Impact of Gender and Parent of Origin on the Phenotypic Expression of Hereditary Nonpolyposis Colorectal Cancer in a Large Newfoundland Kindred With a Common MSH2 Mutation

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PURPOSE: This study was designed to provide precise estimates of death and cancer risks, by gender and parent of origin, in hereditary nonpolyposis colorectal cancer independent of mutation, geographic variation, and ascertainment bias. METHODS: A group of 12 families with a founder MSH2 mutation (nucleotide 943+3, A → T) causing hereditary nonpolyposis colorectal cancer was identified in Newfoundland. Genetic testing was offered to those at 50 percent risk of inheriting the mutation. Medical records were reviewed to identify cancer types, age at onset of cancer, and age at death. Ascertainment bias was limited by analyzing only sibships with good ascertainment of genetic status (≥50 percent of sibships had known genetic status). RESULTS: Of 302 family members with hereditary nonpolyposis colorectal cancer or at 50 percent risk, 151 (50 percent) were considered to be mutation carriers, 96 (32 percent) were mutation negative, and 55 (18 percent) were of unknown mutation status. By age 50 years, 72 percent of males and 72 percent of females who were hereditary nonpolyposis colorectal cancer mutation carriers had developed cancer. The age-related risks of colorectal cancer or of death of cancer were significantly higher in males than in females (relative risk = 2.8, \(P = 0.0001\) and relative risk = 2.1, \(P = 0.01\), respectively). The mutation was transmitted by the mother more frequently than the father. Females who inherited the mutation from their father had an increased risk of developing colorectal cancer (relative risk = 2.5, \(P = 0.05\)) and of dying of cancer (relative risk = 2.7, \(P = 0.04\)) compared with females who inherited the mutation from their mother. CONCLUSIONS: Investigation of large kindreds from the same geographic area who share the same MSH2 mutation and in whom family members have been identified with little ascertainment bias suggests that the risks for colorectal cancer and death of cancer are higher for male mutation carriers than for females and that females who inherit the mutation from their father are at higher risk of colorectal cancer than females who inherit the mutation from their mother. [Key words: Hereditary nonpolyposis colorectal cancer; Genetics; Cancer risk; Newfoundland]


Colorectal cancer (CRC) is the second most common cause of cancer-related death in North America.1 From 5 to 10 percent of CRCs are caused by a hereditary predisposition.2–4 One form, hereditary nonpolyposis colorectal cancer (HNPPC), is an autosomal dominant CRC syndrome characterized by early onset and increased lifetime risk for metachronous colorectal tumors and extracolonic tumors, including those of the endometrium, ovary, stomach, ureter, upper biliary tract, skin, small bowel, and brain.5–7

Mismatch repair genes aid in mismatch repair during DNA replication, and mutations in these genes result in genome-wide microsatellite instability.8 Germline mutations in at least six mismatch repair genes have been shown in HNPPC kindreds world-
Mutations in the mismatch repair genes MSH2 and MLH1 account for up to 90 percent of HNPCC kindreds that fulfill the Amsterdam criteria. Many of the mutations are unique, but founder mutations have been identified in genetic isolates.

An extensive Newfoundland family (Family C) was critical to the original study that demonstrated linkage between hereditary CRC and the locus on 2p. A germline mutation in the DNA mismatch repair gene MSH2 was identified. This was a point mutation, A → T at nucleotide (nt) 943 + 3 (A → T nt 943 + 3) in the 3' splice site of exon 5, which results in the deletion of exon 5 and a truncated protein. Other Newfoundland HNPCC families were tested for this mutation, and a total of 12 independently ascertained families, all with ancestors from the same geographic region as Family C, were shown to carry this mutation. A common haplotype of markers linked to the MSH2 locus suggested a founder effect for the Newfoundland families. This same mutation has been identified in several North American, European, and Asian families, but haplotype analysis indicates recurrent mutational events in these other families.

We identified 447 individuals who were affected or who were at 50 percent risk from among the 12 families in which this same MSH2 mutation is segregating. Mutation testing was offered to all individuals at 50 percent risk of inheriting this mutation. Precise estimation of death and cancer risk in this extremely large kindred with an identical mutation should facilitate the identification of genetic modifiers and factors that determine phenotypic expression, unhindered by differences in methods of ascertainment, geographic variations in environmental factors, and allelic heterogeneity. Furthermore, these data have important implications for carriers of this mutation with respect to clinical screening, counseling, and management of cancers.

**PATIENTS AND METHODS**

**Family Ascertainment**

More than 80 families with hereditary CRC have been referred to the Medical Genetics Clinic at Memorial University, St. John’s, Newfoundland, Canada. Twelve of these families were confirmed as having HNPCC and carrying the MSH2 mutation A → T nt943 + 3. Extensive archival research traced the ancestors to the northern coast of Newfoundland, and a common ancestor was identified for four families. Ancestors of the remaining 8 families came from a geographic area within a radius of 40 km. Eight families have a common haplotype of five microsatellite markers over a minimum 5.9-centimorgan region that spans D2S391 to D2S123. Two families had a different haplotype distal to MSH2 but had an identical haplotype for three centromeric markers. Because of geographic proximity in an isolated area, because they have the same mutation, and because they have similar haplotypes, it is likely that all of these families have a common ancestor. Consanguinity has been recognized in some branches of this extended kindred (usually second cousin or more distant relationship); however, no marriages have been identified in which both members of the couple carry this MSH2 mutation. Thus, no homozygotes for this mutation have been identified in the present study.

**Diagnosis**

To determine the phenotype, up to 5 generations of each family were identified that comprised 447 individuals who were affected or at 50 percent risk of inheriting the mutation and for whom data were available concerning survival. When the mutation was identified for each family, genetic testing was offered to all family members at 50 percent risk, as part of the Clinical Genetics service. This mutation was originally detected by in vitro coupled transcription/translation and sequencing. A clinical service test for this particular mutation was then developed that involved polymerase chain reaction amplification of exon 5 and the flanking intron regions of MSH2, followed by sequencing of this fragment. The normal DNA fragment with A at nt943 + 3 can be distinguished from the mutant DNA fragment with T at this nucleotide with this method.

More than 80 percent of those at risk requested genetic testing. Appropriate counseling was provided before and after testing. Individuals were considered carriers if 1) they were shown by DNA analysis to have the mutation, 2) they presented with an HNPCC tumor and were known to be at 50 percent risk of having the mutation, or 3) they were obligate carriers because their children had the mutation. Mutation-negative status was confirmed by DNA analysis. The remaining family members were considered to have unknown mutation status. Forty-seven of the 447 family members had been included in the previous study.