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## HUMAN GENOME EPIDEMIOLOGY (HuGE) REVIEWS

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### Mismatch Repair Genes *hMLH1* and *hMSH2* and Colorectal Cancer: A HuGE Review

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Evidence to support a role for the mismatch repair genes human mutL homolog 1 (*hMLH1*) and human mutS homolog 2 (*hMSH2*) in the etiology of colorectal cancer has come from linkage analysis, segregation studies, and molecular biologic analysis. More recently, carriers of potentially pathogenic mutations in the *hMLH1/hMSH2* genes have consistently been shown to be at a greatly increased risk of developing colorectal cancer compared with the general population. When considered together, the available evidence shows a strong, consistent, and biologically plausible association between mismatch repair gene mutations and colorectal cancer. The penetrance of mutations in *hMLH1/hMSH2* is incomplete and is significantly higher in males (approximately 80%) than in females (approximately 40%). To date, evidence for gene-gene or gene-environment interactions is limited, although preliminary studies have revealed a number of avenues that merit exploration. Population screening for mutation carriers is not currently a feasible option, and mutation analysis remains restricted to either relatives of mutation carriers or colorectal cancer cases selected on the basis of phenotype.

colorectal neoplasms; epidemiology; genetic screening; germ-line mutation; *hMLH1*; *hMSH2*; penetrance; survival

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Abbreviations: *hMLH1*, human mutL homolog 1; *hMSH2*, human mutS homolog 2; HNPCC, hereditary nonpolyposis colorectal cancer; MSI, microsatellite instability.

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#### INTRODUCTION

The mismatch repair genes human mutL homolog 1 (*hMLH1*) and human mutS homolog 2 (*hMSH2*) are integral components of the DNA mismatch repair pathway. So far, over 200 allelic variants have been identified for each gene, and the majority of these have been reported to be patho-

genic in terms of colorectal cancer. The primary objectives of this review are to describe what is known about *hMLH1* and *hMSH2* and their variants in different populations and to examine the evidence implicating these genes as risk factors in the development of colorectal cancer. Relevant Internet sites are listed in appendix 1.

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**TABLE 1. Commonly observed pathogenic mutations in persons with colorectal cancer**

Exon	Nucleotide change	DNA change	No. of kindreds	Founder effect?	No. from founder population
<i>hMLH1</i> *					
Exon 16		3.5-kilobase deletion	63	Finland	62
Exon 16	Deletion of AAG at nucleotide 1846	In-frame deletion in lysine codon 616	21		
IVS* 5	g-a at nucleotide 4541	Out-of-frame deletion in exon 6 codon 152182	18	Finland	15
Exon 16	AA-GC at nucleotide 1852	Lys618Ala	15		
Exon 4	C-T at nucleotide 350	Thr117Met	12		
Exon 19	G-A at nucleotide 2146	Val716Met	12		
Exon 13	Insertion of C at nucleotide 1490	Frameshift from codon 497	10		
Exon 4	T-G at nucleotide 320	Ile107Arg	7	Finland	7
Exon 13	C-T at nucleotide 1459	Arg487STOP	7		
Exon 17	C-T at nucleotide 1975	Arg659STOP	7		
Exon 19	G-A at nucleotide 2141	Trp714STOP	6		
Exon 8	C-T at nucleotide 676	Arg226STOP	6		
Exon 2	G-A at nucleotide 199	Gly67Arg	5		
Exon 2	C-T at nucleotide 184	Gln62STOP	5		
IVS 14	4-base-pair insertion/3-base-pair deletion at nucleotide 1667+2	Silencing of allele	5	Denmark	4
<i>hMSH2</i> *					
IVS 5	a-t at nucleotide 942+3	In-frame deletion in exon 5	46	Newfoundland	10
Exon 6	G-A at nucleotide 965	Gly322Asp	32		
Exon 12	Deletion of AAT at nucleotide 1786	In-frame deletion in asparagine codon 596	11		
Exon 7	C-T at nucleotide 1216	Arg406STOP	6		

\* *hMLH1*, human mutL homolog 1; IVS, intervening sequence; *hMSH2*, human mutS homolog 2.

## GENERAL METHODOLOGY

### Search strategy

The MEDLINE (National Library of Medicine), EMBASE (Excerpta Medica), and CANCERLIT (National Cancer Institute) databases were searched for papers published before December 31, 2001, using the keywords *hMSH2* and *hMLH1*. Relevant papers were identified, critically appraised, and entered into a Reference Manager (ISI ResearchSoft, Berkeley, California) database. In addition, PubMed was searched via Reference Manager, by author name, for papers from research groups that had published several times on this subject. Finally, the database thus created was cross-referenced with papers cited in the International Collaborative Group on Hereditary Nonpolyposis Colorectal Cancer database of mutations (1).

For the "Gene variants" section, we considered a total of 109 papers, which were identified by the above strategy and fulfilled the following selection criteria: 1) complete mutation analysis had been performed on more than five patients with colorectal cancer and 2) there was sufficient detail on the molecular nature of the genetic alteration. Details on all gene variants described in these published papers are given in the first supplementary table, which is posted on the website of the Human Genome Epidemiology Network ([\[www.cdc.gov/genomics/hugenet/default.htm\]\(http://www.cdc.gov/genomics/hugenet/default.htm\)\), as well as on the \*Journal's\* website \(<http://aje.oupjournals.org/>\).](http://</a></p>
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For the "Associations" section, the above strategy led to the identification of eight studies that had conducted an analysis of the risk of developing colorectal cancer among carriers of mismatch repair gene mutations and 77 papers that included results of complete mutation analysis performed on more than five colorectal cancer patients selected on the basis of family history, microsatellite instability (MSI), or age of onset. These studies are summarized in table 2 and the second supplementary table, respectively. Many papers included information relevant to both gene variants and associations.

### Classification of gene variants

For the purposes of this review, we classified gene variants into one of four categories. These categories are loosely based on the definitions given below, modified according to clinical observations.

1. Pathogenic mutation—generally frameshifts, nonsense mutations, and splice variants
2. Probable pathogenic mutation—generally nonconservative amino acid changes

3. Probable polymorphism—generally conservative changes, often observed in controls
4. Definite polymorphism—synonymous variants

## GENE

### *hMSH2*

The *hMSH2* gene is located at chromosome 2p21, an area initially identified as an important candidate region for genes involved in hereditary nonpolyposis colorectal cancer (HNPCC) syndrome by genetic linkage analysis within large affected families (2, 3).

The *hMSH2* protein product is a component of the DNA mismatch repair pathway, the role of which is well established in bacteria and yeast. *hMSH2* can form a heterodimer with one of two other mismatch repair proteins, *hMSH6* or *hMSH3*. This protein complex recognizes and binds any errors that may have occurred during DNA replication, and a larger protein complex is then recruited to excise the incorrect daughter sequence and replace it with the correct sequence, using the parental strand as a template. In *Escherichia coli*, mutS has been implicated in both short- and long-patch repair systems (4).

### *hMLH1*

The *hMLH1* gene is located at chromosome 3p21–23, an area also identified by genetic linkage analysis as an important candidate region within large HNPCC families that are not connected with the chromosome region 2p21–22 (5, 6).

The *hMLH1* protein product is another component of the DNA mismatch repair pathway, and it has been shown to form a heterodimer with *hMLH3*, *hPMS2*, or *hPMS1*. The *hMLH1* protein has no known enzymatic activity and probably acts as a “molecular matchmaker,” in that it recruits other DNA repair proteins to the mismatch repair complex. Again, the bacterial homolog of *hMLH1* has been implicated in both short- and long-patch repair (4).

## GENE VARIANTS

One conclusion generated by early attempts to identify precise genetic alterations in *hMLH1* and *hMSH2* was that variants in these genes are extremely heterogeneous. All 16 exons of the *hMSH2* gene and 19 exons of the *hMLH1* gene have been found to contain pathogenic mutations.

