A Founder Mutation of the MSH2 Gene and Hereditary Nonpolyposis Colorectal Cancer in the United States

Henry T. Lynch, MD
Stephanie M. Coronel, MPH
Ross Okimoto, BS
Heather Hampel, MS, CGC
Kevin Sweet, MS, CGC
Jane F. Lynch, BSN
Ali Barrows, BS
Juul Wijnen, PhD
Heleen van der Klift, MS
Patrick Franken, HLO
Anja Wagner, PhD, MD
Riccardo Fodde, PhD
Albert de la Chapelle, MD, PhD

Context  Hereditary nonpolyposis colorectal cancer (HNPCC), also known as Lynch syndrome, is caused by mutations in the mismatch repair genes and confers an extraordinarily high risk of colorectal, endometrial, and other cancers. However, while carriers of these mutations should be identified, counseled, and offered clinical surveillance, at present the mutations are not tested for in mutation analyses.

Objective  To describe the prevalence of a large genomic deletion encompassing exons 1 to 6 of the MSH2 gene that is widespread in the US population as a result of a founder effect.

Design, Setting, and Patients  Ongoing genealogical and historical study conducted to assess the origin and spread of an MSH2 mutation previously identified in 9 apparently unrelated families with putative HNPCC and living in widely different geographic locations in the United States.

Main Outcome Measures  Classification of family members as carriers or noncarriers of the MSH2 mutation; spread of the mutation across the continental United States.

Results  To date, 566 family members of the 9 probands have been identified to be at risk and counseled; 137 of these have been tested, and 61 carry the founder mutation. Three families have been genealogically shown to descend from a German immigrant family that arrived and first settled in Pennsylvania in the early 1700s. Movements of branches of the family from Pennsylvania through North Carolina, Alabama, Kentucky, Missouri, Iowa, Nebraska, Utah, Texas, and California have been documented, and carriers of the mutation have already been diagnosed in 14 states. In contrast, the deletion was not found among 407 European and Australian families with HNPCC.

Conclusion  The postulated high frequency and continent-wide geographic distribution of a cancer-predisposing founder mutation of the MSH2 gene in a large, outbred (as opposed to genetically isolated) population, and the ease with which the mutation can be detected, suggest that the routine testing of individuals at risk for HNPCC in the United States should include an assay for this mutation until more is learned about its occurrence.

JAMA. 2004;291:718-724  www.jama.com

Author Affiliations: Department of Preventive Medicine, Creighton University School of Medicine, Omaha, Neb (Dr H. T. Lynch; Miss Coronel, J. F. Lynch, and Barrows; and Mr Okimoto); Human Cancer Genetics Program, Comprehensive Cancer Center, The Ohio State University, Columbus (Ms Hampel, Mr Sweet, and Dr de la Chapelle); Department of Human Genetics, Sylvius Laboratory, Leiden University, Leiden, the Netherlands (Drs Wijnen and Fodde, Ms van der Klift, and Mr Franken); and Department of Clinical Genetics, Erasmus University Medical Centre, Rotterdam, the Netherlands (Dr Wagner). Mr Franken and Dr Fodde are currently affiliated with the Department of Pathology, Josepheine Nefkens Institute, Erasmus University Medical Centre.

Corresponding Author: Henry T. Lynch, MD, Department of Preventive Medicine, Creighton University School of Medicine, 2500 California Plaza, Omaha, NE 68178 (htlynch@creighton.edu).
MSH2 MUTATION AND HEREDITARY NONPOLYPOSIS COLORECTAL CANCER

match repair genes: MSH2, MLH1, MSH6, MSH3, and PMS2. More than 400 different pathogenic mutations have been registered in the international database of mutations in HNPCC kindreds (available at http://www.ncbi.nlm.nih.gov). Detection of mutations is usually performed by sequencing. In the context of the present report it is noteworthy that by sequencing and other commonly used genetic testing methods, 1 class of mutations, ie, large structural rearrangements such as large deletions, is difficult to detect. These mutations can be readily detected by Southern hybridization, multiplex ligation-dependent probe amplification, and after conversion to haploidy.10,11

