

Mutations associated with HNPCC predisposition – Update of ICG-HNPCC/INSiGHT mutation database

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Abstract. In 1994, the International Collaborative Group on Hereditary Nonpolyposis Colorectal Cancer (ICG-HNPCC) established an international database of mutations identified in families with Lynch (HNPCC) syndrome. The data are publicly available at <http://www.nfdht.nl>. The information stored in the database was systematically analyzed in 1997, and at that time, 126 different predisposing mutations were reported affecting the DNA mismatch repair genes MSH2 and MLH1 and occurring in 202 families. In 2003, the ICG-HNPCC and the Leeds Castle Polyposis Group (LCPG) merged into a new group, INSiGHT (International Society for Gastrointestinal Hereditary Tumors). The present update of the database of DNA mismatch repair gene mutations of INSiGHT includes 448 mutations that primarily involve MLH1 (50%), MSH2 (39%), and MSH6 (7%) and occur in 748 families from different parts of the world.

Keywords: Hereditary nonpolyposis colorectal cancer, MLH1, MLH3, MSH2, MSH6, PMS1, PMS2

1. Introduction

Hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) is a multi-organ cancer syndrome that is associated with heritable defects in DNA mismatch repair (MMR). Mutations in four MMR genes, MSH2, MLH1, MSH6, and PMS2, have been convincingly linked to HNPCC susceptibility (Table 1). Two further genes, MLH3 and PMS1, have also been implicated in HNPCC predisposition, but their roles are less clear. Germline mutations in MMR genes give rise to characteristic clinical phenotypes, including a defined spectrum of cancers that show microsatellite instability (MSI) as a manifestation of MMR deficiency [22]. Apart from MMR genes, HNPCC-like phe-

notypes in occasional families may be due to germline mutations in a variety of other genes, including the APC gene variants I1307K and E1317Q [9], TGF β RII [21], CHD1 [30], EXO1 [44], and MYH [2]. With rare exceptions (EXO1), tumors from such families do not display MSI. As HNPCC is traditionally viewed as a MMR deficiency syndrome, the latter genes are not included in the present review or in the mutation database.

Information of mutations and polymorphisms detected in the MMR genes listed in Table 1 are available in the database maintained by the previous International Collaborative Group on Hereditary Nonpolyposis Colorectal Cancer (ICG-HNPCC), current INSiGHT. Since the establishment of the database in 1994, the data deposited in it (available at <http://www.nfdht.nl>) have steadily increased from 126 mutations in the original report [28] to 448 mutations at present. Individual investigators have contributed most of the information (an electronic mutation submission form is available at the Internet address given above); the rest has been retrieved from published literature reports. While a ma-

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Table 1
HNPCC-associated human MMR genes

Gene	Chromosomal location	No. of exons
MSH2	2p21	16
MLH1	3p21-23	19
MSH6	2p21	10
PMS2	7p22	15
MLH3	14q24.3	12
PMS1	2q31-q33	Not determined

Table 2

Number of mutations considered pathogenic that have been deposited in the ICG-HNPCC/INSiGHT mutation database (<http://www.nfdht.nl>) as of July 31st, 2003

Gene	Number of mutations (%)	
MSH2	175	(39%)
MLH1	225	(50%)
MSH6	32	(7%)
PMS2	5	(1%)
MLH3	16	(3%)
PMS1	1	(< 1%)
Total	448	(100%)

majority of initial submissions focused on unequivocally pathogenic changes (nonsense or frameshift mutations) that were present in large HNPCC families, an increasing proportion of mutations currently deposited consist of single amino acid substitutions (missense changes) that occur in small or atypical families. Although the database information is not intended for diagnostic use, it provides useful background data for both clinicians and basic researchers, and proper assignment of pathogenicity is therefore an important challenge for both the depositors and users of the database.