At present, there are no standard criteria for classifying variants as pathogenic mutations or polymorphisms, and consequently there is considerable variation in interpretation by different researchers. In general, categorization of alterations is based on the predicted effect on protein, with segregation of the mutation with colorectal cancer in the kindred in question and/or analysis of control subjects for that specific mutation also being considered when possible. However, the functional consequences of many mutations are difficult to predict accurately. It has been suggested that even alterations that do not affect the amino acid sequence could lead to aberrant splicing, and that the position of the mutation may be more significant than the type (7). In vitro

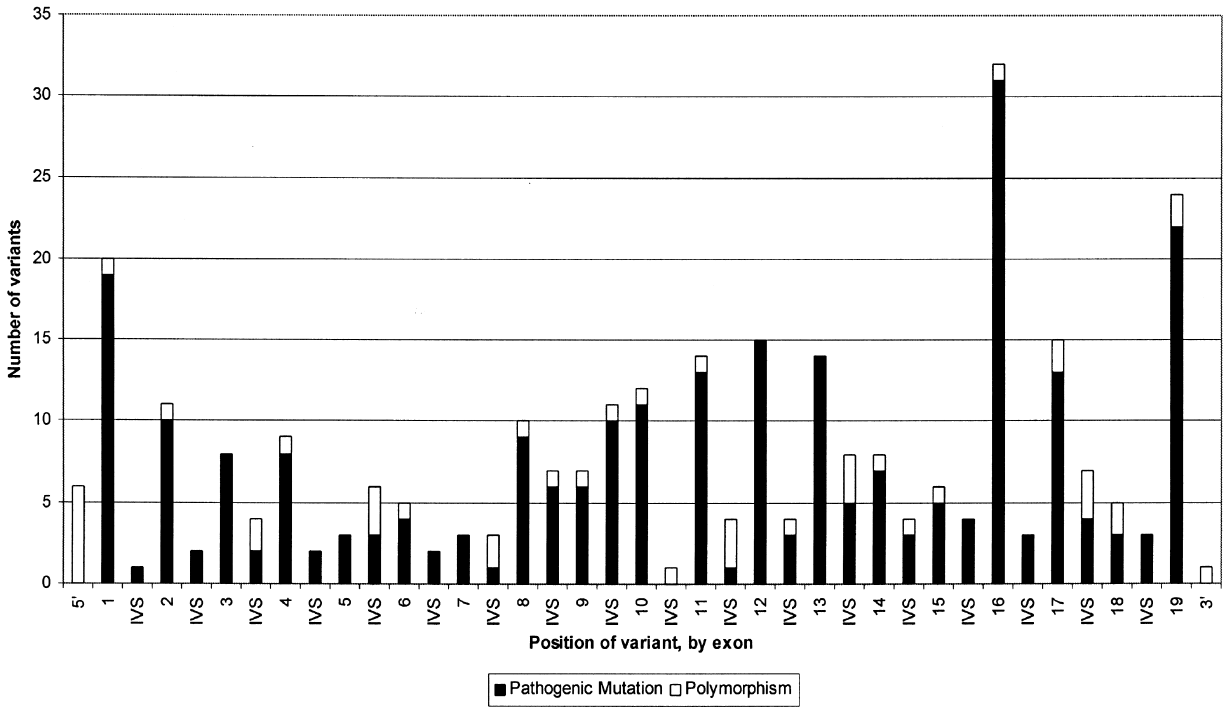
functional assays have been developed and applied to the task of determining the pathogenicity of missense mutations (8–10) and may eventually facilitate accurate classification of such changes.

The first supplementary table lists all of the gene variants identified as part of this review, illustrating the extreme range of mutations identified and the fact that the observed spectrum of mutation is not entirely uniform. Figures 1–4 summarize some features of this table. Figures 1 and 2 illustrate the distributions of unique gene variants that have been fully characterized at the molecular level in *hMLH1* and *hMSH2*, respectively, according to their position on the gene. Figures 3 and 4 are designed to show the actual numbers of families in which pathogenic mutations have been identified.

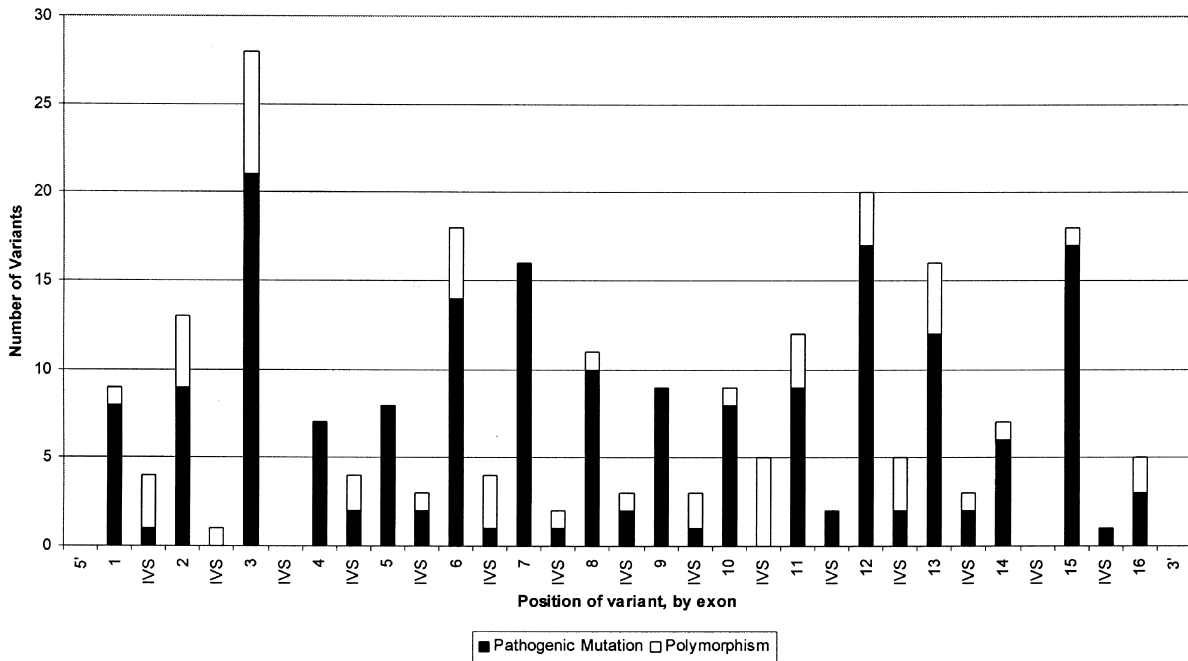
In total, 259 different pathogenic mutations, as defined above, have been identified in *hMLH1*, along with 45 polymorphisms. In *hMSH2*, 191 different pathogenic mutations and 55 polymorphisms have been characterized so far. This high degree of heterogeneity is similar to that found in the breast cancer genes *BRCA1* and *BRCA2*, in each of which over 400 gene variants have been reported. When considering the range and type of gene variants listed in the first supplementary table, there are several important sources of bias that merit consideration. Firstly, a significant publication bias is likely to exist in favor of apparently pathogenic alterations. Highly penetrant mutations are also likely to be overrepresented, since many studies involved conducting mutation analysis in patients selected on the basis of a strong family history of colorectal cancer. Secondly, genomic deletions in mismatch repair genes appear to occur relatively commonly, particularly in *hMSH2*, and such variants are not detected by many of the techniques commonly used for mutation analysis (11).

It is evident from the above figures that certain specific mutations have been identified in more than one kindred. Indeed, some mutations are found with a relatively high frequency. The most commonly observed mutations are summarized in table 1, which displays all mutations identified in more than four ostensibly independent kindreds.