Among patients who meet the diagnostic criteria for HNPCC, about 40% to 60% test positive for mutation, demonstrated by a germline mutation in 1 of the DNA mismatch repair genes; more than 90% of these mutations are in MLH1 or MSH2, while approximately 10% are MSH6 mutations.12 Up to 15% of all disease-causing mutations in patients with HNPCC are believed to be large deletions, especially in MSH2.8

A founder mutation arises in a single individual whose offspring each have a 50% chance of inheriting the mutation. The fate of the mutation in the subsequent generations will depend on 2 main factors, namely, selection and chance. If the mutation leads to selective advantage, it may increase in frequency. If it leads to selective disadvantage, eg, reduced reproduction, it may disappear.

In the absence of selection, chance events known as genetic drift can greatly influence its prevalence in the population. Typically the incidence of founder mutations can be increased at population bottlenecks. If, for instance, a mutation occurs with a low incidence (eg, 1:1000) in a mixed population, and if 10 people were to emigrate from this population to an uninhabited island, and if 1 of the 10 were to have the mutation, then a 100-fold enrichment would occur at the founding. In the new population, genetic drift will determine whether the frequency of 1:10 at the founding increases or decreases, or perhaps remains unchanged. The more isolated the population, the more pronounced will be the effects of genetic drift. For these reasons most founder mutations of this type have been described in populations that have remained isolated while growing rapidly. Prime examples are the Finns (“founded” [ie, the main bottleneck occurred] approximately 2000 years ago13), Icelanders (approximately 1100 years ago14), Ashkenazi Jews (600-800 years ago15), and French Canadians and Amish (250-400 years ago16). Hereditary breast cancer in the Ashkenazi Jews, with cancer-predisposing founder mutations in BRCA1 (185delAG and 5382insC) and BRCA2 (6174delT), is a recent well-published example of a founder effect.17,18

At least 5 examples of founder mutations have been described in cases with HNPCC. In Finns, a 3.5-kilobase (kb) deletion of MLH1 comprising exon 16 accounts for as many as half of all cases of HNPCC, and a splice-site mutation affecting exon 6 of MLH1 accounts for 15% to 20% of all cases.10 A splice-site mutation affecting exon 5 of MSH2 was first detected in a large kindred in Newfoundland and later turned out to be widespread in that population through a founder effect.21 Interestingly, this mutation has been observed in many other populations as well, and actually arises de novo with appreciable frequency22,23; thus, this mutation is a recurrent one worldwide but its spread in Newfoundland is by the founder mechanism. A nonsense mutation leading to a stop codon in exon 19 of MLH1 is widespread in the Valais region of Switzerland.24 A fifth founder mutation, 1906G→C in MSH2, is a major contributor to HNPCC in the Ashkenazi Jewish population, where it may account for as many as one third of all cases.

The identification of a deletion encompassing exons 1 to 6 of the MSH2 gene in 7 seemingly unrelated families with HNPCC from Creighton University’s hereditary cancer resource suggested the existence of a founder mutation in the United States and prompted the screening of an additional cohort of 11 families with HNPCC from Ohio State University’s resource, where this deletion was identified in 2 additional cases.26 Because of the extremely high risk for colorectal (80%-85%), endometrial (56%), ovarian (12%), and a number of other cancers in patients with HNPCC, it is desirable to diagnose carriers of these mutations in order to identify high-risk individuals needing targeted clinical surveillance. Our purpose is to describe the existence of a mutation that is widespread in the US population as a result of a founder effect and its implications for the early detection and prevention of cancers associated with HNPCC.

METHODS

This study was approved by the institutional review boards of Creighton University and the Ohio State University. Family members provided informed consent for separate institutional review board–approved colon cancer genetics studies at each participating institution. Genealogical, medical, and pathological reports were collected for members of the 9 extended families over a period of more than 30 years. Each family displayed inheritance patterns of cancer consonant with HNPCC, in concert with the presence of the mutation.

