Mismatch repair gene mutation spectrum in the Swedish Lynch syndrome population

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Abstract. Lynch syndrome caused by constitutional mismatch-repair defects is one of the most common hereditary cancer syndromes with a high risk for colorectal, endometrial, ovarian and urothelial cancer. Lynch syndrome is caused by mutations in the mismatch repair (MMR) genes i.e., *MLH1*, *MSH2*, *MSH6* and *PMS2*. After 20 years of genetic counseling and genetic testing for Lynch syndrome, we have compiled the mutation spectrum in Sweden with the aim to provide a population-based perspective on the contribution from the different MMR genes, the various types of mutations and the influence from founder mutations. Mutation data were collected on a national basis from all laboratories involved in genetic testing. Mutation analyses were performed using mainly Sanger sequencing and multiplex ligation-dependent probe amplification. A total of 201 unique disease-predisposing MMR gene mutations were identified in 369 Lynch syndrome families. These mutations affected *MLH1* in 40%, *MSH2* in 36%, *MSH6* in 18% and *PMS2* in 6% of the families. A large variety of mutations were identified with splice site mutations being the most common mutation type in *MLH1* and frameshift mutations predominating in *MSH2* and *MSH6*. Large deletions of one or several exons accounted for 21% of the mutations in *MLH1* and *MSH2* and 22% in *PMS2*, but were rare (4%) in *MSH6*. In 66% of the Lynch syndrome families the variants identified were private and the effect from founder mutations was limited and predominantly related to a Finnish founder mutation that accounted for 15% of the families with mutations in *MLH1*. In conclusion, the Swedish Lynch syndrome mutation spectrum is diverse with private MMR gene mutations in two-thirds of the families, has a significant contribution from internationally recognized mutations and a limited effect from founder mutations.

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Introduction

A growing number of disease-predisposing genes are identified and contribute to the complex hereditary colorectal cancer landscape (1). An identifiable cause of cancer predisposition can be demonstrated in 5% of colorectal cancer. Lynch syndrome is the most common hereditary colorectal cancer subtype with an estimated incidence of 1/1,200-1/660 (2). Germline mismatch-repair (MMR) gene mutations give rise to two phenotypic syndromes, i.e., the autosomal dominant, adult-onset Lynch syndrome and the recessive, childhood-onset constitutional mismatch repair deficiency (CMMRD) syndrome (3). Worldwide, more than 1,300 disease-predisposing MMR gene sequence variants have been reported (4). The estimated contribution from the different MMR genes to Lynch syndrome is ~50% *MLH1*, ~40% *MSH2* (5), 7-20% *MSH6* (5-8) and <5% *PMS2* (9). Mutations in the *EPCAM* gene, located upstream of *MSH2*, represent an additional cause that is estimated to contribute to 1-3% of the disease-predisposing mutations (10-12). Founder effects, i.e., mutations that are overrepresented within a geographically or ethically isolated population, have been described in several populations, such as in the different Scandinavian populations (8,13,14) and in the Ashkenazi Jewish population (15-18).

Lynch syndrome is a multi-tumor syndrome and although the highest risks of cancer apply to colorectal, endometrial, ovarian and urinary tract cancer, a number of less common tumor types, such as cancer of the small bowel, brain tumors and skin tumors, have been linked to the syndrome (19). Different disease characteristics have been ascribed to mutations in the different MMR genes with a predominance of colorectal cancer in *MLH1* and *PMS2* mutation carriers, a high risk of extracolonic cancer in *MSH2* mutation carriers and a high risk of gynecologic cancer in *MSH6* mutation carriers. Compared to *MLH1* and *MSH2* mutation carriers, a later age at onset and a reduced penetrance has been described in *MSH6* (20,21) as well as in *PMS2* mutation carriers (9,22). The overall life-time risk of cancer at age 70 is estimated to be 70% (23). Age at onset is on average 20 years earlier than sporadic tumors, although the different tumor types show characteristic peak ages and phenotypes are highly variable, also within Lynch syndrome families. Identification of individuals and families with Lynch syndrome is challenging since family history has suboptimal sensitivity and the syndrome includes a broad tumor spectrum and variable penetrance and age at onset. However, reflex testing for MMR status is increasingly applied in colorectal cancer and is also discussed for endometrial cancer and will increase the likelihood of identifying individuals at increased risk in the future (24).

After 20 years of molecular diagnostics for Lynch syndrome, we compiled mutation data from the Swedish Lynch syndrome population with the aim to define the mutation spectrum, clarify the contribution from the different MMR genes, identify potential founder mutations and contribute to the world-wide data on Lynch syndrome mutations.

Patients and methods

In Sweden, general guidelines for referral of cases with suspected hereditary colorectal cancer to genetic counseling include families/individuals with three or more cases of colorectal cancer or other Lynch syndrome-associated tumors with one family member diagnosed before the age of 50 (in line with the Amsterdam criteria except for the requirement of two first-degree relatives) or a single case of colorectal cancer diagnosed before the age of 50. In addition, clinicians have referred families suspected of Lynch syndrome based on the development of Lynch syndrome-associated tumor types. Reflex testing for MMR defects in colorectal cancer has not been implemented in Swedish pathology laboratories. Targeted analysis for MMR status, typically using four-protein immunohistochemical MMR staining and/or analysis for microsatellite instability (MSI) were applied for pre-screening in most cases.

All individuals/families genetically tested and found to carry MMR gene alterations classified as disease-predisposing genetic variants or a variant of uncertain significance between January 1994 and December 2014 were eligible for the study. Mutation data were collected from the six laboratories and/or oncogenetic clinics at the University hospitals in Umeå, Uppsala, Stockholm, Linköping, Gothenburg and Lund, responsible for genetic diagnostics. The Ethics Committee at Karolinska Institutet approved the study, which followed the tenets of the Declaration of Helsinki. All patients provided oral or written informed consent for genetic diagnostics.

Genetic screening of the proband/affected family member was performed using mainly Sanger DNA sequencing or massive parallel sequencing and the analyses were combined with multiplex ligation-dependent probe amplification (MLPA, P003 and P072; MRC-Holland, Amsterdam, The Netherlands) for the detection of large deletions or duplications.

All variants reported were classified at the nucleotide and protein levels according to the Human Genome Variation Society (HGVS) nomenclature (25). As reference sequences NM_000249, NM_000251, NM_000179 and NM_000535 were used. All sequence variants were then adjusted to the classification used in the InSiGHT database (http://insight-group.org/variants/database/). Variants previously not described in the InSiGHT database were, whenever possible, classified according to the InSiGHT VIC rules (4). Frequency data for certain variants were obtained from the ExAc database using the Alamut software (Alamut Visual, v. 2.7, Interactive Biosoftware, Rouen, France). Variants with a classification of 1 (benign) or 2 (likely benign) are not included (4).

Results

In Sweden, the Lynch syndrome cohort consisted of 369 families with disease-predisposing mutations. These families were found to carry mutations in *MLH1* (n=149), *MSH2* (n=132, including one family with a deletion of the *EPCAM* gene), *MSH6* (n=67) and *PMS2* (n=21) (Table I). The contributions from the different MMR genes were *MLH1* 40%, *MSH2* 36%, *MSH6* 18% and *PMS2* 6% (Fig. 1A). In total, 201 unique alterations were identified, including 48 missense sequence variants, 31 nonsense variants, 43 insertions/deletions, 35 splice site variants and 36 whole exon/exons deletions/duplications. Splice site alterations were the most common mutation type in *MLH1*, frameshift mutations predominated in *MSH2* and *MSH6* and missense variants were most frequent in *PMS2* (Fig. 1B). Copy number variations, i.e., deletions or duplications of whole exon/exons, constituted 21% of the mutations in *MLH1*, 22% in *MSH2* including *EPCAM*, 4% in *MSH6* and 22% in *PMS2* (Fig. 1B).

