Genetic Counseling in a Navajo Hereditary Nonpolyposis Colorectal Cancer Kindred

Henry T. Lynch, M.D.[1]
Tom Drouhard, M.D.[2]
Hans F. A. Vasen, M.D., Ph.D.[3]
Jennifer Cavalieri, R.N.[1]
Jane Lynch, B.S.N.[1]
Suzanne Nord, B.S.[1]
Thomas Smyrk, M.D.[6]
Stephen Lanspa, M.D.[4]
Patricia Murphy, Ph.D.[5]
Kathy L. Whelan, B.S.[5]
June Peters, M.S.[7]
Albert de la Chapelle, M.D.[8]

[1] Department of Preventive Medicine, Creighton University School of Medicine, Omaha, Nebraska.

[2] Tuba City Indian Medical Center, Tuba City, Arizona.

[3] The Netherlands Foundation for the Detection of Hereditary Tumours, Leiden, The Netherlands.

[4] Department of Gastroenterology, Creighton University School of Medicine, Omaha, Nebraska.

[5] OncorMed Laboratories, Gaithersburg, Maryland.

[6] Department of Pathology, Clarkson Hospital, Omaha, Nebraska.

[7] Consultant, Human Genome Research Project, NIH, Medical Genetics Branch, Bethesda, Maryland.

[8] Faculty of Medicine; University of Helsinki, Helsinki, Finland.

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Address for reprints: Henry T. Lynch, M.D., Department of Preventive Medicine, Creighton University School of Medicine, 2500 California Plaza, Omaha, NE 68178.

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BACKGROUND. Cross-cultural genetic counseling was provided to an extended Navajo Indian family in which the MLH1 gene mutation for hereditary nonpolyposis colorectal cancer (HNPCC) had been identified. The family had been observed by the authors since 1983 and over the years had been provided with intensive education regarding the natural history of HNPCC as well as recommendations for cancer surveillance and management that was responsive to this natural history.

METHODS. Following identification of the MLH1 mutation, DNA from family members was evaluated by a reference laboratory (OncorMed, Gaithersburg, MD), where sequences were checked in both the forward and reverse directions against the published sequence for MLH1. The 4bp deletion beginning at the first nucleotide of codon 727 was easily visualized in the heterozygous condition in both affected and predispositional individuals. The family was reeducated as a group and then provided further education individually during genetic counseling sessions, at which time they were appraised of potential penalties, such as insurance and employer discrimination, and psychological sequelae that could result from knowledge of the MLH1 mutation. Strict confidentiality of this information was assured.

RESULTS. DNA testing was performed on 51 family members. Twenty-three individuals were counseled, seven of whom were positive for MLH1. Reactions ranged from full acceptance of the genetic implications to traditional Navajo reasoning such as the family had been cursed.

CONCLUSIONS. DNA-based genetic counseling requires compassion and empathy, coupled with intensive preeducation regarding potential penalties and advantages that might emanate from this knowledge. Special care must be given to patients' culture, beliefs, and traditions. *Cancer* 1996; 77:30–5.
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KEYWORDS: cross-cultural genetic counseling, Navajo, MLH1 mutation, hereditary nonpolyposis colorectal cancer.

Colorectal cancer (CRC) occurs infrequently in Native Americans.[1–5] To the authors' knowledge, the hereditary variant of CRC, with the exception of a single Navajo family who are the subject of this report, has never been documented. This investigation of this large Navajo kindred has extended over more than a decade and culminated in their diagnosis of hereditary nonpolyposis colorectal cancer (HNPCC)[6] and the identification of a mutation in the culprit MLH1 gene.[7]

An account of what is believed to be the first cross-cultural example of genetic counseling for hereditary cancer based on DNA findings in this family will be presented.

METHODS

The family was referred to the authors by a surgeon (T.D.) in 1982 because of an apparent excess of CRC among the first-degree relatives of the

proband. Informed consent was given by Creighton's Institutional Review Board for this study. In 1983, a visit to the family's reservation to educate family members about the natural history and genetic risk factors in HNPCC was made after utilizing the Public Health Hospital facilities at Tuba City, Arizona. Individuals who were at 50% risk for cancer, based on their position in the pedigree, were encouraged to begin colonoscopy or barium enema screening by age 25 and have this procedure performed every 2 to 3 years. Colonoscopy equipment was not available at the Public Health Hospital, but barium enemas could be performed. However, none of the high-risk family members followed this recommendation. A program for guaiac testing of stool was also offered, but the response was not enthusiastic.