Genealogical Studies

Available key family members from each of these mutation-verified HNPCC families were personally interviewed when possible and/or completed detailed family history questionnaires. In order to decipher the relationships among the 9 families, genealogical records from 3 databases were intensively searched: the Church of Jesus Christ of Latter-Day Saints Family Historical Library, Ancestry.com, and a German family genealogy database. These databases contained information from individual family annals as well as from federal and state census records. Dates of birth, death, and marriage were recorded, as were places of family origin, temporary or permanent residential settlements, west-
ward migration patterns, immigration records, land ownership, personal wills, and religious affiliations.

Haplotype Analysis
The exact breakpoints of the MSH2 deletion mutation have been described previously. By the detailed nucleotide nomenclature the deletion can be characterized as g.5330483_5349647del19165 (NT034483). The 5’ breakpoint is 1 kb upstream of exon 1 and the 3’ breakpoint is in intron 6. Totally the deletion comprises 16 kb of genomic DNA, including exons 1 to 6. The fact that the breakpoint was exactly the same at the nucleotide level in the different families is evidence that the mutation has a common origin. In addition, we used numerous polymorphic markers to construct haplotypes that proved the common origin of the mutation in all 9 families.

Detection Method
As a diagnostic tool, polymerase chain reaction analysis of genomic DNA produces a unique 1.7-kb band when primers 5’-GCTGAATTTAGGTGTGGAAC-3’ and 5’-AAGCATCACAGTTACTGTG-3’ are used. The experimental conditions are as recommended in the Expand Long Template polymerase chain reaction system (Roche Diagnostics Corp, Indianapolis, Ind).

Southern Blot Analysis
Southern blot analysis of MSH2 was performed as previously described with XbaI, HindIII, NsiI, EcoRI, and BclI genomic DNA digests followed by hybridization with 3 overlapping cDNA probes (encompassing exons 1-7, exons 7-12, and exons 10-16). The American founder deletion is characterized by an aberrant EcoRI fragment of approximately 14 kb and by an approximately 13-kb BclI aberrant band, after hybridization with the MSH2 probe encompassing exons 7 to 12.

European and Australian HNPCC Studies
A total of 407 European and Australian families with HNPCC were screened for the presence of the American founder deletion. The Dutch HNPCC cohort (250 families) was collected through the Dutch Foundation for the Detection of Hereditary Tumors and through various Dutch clinical genetics centers. The European cohort (99 families; 58 residing in Germany and 41 residing elsewhere) was made up of patients with HNPCC participating in the Concerted Action Polyp Prevention 2 (CAPP2) study. The remaining cohorts were made up of families from Italy (n=12), Australia (n=31), and Norway (n=15). Of the 407 European and Australian families, 37 had large genomic rearrangements; none had the American founder mutations. The HNPCC families described above were selected because they tested negative for point mutations in MSH2, MSH6, and MLH1 after denaturing gradient gel electrophoresis analysis or direct sequencing.

RESULTS
During the genetic testing portion of our family studies process, it was discovered that 9 families (Families A-I) from across the United States shared the identical deletion of exons 1 through 6 of the MSH2 gene and a unique haplotype of the region. Currently, a total of 566 high-risk individuals from these 9 families have been ascertained, and DNA testing for the mutation has been performed in 137 individuals. Of these, 61 individuals, residing in 14 states, were carriers of the mutation, while the remaining 76 individuals did not carry it.