The Swedish Lynch syndrome sequence variant spectrum is broad with 133 of the 201 (66%) alterations being private, i.e., observed in a single family, 26% observed in 2-3 families, and 18 variants observed in ≥4 families (Table II). In relation to the different genes, private mutations accounted for 46/71 *MLH1* variants, 49/76 *MSH2* variants (including the *EPCAM* deletion), 31/45 *MSH6* variants and 6/9 *PMS2* variants. Of the 201 unique variants, 137 were present in the InSiGHT LOVD with a classification made by an expert panel for 136 of these variants (4) (http://insight-group.org/variants/database/). For the remaining 64 sequence variants, 31 could, based on the predicted protein consequence from the sequence alteration, be classified as class 3-5 according to the five tier system (4).

Alterations observed in 4 or more families (Table II), i.e., recurrent alterations, included the *MLH1* sequence variations c.62C>T, c.131C>T, deletion of exon 6 (c.454-?_545+?del), c.546-2A>G, deletion of exon 11 (c.885-?_1038+?del), deletion of exon 16 (c.1732-?_1896+?del) and the c.2059C>T variation. These variants have previously been recognized in Lynch syndrome families and are classified as disease-predisposing. In *MSH2*, recurrent alterations included deletion of exons 1-6

Table I. List of sequence variants in Swedish families with Lynch syndrome.

Variant no.	Gene	Sequence variant	Type of variant/ comment	Change at protein level	InSiGHT classification	Refs.
1	*MLH1*	c.-7C>T	Other		Class 3	
2	*MLH1*	c.1-?_306+?del	Deletion exons 1-3		Class 5	
3	*MLH1*	c.1-?_306+?del	Deletion exons 1-3		Class 5	
4	*MLH1*	c.1-?_306+?del	Deletion exons 1-3		Class 5	
5	*MLH1*	c.1-?_1731+?del	Deletion exons 1-15		Class 5	(26)
6	*MLH1*	c.1-?_2271+?del	Whole gene deletion		Class 5	
7	*MLH1*	c.1-?_2271+?del	Whole gene deletion		Class 5	
8	*MLH1*	c.1-?_2271+?del	Whole gene deletion		Class 5	
9	*MLH1*	c.19G>T	Missense	p.(Val7Phe)		
10	*MLH1*	c.62C>T	Missense	p.(Ala21Val)	Class 4	(27)
11	*MLH1*	c.62C>T	Missense	p.(Ala21Val)	Class 4	
12	*MLH1*	c.62C>T	Missense	p.(Ala21Val)	Class 4	
13	*MLH1*	c.62C>T	Missense	p.(Ala21Val)	Class 4	
14	*MLH1*	c.104T>G	Missense	p.(Met35Arg)	Class 5	(26)
15	*MLH1*	c.117-?_207+?del	Deletion exon 2	p.(Cys39*)	Class 5	
16	*MLH1*	c.117-?_207+?del	Deletion exon 2	p.(Cys39*)	Class 5	
17	*MLH1*	c.117-?_207+?del	Deletion exon 2	p.(Cys39*)	Class 5	
18	*MLH1*	c.131C>T	Missense	p.(Ser44Phe)	Class 5	(26)
19	*MLH1*	c.131C>T	Missense	p.(Ser44Phe)	Class 5	(26)
20	*MLH1*	c.131C>T	Missense	p.(Ser44Phe)	Class 5	(26)
21	*MLH1*	c.131C>T	Missense	p.(Ser44Phe)	Class 5	
22	*MLH1*	c.131C>T	Missense	p.(Ser44Phe)	Class 5	
23	*MLH1*	c.199G>A	Missense	p.(Gly67Arg)	Class 5	(26)
24	*MLH1*	c.202dup	Frameshift	p.(Ile68Asnfs*11)	(Class 5)	
25	*MLH1*	c.203T>A	Missense	p.(Ile68Asn)	Class 4	(27)
26	*MLH1*	c.203T>A	Missense	p.(Ile68Asn)	Class 4	
27	*MLH1*	c.208-1G>A	Aberrant splicing		Class 5	
28	*MLH1*	c.208-2A>G	Aberrant splicing		Class 5	(26)
29	*MLH1*	c.298C>T	Nonsense	p.(Arg100*)	Class 5	(26)
30	*MLH1*	c.306+1G>A	Aberrant splicing	p.(Lys70_Glu102del)	Class 4	
31	*MLH1*	c.306+1G>A	Aberrant splicing	p.(Lys70_Glu102del)	Class 4	
32	*MLH1*	c.306+3A>C	Aberrant splicing		Class 3	(26)
33	*MLH1*	c.307-?_1038+?del	Deletion exons 4-11	p.(Ala103Argfs*8)	Class 5	(26)
34	*MLH1*	c.307-?_545+?del	Deletion exons 4-6	p.(Ala103Valfs*9)	Class 5	
35	*MLH1*	c.307-?_677+?del	Deletion exons 4-8		(Class 5)	
36	*MLH1*	c.34G>T	Missense	p.(Asp12Tyr)		
37	*MLH1*	c.350C>T	Missense	p.(Thr117Met)	Class 5	
38	*MLH1*	c.350C>T	Missense	p.(Thr117Met)	Class 5	
39	*MLH1*	c.409G>A	Missense	p.(Ala137Thr)		
40	*MLH1*	c.409G>A	Missense	p.(Ala137Thr)		
41	*MLH1*	c.454-?_545+?del	Deletion exon 6	p.(Glu153Phefs*8)	Class 5	
42	*MLH1*	c.454-?_545+?del	Deletion exon 6	p.(Glu153Phefs*8)	Class 5	
43	*MLH1*	c.454-?_545+?del	Deletion exon 6	p.(Glu153Phefs*8)	Class 5	26)
44	*MLH1*	c.454-?_545+?del	Deletion exon 6	p.(Glu153Phefs*8)	Class 5	
45	*MLH1*	c.454-13A>G	Aberrant splicing		Class 3	(26)
46	*MLH1*	c.454-1G>A	Aberrant splicing	p.(Glu153Phefs*8)	Class 5	
47	*MLH1*	c.454-1G>A	Aberrant splicing	p.(Glu153Phefs*8)	Class 5	(26)
48	*MLH1*	c.546-2A>G	Aberrant splicing	p.(Arg182Serfs*6)	Class 5	(26)
49	*MLH1*	c.546-2A>G	Aberrant splicing	p.(Arg182Serfs*6)	Class 5	
50	*MLH1*	c.546-2A>G	Aberrant splicing	p.(Arg182Serfs*6)	Class 5	

Table I. Continued.