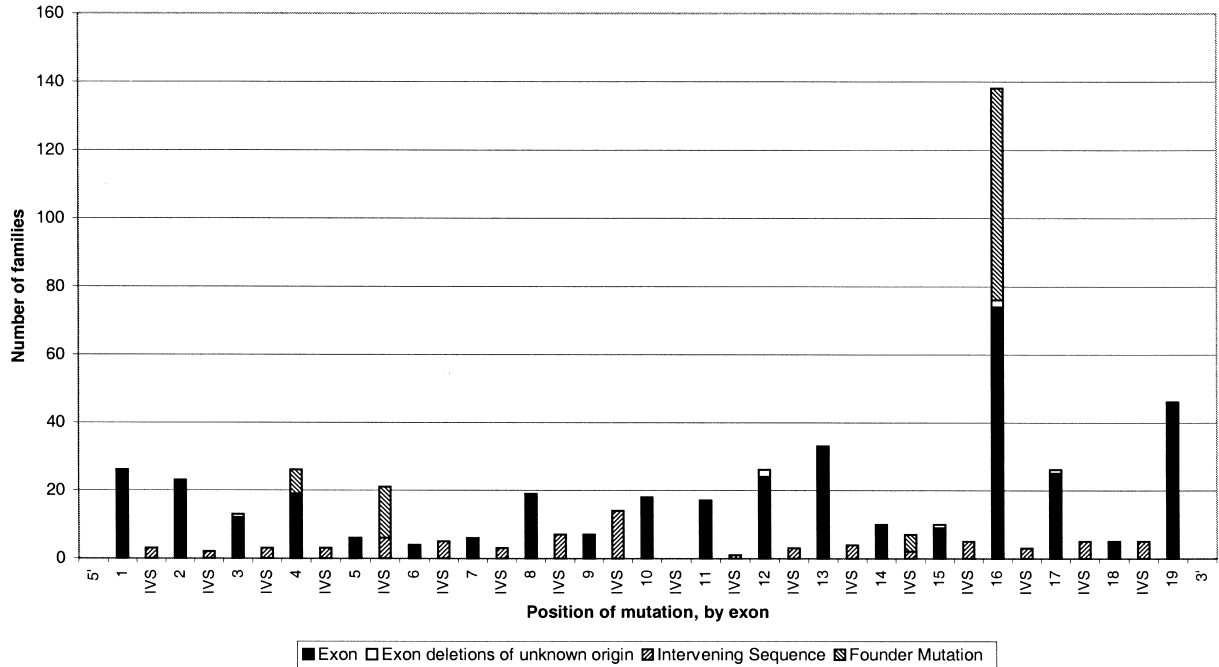
The observed spectrum of gene variants may be largely due to genuine differences in the mutability of specific nucleotides or sequences within the gene, but in some cases variants identified in apparently unrelated kindreds can be traced to a common ancestor. Such “founder effects” have been identified in the Finnish population, where two specific founder mutations in *hMLH1* account for the vast majority of families in which mismatch repair gene mutations have been identified (12, 13). Another *hMLH1* founder effect is evident in the Danish population (14). The extent to which founder effects are responsible for other frequently detected alterations is not entirely clear from the data currently available, and it is likely that some of the kindreds included in the first supplementary table share a common ancestor. Interestingly, the intervening sequence 5 variant A-T at nucleotide 942+3 has been shown to occur as a founder mutation in Newfoundland (15), but another study found no evidence for a common haplotype in 10 carriers of this variant, of various origins, and concluded that the mutation also arises frequently de novo (16). This



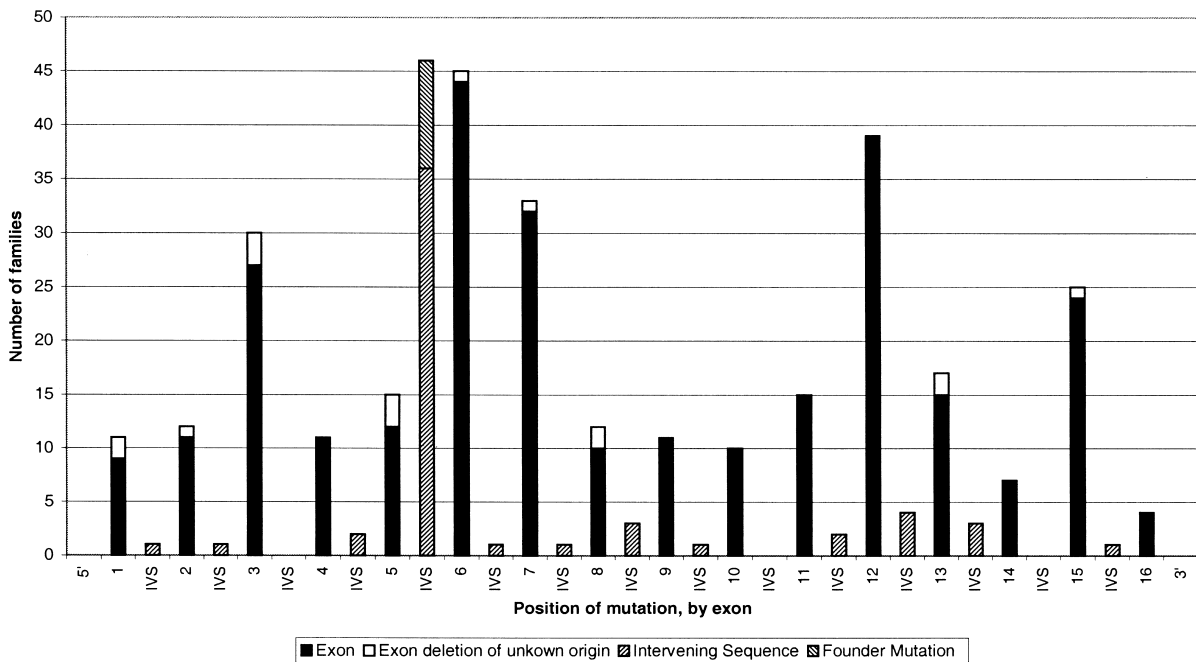
**FIGURE 1.** Distribution of unique gene variants in the mismatch repair gene *hMLH1*. The figure illustrates the distribution of all unique gene variants that have been identified and fully characterized in mutation analysis studies of colorectal cancer patients. Variants designated as categories 1, 1/2, 2, and 2/3 in the first supplementary table are considered to be pathogenic for the purpose of this summary figure, and all other variants are described as polymorphisms. Exon deletions in which the underlying molecular variant was not known were excluded. IVS, intervening sequence.



**FIGURE 2.** Distribution of unique gene variants in the mismatch repair gene *hMSH2*. The figure illustrates the distribution of all unique gene variants that have been identified and fully characterized in mutation analysis studies of colorectal cancer patients. Variants designated as categories 1, 1/2, 2, and 2/3 in the first supplementary table are considered to be pathogenic for the purpose of this summary figure, and all other variants are described as polymorphisms. Exon deletions in which the underlying molecular variant was not known were excluded. IVS, intervening sequence.



**FIGURE 3.** Distribution of gene variants in the mismatch repair gene *hMLH1* by the number of families affected. The figure illustrates the distribution of pathogenic mutations according to the actual number of families in which a pathogenic mutation has been identified. These figures include all pathogenic mutations as defined in figure 1, plus exon deletions of unspecified origin. Deletions of more than one exon were excluded. Families are deemed to have a “founder mutation” if they have a mutation which has been shown to have a founder effect in the same population.



