys301GlufsX11	U88612	2	MSH2/MSH6	II	2, 4 & 5	¶¶
MSH6e4	c.1405delT	p.Tyr469IlefsX11	S254	9	normal†	II	5	¶¶
MSH6e4	c.1943delG	p.Ser648MetfsX5	H2327	3	MSH2/MSH6	0	1, 2 & 4	¶¶
MSH6e4	c.2604delG	p.Met868IlefsX5	D1731	3	MSH6	II	2 & 5	¶¶
MSH6e5	c.3195_3199delCTATA	p.Asn1065LysfsX4	D2115	5	MSH6	II	1, 4 & 5	LOVD
MSH6e5	c.3261dupC	p.Phe1088LeufsX5	H1408	1	MSH6	I & II	1, 4 & 5	LOVD
			S631	7	MSH6	0	1 & 2	
			S1108	4	MSH6	II	1, 2 & 4	
			T02	2	MSH2/MSH6	I & II	1, 2, 4 & 5	
MSH6e5	3261delC	p.Phe1088ProfsX2	D867	8	MSH6	0	2 & 5	LOVD
MSH6e6	c.3514dupA	p.Arg1172LysfsX4	U94618	1	MSH6	II	2	LOVD
MSH6e9	c.3804dupA	p.Cys1269MetfsX5	U61010	7	MSH2/MSH6	0	2 & 5	LOVD
			U98731	2	MSH6	II	2, 4 & 5	
			U1000922	2	MSH6	0	0	
			U1003522	1	MSH6	0	1 & 5	
			D1151	4	MSH6	0	2 & 5	
			S889	1	MSH6	0	2	
MSH6e9	c.3832_3845del14	p.Pro1278_1282delfsX6	U1000116	1	MSH6	II	4 & 5	¶¶
PMS2e7	c.736_741delCCCCCT insTGTGTGTGAAG	p.Pro246CysfsX2	U97751	1	PMS2	0	1 & 5	LOVD
PMS2e14	c.2382dupT	p.Gly795TrpfsX29	T92	3	PMS2	0	2 & 5	¶¶
Splice defect								
MLH1int9	c.790+1G→A	Skipping of exon 9-10	H285	2	ND	0	1, 4 & 5	LOVD
MLH1int9	c.791−2A→G	Splice defect	T04 (S639 & H1547))	8	MLH1/PMS2	I & II	1, 2, 4 & 5	LOVD
MLH1e10	c.793C→T	p.Arg265Cys‡	D490	10	MLH1/PMS2	I & II	1, 2, 4 & 5	LOVD
MLH1e15	c.1731G→C	Skipping of exon 15§	U1001245	3	MLH1/PMS2	I	1, 2, 4 & 5	LOVD (c.1731G→A)
MLH1int15	c.1731+1G→C	Splice defect	D1532	1	MLH1/PMS2	II	1, 2, 4 & 5	LOVD
MSH2e5	c.815C→T	r.(=)+(=; 793_942del)¶	S403	5	MSH2/MSH6	0	2, 4 & 5	LOVD
MSH2int5	c.942+3A→T	r.(=)+(793_942del)¶	H07	5	ND	II	1, 2, 4 & 5	LOVD
			H892*	3	MSH2/MSH6	II	1, 2, 4 & 5	
			H1503 (S551)	4	MSH2/MSH6	0	1, 2, 4 & 5	
			H1598 (S583)	3	MSH2/MSH6	II	1, 2, 4 & 5	
			H2215	4	MSH2/MSH6	I & II	1, 2, 4 & 5	
			H2280	1	MSH2/MSH6	0	1, 2, 4 & 5	
			U101185	1	MSH2/MSH6	II	1, 4 & 5	
			T059	3	MSH2/MSH6	0	1, 4 & 5	
			T073	9	MSH2/MSH6	0	1, 2, 4 & 5	
			D637	3	MSH2/MSH6	0	4 & 5	
			D1211	11	MSH2/MSH6	I & II	1, 2, 4 & 5	
			D4522	2	MSH2/MSH6	0	1, 2 & 5	
MSH2int6	c.1076+1G→A	Skipping of exon 6	H1903	5	MSH2/MSH6	0	1, 4 & 5	LOVD
			D1773	5	MSH2/MSH6	0	2 & 5	
MSH2int7	c.1277−2A→G	r.(=, 1277_1386del)¶	S577	9	MSH2/MSH6	I & II	1, 2 & 4	LOVD