A second visit to the reservation was made in 1989. Further education about HNPCC and its cancer control objectives were provided to approximately 50 family members. In addition, research colonoscopies were performed by gastroenterologists on nine high-risk family members.[8] DNA was obtained from peripheral blood lymphocytes to test for linkage. Following exclusion of the 2p chromosome locus, the authors focused on chromosome 3p and employed 12 microsatellite markers from this locus for pairwise linkage analysis. Linkage to the MLH1 locus was demonstrated by lod score values above 2 for the closest flanking markers, where the highest pairwise lod scores were 2.88 at $\theta = 0.0$ for D3S1619 (1 cm distal to MLH1) and 2.80 at $\theta = 0.0$ for D3S1561 (0 cm to MLH1). Sequence analysis of the MLH1 gene revealed a 4-bp deletion beginning at the first nucleotide of codon 727, which predicted a frameshift and addition of new amino acids to the carboxy-terminal end of the protein.[9] The mutation was identified in two affected individuals from separate branches of the family.

Reference Laboratory

These MLH1 mutation findings were reconfirmed at a reference laboratory (OncorMed, Gaithersburg, MD).

DNA Purification

Frozen white blood cell pellets were resuspended in 1× phosphate-buffered saline, and DNA was extracted using the QIAamp Blood Kit (Cat. no. 29104, Qiagen, Inc., Chadsworth, CA). A protease solution and a chaotropic salt buffer were added to the tube, which was then mixed and incubated at 70°C. Ethanol was added to the tube, and the lysate was transferred to a QIAamp spin column and collection tube. The column was centrifuged and washed twice with an ethanol buffer. The DNA was eluted off of the column into a clean tube with distilled water.

Identification of Mutations

Polymerase chain reaction was used to amplify exon 19 of the MLH1 gene from sample genomic DNA. The reaction products were purified using the QIAquick Spin PCR Purification Kit (Cat. no. 28104, Qiaagen, Inc.). Cycle sequencing was performed on the purified reaction products using the PRISM™ Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit (Cat. no. 401628, ABI, Foster City, CA). Unincorporated dye was removed with Centri Sep spin columns (Cat. no. PSR00105, Princeton Separations, Adelphia, NJ). The dye-labeled reaction products were separated on acrylamide gels; data were collected and analyzed using automated sequencers and software (Model 377, ABI). Sequences were checked in both the forward and reverse directions against the published sequence for MLH1. The 4bp deletion beginning at the first nucleotide of codon 727 was easily visualized in the heterozygous condition in both affected and predispositional individuals.

Genetic Counseling

A third visit was made in 1995 to reinforce the educational information and to perform individual genetic counseling to reveal risk status based on the MLH1 mutation data. DNA testing had been performed on 51 family members when linkage was performed. Of these individuals, 43 were direct line blood relatives and 8 were married-in, unrelated spouses. Of the 43 blood relatives, 14 were gene mutation positive, and 29 were negative.

The educational session provided information on the genetics of HNPCC, its natural history, surveillance, and management strategies and the advantages and disadvantages for the family in knowing their DNA status. If they elected to receive their individual DNA results, a permission form containing this educational information was read and a signature was affixed. When there was a language barrier, the material was explained in the Navajo language. Specifically, the information reiterated that, if positive for MLH1, CRC screening (colonoscopy) for early cancer detection or prophylactic subtotal colectomy could be employed. If found to be negative for this germline mutation, they were advised that their screening strategy would revert to that for the general population in accordance with the recommendations by the American Cancer Society.

Potential emotional consequences were reviewed. For example, if found to be negative for MLH1, they were told that they could experience feelings of survival guilt; or if MLH1 positive, they could feel anxious or depressed. They could also encounter intrafamily strife. It was explained that they could face the potential of denial or higher rating of their health and/or life insurance policies.

Prior to revealing their DNA status, the counselees were queried about their expectations of being positive or negative and how this knowledge might alter their lives. The genetic counseling was performed on an individual basis or, when the counselee requested, one or

more close relatives or a spouse accompanied the counselee for emotional support.