**FIGURE 4.** Distribution of gene variants in the mismatch repair gene *hMSH2* by the number of families affected. The figure illustrates the distribution of pathogenic mutations according to the actual number of families in which a pathogenic mutation has been identified. These figures include all pathogenic mutations as defined in figure 2, plus exon deletions of unspecified origin. Deletions of more than one exon were excluded. Families are deemed to have a “founder mutation” if they have a mutation which has been shown to have a founder effect in the same population.

example underlines the notion that observations of mutation frequency are the result of both the probability of a mutation at a given nucleotide and the demographic history of the population in question.

Overall, little ethnic or population variation is apparent from the available gene variant data. However, the current biases towards highly penetrant mutations are such that the effect of the identified mutation is likely to transcend any population differences. Clearly, there is a need for accurate and extensive population-based data before any population differences in the spectrum and frequency of mismatch repair gene variants become apparent.

There is no clear evidence to suggest that any specific mismatch repair gene mutation produces a specific phenotype of colorectal cancer, although it has been suggested that some differences exist between the spectrum of extracolonic cancers associated with *hMSH2* mutations in comparison with *hMLH1* mutations (17, 18).

## DISEASE

Colorectal cancer is a major public health problem worldwide, with a current annual incidence approaching 950,000 cases (19). Colorectal cancer is more common in males than in females, and in both sexes the incidence rate increases with advancing age. Incidence rates vary globally and are about four times higher in developed countries than in developing countries (20). While incidence rates do vary according to ethnicity (21), there is compelling evidence that the observed variation between countries is primarily due to the role of environmental factors. This hypothesis is supported by the rising incidence of colorectal cancer in populations undergoing rapid economic development, with associated "westernization" of diet and lifestyle. Further evidence for a strong environmental influence comes from migrant data; despite the relatively low incidence of colorectal cancer in Japan, incidence rates in Hawaiian Japanese are among the highest in the world (22).

Considerable effort and resources have been expended with the aim of elucidating the precise dietary and other variables responsible for the observed environmental influences on colorectal cancer incidence. A report commissioned by the World Cancer Research Fund and the American Institute for Cancer Research concluded that evidence was sufficient to suggest that colorectal cancer risk could be substantially reduced by adhering to a diet high in vegetables and low in meat, together with regular physical activity and avoidance of alcohol (23). Other reviews have reached similar conclusions (24), but some studies have failed to provide evidence to uphold the hypothesis that dietary modification can prevent colorectal cancer. Clinical intervention studies (25, 26) and observational cohort studies (27), as well as studies utilizing animal models (28, 29), have shown no evidence of polyp prevention related to diet. Nonetheless, polyp prevention may not be the best endpoint, so results of further clinical studies with cancer prevention as the endpoint are awaited.

Both epidemiologic evidence and experiments utilizing murine models have suggested that nonsteroidal antiinflammatory drugs have antitumor properties that may prevent

colorectal cancer. Sulindac has been shown to inhibit tumor growth in experimental systems and to reduce adenoma counts in humans with familial adenomatous polyposis (30), as has a recent study of the specific cyclooxygenase-2 inhibitor celecoxib (31).

A number of case-control and cohort studies have reported an association between hormone replacement therapy and colorectal cancer, with the majority of these providing evidence in favor of a protective effect (24). Accumulating evidence also implicates obesity as a risk factor for colorectal cancer (32), and a positive association may exist between colorectal cancer and diabetes (33, 34). The weight of evidence also suggests that smoking may be a significant risk factor (35).

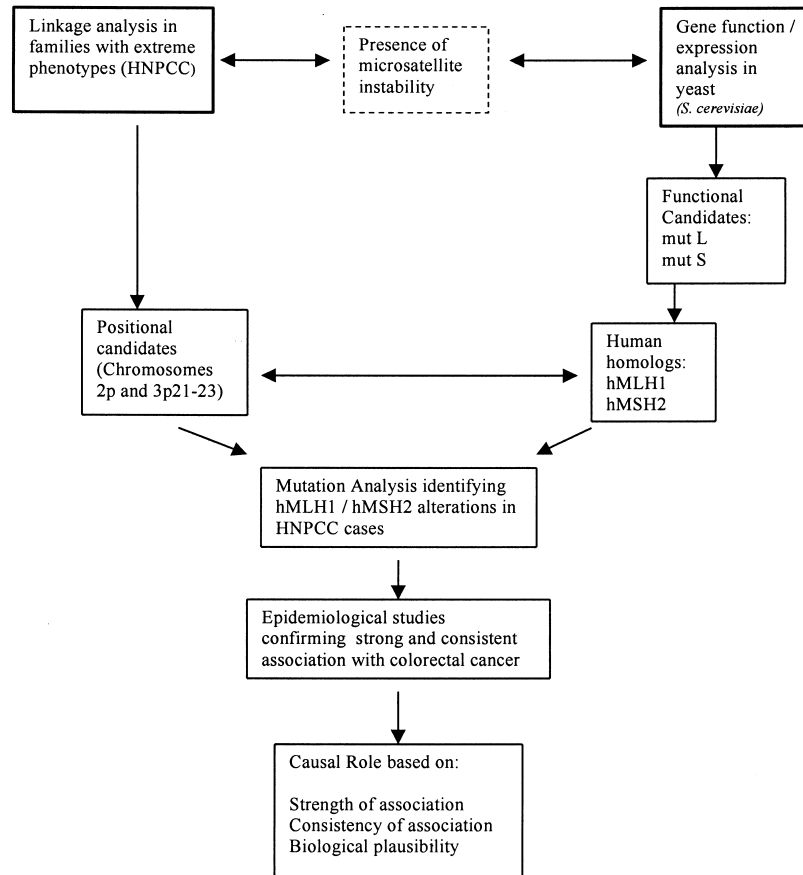
Colorectal cancer is a multifactorial condition, and while environmental factors are clearly important in the etiology of the disease, there is a significant input from genetic factors. A recent study of twins provided evidence suggesting that about 35 percent of all colorectal cancer cases have a genetic component (36), and first-degree relatives of colorectal cancer patients are well-recognized to have a 2- to 4-fold increased risk of developing the disease themselves. The genetic factors involved are poorly understood and may include recessive genes, pathogenic mutations of low penetrance, and complex gene-gene and gene-environment interactions.

In addition to the less obvious genetic factors, two autosomally inherited cancer syndromes account for a significant minority of colorectal cancer cases. Familial adenomatous polyposis is a rare syndrome caused by mutations in the adenomatous polyposis coli gene and is characterized by the presence of multiple adenomas. In the HNPCC syndrome, affected kindreds have an unusually high occurrence of colorectal and certain extracolonic cancers, with a relatively early age of onset. HNPCC has traditionally been diagnosed on the basis of family history, and the various criteria used for defining HNPCC are summarized in appendix 2. For research purposes, the Amsterdam criteria are the most widely used, and by this definition of HNPCC, the syndrome may account for 2–5 percent of all colorectal cancer cases.

It has been established that a large proportion of families diagnosed with HNPCC harbor potentially pathogenic mutations in mismatch repair genes. Of the mutations identified so far, over 90 percent occur in *hMLH1* and *hMSH2*. HNPCC families in which mutations in *hMLH1* and *hMSH2* are not identified may harbor pathogenic mutations in other mismatch repair genes, such as *hMSH6* and *hPMS2*, or in genes as yet unidentified. Pathogenic mutations in *hMLH1* or *hMSH2* have also been identified in kindreds that do not meet the traditional criteria for diagnosis of HNPCC. This observation may be due to the inherent misclassification bias involved in diagnosing a condition on the basis of family history alone, particularly in small families.