Continued

Table 1 Continued

Type of mutation/ gene	Mutation	Effect of mutation (verified or predicted)	Family No	No of mut+	IHC (missing protein)	Inclusion criteria: Amsterd.‡‡	Inclusion criteria: BII §§	Ref.
			S612	4	MSH2/MSH6	0	1, 2 & 4	
			D671	2	MSH2/MSH6	II	1, 2, 4 & 5	
MSH2int10	c.1661+1G→A	Splice defect	D470	2	MSH2/MSH6	II	1, 2, 4 & 5	LOVD
MSH2e11	c.1759G→C	r.(=, 1662_1759del)§ ¶	S959	2	MSH2/MSH6	0	1, 2 & 4	LOVD
MSH2int11	c.1759+2T→A	Deletion exon 12, 13	D971	1	MSH2/MSH6	I & II	1, 2, 4 & 5	LOVD
MSH2e12	c.1979A→G	r.(=, 1979_2005del)¶	U74987	1	MSH2/MSH6	0	1, 2, 4 & 5	31
MSH2int12	c.2006−1G→C	splice defect	D2013	3	MSH2/MSH6	I & II	1, 2, 4 & 5	LOVD
MSH2int15	c.2634+1G→T	r.(=, 2459_2634del)	H246/275	10	ND	I & II	1, 4 & 5	LOVD
MSH6int7	c.3647−2A→C	r.(=, 3646_3647ins3646+1_3646+492)¶	S819 (U100998&U104021)	10	MSH6	II	1, 2 & 4	LOVD
			D686	7	MSH6	II	0	
PMS2intr5	c.537+1G→T	Splice defect	H3118	2	PMS2	0	1	¶¶
PMS2int9	c.989−1G→T	r.(=)+(989_1144del, 989_1015del)¶	S90	4	normal (MSI)**	0	1, 2 & 4	22
			S335	1	normal (MSI)**	II	1, 2	
			S350	4	PMS2	I & II	1 & 4	
			S1147	2	normal (MSI)**	0	1	
			D3786	5	PMS2	I & II	1, 2, 4 & 5	
Stop codon								
MLH1e2	c.184C→T	p.Gln62X	H321	8	ND	I & II	1, 4 & 5	LOVD
			H480	4	ND	I & II	1, 2, 4 & 5	
			H487	3	MLH1/PMS2	I & II	1, 2, 4 & 5	
			D498	9	MLH1/PMS2	I & II	1, 2, 4 & 5	
			D874	4	MLH1/PMS2	I & II	1, 2, 4 & 5	
			D1704	5	MLH1/PMS2	I & II	1, 2, 4 & 5	
MSH2e1	c.142G→T	p.Glu48X	U1101385	1	MSH2/MSH6	0	1 & 5	LOVD
			U101386	1	MSH2/MSH6	II	1, 4 & 5	
MSH2e1	c.181C→T	p.Gln61X	D3959	3	MSH2/MSH6	II	1 & 5	LOVD
MSH2e2	c.226C→T	p.Gln76X	D271	2	MSH2/MSH6	0	1, 2, 4 & 5	LOVD
MSH2e12	c.1857T→G	p.Tyr619X	D3648	4	MSH2/MSH6	0	1, 2, 4 & 5	LOVD
MSH2e13	c.2038C→T	p.Arg680X	U59124	1	MSH2/MSH6	I	1, 2, 4 & 5	LOVD
			D414	7	MSH2/MSH6	I & II	1, 2, 4 & 5	
MSH2e14	c.2275G→T	p.Gly759X	D1661	4	MSH2/MSH6	0	1, 2, 4 & 5	LOVD
MSH6e3	c.467C→G	p.Ser156X	D1651	1	MSH6	0	5	LOVD
MSH6e4	c.718C→T	p.Arg240X	D4216	2	MSH6	0	1, 2, 4 & 5	LOVD
MSH6e4	c.1444C→T	p.Arg482X	S407	5	MSH2/MSH6	I & II	1, 2 & 4	LOVD
			S1003	10	MSH6	0	5	
MSH6e4	c.1483C→T	p.Arg495X	S363	5	MSH2/MSH6	II	2 & 4	LOVD
MSH6e4	c.2731C→T	p.Arg911X	D1316	7	MSH6	0	1, 2 & 5	LOVD
MSH6e9	c.3991C→T	p.Arg1331X	H1522	5	ND	0	1, 2 & 4	LOVD
			D1826	10	MSH6	0	5	
Exon deletion								
MLH1	c.546-?_790+?del	del exon 7−9	S499 (H1102)	11	MLH1/PMS2	I & II	1, 2, 4 & 5	LOVD
			D2020	4	MLH1/PMS2	I & II	1, 2, 4 & 5	
MLH1	c.1732-?_1896+?del	del exon 16	H2094	1	ND	I	1, 4 & 5	LOVD
MSH2	c.1-?_366+?del	del exon 1−2	S541	2	MSH2/MSH6	0	1, 2 & 4	LOVD
MSH2	c.1-?_1076+?del	del exon 1−6	H592	3	MSH2/MSH6	0	1, 2, 4 & 5	LOVD
			D1718	4	MSH2/MSH6	0	1, 2, 4 & 5	
MSH2	c.1-?_1276+?del	del exon 1−7	U81431	2	MSH2/MSH6	I & II	1, 2, 4 & 5	LOVD
MSH2	c.1-?_1661+?del	del exon 1-10	D3824	3	MSH2/MSH6	0	1 & 5	LOVD
MSH2	c.212-?_1276+?del	del exon 2-7	H346	3	ND	I & II	1, 2, 4 & 5	LOVD
			H496	1	MSH2/MSH6	II	1, 4 & 5	
			H1110 (S604)	9	MSH2/MSH6	II	1, 2, 4 & 5	
			S81	5	MSH2/MSH6	I & II	1, 4 & 5	
			S281(U1002732)	8	MSH2/MSH6	I & II	1, 2, 4 & 5	
			S649	6	MSH2/MSH6	II	1, 2 & 4	
			D2107	7	MSH2/MSH6	I & II	1, 2, 4 & 5	
MSH2	c.367-?_645+?del	del exon 3	H400	4	MSH2/MSH6	I & II	1, 4 & 5	LOVD
In-frame deletion								
MSH2e3	c.571_573delCTC	p.Leu191del	H1294	1	MSH2/MSH6	I & II	1, 2, 4 & 5	¶¶
			H2544	1	MSH2/MSH6	0	1 & 5	
			H3517	1	ND	0	4	