RESULTS

During the 2-day 1995 visit, 44 family members came in small groups (5–10 individuals per group) for educational information sessions, each lasting approximately 1 hour. Twenty-three of these 44 individuals received DNA-based genetic counseling (Figure 1). MLH1 mutations and their association with cancer genetic risk status are found in Table 1. Sensitivity and specificity approached 100% in each case.

The counseling sessions revealed that most family members became aware of the cancer occurrences in the family when they were adolescents or young adults, usually when a close relative became affected. When asked when they became aware of a familial or hereditary pattern of the cancer occurrences in their family, the majority of family members noted the time of our first visit in 1983.

Many family members believed the family's fate was influenced by factors such as fear, a taboo, or a curse. One older man believed his rectal bleeding and subsequent CRC was precipitated by his "peeing on a spider." His explanation for the hereditary colon cancer was that the family had been cursed in previous generations. Another man believed the cancer in the family was not widely discussed because "simply thinking about this or discussing it would magnify the risk." The modern Navajo culture appears to continue to embrace traditional values but also seems open to the augmentation of modern medical techniques to arrive at their highly sought after "harmony."

Four family members, when asked about their surveillance practices, denied their heightened cancer genetic risk: "Hereditary risk isn't an immediate danger," "I feel healthy and see no need to actively pursue medical surveillance," "I'm too young" (age 28), and "I was never advised to undergo screening."

One 71-year-old unaffected woman who had affected siblings and children and who was positive for MLH1 believed her cancer risk to be only 10%. Despite our educational efforts, she continued to demonstrate lack of understanding of the hereditary transmission pattern.

Several family members who had not undergone gene testing wanted their children tested. Genetic transmission was again discussed to explain that the children would not need DNA testing unless the parent was gene positive.

While waiting for counseling, a 31-year-old woman appeared apprehensive and anxious as did her 37-year-old sister. They requested to be counseled together to provide emotional support to each other. They said the information would be dealt with regardless of their DNA findings, and even if seen separately, they would share the information with each other immediately. They were counseled together. The younger sister, who estimated her genetic risk to be 50%, was found to be positive for the gene. She was shocked to learn that she was positive and began crying uncontrollably. She stated that she was afraid for herself and for her children and has requested follow-up family counseling with her primary physician and a psychiatrist. Her sister was negative and was obviously relieved but was extremely supportive of her sister.

One family member who was at 50% risk left the reservation after high school and had received a degree in business. He believed that this more "worldly" experience gave him greater insight into the family cancer problem. He "took the bull by the horns" and pursued surveillance based on his own perception of his genetic risk and "didn't need doctors to put it all together." He was negative for the gene.

Certain interesting vignettes are found in this pedigree for which there is no satisfactory explanation. For example, there are fewer mutation-positive individuals in the pedigree than the 1:1 segregation would predict. This may be due to chance or some bias in the sampling scheme.

There was a wide range in age at onset of cancer among mutation-positive individuals. Specifically, a male manifested CRC at age 74 (III-5), whereas his son (IV-9) presented with CRC at age 23. This could be due to variable expressivity of the MLH1 mutation.

This family, given their Native American status, has access to a local clinic where they can receive medical care without cost. Family members without financial resources may apply for Medicaid benefits, and senior citizens are eligible for Medicare. Private insurance coverage is held by a minority of family members. Possible denial or higher rating of insurance did not seem to concern them.

All but 1 of the 23 individuals counseled appeared to understand what was being told to them. The family members who were given negative results exhibited profound relief and happiness. Those individuals who were given positive results were for the most part stoic, with the exception of the one individual, who was highly emotional, agitated, and later requested additional supportive counseling.

DISCUSSION

Weil and Mittman[10] discussed the need for genetic counselors to learn how to deal effectively with cultural diversity as population shifts occur in the United States. For example, census figures in the United States indicate that between 1970 and 1990 the Latino population increased by 145% and the Pacific Asian population increased by 375%. It is estimated that almost one in three Americans are either foreign born or are members of ethnic minority groups. Thus, there is a need for genetic counselors to be