## ASSOCIATIONS

Evidence implying and supporting a causal role for *hMLH1/hMSH2* in colorectal cancer comes from both epidemiologic studies and laboratory-based molecular studies, as summarized in figure 5. Initially, linkage studies revealed



**FIGURE 5.** Pathways of epidemiologic and biologic research identifying and confirming the causal role of the mismatch repair genes *hMLH1* and *hMSH2* in colorectal cancer. HNPCC, hereditary nonpolyposis colorectal cancer; *hMLH1*, human mutL homolog 1; *hMSH2*, human mutS homolog 2.

that disease expression in a proportion of HNPCC kindreds was linked to either chromosome 2p21 (2, 3, 37) or chromosome 3p21–23 (5, 6, 37, 38).

The connection between the HNPCC syndrome and mismatch repair arose from the observation that the majority of tumors from HNPCC families exhibited a replication error phenotype, a feature resulting from instability of microsatellite repeats during replication that is found only in a minority of “sporadic” colorectal cancer cases (39, 40). Previous molecular studies in the yeast *Saccharomyces cerevisiae* had led to the identification of a group of genes, known as mismatch repair genes, that were involved in maintaining the fidelity of DNA replication. Defects in yeast mismatch repair genes led to MSI, prompting formulation of the hypothesis that human homologs of these genes were involved in the HNPCC syndrome (41). Subsequently, several such homologs were identified, and two of them, *hMLH1* and *hMSH2*, were shown to reside on chromosomes 3p21–23 and 2p21, respectively (2, 42–44). Further supportive evidence came from the observation that pathogenic mutations in *hMLH1* or *hMSH2* could be identified and shown to segregate with disease in a high proportion of

kindreds that had shown linkage to the corresponding chromosome (2, 43, 44).

The heterogeneity of mutations in mismatch repair genes means that screening for mutations in these genes is a lengthy and complicated process. Consequently, for purely economic, practical, and ethical reasons, mutation analysis has been carried out almost exclusively among colorectal cancer patients, particularly those identified as being at high risk of harboring mutations. Only two studies identified in this review conducted mutation analysis among control subjects. Farrington et al. (45) found that none of 26 Scottish blood donors harbored previously identified mutations, although four variants of unknown significance were found. This was compared with the identification of potentially pathogenic mutations in 14 of 50 colorectal cancer patients diagnosed at less than 30 years of age. Similarly, no pathogenic mutations were reported in an analysis of 73 population controls from Utah (46).

Thus, the practical restrictions on mutation analysis, coupled with the low population prevalence of mismatch repair gene mutations and the fact that such mutations are found only in a minority of colorectal cancer patients, has

TABLE 2. Findings of risk analysis studies of colorectal cancer

Published reference	Area of study	Ascertainment of index cases	No. of index cases	Ascertainment of mutation carriers	No. of mutation carriers	Penetrance* in mutation carriers	Source of data for comparison	Risk in comparison group	Standardized incidence ratio/relative risk
Aarnio et al. (47)	Finland	Members of HNPCC† kindreds previously shown to have an <i>hMLH1</i> † or <i>hMSH2</i> † gene mutation.	50	Test-positive or obligate carriers.	360	Males = 100%; females = 54% (to age 70 years)	Finnish Cancer Registry data, 1991–1995	N/A†	Females + males = 68 (95% CI†: 56, 81)
Dunlop et al. (56)	Scotland	Colorectal cancer cases aged ≤30 years identified through the Scottish National Cancer Registry between 1970 and 1993, excluding those with a family history fulfilling the Amsterdam criteria.	6	Relatives were traced, tested for mutation status where possible, and classified accordingly.	67	Males = 74%; females = 30% (to age 70 years)	United Kingdom cumulative incidence data published by EUCAN† (91)	Males = 2.53%; females = 1.67% (to age 70 years)	Males = 29‡ Females = 18‡
Aarnio et al. (92)	Finland	Families that fulfilled the Amsterdam criteria. In 24 of these, mutation analysis had demonstrated the segregation of <i>hMLH1</i> or <i>hMSH2</i> .	40	Cases of any cancer in relatives were identified and were included if adequate documentation was available "with the presumption that all tumor patients were HNPCC gene carriers."	293	Females + males = 78% (lifetime)	Finnish cumulative incidence data published by EUCAN (91)	Females + males = 2.6% (to age 75 years)	Females + males = 30‡
Vasen et al. (55)	Netherlands	Families that fulfilled the Amsterdam criteria, identified through the Netherlands HNPCC registry and found to have a mutation in <i>hMLH1</i> or <i>hMSH2</i> .	19	Relatives were traced and tested for mutation carrier status where possible.	210	Males = 92%; females = 83% (to age 75 years)	Netherlands cumulative incidence data published by EUCAN (91)	Males = 4.41%; females = 3.28% (to age 75 years)	Males = 21‡ Females = 25‡
Vasen et al. (54)	Netherlands and Norway	Kindreds registered with the Netherlands HNPCC registry ( <i>n</i> = 193) or suspected HNPCC families from the Clinical Genetic Centre, Radium Hospital, Norway ( <i>n</i> = 58).	34 <i>hMLH1</i> carriers; 40 <i>hMSH2</i> carriers	Mutation carrier status was assigned to one of three groups: 1) tested carriers; 2) relatives with colorectal or endometrial cancer (excluding those tested negative); and 3) obligate carriers.	362 <i>hMLH1</i> carriers; 301 <i>hMSH2</i> carriers	<i>hMLH1</i> : Males = 65%; females = 55%; females + males = 60% <i>hMSH2</i> : Males = 73%; females = 54%; females + males = 65% (to age 70 years)	Netherlands cumulative incidence data published by EUCAN (91)	Males = 2.81%; females = 2.17% (to age 70 years)	<i>hMLH1</i> : Males = 23‡ Females = 25‡ <i>hMSH2</i> : Males = 26‡ Females = 25‡
Froggatt et al. (93)	England	Families that fulfilled the Amsterdam criteria, with mutations in <i>hMLH1</i> or <i>hMSH2</i> .	8	Subjects with mutations were included in the analysis. No further details were given.	50 ( <i>hMLH1</i> : <i>n</i> = 23; <i>hMSH2</i> : <i>n</i> = 27)	<i>hMLH1</i> : Females + males = 67% <i>hMSH2</i> : Females + males = 62%	United Kingdom cumulative incidence data published by EUCAN (91)	Females + males = 3.16%	<i>hMLH1</i> : 21‡ <i>hMSH2</i> : 20‡

Table continues

meant that traditional cohort and case-control study designs have not been feasible. However, despite this lack of conventional epidemiologic evidence, subsequent studies have provided convincing evidence to support the hypothesis that mismatch repair gene mutations cause a subset of colorectal cancer cases.

The most compelling supportive evidence comes from studies which demonstrate that mutation carriers are at greatly increased risk of developing colorectal cancer in comparison with the general population. Such studies are summarized in table 2. Aarnio et al. (47) calculated a stan-

dardized incidence ratio of 68 (95 percent confidence interval: 56, 81) for Finnish carriers of *hMLH1* or *hMSH2* mutations. In the other studies identified in table 2, researchers did not make a formal calculation of the standardized incidence ratio, but approximate estimates utilizing appropriate cancer registry data consistently show that the risk of colorectal cancer in mutation carriers is greatly in excess of the corresponding risk in the general population (see table 2). The relative risk of 8.1 (95 percent confidence interval: 3.5, 15.9) for first-degree relatives of mutation carriers observed by Millar et al. (48) is consistent with a risk