Continued

Table 1 Continued

Type of mutation/ gene	Mutation	Effect of mutation (verified or predicted)	Family No	No of mut+	IHC (missing protein)	Inclusion criteria: Amsterd.‡‡	Inclusion criteria: BII §§	Ref.
			U90087	1	MSH2/MSH6	II	1, 2, 4 & 5	
			U1000173	2	MSH2/MSH6	II	1, 4 & 5	
			T382	3	MSH2/MSH6	0	1, 4 & 5	
MSH2e12	c.1786_1788delAAT	p.Asn596del	D554	19	MSH2/MSH6	I & II	1, 2, 4 & 5	LOVD
			D853	5	MSH2/MSH6	I & II	1, 2, 4 & 5	
			D3618	5	MSH2/MSH6	I & II	1, 2, 4 & 5	
			D3667	3	MSH2/MSH6	I & II	1, 2, 4 & 5	
			D3707	7	MSH2/MSH6	I & II	1, 2, 4 & 5	
			D4202	2	MSH2/MSH6	II	1, 2, 4 & 5	
MSH6e4	c.2302_2304delCCT	p.Pro768del	H801	2	MSH2/MSH6	0	1, 2, 4 & 5	LOVD
		4/14	H2160	5	ND	0	2, 4 & 5	
			S149	5	MSH2/MSH6	0	1 & 5	
			S647	3	MSH2/MSH6	II	2	
Missense								
MLH1e3	c.245C→T	p.Thr82Ile	S420††	6	normal (MSI)**	II	1, 4 & 5	LOVD
MLH1e16	c.1823C→A	p.Ala608Asp	S581††	2	MLH1/PMS2	II	1, 2 & 4	LOVD
MSH6e4	c.2906A→G	p.Tyr969Cys	D2955††	6	MSH6	I & II	1, 2, 4 & 5	LOVD

*Two pathogenic mutations in two branches in the same family.
†No indications from IHC or microsatellite instability.
‡Reported to affect splicing and stability.
§Last nucleotide in exon; reported to cause skipping of exon.
¶Shown in present study to give aberrant splicing.
**Normal protein expression, but microsatellite instability.
††Cosegregation with disease.
‡‡Amsterdam I and/or Amsterdam II.
§§Bethesda II (revised), see text for details.
¶¶Not found to be reported in databases.
IHC, immunohistochemical analysis; LOVD, Leiden Open Variation Database (http://www.insight-group.org/mutations/); mut+, mutation carriers; ND, not done.