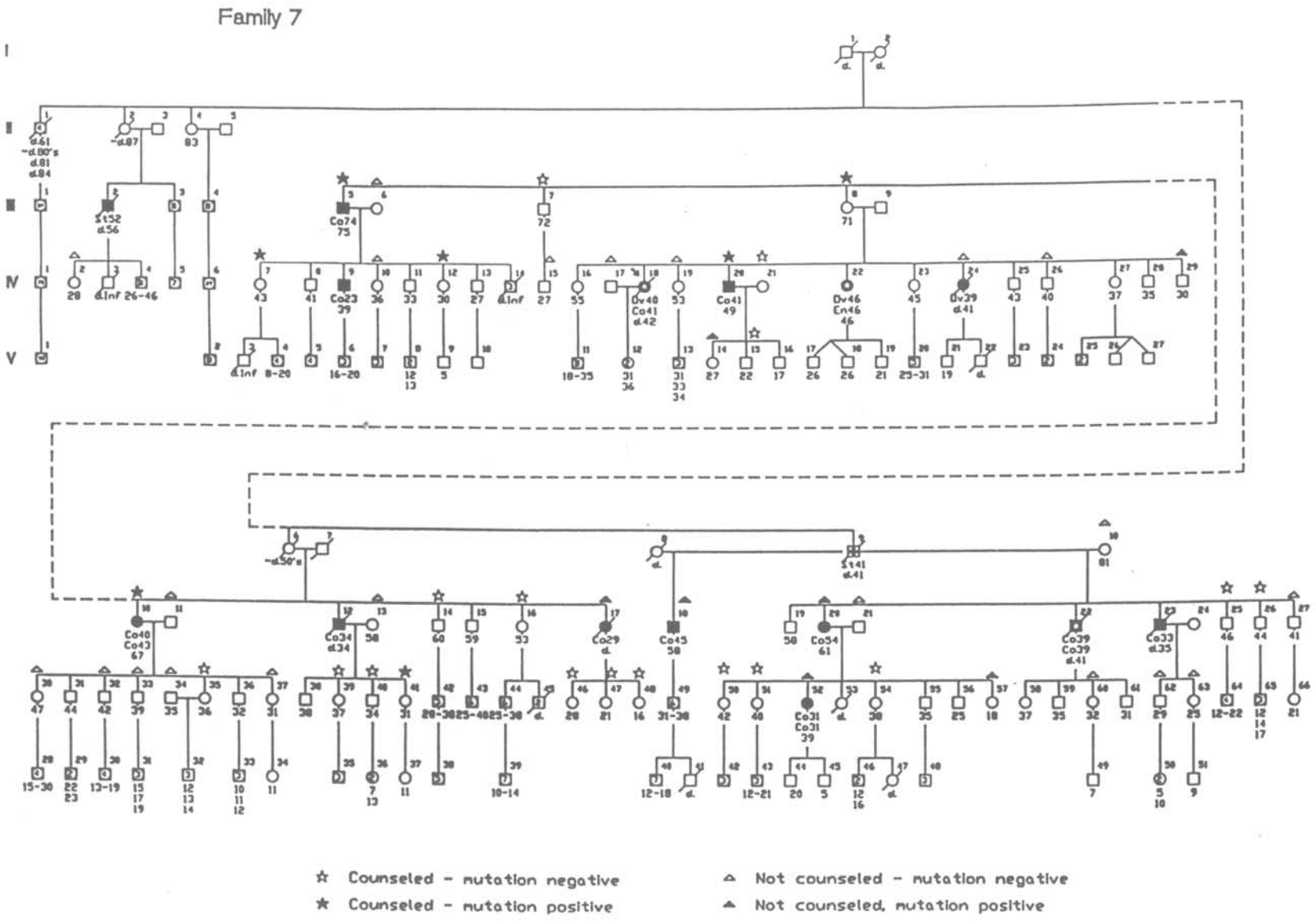

FIGURE 1. Pedigree of an extended HNPCC family with MLH1 germ-line mutation.

TABLE 1
MLH Mutations and Cancer Genetic Risk

	Risk category		
Result	0%	50%	100%
Mutation present	0/8	7/30	6/6*
Mutation absent	8/8*	23/30	0/6

* The sensitivity and specificity of this assay under the current conditions approach 100% in each case.

exposed to the ethnocultural issues that have an impact on these diverse population groups, so that during the genetic counseling the counselor will have a self-awareness and empathy for transference/countertransference issues. Thus, this "ethnocultural approach may be seen as an expansion of the psychological or psychosocial orientation from monocultural to multicultural."[10] Stereotypes, fears, prejudices, assumptions, and beliefs that emanate from individuals who are members of specific minorities must be respected. Unfortunately, insight into these ethnocultural differences are not adequately recognized. The counselor may, because of her/his own cultural beliefs, unconsciously assume a condescending, superior attitude about the interpersonal interactions and health beliefs of the counselee. Several Navajo patients had just these concerns.