2. Mutations and polymorphisms

As of July 31, 2003 the database contains information of 448 germline alterations that are likely to be pathogenic and are referred to as mutations (Table 2). Of these, 50% affect MLH1, 39% MSH2, and 7% MSH6, while the share of the remaining genes is less than 5%. According to available reports, germline mutations in PMS2 are rare in classical HNPCC families [20] and are primarily associated with the Turcot syndrome variant [4,14,23]. The role of MLH3 as an HNPCC susceptibility gene relies on two population-based reports, one based on Dutch [45] and the other one on Swedish [19] HNPCC or colon cancer families. Among a total of 16 germline variants that were absent in the normal population, only 2 were frameshift mutations, whereas the remaining ones were of the missense type with uncertain pathogenic significance. Moreover,

while some MLH3 mutations showed co-segregation with disease in the families studied, others did not [19]. More data are clearly needed for a reliable evaluation of the significance of MLH3 in HNPCC predisposition. Finally, a single PMS1 germline mutation has been reported [25], a nonsense mutation occurring in a patient with colon and other cancers from a family meeting the Amsterdam I criteria for HNPCC [34]. However, re-examination of the same family revealed an additional mutation in MSH2 (large deletion encompassing exons 1–7), and only the MSH2 mutation co-segregated with colon cancer [20]. Thus, there is presently no convincing evidence that germline mutation of PMS1 causes predisposition to HNPCC-type cancers; however, a possible role of PMS1 as a susceptibility gene for some other cancers cannot be excluded [20].

The 448 MMR gene alterations detected in the germline to date occur in 748 families from different parts of the world (Table 3). While most MSH2- and MLH1-associated families meet the stringent Amsterdam I criteria for HNPCC, mutations in the remaining genes are mainly associated with Amsterdam I-negative families.

In addition to sequence changes considered pathogenic, the database also contains information of apparently nonpathogenic sequence variants and polymorphisms. Emphasis is given on those variants for which allele frequencies in the population have been determined. The database currently lists 108 non-pathogenic alterations (28 for MSH2, 27 for MLH1, 43 for MSH6, 5 for PMS2, and 5 for MLH3).

It may be difficult to determine the pathogenicity of missense and in-frame alterations as well as some splice changes. A few changes have been reported both as pathogenic mutations and as innocuous sequence variants to the database (G322D in MSH2, and V326A and K618A in MLH1). For missense mutations, the most important theoretical criteria in support of pathogenicity include nonconservative nature of the amino acid change, evolutionary conservation of the amino acid, absence in the normal population, cosegregation with disease, and association with MSI or lack of specific protein in tumor tissue. Most missense mutations of MSH2 and MLH1 meet one or several of these criteria. For mutations for which functional data are available [6, 11,12,16,27,31,33] there is generally a good agreement between theoretical predictions and functional classification.

Table 3
Fulfilment (+ or –) of Amsterdam I criteria among families associated with MMR gene mutations

Gene	Number of associated families			Total (%)
	Amsterdam I+	Amsterdam I–	Not specified	
MSH2	143	92	50	285 (38%)
MLH1	253	71	77	401 (54%)
MSH6	7	26	4	37 (5%)
PMS2	1	4	0	5 (< 1%)
MLH3	0	19	0	19 (3%)
PMS1	1	0	0	1 (< 1%)
Total	405 (54%)	212 (28%)	131 (18%)	748 (100%)

3. Sites and types of mutation

The exonic distribution of germline mutations along the MSH2, MLH1, and MSH6 genes is shown in Fig. 1. As a rule, the mutations are scattered throughout the genes, with some hot spot areas in exons 12 and 3 for MSH2, exons 16 and 1 for MLH1, and exon 4 (the largest exon) for MSH6. Apart from exons and intron/exon borders, a few point mutations have been identified in the promoter regions of MSH2 [32] and MLH1 [10], and at least for some, functional data are available to support their pathogenicity.

A majority of HNPCC-associated MMR gene alterations are frameshift or nonsense mutations that lead to truncated proteins (Fig. 2). For MLH1 and MSH6, missense mutations are also common constituting more than one-third of all mutations in these genes. Most alterations observed in MLH3 in HNPCC- or HNPCC-like families are of the missense type. Pathogenicity of HNPCC-associated mutations typically results from the loss of important interaction domains (truncating mutations) or changes in the local structure or conformation (missense mutations) that impair the ability of the proteins to interact with their partners or other components of the MMR pathway, or otherwise properly accomplish MMR or other functions these proteins are responsible for [7,15].

4. Unique vs. recurrent mutations and the role of ethnicity

Most mutations reported to the database (362/448, 81%) are unique, i.e. specific to each family. This observation together with the fact that the mutations are distributed throughout the genes (see above) means that for the detection of a predisposing mutation in a new family, the entire genes generally need to be screened. However, a few recurrent mutations are known that occur in HNPCC families all over the world (Table 4).

Yet, the overall share of the three most common MSH2 and MLH1 mutations, if counted together, is only 13% of all mutation-positive families (95/748).