Details on the prevalence of each mutation are given in table 1. Forty-nine of the 69 mutations were exclusively found in one family. There were no mutations that were frequent enough to have a significant effect on the distributions observed between the different genes. The most recurrent mutation, *MSH2* c.942+3A→T, was found in 12 apparently unrelated families (49 people) from different geographical locations and has been described in other populations.[19–21] It has possibly been introduced more than once into our population. The majority of the remaining recurrent mutations could be traced to confined geographical areas and were considered to be branches from a common origin (founder mutations). Families with deleterious *PMS2* mutations were limited. One single mutation (c.989−1G→T)[22] accounted for the majority of *PMS2* mutation-carrying kindreds, all from the same (small) area. Average numbers of demonstrated mutation carriers per family were similar for all mutations; details are shown in table 2.

Tumour tissue was available for IHC and MSI analysis for most of the families later demonstrated to have an MMR mutation. The IHC results are shown in table 1. All but five families showed abnormal IHC corresponding to the gene mutated. Tumours from three of five kindreds with the founder *PMS2* splice variant, c.989−1G→T, mentioned above expressed *PMS2* normally but showed MSI (MSI-high). Similarly, tumour tissue from one family with a missense mutation in *MLH1* (c.245C→T (p.Thr82Ile)) showed normal IHC and MSI (MSI-high). Apparently normal expression of *MLH1* indicated by IHC in MSI-high tumours is in agreement with another report.[23] In the family with the c.1405delT, in *MSH6*, the tumour showed normal IHC and was microsatellite stable (MSS).

The mutation-positive families that fulfilled the various clinical criteria when reclassified are detailed in table 2. Thirty-eight per cent of *MSH2* families, 12% of *MSH6* families, 78% of *MLH1* families and 25% of *PMS2* families met the AMSI criteria. Corresponding sensitivity for the AMSII criteria for identifying mutations in the different genes were 62%, 48%, 87% and 38%. Similarly, each of the clinical Bethesda criteria had low sensitivity for identifying *MSH6* and *PMS2* mutations.

DISCUSSION

In this study in which all national activity was compiled, we found that most families with *MLH1* mutations were identified by any of the clinical criteria used. The criteria that included extracolonic cancers (AMSII) identified two out of three *MSH2* mutations, whereas *MSH6* mutations were not identified with reasonable sensitivity by any of the single clinical criteria. As these results were obtained after expanding all mutation-

Table 2 Summary of deleterious variants according to gene, number of mutation carriers and which clinical criteria are fulfilled

Gene	No of families	No of mut+	Fraction of mutations (in %)	No of mut+/family	AMSI	AMSII	BII_1	BII_2	BII_4	BII_5
MSH2	65	248	50	3.82	25 (10.38)	40 (10.62)	61 (10.94)	46 (10.71)	58 (10.89)	57 (10.88)
MSH6	33	146	26	4.42	4 (10.12)	16 (10.48)	15 (10.45)	24 (10.72)	16 (10.48)	22 (10.67)
MLH1	23	98	18	4.26	18 (10.78)	20 (10.87)	23 (11.00)	20 (10.87)	23 (11.00)	23 (11.00)
PMS2	8	22	6	2.75	2 (10.25)	3 (10.38)	7 (10.88)	4 (10.50)	3 (10.38)	3 (10.38)
Total	129	514	100	3.98						

AMSI/II, Amsterdam I/II criteria; BII, Bethesda II criteria; mut+, mutation carriers.

carrying kindreds, we consider them to be maximum estimates. The sensitivities for detecting mutation-positive families upon referral were lower.