Variant no.	Gene	Sequence variant	Type of variant/ comment	Change at protein level	InSiGHT classification	Refs.
51	*MLH1*	c.546-2A>G	Aberrant splicing	p.(Arg182Serfs*6)	Class 5	
52	*MLH1*	c.546-2A>G	Aberrant splicing	p.(Arg182Serfs*6)	Class 5	
53	*MLH1*	c.546-2A>G	Aberrant splicing	p.(Arg182Serfs*6)	Class 5	
54	*MLH1*	c.546-2A>G	Aberrant splicing	p.(Arg182Serfs*6)	Class 5	
55	*MLH1*	c.546-2A>G	Aberrant splicing	p.(Arg182Serfs*6)	Class 5	
56	*MLH1*	c.546-2A>G	Aberrant splicing	p.(Arg182Serfs*6)	Class 5	
57	*MLH1*	c.546-2A>G	Aberrant splicing	p.(Arg182Serfs*6)	Class 5	
58	*MLH1*	c.546-2A>G	Aberrant splicing	p.(Arg182Serfs*6)	Class 5	
59	*MLH1*	c.546-2A>G	Aberrant splicing	p.(Arg182Serfs*6)	Class 5	
60	*MLH1*	c.588+1del	Aberrant splicing		Class 4	
61	*MLH1*	c.589-?_790+?dup	Duplication exons 8-9			
62	*MLH1*	c.665del	Frameshift	p.(Asn222Metfs*7)	Class 5	(26)
63	*MLH1*	c.665del	Frameshift	p.(Asn222Metfs*7)	Class 5	
64	*MLH1*	c.676C>T	Nonsense	p.(Arg226*)	Class 5	
65	*MLH1*	c.677+1G>T	Aberrant splicing		Class 5	(26)
66	*MLH1*	c.677G>A	Aberrant splicing	p.(Gln197Argfs*8)	Class 5	
67	*MLH1*	c.679_689del	Frameshift	p.(Glu227Asnfs*4)	(Class 5)	
68	*MLH1*	c.790+1G>C	Aberrant splicing	p.(Glu227_Ser295del)	Class 4	
69	*MLH1*	c.790+1G>C	Aberrant splicing	p.(Glu227_Ser295del)	Class 4	
70	*MLH1*	c.793C>T	Aberrant splicing	p.(His264Leufs*2)	Class 5	(26)
71	*MLH1*	c.793C>T	Aberrant splicing	p.(His264Leufs*2)	Class 5	
72	*MLH1*	c.793C>T	Aberrant splicing	p.(His264Leufs*2)	Class 5	
73	*MLH1*	c.885-?_1038+?del	Deletion exon 11	p.(Ser295Argfs*21)	Class 5	(26)
74	*MLH1*	c.885-?_1038+?del	Deletion exon 11	p.(Ser295Argfs*21)	Class 5	
75	*MLH1*	c.885-?_1038+?del	Deletion exon 11	p.(Ser295Argfs*21)	Class 5	
76	*MLH1*	c.885-?_1038+?del	Deletion exon 11	p.(Ser295Argfs*21)	Class 5	
77	*MLH1*	c.885-?_1409+?del	Deletion exons 11-12		(Class 5)	
78	*MLH1*	c.885-?_1409+?del	Deletion exons 11-12		(Class 5)	
79	*MLH1*	c.955G>A	Missense	p.(Glu319Lys)	Class 3	
80	*MLH1*	c.958G>T	Nonsense	p.(Glu320*)	(Class 5)	
81	*MLH1*	c.1050del	Frameshift	p.(Gly351Aspfs*16)	Class 5	
82	*MLH1*	c.1219C>T	Nonsense	p.(Gln407*)	(Class 5)	
83	*MLH1*	c.1225C>T	Nonsense	p.(Gln409*)	Class 5	(26)
84	*MLH1*	c.1309_1310del	Frameshift	p.(Pro437Cysfs*2)	(Class 5)	
85	*MLH1*	c.1379A>C	Missense	p.(Glu460Ala)		
86	*MLH1*	c.1379A>C	Missense	p.(Glu460Ala)		
87	*MLH1*	c.1379A>C	Missense	p.(Glu460Ala)		
88	*MLH1*	c.1459C>T	Nonsense	p.(Arg487*)	Class 5	(26)
89	*MLH1*	c.1559-?_1730+?del	Deletion exons 14-15	p.(Val520Glyfs*7)	Class 5	(26)
90	*MLH1*	c.1559-?_2271+?del	Deletion exons 14-19		Class 5	
91	*MLH1*	c.1564C>T	Missense	p.(Arg522Trp)		
92	*MLH1*	c.1609C>T	Nonsense	p.(Gln537*)	Class 5	
93	*MLH1*	c.1667+2_1667+8delinsATTT	Aberrant splicing		Class 5	
94	*MLH1*	c.1667+2_1667+8delinsATTT	Aberrant splicing		Class 5	
95	*MLH1*	c.1668-1G>T	Aberrant splicing		Class 4	
96	*MLH1*	c.1730C>T	Missense	p.(Ser577Leu)		
97	*MLH1*	c.1730C>T	Missense	p.(Ser577Leu)		
98	*MLH1*	c.1731G>A	Aberrant splicing	p.(Ser556Argfs*14)	Class 5	
99	*MLH1*	c.1732-?_1896+?del	Deletion exon 16	p.(Pro579_Glu633del)	Class 5	(27)
100	*MLH1*	c.1732-?_1896+?del	Deletion exon 16	p.(Pro579_Glu633del)	Class 5	(26)
101	*MLH1*	c.1732-?_1896+?del	Deletion exon 16	p.(Pro579_Glu633del)	Class 5	(26)

Table I. Continued.