Typically, recurrent mutations arise de novo in different background haplotypes. However, some are associated with shared haplotypes between different families suggesting a common ancestral origin (founding mutations). Founding mutations are characteristic of isolated populations, such as the Ashkenazi Jews or Finns, and provide important targets for mutation diagnostics in these particular populations. Thus, a missense mutation (G>C at nucleotide 1906 in exon 12 of MSH2, designated as A636P) accounts for one-third of HNPCC in Ashkenazi Jewish families that fulfil the Amsterdam criteria I [34] or II [37]; it is infrequent or absent in other populations [8]. Similarly, two founding mutations in MLH1, a 3.5-kb genomic deletion affecting exon 16 (“Mutation 1”) and a splice acceptor site mutation (g>a at 454-1) of exon 6 (“Mutation 2”), together account for 63% of all mutations identified in Finnish HNPCC families [24,26]. So far, neither of these two mutations has been shown to occur in families of non-Finnish origin. Recently, a genomic deletion encompassing MSH2 exons 1–6 was found to represent a founder mutation among North American kindreds, accounting for 10% of the studied cohort [39]. In analogy to the Finnish MLH1 “Mutation 1”, breakpoint analysis suggested the origin of the MSH2 deletion as a result of Alu-mediated recombination. Finally, some mutations may arise de novo in some populations and represent founding mutations in others. For example, the recurrent MSH2 intron 5 splice site mutation (Table 4) has multiple origins based on haplotype analysis with MSH2-linked markers, but is a founding mutation in Newfoundland, Canada [5].

5. Genotype-phenotype correlations

Based on available information, classical HNPCC is mostly associated with mutations in MSH2 or MLH1