In concert with genealogical studies that identified geographic and ancestral relationships, we established a 13-generation lineage that was ultimately traced back to a single couple, the progenitors, who migrated from Hesse, Germany, and settled in Pennsylvania in the early 1700s. The first 3 generations of this family are illustrated in Figure 1. The progenitor couple (II-1 and II-2) had 11 children, 2 of whom (III-2 and III-10) have been determined to be obligate carriers of the MSH2 deletion based on the direct links of Family E, F, and G to their descendants.
Currently, it is unclear from which parent individuals III-2 and III-10 inherited the mutation (Figure 1). However, if it was inherited from their father (II-1), there are even more at-risk individuals in the United States, since the progenitor couple (II-1 and II-2) immigrated from Germany with his parents and 5 siblings but none of her (II-2) relatives. Figure 1 shows the large number of possible mutation carriers in generation II under the assumption that II-1 was the carrier.

Figure 2 gives an idea of the truly enormous numbers of potential mutation carriers irrespective of whether II-1 or II-2 was the carrier parent. At minimum, it is clear that the 9 siblings of the 2 obligate carriers were at 50% risk for inheriting the founder mutation. Furthermore, as illustrated in Figure 2, the obligate carriers (III-2, III-10) had 12 known children, each of whom were also at 50% risk; the 9 at-risk siblings (III-1, III-3 through III-9, III-11) had 35 known children, each of whom had a 25% chance of inheriting the mutation.

Figure 2 also depicts the remainder of the genealogical record emanating from the progenitor couple. It not only illustrates the direct lineal relationships that were established connecting families E, F, and G to our putative progenitors, but also illustrates the extensive number of additional family members potentially at high risk for carrying the MSH2 deletion. The remaining 6 families, while harboring the founder mutation, have not been unequivocally linked genealogically. These families may descend from

**Figure 2. Pedigree of the Founder HNPCC Family**

This pedigree depicts the potential number of individuals at risk for carrying the mutation. For example, 9 of the 10 children of individual III-2 had a total of 61 children, of which 23 had 43 children. HNPCC indicates hereditary nonpolyposis colorectal cancer; ind, individual(s). Dashed lines indicate earlier generations that were unable to be traced.
female family members who married and whose descendants have a variety of surnames that have been difficult to trace with certainty.

Thus, while we cannot yet provide the established lineal or collateral relationships connecting families A through D, H, and I to our progenitors, we do provide evidence of their ancestral interactions with respect to time, geographical location, and genetic mutation.

FIGURE 3 is a succinct algorithm that depicts the relative time frame that 8 of the 9 families coexisted with the progenitor couple or with their descendants. Currently, the time frame when family H coexisted with the progenitor couple is unclear.

The map of the United States with the respective migration pattern of each of the 9 HNPCC families is depicted in FIGURE 4. This map denotes the migration patterns of the descendants of the progenitors. Note that families A, B, C, D, H, and I reside in regions along the migratory path.

To further test the hypothesis that this deletion is an American founder mutation, we screened 407 European and Australian families with HNPPCC. Southern analysis of the MSH2 gene among these families did not reveal the presence of the aberrant EcoRI and BclI fragments characteristic of the American founder mutation. However, 24 other MSH2 deletion types accounting for 37 cases were identified.

**COMMENT**

Studies using genealogical, clinical, and molecular genetic technology have enabled us to confirm the common heritage of these 9 families with HNPCC, each of which had been identified independently in diverse geographic areas of the United States (Figure 3). They were then eventually tracked to their progenitors’ origin in Germany in the early 18th century (Figures 1 and 2). The presence of an identical MSH2 del exon 1 to 6 mutation in each family and a shared haplotype provided substantial evidence in support of the founder nature of this phenomenon. Unlike previous reports of founder mutations in hereditary cancer syndromes, this study involved families in a genetically heterogeneous population, spread over a wide geographic area.

**Time of Origin and Spread of Founder Mutations**

Two MLH1 founder mutations causing HNPCC in Finland were found to have arisen or been introduced approximately 1000 years ago and approximately 250 years ago, respectively. Calculations of the “age” of a mutation depend on extensive haplotype analyses and are not very precise. In the case of the American founder mutation we describe here, all the evidence suggests that the founding (ie, the bottleneck of 1 immigrant introducing it on the American continent) took place 12 or 13 generations ago, in 1727.