Variant no.	Gene	Sequence variant	Type of variant/ comment	Change at protein level	InSiGHT classification	Refs.
102	*MLH1*	c.1732-?_1896+?del	Deletion exon 16	p.(Pro579_Glu633del)	Class 5	(26)
103	*MLH1*	c.1732-?_1896+?del	Deletion exon 16	p.(Pro579_Glu633del)	Class 5	(26)
104	*MLH1*	c.1732-?_1896+?del	Deletion exon 16	p.(Pro579_Glu633del)	Class 5	(26)
105	*MLH1*	c.1732-?_1896+?del	Deletion exon 16	p.(Pro579_Glu633del)	Class 5	(26)
106	*MLH1*	c.1732-?_1896+?del	Deletion exon 16	p.(Pro579_Glu633del)	Class 5	
107	*MLH1*	c.1732-?_1896+?del	Deletion exon 16	p.(Pro579_Glu633del)	Class 5	
108	*MLH1*	c.1732-?_1896+?del	Deletion exon 16	p.(Pro579_Glu633del)	Class 5	
109	*MLH1*	c.1732-?_1896+?del	Deletion exon 16	p.(Pro579_Glu633del)	Class 5	
110	*MLH1*	c.1732-?_1896+?del	Deletion exon 16	p.(Pro579_Glu633del)	Class 5	
111	*MLH1*	c.1732-?_1896+?del	Deletion exon 16	p.(Pro579_Glu633del)	Class 5	
112	*MLH1*	c.1732-?_1896+?del	Deletion exon 16	p.(Pro579_Glu633del)	Class 5	(26)
113	*MLH1*	c.1732-?_1896+?del	Deletion exon 16	p.(Pro579_Glu633del)	Class 5	
114	*MLH1*	c.1732-?_1896+?del	Deletion exon 16	p.(Pro579_Glu633del)	Class 5	
115	*MLH1*	c.1732-?_1896+?del	Deletion exon 16	p.(Pro579_Glu633del)	Class 5	
116	*MLH1*	c.1732-?_1896+?del	Deletion exon 16	p.(Pro579_Glu633del)	Class 5	
117	*MLH1*	c.1732-?_1896+?del	Deletion exon 16	p.(Pro579_Glu633del)	Class 5	
118	*MLH1*	c.1732-?_1896+?del	Deletion exon 16	p.(Pro579_Glu633del)	Class 5	
119	*MLH1*	c.1732-?_1896+?del	Deletion exon 16	p.(Pro579_Glu633del)	Class 5	
120	*MLH1*	c.1732-?_1896+?del	Deletion exon 16	p.(Pro579_Glu633del)	Class 5	
121	*MLH1*	c.1732-?_1896+?del	Deletion exon 16	p.(Pro579_Glu633del)	Class 5	
122	*MLH1*	c.1732-?_2271+?del	Deletion exons 16-19		Class 5	
123	*MLH1*	c.1732-2A>T	Aberrant splicing	p.(Pro579_Glu633del)	Class 5	
124	*MLH1*	c.1769del	Nonsense	p.(Leu590*)	Class 5	(26)
125	*MLH1*	c.1769del	Nonsense	p.(Leu590*)	Class 5	
126	*MLH1*	c.1772_1775del	Frameshift	p.(Asp591Valfs*24)	Class 5	(26)
127	*MLH1*	c.1772_1775del	Frameshift	p.(Asp591Valfs*24)	Class 5	
128	*MLH1*	c.1812dup	Frameshift	p.(Glu605Argfs*5)	Class 5	
129	*MLH1*	c.1852_1854del	Other	p.(Lys618del)	Class 5	(27)
130	*MLH1*	c.1852_1854del	Other	p.(Lys618del)	Class 5	
131	*MLH1*	c.1896+1G>T	Aberrant splicing		Class 4	
132	*MLH1*	c.1939G>A	Missense	p.(Val647Met)	Class 3	
133	*MLH1*	c.1943C>T	Missense	p.(Pro648Leu)	Class 5	
134*	*MLH1*	c.1989G>A	Aberrant splicing		Class 4	
135	*MLH1*	c.2038T>C	Missense	p.(Cys680Arg)	Class 5	
136	*MLH1*	c.2059C>T	Missense	p.(Arg687Trp)	Class 5	(26)
137	*MLH1*	c.2059C>T	Missense	p.(Arg687Trp)	Class 5	
138	*MLH1*	c.2059C>T	Missense	p.(Arg687Trp)	Class 5	
139	*MLH1*	c.2059C>T	Missense	p.(Arg687Trp)	Class 5	(26)
140	*MLH1*	c.2059C>T	Missense	p.(Arg687Trp)	Class 5	
141	*MLH1*	c.2059C>T	Missense	p.(Arg687Trp)	Class 5	
142	*MLH1*	c.2059C>T	Missense	p.(Arg687Trp)	Class 5	
143	*MLH1*	c.2059C>T	Missense	p.(Arg687Trp)	Class 5	
144	*MLH1*	c.2059C>T	Missense	p.(Arg687Trp)	Class 5	
145	*MLH1*	c.2059C>T	Missense	p.(Arg687Trp)	Class 5	
146	*MLH1*	c.2076dup	Nonsense	p.(Glu693*)	(Class 5)	
147	*MLH1*	c.2103+1G>A	Aberrant splicing		Class 5	
148	*MLH1*	c.2104-11_2104-10delinsA	Aberrant splicing		Class 3	(26)
149	*MLH1*	c.2141G>A	Nonsense	p.(Trp714*)	Class 5	
150	*MSH2*	c.1-?_1076+?del	Deletion exons 1-6		Class 5	
151	*MSH2*	c.1-?_1076+?del	Deletion exons 1-6		Class 5	
152	*MSH2*	c.1-?_1076+?del	Deletion exons 1-6		Class 5	

Table I. Continued.

Variant no.	Gene	Sequence variant	Type of variant/ comment	Change at protein level	InSiGHT classification	Refs.
153	*MSH2*	c.1-?_1076+?del	Deletion exons 1-6		Class 5	
154	*MSH2*	c.1-?_1076+?del	Deletion exons 1-6		Class 5	(26)
155	*MSH2*	c.1-?_1076+?del	Deletion exons 1-6		Class 5	(26)
156	*MSH2*	c.1-?_1076+?del	Deletion exons 1-6		Class 5	
157	*MSH2*	c.1-?_1076+?del	Deletion exons 1-6		Class 5	
158	*MSH2*	c.1-?_1076+?del	Deletion exons 1-6		Class 5	
159	*MSH2*	c.1-?_1076+?del	Deletion exons 1-6		Class 5	
160	*MSH2*	c.1-?_1276+?del	Deletion exons 1-7		Class 5	(26)
161	*MSH2*	c.1-?_1276+?del	Deletion exons 1-7		Class 5	
162	*MSH2*	c.1-?_1386+?del	Deletion exons 1-8		Class 5	
163	*MSH2*	c.1-?_1386+?del	Deletion exons 1-8		Class 5	(26)
164	*MSH2*	c.1-?_1386+?del	Deletion exons 1-8		Class 5	
165	*MSH2*	c.1-?_1386+?del	Deletion exons 1-8		Class 5	
166	*MSH2*	c.1-?_1386+?del	Deletion exons 1-8		Class 5	
167	*MSH2*	c.1-?_1386+?del	Deletion exons 1-8		Class 5	
168	*MSH2*	c.1-?_1386+?del	Deletion exons 1-8		Class 5	
169	*MSH2*	c.1-?_1661+?del	Deletion exons 1-11		Class 5	
170	*MSH2*	c.17_20del	Frameshift	p.(Lys6Argfs*57)	(Class 5)	
171	*MSH2*	c.138C>G	Missense	p.(His46Gln)	Class 3	
172	*MSH2*	c.183G>T	Missense	p.(Gln61His)		
173	*MSH2*	c.187del	Nonsense	p.(Val63*)	Class 5	
174	*MSH2*	c.204del	Frameshift	p.(Pro69Argfs*15)	Class 5	(26)
175	*MSH2*	c.212-?_366+?del	Deletion exon 2	p.(Ala72Phefs*9)	Class 5	(26)
176	*MSH2*	c.212-?_366+?del	Deletion exon 2	p.(Ala72Phefs*9)	Class 5	
177	*MSH2*	c.212-?_1276+?del	Deletion exons 2-7	p.(Ala72_Gly426del)	Class 5	
178	*MSH2*	c.366+1G>C	Aberrant splicing	p.(Ala72Phefs*9)	Class 4	
179	*MSH2*	c.416delA	Frameshift	p.(Asn139Metfs*35)	Class 5	
180	*MSH2*	c.499G>C	Missense	p.(Asp167His)	Class 3	
181	*MSH2*	c.508C>T	Nonsense	p.(Gln170*)	Class 5	
182	*MSH2*	c.518_519del	Frameshift	p.(Leu173Argfs*4)	(Class 5)	
183	*MSH2*	c.557A>G	Missense	p.(Asn186Ser)	Class 3	
184	*MSH2*	c.646-?_1076+?del	Deletion exons 4-6	p.(Ile217Glufs*28)	Class 5	
185	*MSH2*	c.646-?_1076+?del	Deletion exons 4-6	p.(Ile217Glufs*28)	Class 5	
186	*MSH2*	c.646-1G>A	Aberrant splicing			
187	*MSH2*	c.793-?_1076+?del	Deletion exons 5-6	p.(Val265Ilefs*29)	Class 5	
188	*MSH2*	c.793-1G>A	Aberrant splicing			
189	*MSH2*	c.811_814del	Frameshift	p.(Ser271Argfs*2)	Class 5	(26)
190	*MSH2*	c.892C>T	Nonsense	p.(Gln298*)	Class 5	(26)
191	*MSH2*	c.942+1G>T	Aberrant splicing		Class 4	
192	*MSH2*	c.942+3A>T	Aberrant splicing	p.(Val265_Gln314del)	Class 5	(26)
193	*MSH2*	c.942+3A>T	Aberrant splicing	p.(Val265_Gln314del)	Class 5	(26)
194	*MSH2*	c.942+3A>T	Aberrant splicing	p.(Val265_Gln314del)	Class 5	
195	*MSH2*	c.942+3A>T	Aberrant splicing	p.(Val265_Gln314del)	Class 5	
196	*MSH2*	c.942+3A>T	Aberrant splicing	p.(Val265_Gln314del)	Class 5	
197	*MSH2*	c.942+3A>T	Aberrant splicing	p.(Val265_Gln314del)	Class 5	
198	*MSH2*	c.942+3A>T	Aberrant splicing	p.(Val265_Gln314del)	Class 5	
199	*MSH2*	c.942G>A	Aberrant splicing	p.(Val265_Gln314del)	Class 5	
200	*MSH2*	c.989T>C	Missense	p.(Leu330Pro)	Class 4	
201	*MSH2*	c.997T>A	Missense	p.(Cys333Ser)		
202	*MSH2*	c.1077-?_1276+?del	Deletion exon 7	p.(Leu360Lysfs*16)	Class 5	
203	*MSH2*	c.1077-?_1276+?del	Deletion exon 7	p.(Leu360Lysfs*16)	Class 5	