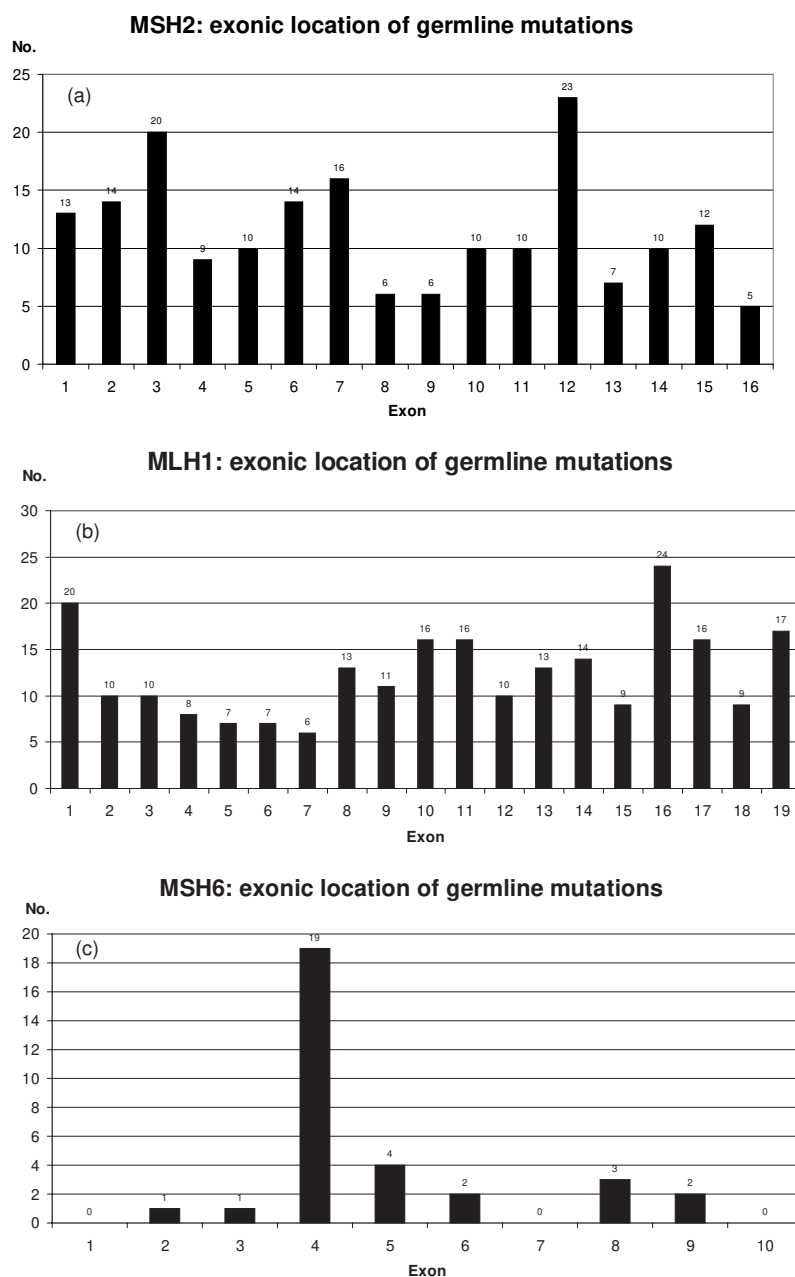


Fig. 1. (a-c). Distribution of germline mutations in different exons of the MMR genes.

(often referred to as “major” MMR genes) especially in families that meet the Amsterdam criteria, and mutations in these genes or MSH6 in families that do not meet these criteria (Table 3). In the Muir-Torre variant, which is characterized by the occurrence of sebaceous gland tumors together with HNPCC-type malignancy, MSH2 is primarily affected (ref. 18 and <http://www.nfdht.nl>). The genetic basis of Turcot syn-

drome (featured by the coexistence of primary brain tumor and colorectal adenoma or carcinoma) is heterogeneous and involves the MMR genes MLH1 and PMS2 or the APC gene (ref. 14 and <http://www.nfdht.nl>).

Consistent with the observed vertical transmission of cancer susceptibility in HNPCC families (autosomal dominant inheritance), one copy of a MMR gene is mutant and the other one wild type in the germline of any