The most sensitive single clinical criterion for identifying *MSH6* mutation carriers was the presence of two independent primary cancers (BII_2) (table 2). This information is, however, awaiting detailed validation of diagnoses in the families and may not be easily obtainable when interviewing a family member.

We have recently reported that, when applied to a consecutive series of unselected patients with CRC, the sensitivities of AMSII and BII criteria were as low as 25% and 50%, respectively.[12] Moreover, awareness of hereditary cancer among clinicians involved in diagnosis and treatment of CRC is low, and families actually meeting the criteria may not be identified.[24] These points highlight the challenges associated with using family history for detecting families with MMR mutations. Our combined findings support the suggestion by the Mallorca group to apply IHC and/or MSI analysis to all CRCs to identify MMR mutation carriers.[25] As *MSH6* mutation carriers are likely to develop extracolonic cancers, it may be justified to suggest that all cancer phenotypes associated with Lynch syndrome should be subjected to IHC and/or MSI analysis and subsequent DNA mutation analysis. Until such studies have been performed, we remain cautious when discussing the prevalence of *MSH6* mutations. Correspondingly, the current estimates of penetrance/expression of *MSH6* mutations may be (partly) derived from families fulfilling current clinical criteria.[26 27] These estimates may be misleading, as they may reflect the criteria used to select the families from which the estimates were derived.

MLH1 mutations were less common than assumed from previous reports,[8] and *MSH2* mutations accounted for almost half of all kindreds with a mutation. Despite the fact that the criteria used were insensitive for detecting *MSH6* mutations, the number of *MSH6* mutation-carrying kindreds were higher than *MLH1* mutation-carrying kindreds.

None of the mutations were common enough to affect the distribution significantly, with respect to neither number of mutation-carrying kindreds nor number of mutation-carrying people. Despite the difference in prevalence of mutations in the different genes, the mean number of mutation carriers per family was similar for all the genes.

A Danish study reported a relatively high prevalence of *MSH6* mutations.[20] If this were due to similarities between these neighbouring populations, we would have expected to detect founder mutation(s) in both populations, but this was not the case. The reason for the similar results may be the study designs. Both studies applied wide criteria for IHC and MSI analysis.

The number of carriers of *PMS2* mutations was insufficient for sophisticated statistical analysis. Part of the explanation may be that testing for *PMS2* mutations has not been available for as long as testing for mutations in the other genes. Also, most of the few *PMS2* mutation carriers were included in several branches of one old family. Some of these branches were not identified by IHC, but the tumours displayed MSI (MSI-high). Thus, by performing only IHC and not MSI analysis to prescreen for mutation testing, a few mutations may have been missed. This indicates that MSI analysis is of importance if IHC shows normal expression of all MMR genes. MMR mutations cannot be excluded if neither analysis has been performed. Technical problems involved in DNA *PMS2* mutation analyses are well known.[28–30] Current procedures (including both technical aspects and clinical criteria) may be insufficient to detect *PMS2* mutations, and current estimates of prevalence of *PMS2* mutations may be too low.

IHC was used as a selection criterion for mutation analysis and could not be scored as a result. The result of all efforts to examine selected families without abnormal IHC or MSI for mutations was the identification of a single mutation-carrying family (family S254, *MSH6* c.1405delT). The study was not designed to assess sensitivity of IHC/MSI, and we will not discuss this further. Our impression from other reports is, however, that IHC and MSI analyses are more sensitive than any clinical criteria for identifying kindreds carrying *MSH2* or *MSH6* mutations, in particular,[11 12] and the present report is in keeping with that notion.

In conclusion, we observed that 87% of families with an *MLH1* mutation, 62% with an *MSH2* mutation, but less than half of families with an *MSH6* or *PMS2* mutation were identified by the AMSII criteria. Each of the clinical Bethesda criteria when considered individually also showed low sensitivity. We have, however, previously demonstrated that these criteria were neither sensitive nor specific in an unselected series of CRC cases. Our combined observations indicate that the prevalence of *MSH6* mutations may be higher than currently assumed, and their penetrance and expression may differ from what is currently assumed. These findings are in keeping with the Mallorca guidelines, which recommend that MSI analysis and/or IHC should be performed on all CRCs.[25] In addition, we suggest that such testing should be applied to all incident cancers in the Lynch syndrome tumour spectrum to increase the rate of detection of MMR mutation carriers.

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