Table I. Continued.

Variant no.	Gene	Sequence variant	Type of variant/ comment	Change at protein level	InSiGHT classification	Refs.
204	*MSH2*	c.1077-?_1386+?dup	Duplication exons 7-8		Class 3	(26)
205	*MSH2*	c.1077-?_1386+?dup	Duplication exons 7-8		Class 3	
206	*MSH2*	c.1077-?_1661+?del	Deletion exons 7-10	p.(Arg359_Asn553del)	Class 5	(27)
207	*MSH2*	c.1077-?_1661+?del	Deletion exons 7-10	p.(Arg359_Asn553del)	Class 5	
208	*MSH2*	c.1077-?_1661+?del	Deletion exons 7-10	p.(Arg359_Asn553del)	Class 5	
209	*MSH2*	c.1077-?_1661+?del	Deletion exons 7-10	p.(Arg359_Asn553del)	Class 5	
210	*MSH2*	c.1077-?_1661+?del	Deletion exons 7-10	p.(Arg359_Asn553del)	Class 5	
211	*MSH2*	c.1077-?_1661+?del	Deletion exons 7-10	p.(Arg359_Asn553del)	Class 5	
212	*MSH2*	c.1077-1G>A	Aberrant splicing			
213	*MSH2*	c.1077-1G>A	Aberrant splicing			
214	*MSH2*	c.1097_1098insA	Frameshift	p.(Phe366Leufs*23)	Class 5	
215	*MSH2*	c.1097_1098insA	Frameshift	p.(Phe366Leufs*23)	Class 5	(26)
216	*MSH2*	c.1097_1098insA	Frameshift	p.(Phe366Leufs*23)	Class 5	
217	*MSH2*	c.1147C>T	Nonsense	p.(Arg383*)	Class 5	
218	*MSH2*	c.1147C>T	Nonsense	p.(Arg383*)	Class 5	
219	*MSH2*	c.1162-?_2805+?del	Deletion exons 11-16		(Class 5)	
220	*MSH2*	c.1164C>G	Missense	p.(Asn388Lys)		
221	*MSH2*	c.1165C>T	Nonsense	p.(Arg389*)	Class 5	
222	*MSH2*	c.1165C>T	Nonsense	p.(Arg389*)	Class 5	
223	*MSH2*	c.1204del	Frameshift	p.(Gln402Lysfs*10)	Class 5	
224	*MSH2*	c.1204del	Frameshift	p.(Gln402Lysfs*10)	Class 5	
225	*MSH2*	c.1216C>T	Nonsense	p.(Arg406*)	Class 5	(26)
226	*MSH2*	c.1225C>T	Nonsense	p.(Gln409*)	(Class 5)	
227	*MSH2*	c.1226_1227del	Frameshift	p.(Gln409Argfs*7)	Class 5	(26)
228	*MSH2*	c.1237del	Frameshift	p.(Gln413Asnfs*25)	(Class 5)	
229	*MSH2*	c.1275A>G	Aberrant splicing	p.(=, Ile411_Gly426del)	Class 3	
230	*MSH2*	c.1277-?_1386+?del	Deletion exon 8	p.(Lys427Glyfs*4)	Class 5	(26)
231	*MSH2*	c.1277-?_1386+?del	Deletion exon 8	p.(Lys427Glyfs*4)	Class 5	
232	*MSH2*	c.1373T>G	Nonsense	p.(Leu458*)	Class 5	(26)
233	*MSH2*	c.1387-?_1661+?del	Deletion exons 9-10	p.(Val463Glnfs*7)	Class 5	
234	*MSH2*	c.1447_1448del	Frameshift	p.(Glu483Asnfs*4)	Class 5	(26)
235	*MSH2*	c.1447_1448del	Frameshift	p.(Glu483Asnfs*4)	Class 5	
236	*MSH2*	c.1447G>T	Nonsense	p.(Glu483*)	Class 5	(26)
237	*MSH2*	c.1447G>T	Nonsense	p.(Glu483*)	Class 5	
238**	*MSH2*	c.1484C>T	Missense	p.(Thr495Ile)		
239	*MSH2*	c.1490_1492del	Other	p.(Ile497del)		
240	*MSH2*	c.1520del	Frameshift	p.(Pro507Leufs*19)	(Class 5)	
241	*MSH2*	c.1587del	Frameshift	p.(Glu530Lysfs*13)	Class 5	
242	*MSH2*	c.1587del	Frameshift	p.(Glu530Lysfs*13)	Class 5	
243	*MSH2*	c.1587del	Frameshift	p.(Glu530Lysfs*13)	Class 5	
244	*MSH2*	c.1661+5G>C	Aberrant splicing	p.(Gly504Alafs*3)	Class 3	
245	*MSH2*	c.1662-?_2805+?del	Deletion exons 11-16		Class 5	
246	*MSH2*	c.1703C>G	Missense	p.(Thr568Arg)		
247	*MSH2*	c.1759G>C	Aberrant splicing	p.(Ser554Argfs*11)	Class 5	
248	*MSH2*	c.1777C>T	Nonsense	p.(Gln593*)	Class 5	
249	*MSH2*	c.1777C>T	Nonsense	p.(Gln593*)	Class 5	
250	*MSH2*	c.1786_1788del	Other	p.(Asn596del)	Class 5	
251	*MSH2*	c.1786_1788del	Other	p.(Asn596del)	Class 5	
252	*MSH2*	c.1786_1788del	Other	p.(Asn596del)	Class 5	
253	*MSH2*	c.1786_1788del	Other	p.(Asn596del)	Class 5	
254	*MSH2*	c.1786_1788del	Other	p.(Asn596del)	Class 5	

Table I. Continued.