**TABLE 2. Continued**

Published reference	Area of study	Ascertainment of index cases	No. of index cases	Ascertainment of mutation carriers	No. of mutation carriers	Penetrance* in mutation carriers	Source of data for comparison	Risk in comparison group	Standardized incidence ratio/relative risk
Millar et al. (48)	Canada	Women with both colorectal cancer and endometrial cancer before age 70 years, identified through the Ontario Cancer Registry and/or the tumor registry at Princess Margaret Hospital, Toronto, and harboring <i>hMLH1</i> or <i>hMSH2</i> mutations.	7	First-degree relatives were identified. Carrier status was not determined.	N/A	N/A	Ontario provincial cancer rate		First-degree relatives of mutation carriers 8.1 (95% CI: 3.5, 15.9); first-degree relatives of mutation-negative probands: 2.8 (95% CI: 1.7, 4.5)
Lin et al. (53)	United States	Kindreds were known to have mutations in <i>hMLH1</i> ( <i>n</i> = 2) or <i>hMSH2</i> ( <i>n</i> = 2). No further detail was given on how these kindreds were ascertained.	4	Mutation carriers were identified by testing ( <i>n</i> = 78) or determined to be obligate carriers ( <i>n</i> = 27).	105	<i>hMLH1</i> : Males = 94%; females = 63%; females + males = 84% <i>hMSH2</i> : Males = 96%; females = 39%; females + males = 71%	N/A		N/A

\* Males: penetrance in males only; Females: penetrance in females only; Females + Males: penetrance in group comprising both sexes.  
 † HNPCC, hereditary nonpolyposis colorectal cancer; *hMLH1*, human mutL homolog 1; *hMSH2*, human mutS homolog 2; N/A, not applicable; CI, confidence interval; EUCAN, European Network of Cancer Registries.  
 ‡ Where EUCAN data have been used for comparison, the estimate of the standardized incidence ratio is a crude one and does not take into account the age structure of the mutation carrier group. Because of the approximate nature of this comparison, we did not consider it appropriate to calculate confidence intervals for these estimates.

that is an order of magnitude greater in mutation carriers than in noncarriers.

The clinical presentation of colorectal cancer among mutation carriers appears to differ from that found among persons with sporadic cases in several respects, an observation that indirectly supports the hypothesis that mutations in mismatch repair genes account for a distinct subset of colorectal cancer cases. The most obvious clinical characteristic associated with colorectal cancer among mismatch repair gene mutation carriers is familial aggregation. Part *a* of the second supplementary table, which is available on the website of the Human Genome Epidemiology Network (<http://www.cdc.gov/genomics/hugenet/default.htm>) and the *Journal's* website (<http://aje.oupjournals.org/>), provides details on mutation analysis studies conducted among patients selected on the basis of family history. The results of these studies are summarized in tables 3 and 4. The observed prevalence of potentially pathogenic mutations in individ-

uals meeting the Amsterdam criteria is remarkably consistent across different populations (table 4).

MSI is evident in 12–15 percent of sporadic colorectal cancer cases, compared with over 90 percent of cases defined, according to the Amsterdam criteria, as being from HNPCC kindreds (49). MSI is currently thought to result from defective mismatch repair, although evidence to support this hypothesis is limited by two factors. Firstly, the vast majority of studies that examine mutations in MSI-positive patients concentrate on HNPCC families, introducing considerable bias. Secondly, few investigators look systematically for mutations in patients with MSI-negative tumors. Interestingly, when this has been done, there have been a few instances in which tumors from patients with identified mutations in *hMLH1* or *hMSH2* have not exhibited the MSI phenotype (45, 50, 51). Analysis of all published results from one research group showed that, among kindreds with suspected HNPCC, germline mutations could be detected in

**TABLE 3. Association between the extent of family history of colorectal cancer and the prevalence of mismatch repair gene mutations**

Family history criteria*	No. of studies	No. of index cases	<i>hMLH1</i> mutation carriers		<i>hMSH2</i> mutation carriers		Published references (ref. no. from current review)
			No.	%	No.	%	
Fulfillment of the Amsterdam criteria	27	534	145	27.2	87	16.3	(18, 50, 57, 93–116)
Strong family history not fulfilling the Amsterdam criteria	25	494	46	9.3	43	8.7	(18, 50, 57, 94, 96–101, 103–106, 108–118)

\* See appendix 2.

16 out of 22 colorectal cancer patients with MSI-positive tumors, as compared with one out of 37 mutations in MSI-negative patients (52). The presence of mutations in MSI-negative cases may reflect mechanisms of tumorigenesis in people with mismatch repair gene mutations that do not require mutation instability. Mutation analysis studies involving patients selected on the basis of MSI are summarized in the second supplementary table, part *b*.

The association between early age of colorectal cancer onset and *hMLH1/hMSH2* gene mutations is often confounded by the fact that the selection criteria have included family history, but a few studies have performed mutation analysis on patients selected solely on the basis of

early age of onset. As is illustrated in table 5, these studies demonstrate a trend towards a higher pathogenic mutation detection rate in individuals diagnosed at a relatively young age, an observation that is consistent with the hypothesis that these genes are involved in colorectal cancer tumorigenesis. Details on the studies considered in table 5 can be found in the second supplementary table, part *c*.

### Penetrance

While it has become widely accepted that mutations in the mismatch repair genes *hMLH1* and *hMSH2* play a causal role in a subset of colorectal cancer cases, the precise pene-

**TABLE 4. Results of mutation analysis in patients fulfilling the Amsterdam criteria\* for colorectal cancer, by geographic origin**

Country	No. of index cases	<i>hMLH1</i> mutation carriers	<i>hMSH2</i> mutation carriers	Published reference
<i>Asia</i>				
Japan	15	1	8	Bai et al. (94)
Japan	11	5	0	Miyaki et al. (107)
Japan	4	0	1	Nomura et al. (109)
Korea	25	8	0	Han et al. (100)
Total	55	14 (25.5%)	9 (16.4%)	
<i>Europe</i>				
Russia/Moldavia	7	1	3	Maliaka et al. (106)
Sweden	21	5	1	Tannergard et al. (112) and Wahlberg et al. (113)
Sweden	7	1	0	Liu et al. (104)
Switzerland	10	3	3	Buerstedde et al. (95)
Switzerland	15	6	4	Heinimann et al. (57)
Switzerland	14	10	0	Hutter et al. (101)
Italy	14	4	3	Pensotti et al. (110)
Italy	18	1	2	de Leon et al. (98)
Italy	17	5	2	Viel et al. (119)
Italy	17	2	3	Curia et al. (97)
Italy	13	3	3	Calistri et al. (96)
France	10	3	2	Dieumegard et al. (99)
France	3	2	0	Wang et al., 1997 (114)
France	22	11	3	Wang et al., 1999 (120)
Holland and Norway	92	25	16	Wijnen et al. (116)
Germany	57	11	4	Lamberti et al. (103)
England	17	3	5	Froggatt et al. (93)
Total	344	96 (27.9%)	54 (15.7%)	
<i>Australia</i>				
Australia	18	4	2	Kohonen-Corish et al. (102)
Australia	33	11	9	Scott et al. (18)
Total	51	15 (29.4%)	11 (21.6%)	
<i>North America</i>				
USA	12	4	2	Luce et al. (105)
USA	28	10	1	Syngal et al. (111)
Canada	14	2	5	Bapat et al. (50)
Total	54	16 (29.6%)	8 (14.8%)	

\* See appendix 2.

TABLE 5. Association between age at onset of colorectal cancer and mismatch repair gene mutations

Age range (years)	No. of studies	No. of index cases	hMLH1 mutation carriers		hMSH2 mutation carriers		Published reference(s)
			No.	%	No.	%	
<30	1	50	7	14	7	14	Farrington et al. (45)
<40	1	12	1	8.3	1	8.3	Syngal et al. (111)
<45	1	38	1	2.6	2	5.3	Fornasarig et al. (121)
<50	6	135	6	4.4	6	4.4	Dieumegard et al. (99), Montera et al. (122), Tomlinson et al. (123), Wang et al., 1997 (114), Wang et al., 1999 (120), Weber et al. (124), and Yuan et al. (118)

trance of these mutations remains unknown. A number of studies, summarized in table 2, have addressed this issue. Results are presented differently for each study, so direct comparison is difficult. One consistent finding is that risk is higher among male mutation carriers (approximately 80 percent by age 70 years) than among females (approximately 40 percent by age 70 years), an observation with important implications for patient management and surveillance. Observed differences in penetrance between carriers of *hMLH1* or *hMSH2* mutations (53, 54) await confirmation in future studies.