For the sake of clarity it might be emphasized that one cannot determine exactly when the mutation arose. It could not have occurred later than generation II, as at least 2 members of generation III were obligate mutation carriers (Figure 1). Conversely, it could have existed in the German population for un-
known numbers of generations before the American founding event. We screened 407 families with HNPCC by Southern hybridization of the MSH2 and MLH1 genes and found 37 with a deletion or other genomic rearrangement. Among these, there were many deletions of MSH2, including deletions encompassing exons 1 to 6. More importantly, however, by polymerase chain reaction analysis, none of these were the same as the American founder mutation. The series of families described in the “Methods” section is by far the largest series of HNPCC cases hitherto studied systematically for genomic rearrangements; therefore, the absence of the American founder mutation is significant. While the existence of the American founder mutation in Europe cannot be excluded, it is certainly unlikely to be common.

**Diagnostic Significance and Prevalence of Founder Mutations**

In the case of Finland, the 2 founder mutations described above (ie, 3.5-kb MLH1 del exon 16; splice-site mutation of MLH1 at exon 6) are so prevalent in specific regions of Finland (50% and 20%, respectively, of all cases of HNPCC) that their detection has been used as a first step in primary screening for HNPCC.32,33 We argue here that the American founder deletion of MSH2 may turn out to account for a significant proportion of all cases of HNPCC in the white US population. This hypothesis is based on 2 facts. First, we have already diagnosed 61 individuals from 9 ostensibly unrelated families by studying families with clinically diagnosed HNPCC at just 2 Midwestern oncology services. These families all reside in proximity of the historically proven westward movement of the progenitors’ descendants. In addition to these families, additional codescendants of the progenitors presumably reside in locales along this path (eg, Pennsylvania, North Carolina, Alabama) that remain to be explored in this regard. Second, importantly, the earliest generations of the pedigree (Figure 1) show that there were scores of individuals at risk whose descendants are not known to us. The a priori risk of having the mutation is 50% in all siblings and children of a mutation carrier, so evidence supports the notion that the affected families we have encountered represent but a fraction of all that exist. The specific mutation observed here, being a large deletion, would not be detected in a routine HNPCC mutation analysis as currently performed in most laboratories.

In our studies of HNPCC to date, this MSH2 mutation was the most frequently observed specific alteration.34 An assay for this specific mutation should be added to routine MSH2 testing in the United States. Previously tested families with HNPCC for which no mutation was found should be retested for this specific mutation. Should they test positive for the MSH2 del exon 1 to 6 mutation, affected patients may then benefit from highly targeted screening and management programs for HNPCC-associated cancers.3,35

Our documentation of a founder phenomenon in 9 independently ascertained American families with HNPCC who trace their origin to Germany, and who heretofore were not known to have been related to each other, has important heuristic interest. Their public health impact from the cancer prevention standpoint harbors potential implications for cancer control.

In addition to the cancer control implications, knowing the molecular breakpoints for the founder mutation may also reduce the economic barriers to consumers and third-party payers. Reyes et al,36 in their discussion of selection strategies for genetic testing of patients with HNPCC, already point out that molecular testing for HNPCC is becoming a standard of care, and is cost-effective when compared with the absence of such genetic testing. Thus, if the detection of this founder mutation is used in the primary screening for HNPCC, the cost-effectiveness of DNA screening may increase even more.
MSH2 MUTATION AND HEREDITARY NONPOLYPOSIS COLORECTAL CANCER

In conclusion, the current strategy for DNA screening for HNPCC-associated mutations has been based on the observation that there are no common mutations except in rare, relatively small and homogeneous populations with important founder mutations. Our report highlights the fact that common mutations may exist in large and diverse populations. It reinforces the continuing need for genetic centers to share information on mutation frequencies via databases and publications, and for clinical testing centers to translate new findings into improved DNA testing protocols.

Author Contributions: Dr H. T. Lynch, as principal investigator of this study, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analyses.

References