Variant no.	Gene	Sequence variant	Type of variant/ comment	Change at protein level	InSiGHT classification	Refs.
255	*MSH2*	c.1786_1788del	Other	p.(Asn596del)	Class 5	
256	*MSH2*	c.1807G>A	Missense	p.(Asp603Asn)	Class 3	
257	*MSH2*	c.1858_1859dup	Frameshift	p.(Arg621Tyrfs*15)	Class 5	
258	*MSH2*	c.1881dup	Frameshift	p.(Gly628Argfs*16)	(Class 5)	
259	*MSH2*	c.1906G>C	Missense	p.(Ala636Pro)	Class 5	
260	*MSH2*	c.1906G>C	Missense	p.(Ala636Pro)	Class 5	
261	*MSH2*	c.1906G>C	Missense	p.(Ala636Pro)	Class 5	
262	*MSH2*	c.1943T>A	Missense	p.(Ile648Asn)		
263	*MSH2*	c.1982_1985del	Frameshift	p.(Lys661Argfs*23)	Class 5	
264	*MSH2*	c.1986_1987del	Frameshift	p.(Gln662Hisfs*13)	Class 5	
265	*MSH2*	c.1986del	Frameshift	p.(Met663Cysfs*22)	Class 5	(27)
266	*MSH2*	c.2006-?_2634+?del	Deletion exons 13-15		(Class 5)	(26)
267	*MSH2*	c.2038C>T	Nonsense	p.(Arg680*)	Class 5	(26)
268	*MSH2*	c.2038C>T	Nonsense	p.(Arg680*)	Class 5	
269	*MSH2*	c.2131C>T	Nonsense	p.(Arg711*)	Class 5	(26)
270	*MSH2*	c.2131C>T	Nonsense	p.(Arg711*)	Class 5	
271	*MSH2*	c.2164G>A	Missense	p.(Val722Ile)	Not classified	
272	*MSH2*	c.2228_2231del	Nonsense	p.(Ser743*)	Class 5	(26)
273	*MSH2*	c.2234_2236del	Other	p.(Ile747del)		
274	*MSH2*	c.2234_2236del	Other	p.(Ile747del)		
275	*MSH2*	c.2275G>T	Nonsense	p.(Gly759*)	Class 5	
276	*MSH2*	c.2420C>G	Missense	p.(Thr807Ser)	Class 3	
277	*MSH2*	c.2635-1G>A	Aberrant splicing	p.(Gln879Valfs*12)	Class 4	
278	*MSH2*	c.2635-1G>A	Aberrant splicing	p.(Gln879Valfs*12)	Class 4	
279	*MSH2*	c.2680dup	Frameshift	p.(Met894Asnfs*5)		
280	*MSH2*	c.2680dup	Frameshift	p.(Met894Asnfs*5)		
281	*EPCAM*	c.185-?_945+?del	Deletion exons 3-9			
282	*MSH6*	c.261-?_457+?dup	Duplication exon 2		Class 3	
283	*MSH6*	c.463A>G	Missense	p.(Lys155Glu)		
284*	*MSH6*	c.773T>C	Missense	p.(Ile258Thr)		
285	*MSH6*	c.900dup	Frameshift	p.(Lys301Glufs*11)	(Class 5)	
286	*MSH6*	c.1346T>C	Missense	p.(Leu449Pro)	Class 5	(27)
287	*MSH6*	c.1346T>C	Missense	p.(Leu449Pro)	Class 5	
288	*MSH6*	c.1346T>C	Missense	p.(Leu449Pro)	Class 5	
289	*MSH6*	c.1346T>C	Missense	p.(Leu449Pro)	Class 5	
290	*MSH6*	c.1407T>A	Nonsense	p.(Tyr469*)	(Class 5)	
291	*MSH6*	c.1444C>T	Nonsense	p.(Arg482*)	Class 5	
292	*MSH6*	c.1483C>T	Nonsense	p.(Arg495*)	Class 5	
293	*MSH6*	c.1499dup	Frameshift	p.(His501Thrfs*6)	(Class 5)	
294**	*MSH6*	c.1649del	Frameshift	p.(Ser550Leufs*21)	(Class 5)	
295	*MSH6*	c.1691C>G	Nonsense	p.(Ser564*)	(Class 5)	
296	*MSH6*	c.1691C>G	Nonsense	p.(Ser564*)	(Class 5)	
297	*MSH6*	c.1857A>C	Missense	p.(Glu619Asp)	Class 3	
298	*MSH6*	c.1943del	Frameshift	p.(Ser648Metfs*6)	(Class 5)	
299	*MSH6*	c.2062_2063del	Frameshift	p.(Val688Leufs*9)	Class 5	
300	*MSH6*	c.2194C>T	Nonsense	p.(Arg732*)	Class 5	
301	*MSH6*	c.2299A>T	Missense	p.(Thr767Ser)		
302	*MSH6*	c.2302_2304del	Other	p.(Pro768del)	Class 3	(26)
303	*MSH6*	c.2302_2304del	Other	p.(Pro768del)	Class 3	
304	*MSH6*	c.2302_2304del	Other	p.(Pro768del)	Class 3	
305	*MSH6*	c.2608A>G	Missense	p.(Lys870Glu)		

Table I. Continued.