A study by Aarnio et al. (47) classified relatives of clinically defined HNPCC cases as being at a 25 percent, 50 percent, or 100 percent risk of being mutation carriers and calculated the cumulative incidence of colorectal cancer up to age 70 years as being 100 percent and 54 percent for males and females, respectively. A potential source of bias in this particular study is the fact that the majority of the probands had one of the Finnish founder mutations. A similar study carried out in Amsterdam Dutch kindreds calculated risk of colorectal cancer among mutation carriers at age 75 years to be 92 percent in males and 83 percent in females (55).

These studies used family history as a selection criterion, an approach that introduces considerable ascertainment bias. Kindreds identified in this way will inherently have an unusually large number of colorectal cancer cases, and estimates of penetrance obtained in this way are likely to be falsely high. Dunlop et al. (56) used an alternative approach to identify mutation-carrying probands from the Scottish population, performing mutation analysis on colorectal cancer patients with a very early age of onset (<30 years). The cumulative incidence of colorectal cancer among relatives proven to be mutation carriers was found to be 74 percent in males and 30 percent in females at age 70 (56).

Note that the identification of families with mismatch repair gene mutations using any phenotypic selection criteria introduces ascertainment bias, and such kindreds may not be representative of all mutation-carrying families in the general population. Thus, there is a considerable need for estimates of penetrance based on systematically collected familial or population data.

## Survival

Prior to the identification of mismatch repair genes, several studies suggested that the prognosis for patients with colorectal cancer due to HNPCC was more favorable than that for patients with sporadic colorectal cancer. Whether improved prognosis is specifically a feature of colorectal cancer in patients harboring mismatch repair gene mutations is not yet clear, although preliminary evidence suggests that this may be the case (57, 58).

A possible explanation for this phenomenon may be that the high frequency of mutations characteristic of mismatch repair-deficient tumors actually restricts tumor growth (58). However, kindreds included in survival analysis studies on the basis of a strong family history of colorectal cancer have, by definition, survived to produce a large family group for analysis. Therefore, these kindreds may not be representative of all mutation carriers, and there is a need for survival data from unselected, population-based cohort studies.

It has also been postulated that mismatch repair deficiency may have an effect on response to chemotherapy. Results are not entirely consistent, but several studies suggest an association between *hMLH1/hMSH2* deficiency in cell lines and resistance to chemotherapeutic agents (59–62).

## INTERACTIONS

While the exact penetrance of specific mutations in *hMLH1* and *hMSH2* is unknown, it is not complete. Consequently, the age-related risk, pathologic features, and outcomes associated with such mutations are subject to modification by other genetic and environmental factors.

The body of epidemiologic data regarding modification of disease resulting from mismatch repair gene mutations is somewhat limited. The effects of known environmental risk factors for colorectal cancer in mutation carriers are largely unstudied, and much of the suggestive evidence for interactions comes indirectly from studies using MSI-positive or clinically defined HNPCC cases as a surrogate for mutation carriers. Furthermore, the apparent presence of a statistical interaction between mismatch repair gene mutations and other genetic or environmental factors does not necessarily imply the existence of a biologic or causal interaction. Therefore, the studies considered below do not constitute evidence for true interactions involving *hMLH1* and *hMSH2*,

although they may prove useful in terms of identifying potential interactions that merit further investigation.

### Gene-environment interactions

Reports by Ruschoff et al. (63) and Yamamoto et al. (64) have suggested that treatment of *hMLH1*- or *hMSH2*-deficient cell lines with nonsteroidal antiinflammatory drugs leads to a significant reduction in the proportion of cells exhibiting MSI, indicating that this phenotypic manifestation of mismatch repair deficiency may be modified by these drugs.

Slattery et al. (65) have presented evidence suggesting that an interaction may exist between MSI and smoking. Compared with patients with MSI-negative tumors, patients with MSI-positive tumors were more likely to be heavy smokers: Odds ratios were 1.6 (95 percent confidence interval: 1.0, 2.5) in men and 2.2 (95 percent confidence interval: 1.4, 3.5) in women (65). These results are supported by those of another recent study (66), and the implication that smoking is specifically associated with a particular subset of colorectal cancer cases is consistent with the weak associations reported between smoking and sporadic colon cancer. It is possible that mismatch repair deficiency is involved in the observed association between smoking and MSI, but further studies involving known mutation carriers will be required to confirm this hypothesis.

Another recent paper by Slattery et al. (67) showed that the risk of MSI-positive colon cancer may be reduced by estrogens and increased by estrogen withdrawal.

Dietary heterocyclic aromatic amines are another risk factor that requires further evaluation. Wu et al. (66) found that patients with MSI-positive tumors had received a relatively high dietary exposure to heterocyclic aromatic amines, an observation that remained significant after adjustment for smoking and red meat intake. This finding is consistent with laboratory studies, which have shown that rats exposed to particular heterocyclic amines showed the trait of MSI (68).

### Gene-gene interactions

Risk of colorectal cancer among female *hMLH1/hMSH2* mutation carriers is approximately half the risk in male mutation carriers (47, 56). In the absence of clear evidence of hormonal influence, the presence of a genetic modifier, X-linked or otherwise, remains a possibility.

The possibility of interaction between mismatch repair genes and other genes known to influence colorectal cancer susceptibility is an area that merits consideration. Initial studies have suggested that genes involved in carcinogen metabolism might modify the phenotypic expression of mismatch repair gene mutations. For example, Moisio et al. (69) demonstrated that a specific polymorphism in the gene encoding the xenobiotic enzyme *N*-acetyltransferase 1 was associated with a lower age of colorectal cancer onset in Finnish HNPCC kindreds with identified mutations in *hMLH1*. Similarly, an alteration in cyclin D1 has been associated with earlier age of onset in HNPCC cases; patients who harbor the mutant cyclin D1 allele develop cancer an

average of 11 years earlier than patients with two wild-type alleles (70).

Murine studies have demonstrated that *MSH2* deficiency accelerates intestinal tumorigenesis in transgenic mice that are heterozygous for a germline mutation in the adenomatous polyposis coli gene (71). Similarly, Toft et al. (72) have used mice mutant for both *MSH2* and p53 to demonstrate interaction between these genes. Additionally, in-vitro studies have suggested that interactions may exist between mismatch repair genes and transforming growth factor- $\beta$  receptor II (73). While these molecular studies demonstrate that gene-gene interactions may be worth further investigation, the above hypotheses have yet to be tested in human populations for relevance to cancer susceptibility.

### LABORATORY TESTING

The heterogeneity of mutation types found in *hMLH1* and *hMSH2* has meant that many different techniques have been employed to test for mutations in these genes. A number of techniques are described below, along with their benefits and disadvantages.

#### In vitro synthesized protein assay

The in vitro synthesized protein assay technique uses an in vitro system to transcribe and translate a large polymerase chain reaction product containing several exons. The translated product is separated on a polyacrylamide gel electrophoresis system, and potential mutations are identified as truncated bands. These may represent a number of mutations that have the effect of altering splicing, therefore producing a translated fragment with certain exons deleted. Out-of-frame deletions or insertions, resulting in frameshifts or splice variants, will also be detected using this method.

In vitro synthesized protein assay does not detect missense mutations, in-frame deletions or insertions, large genomic deletions involving numerous exons, promoter mutations, or mutations that silence the gene. The assay also requires the use of mRNA for the production of a cDNA polymerase chain reaction product.