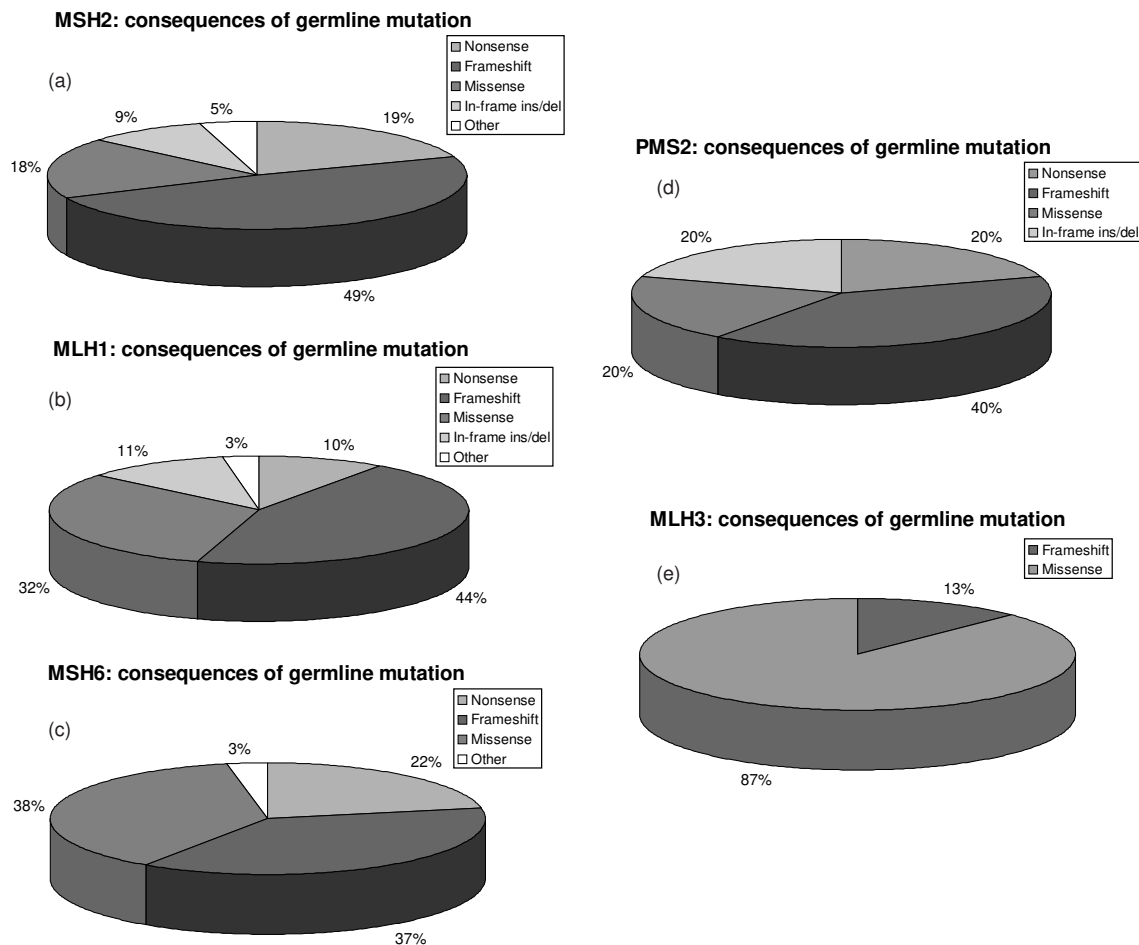


Fig. 2. (a-e). Types of germline mutations in HNPCC-associated MMR genes.

individual who has inherited the susceptibility. Rare instances of homozygosity [29,38,40,41] or compound heterozygosity [4,13] for MMR gene mutations have been described. Homozygosity for MLH1 or MSH2 mutations is associated with atypical tumor spectrum (hematological malignancy and neurofibromatosis type 1) as well as constitutional mutator phenotype. An individual heterozygous for two missense mutations in MLH1 developed breast cancer at age 35 combined with constitutional mutator phenotype, but showed no colon cancer at the time of the latest observation (45 years) [13]. Another individual heterozygous for two truncating PMS2 mutations had a Turcot phenotype and microsatellite instability in both normal and tumor tissue [4]. Remarkably, the parents, who both carried one PMS2 mutation, were clinically unaffected, suggesting recessive inheritance in this exceptional case.

Table 5 summarizes the characteristic clinical features and MSI status associated with mutations in the

different MMR genes. Families with MSH2 or MLH1 mutations mainly display typical HNPCC and high-degree of MSI in tumors (for “grading” of MSI, see ref. 3). Furthermore, compared to carriers of MLH1 mutations, MSH2 mutation carriers appear to be at higher risk for extracolonic cancers [36] and their lifetime risk of developing any cancer may be higher [35]. MSH6 mutations are often associated with atypical HNPCC (characterized by small family size, atypical tumor spectrum, late age at onset, and reduced penetrance) with high- or low-degree MSI in tumors [1,17,42,43]. Moreover, families with MSH6 mutations have a higher risk of developing endometrial cancer than families with MSH2 or MLH1 mutations [42]. Carriers of PMS2 mutations usually show features of Turcot syndrome and – as mentioned [4] – the penetrance of mutations may vary. Finally, as a rule, families with MLH3 mutations do not fulfil the Amsterdam criteria,

Table 4
The most common recurrent mutations that occur in HNPCC families worldwide (irrespective of ethnic origin)

Mutation	Consequence	No. of associated families
MSH2		
a>t at nt. 942 + 3 (intron 5)	Deletion of exon 5 (in frame)	42
Del AAT at nt. 1786, codon 596 (exon 12)	Deletion of an amino acid (Asn) (in frame)	8
C>T at nt. 2038, codon 680 (exon 13)	Arg>stop (nonsense)	7
MLH1		
Del AAG at nt. 1846, codon 616 (exon 16)	Deletion of an amino acid (Lys) (in frame)	20
C>T at nt. 350, codon 117 (exon 4)	Thr>Met (missense)	10
AA>GC at nt. 1852, codon 618 (exon 16)	Lys>Ala missense	8

Table 5
Clinical and MSI phenotypes associated with germline mutations in MMR genes

Gene	Clinical phenotype	MSI phenotype
MSH2	Mostly typical HNPCC, also a major Muir-Torre gene.	MSI-High
MLH1	Mostly typical HNPCC.	MSI-High
MSH6	Typical (minority) or atypical HNPCC (majority) with frequent endometrial cancers.	MSI-High or MSI-Low
PMS2	Turcot syndrome.	MSI-High
MLH3	Atypical HNPCC.	Variable (from MSI-High to no MSI)

and MSI in tumors varies from high-degree MSI [45] to stable microsatellite sequences [19].

6. Conclusion

During its almost a decade of existence, the ICG-HNPCC/INSIGHT mutation database has established its position as an important repository of information for both clinicians and researchers. Knowledge of mutations predisposing to HNPCC provides the basis for studies of mutation mechanisms and consequences, the design of diagnostic strategies, and increased understanding of genotype-phenotype correlations.

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