Variant no.	Gene	Sequence variant	Type of variant/ comment	Change at protein level	InSiGHT classification	Refs.
306	*MSH6*	c.2732G>A	Missense	p.(Arg911Gln)		
307	*MSH6*	c.2732G>A	Missense	p.(Arg911Gln)		
308	*MSH6*	c.2779dup	Frameshift	p.(Ile927Asnfs*8)	(Class 5)	
309	*MSH6*	c.2779dup	Frameshift	p.(Ile927Asnfs*8)	(Class 5)	
310	*MSH6*	c.2780_2781insA	Frameshift	p.(Thr928Tyrfs*7)	(Class 5)	
311	*MSH6*	c.2780_2781insA	Frameshift	p.(Thr928Tyrfs*7)	(Class 5)	
312	*MSH6*	c.2780_2781insA	Frameshift	p.(Thr928Tyrfs*7)	(Class 5)	
313	*MSH6*	c.2780_2781insA	Frameshift	p.(Thr928Tyrfs*7)	(Class 5)	
314	*MSH6*	c.2851_2858del	Frameshift	p.(Leu951Ilefs*12)	Class 5	(26)
315	*MSH6*	c.2851_2858del	Frameshift	p.(Leu951Ilefs*12)	Class 5	
316	*MSH6*	c.2931C>G	Nonsense	p.(Tyr977*)	Class 5	(27)
317	*MSH6*	c.2931C>G	Nonsense	p.(Tyr977*)	Class 5	(27)
318	*MSH6*	c.2931C>G	Nonsense	p.(Tyr977*)	Class 5	
319	*MSH6*	c.2931C>G	Nonsense	p.(Tyr977*)	Class 5	
320	*MSH6*	c.2962C>T	Missense	p.(Arg988Cys)		
321	*MSH6*	c.3053_3054del	Frameshift	p.(Leu1018Hisfs*4)	Class 5	(26)
322	*MSH6*	c.3103C>T	Nonsense	p.(Arg1035*)	Class 5	
323	*MSH6*	c.3173-?_3556+?del	Deletion exons 5-6		(Class 5)	
324	*MSH6*	c.3195_3199del	Frameshift	p.(Asn1065Lysfs*5)	(Class 5)	
325	*MSH6*	c.3226C>T	Missense	p.(Arg1076Cys)	Class 3	
326	*MSH6*	c.3261del	Frameshift	p.(Phe1088Serfs*2)	Class 5	
327	*MSH6*	c.3261del	Frameshift	p.(Phe1088Serfs*2)	Class 5	
328	*MSH6*	c.3261del	Frameshift	p.(Phe1088Serfs*2)	Class 5	
329	*MSH6*	c.3261dup	Frameshift	p.(Phe1088Leufs*5)	Class 5	
330	*MSH6*	c.3268_3274del	Frameshift	p.(Glu1090Lysfs*23)	Class 5	
331	*MSH6*	c.3299C>G	Missense	p.(Thr1100Arg)		
332	*MSH6*	c.3312del	Frameshift	p.(Phe1104Leufs*11)	Class 5	
333	*MSH6*	c.3554_3556+2del	Other	p.(Ser1185_Gly1186delinsCys)		
334	*MSH6*	c.3554_3556+2del	Other	p.(Ser1185_Gly1186delinsCys)		
335	*MSH6*	c.3619_3620del	Frameshift	p.(His1207Phefs*7)	(Class 5)	
336	*MSH6*	c.3647-2A>C	Aberrant splicing	p.(Arg1217Lysfs*13)	Class 5	
337	*MSH6*	c.3674C>T	Missense	p.(Thr1225Met)	Class 3	(26)
338	*MSH6*	c.3674C>T	Missense	p.(Thr1225Met)	Class 3	
339	*MSH6*	c.3801+1del	Aberrant splicing			
340	*MSH6*	c.3848_3850dup	Other	p.(Ile1283dup)		
341	*MSH6*	c.3848_3850dup	Other	p.(Ile1283dup)		
342	*MSH6*	c.3878C>G	Missense	p.(Ala1293Gly)		
343	*MSH6*	c.3974_3983dup	Frameshift	p.(Ser1329Aspfs*15)	(Class 5)	
344	*MSH6*	c.3974_3983dup	Frameshift	p.(Ser1329Aspfs*15)	(Class 5)	
345	*MSH6*	c.3991C>T	Aberrant splicing	p.(Ala1268Glyfs*6)	Class 5	
346	*MSH6*	c.3991C>T	Aberrant splicing	p.(Ala1268Glyfs*6)	Class 5	
347	*MSH6*	c.4001+2T>C	Aberrant splicing	p.(Ala1268Glyfs*6)	Class 5	
348	*MSH6*	c.4001G>A	Missense	p.(Arg1334Gln)	Class 5	
349	*PMS2*	c.1-?_2586+?del	Whole gene deletion		Class 5	
350	*PMS2*	c.24-?_988+?del	Deletion exons 2-9		(Class 5)	
351	*PMS2*	c.24-?_988+?del	Deletion exons 2-9		(Class 5)	
352	*PMS2*	c.24-?_988+?del	Deletion exons 2-9		(Class 5)	
353	*PMS2*	c.24-?_988+?del	Deletion exons 2-9		(Class 5)	
354	*PMS2*	c.686_687del	Frameshift	p.(Ser229Cysfs*19)	(Class 5)	
355	*PMS2*	c.736_741delins11	Frameshift	p.(Pro246Cysfs*3)	Class 5	(26)
356	*PMS2*	c.736_741delins11	Frameshift	p.(Pro246Cysfs*3)	Class 5	(26)

Table I. Continued.

Variant no.	Gene	Sequence variant	Type of variant/ comment	Change at protein level	InSiGHT classification	Refs.
357	*PMS2*	c.736_741delins11	Frameshift	p.(Pro246Cysfs*3)	Class 5	
358	*PMS2*	c.736_741delins11	Frameshift	p.(Pro246Cysfs*3)	Class 5	
359	*PMS2*	c.1437C>G	Missense	p.(His479Gln)	Class 3	
360	*PMS2*	c.1556A>G	Missense	p.(Tyr519Cys)		
361	*PMS2*	c.1559C>T	Missense	p.(Ala520Val)		
362	*PMS2*	c.2113G>A	Missense	p.(Glu705Lys)	Class 3	
363	*PMS2*	c.2113G>A	Missense	p.(Glu705Lys)	Class 3	
364	*PMS2*	c.2113G>A	Missense	p.(Glu705Lys)	Class 3	
365	*PMS2*	c.2113G>A	Missense	p.(Glu705Lys)	Class 3	(26)
366	*PMS2*	c.2113G>A	Missense	p.(Glu705Lys)	Class 3	
367	*PMS2*	c.2113G>A	Missense	p.(Glu705Lys)	Class 3	
368	*PMS2*	c.2113G>A	Missense	p.(Glu705Lys)	Class 3	
369	*PMS2*	c.2520dup	Frameshift	p.(Trp841Leufs*47)	(Class 5)	

Variants marked with * and ** represent variants detected in one individual respectively. Classifications made by the authors are listed in parentheses.

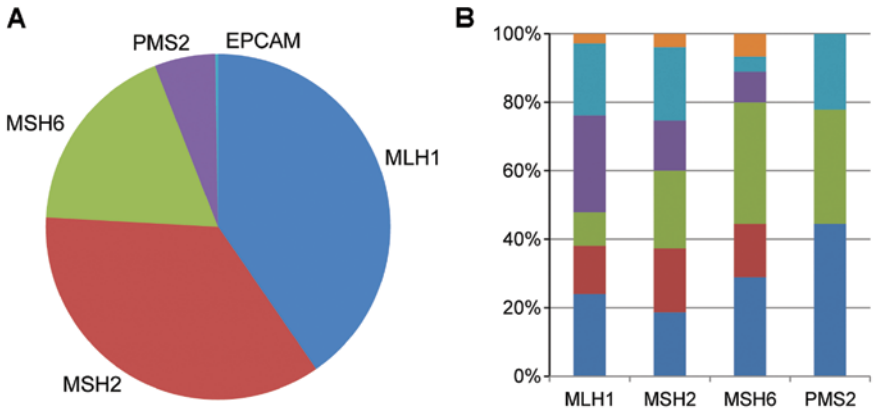

Figure 1. (A) Schematic view of the distribution of the total number of sequence variants in the *MLH1*, *MSH2*, *MSH6*, *PMS2* and *EPCAM* genes from a total of 369 families. (B) Percentage of different sequence variants observed in the *MLH1*, *MSH2*, *MSH6* and *PMS2* genes from a total of 201 variants. The single variant detected in the *EPCAM* gene is not included. The different changes at the amino acid level are shown in different colors: missense variations are shown in blue, nonsense in red, frameshift variants in green, variants affecting splicing in lilac, whole exon deletions/duplications in turquoise and other changes in orange.