#### Genomic sequencing

cDNA sequencing also relies on mRNA being available. It will identify all mutation types except large genomic deletions, promoter mutations, and gene silencing mutations. Genomic sequencing detects even fewer changes than cDNA, but it does have the advantage of only requiring genomic DNA. Table 6 shows a comparison of the sensitivity of the two techniques, in vitro synthesized protein assay and genomic sequencing, as described by Farrington et al. (45).

#### DNA structure techniques

A number of techniques rely on changes in DNA structure created by a mutation. These include denaturing gradient gel electrophoresis (74), including the adaptation of using two-dimensional gel electrophoresis (75), single-strand confor-

**TABLE 6. Sensitivity of mutation detection techniques**

	Sensitivity (%)	Published reference
In vitro synthesized protein assay	69	Farrington et al. (45)
Genomic sequencing	80	Farrington et al. (45)
In vitro synthesized protein assay/genomic sequencing	93	Farrington et al. (45)
Denaturing gradient gel electrophoresis	>67	Fidalgo et al. (125)
Single-strand conformational polymorphism	>67	Fidalgo et al. (125)
Protein truncation test	50	Fidalgo et al. (125)
Heteroduplex analysis	19	Fidalgo et al. (125)
Two-dimensional DNA typing	—*	Sasaki et al. (75)

\* Comparable to that of denaturing gradient gel electrophoresis.

mational polymorphism analysis (76), heteroduplex analysis, and denaturing high-performance liquid chromatography.

Table 6 summarizes the available information regarding the sensitivity of the above techniques. The use of various combinations of techniques may enhance sensitivity, but this is usually impractical. Recently, Yan et al. (77) demonstrated that the conversion of chromosomes from the diploid state to the haploid state, by fusion to a recipient rodent cell line, may facilitate improved sensitivity of current mutation detection techniques.

#### POPULATION TESTING

The population prevalence of *hMLH1/hMSH2* mutation carriers in the Scottish population aged 15–74 years has been estimated at 1 in 3,139 (78). A recent UK National Screening Committee workshop concluded that there is currently no case to offer population screening in an attempt to identify mutation carriers (Rose et al., UK National Screening Committee, unpublished data). Authors in the United States have reached similar conclusions, agreeing that more information regarding the prevalence and penetrance of mismatch repair gene mutations and more evidence of effective intervention strategies are essential prerequisites for implementing screening outside of the research context (79–84).

There are essentially two strategies that could be employed to search for mutations in the context of population screening: searching the entire gene(s) for mutations using the techniques considered above or looking for specific mutations. The latter option is far less expensive and labor-intensive and could be of particular benefit in countries where specific “founder” mutations are prevalent. It may also be possible to apply DNA pooling strategies in this context to enhance efficiency (85). However, this approach is not currently feasible because of the extreme heterogeneity of mismatch repair gene variants and the low allele frequency of individual mutations. The ethical issues inherent in genetic screening, coupled with the poor efficiency and high cost of detecting mutations using current technology, mean that population testing in any form is unlikely to be recommended in the near future.

Another approach to identifying mutation carriers is performing mutation analysis in colorectal cancer patients

deemed to be at high risk of harboring mutations, and subsequently performing “cascade screening” of their relatives. The major issue in the context of a cascade screening program is that of how resources can be efficiently targeted towards the identification of kindreds with *hMLH1/hMSH2* mutations. This issue is considered in detail in an overview of findings from one research group (52), and the sensitivity and specificity of various clinical criteria are considered by Syngal et al. (86).

Currently, most mutation carriers are identified by referral of patients with a family history of colorectal cancer to cancer genetics services. Another option, under investigation in an ongoing program in Scotland, is to search for mismatch repair gene mutations among persons with early-onset colorectal cancer and subsequently perform cascade screening in the relatives of mutation carriers. The phenotypic features of age at onset, family history, and MSI are commonly used selection criteria in mutation analysis studies, as summarized for reference in the second supplementary table.

At the present time, there is no consensus regarding the most efficient approach to identifying mutation carriers. It is clear, however, that further understanding of the role of mismatch repair genes in colorectal cancer has important scientific and clinical implications.

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**APPENDIX 1**

**Internet Sites**

The following Internet sites may be useful to investigators wishing to pursue further study of the above issues.

*Database of Gene Variants and Summary of Mutation Analysis Studies (supplementary tables)*

Human Genome Epidemiology Network <http://www.cdc.gov/genomics/hugenet/default.htm>

*Colorectal Cancer Statistics*

International Agency for Research on Cancer <http://www-dep.iarc.fr/eucan/eucan.htm>  
 Surveillance, Epidemiology, and End Results Program <http://seer.cancer.gov/>

*Genetic Information and Databases*

National Centre for Biotechnology Information <http://www.ncbi.nlm.nih.gov/>  
 Online Mendelian Inheritance in Man <http://www.ncbi.nlm.nih.gov/omim>  
 ICG-HNPCC\* database <http://www.nfdht.nl/>  
 Cambridge Public Health Genetics Unit <http://www.medinfo.cam.ac.uk/phgu/>

*Patient Education and Support*

World Cancer Research Fund <http://www.wcrf.org/>  
 Genetic Health <http://www.genetichhealth.com/>  
 Medicine Online <http://www.meds.com/colon/colon.html>  
 American Cancer Society <http://www.cancer.org/>  
 Cancer Research Campaign <http://www.crc.org.uk/>  
 International Union Against Cancer <http://www.uicc.org/>  
 UK National Screening Committee <http://www.nsc.nhs.uk/>

\* ICG-HNPCC, International Collaborative Group on Hereditary Nonpolyposis Colorectal Cancer.

(Appendix 2 follows)

## APPENDIX 2

## Clinical Criteria for Diagnosis of Hereditary Nonpolyposis Colorectal Cancer

Name of criteria	Specific criteria	Published reference
Amsterdam	Three relatives with colorectal cancer, one of which is a first-degree relative of the other two; colorectal cancer affecting more than one generation; at least one colorectal cancer case diagnosed before age 50 years	Vasen et al. (87)
Modified Amsterdam*	Two colorectal cancer cases in first-degree relatives in very small families that cannot be expanded further; colorectal cancer affecting more than one generation; at least one colorectal cancer case diagnosed before age 55 years Two first-degree relatives affected by colorectal cancer, plus a third relative with an unusually early-onset neoplasm or endometrial cancer	Bellacosa et al. (88)
Japanese†	Three or more colorectal cancer cases among first-degree relatives Two or more colorectal cancers among first-degree relatives and any of the following: diagnosis before age 50 years; right colon involvement; synchronous or metachronous multiple colorectal cancers; association with extracolonic malignancy	Fujita et al. (89)
Bethesda*	Individuals from families that fulfill the Amsterdam criteria Individuals with two HNPCC‡-related cancers, including synchronous and metachronous colorectal cancers or associated extracolonic cancers Individuals with colorectal cancer, plus colorectal cancer and/or HNPCC-related extracolonic cancer and/or colorectal adenoma in a first-degree relative; at least one of the cancers diagnosed before age 45 years and the adenoma diagnosed before age 40 years Individuals with colorectal or endometrial cancer diagnosed before age 45 years Individuals with right-sided colorectal cancer with an undifferentiated histopathologic pattern (solid/cribiform) diagnosed before age 45 years Individuals with signet-ring cell type colorectal cancer diagnosed before age 45 years Individuals with colorectal adenomas diagnosed before age 40 years	Rodriguez-Bigas et al. (90)

\* Fulfillment of all criteria listed in any paragraph in this section is sufficient.

† Cases can be classified as fulfilling either the first set of criteria or the second set and can be diagnosed with hereditary nonpolyposis colorectal cancer if they fulfill either set of criteria.

‡ HNPCC, hereditary nonpolyposis colorectal cancer.