(c.1-?_1076+?del), deletion of exons 1-8 (c.1-?_1386+?del), c.942+3A>T, deletion of exons 7-10 (c.1077-?_1661+?del) and the c.1786_1788del. These variants have previously been reported in Lynch syndrome families from different countries and are classified as disease-predisposing. The deletion of *MSH2* exons 1-6 was the most common recurrent variant identified in a total of 10 families. *MSH6* had a high number of private mutations with only the c.1346T>C, c.2780_81insA and the c.2931>G pathological variants identified in ≥4 families. In *PMS2*, the sequence variant of unknown significance c.2113G>A was the most common variant found in 7/21 families. The deletions of exons 2-9 (c.24-?_988+?del) and c.736_741delins11 were both identified in 4 families. All of the recurrent sequence variants in *MHS6* and *PMS2* have previously been reported.

No recurrent mutation suggestive of a Swedish founder mutation was identified. We did, however, recognize a contribution from other Scandinavian founder mutations in the Swedish population. The Finnish founder mutation *MLH1* c.1732-?_1896+?del was found in 6% of the Swedish Lynch syndrome families and constituted 15% of the *MLH1* families. The Danish founder mutation *MLH1* c.1667+2_1667+8delinsATTT was observed in two families.

Discussion

This study is the first compiled data on the Swedish Lynch syndrome cohort and demonstrates mutations in *MLH1* in 40%, *MSH2* in 36%, *MSH6* in 18% and *PMS2* in 6% of the families (Fig. 1A). The Swedish mutation spectrum is

Table II. List of mutations occurring four or more times in Swedish families with Lynch syndrome.

Gene	DNA variant	Protein effect	No. of families	InSiGHT class
MLH1	c.62C>T	p.(Ala21Val)	4	4
	c.131C>T	p.(Ser44Phe)	5	5
	c.454-?_545+? del	p.(Glu153Phefs*8)	4	5
	c.546-2A>G	p.(Arg182Serfs*6)	12	5
	c.855-?_1038+?del	p.(Ser295Argfs*21)	4	5
	c.1732-?_1896+?del	p.(Pro579_Glu633 del)	23	5
	c.2059C>T	p.(Arg687Trp)	10	5
MSH2	c.1-?_1076+?del	p.?	10	5
	c.1?_1386+?del	p.?	7	5
	c.942+3A>T	p.(Val265_Gln314del)	7	5
	c.1077-?_1661+?del	p.(Arg359_Asn553del)	6	5
	c.1786_1788del	p.(Asn596del)	6	5
MSH6	c.1346T>C	p.(Leu449Pro)	4	5
	c.2780_2781insA	p.(Thr928Tyrfs*7)	4	5
	c.2931C>G	p.(Tyr977*)	4	5
PMS2	c.24-?_988+?del	p.?	4	-
	c.736_741delins11	p.(Pro246Cysfs*3)	4	5
	c.2113G>A	p.(Glu795Lys)	7	3

broad with a total of 201 different mutations of which 66% were private and 9% were classified as recurrent, i.e., found in ≥4 families (Table II). The contribution from the different MMR genes is in line with international reports, which are mainly based on Western populations (4). The predominant types of alterations in *MSH2* and *MSH6* were small insertions/deletions and in *MLH1* splice site variants (Fig. 1B). Whole-exon deletions significantly contributed and accounted for 20-22% of the mutations in *MLH1*, *MSH2* and *PMS2*, but were rare (4%) in *MSH6* (Table I, Fig. 1B). Our data support evidence on a significant contribution from whole-exon deletions in *MSH2* and *PMS2*, and demonstrate a higher rate of large deletions than previously reported in *MLH1* (28,29). Of the 201 sequence variants reported, 137 are available in the InSiGHT database, whereas 64 have not previously been reported.

In Sweden, 80% of the population is of Swedish origin and 20% were either born in another country or born in Sweden by two parents from another country. Among non-Swedish ethnic groups, Finns represent the largest group and during recent decades Sweden has received immigrants from a large number of countries with particularly large contributions from Denmark, Norway, Germany, Chile, former Yugoslavia, Iran, Irak, Eritrea, Somalia and Syria. Strong founder effects have been reported in Finland where two *MLH1* mutations account for 63% of the families with Lynch syndrome (13). The Finnish founder mutation *MLH1* c.1732-?_1896+?del, which leads to deletion of *MLH1* exon 16, was identified in 6% of our Lynch syndrome families and constituted 15% of the *MLH1* families, which is in line with the Finnish ancestry in 5% of the Swedish population. Two families in Sweden carried the Danish founder mutation *MLH1* c.1667+2_1667+8delinsATTT (8). Two of the most frequent mutations in the Swedish population, i.e., the *MLH1* c.546-2A>G and *MSH2* c.1-?_1076+?del (deletion of exons 1-6), have been described as founder mutations in the US (30,31). From the mid 1800's until the early 1920's, 1.5 million Swedes migrated to US and it is therefore plausible that this US founder mutation is of Swedish origin. Regarding the deletion of exons 1-6, the common haplotype found in the US was analyzed in two Swedish samples with the same mutation although the results cannot confirm a common ancestry (30). The *MLH1* c.2059C>T pathogenic variant is also common in the Swedish population.

Several recurrent mutations identified in *MSH2*, e.g. the c.1-?_1076+?del, c.942+3A>T and c.1786_1788del have also been reported from Denmark and in Norway (8). Also several of the *MSH6* mutations identified, such as c.1444C>T, c.1483C>T, c.2302_2304del, c.3647-2A>C, c.3991C>T and c.4001+2T>C have also been observed in several families from Norway and/or Denmark and these mutations may be of Scandinavian origin. In *PMS2* the c.736_741delins11 mutations have been reported from Denmark and Norway and the c.2113G>A transition (class 3) has also been identified in families from Norway.

We did not detect any individuals with CMMRD in our cohort. Two families harbored more than one MMR gene variants. Both of these families did fulfill the Amsterdam criteria. One family of Arabic origin had a *MLH1* c.1989G>A (class 4) variant that affects splicing and a concomitant *MSH6* c.773T>C variant, which has not been reported in the ExAc database. Another family had a *MSH6* c.1649del frameshift variant and a concomitant *MSH2* c.1484C>T variant of unknown significance according to ClinVar. In these families, the *MSH6* c.773T>C and the *MSH2* c.1484C>T variants may represent benign variants.

Identification of individuals with Lynch syndrome is cost effective with significant positive effects on morbidity

and mortality from colorectal cancer (32). In Sweden, Lynch syndrome diagnostics have traditionally been based on individual or physician suspicion of hereditary cancer in which case families have been referred for genetic counseling followed by genetic diagnostics. In total, 369 Lynch syndrome families have been identified. Assuming a carrier frequency in the lower range (1/1,200), at least 8,000 individuals would be estimated to be mutation carriers in the Swedish population of 9.8 million. Though the absolute number of mutation carriers in Sweden is not known, it can be estimated that no more than one-quarter of the mutation carriers have at present been identified. Comparison is also possible with our neighboring country Denmark where Lynch syndrome families are registered on a national basis. Denmark has, relative to the size of the population, identified an additional 60% of Lynch syndrome families (data not shown).

In summary, the Swedish Lynch syndrome cohort with 369 families carries 201 unique alterations, of which 64 have not been previously reported. The mutation spectrum shows the expected contribution from the different MMR genes, underscores the roles of *MSH6* and *PMS2*, which caused 18% and 6% of the mutations in the families, respectively. The cohort reveals a higher contribution from large deletion in *MLH1* than previously reported. An overlap with mutations identified in the other Nordic countries is identified and our data suggest that US founder mutations in *MLH1* and *MSH2* may be of Scandinavian origin.

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