Due to the perceived cross-cultural barriers, pertinent aspects of the natural history and genetics of HNPCC were reviewed with each patient in the form of questions to determine whether the counselee understood the implications contained therein. Questions were pursued to determine the patient's desire for genetic counseling and release of the DNA findings, comprehension of genetic risk status, concerns about insurance and confidentiality, and how this information would be used if a positive hMLH1 carrier status was disclosed.

An attempt was then made to assess the patient's reaction to receiving this information. When the patient was positive for hMLH1, the cancer control implications were reinforced once again. When the patient was negative for hMLH1, he or she was advised that this did not eliminate his or her cancer risk and that it would be prudent to follow the American Cancer Society recommendations. Finally, the patients were advised that all of the resources of the Public Health Hospital in Tuba City, including their physician (T.D.), those of the Indian Health Services, and those of Creighton's Hereditary Cancer Institute would be available to them at any time they wished.

Navajo Indians are a subgroup of the Athabascan linguistic group who migrated to the Southwestern United States from Eastern Alaska and Canada about 1000–1200 A.D.[11] It is estimated that approximately 150,000 Navajo reside in New Mexico and Arizona. Most of them live on reservation lands, where they have undergone relatively little genetic mixing with other racial groups. This racial homogeneity and the generally common environmental exposures of reservation life make it likely that the evaluation of cancer family history and lifestyle among the Navajo could elicit important epidemiological clues about host and environmental interaction in cancer etiology. Prospective studies employing the MLH1 mutation could facilitate such genetic–epidemiological research.

Knowledge of the genetics, inclusive of molecular genetics and natural history of HNPCC, coupled with the recommendations for screening and management are extremely important given the lack of premonitory clinical signs of cancer genetic susceptibility in HNPCC.[12] Such knowledge is mandatory for diagnosis, screening/management, and genetic counseling in HNPCC.

It is important to advise patients who manifest an HNPCC gene that their cancer risk is in the range of 85% to 90% (and not 100%) in accord with the gene's reduced penetrance.[13] Indeed, this may be the case for the 71-year-old unaffected female who was positive for the MLH1 mutation and who had affected siblings and children. The reason for this reduced penetrance remains elusive, but it could be due to lack of exposure to specific environmental factors or to a modifier gene.[14]

An example of such a modifier gene (*Mom1*) is found in the Min mouse model, which provides a good approximation to familial adenomatous polyposis in the human. The Apc gene in the Min model is believed to be the APC homologue gene for FAP. Dove et al.[14] suggested that a modifier gene, namely the *Mom*-1 gene, controls tumor multiplicity in these mice, but the molecular mechanism of action of this modifier locus is not known. It is therefore plausible that a comparable modifier locus exists in HNPCC patients who have the hMLH1 mutation and which thereby may be contributing to its reduced penetrance.

Members of this family had shown extremely poor compliance with the recommendations for colonoscopy or barium enema, procedures that had been recommended at visits in 1983 and in 1989. Cultural differences could have contributed to this poor compliance, but it was hoped that if the family members could understand their enormous cancer risk because of the presence of the MLH1 mutation, this might improve compliance.

It is important to appreciate problems in cross-cultural beliefs when attempting to provide genetic counseling to groups such as this Navajo family. Specifically, they may have perceptions about biology and genetics that differ strikingly from modern scientific medicine. Thus, when providing genetic counseling, one must accept these differences in perceptions, but at the same time relate risk information and surveillance and management

recommendations in a manner that is not only understood but is not upsetting or degrading to the traditional beliefs of that culture. This might best be presented in context with their cultural perceptions by emphasizing a balance of modern medicine and traditional beliefs with a resultant harmonious balance. In the Navajo culture, prediction of future events, such as the possibility of developing colon cancer based on a germ-line mutation, might be viewed either as a curse or, at best, an omen of bad luck. As noted, there was substantial evidence of these interpretations of biology and genetics based on their cultural frame of reference.

For example, the universe as viewed by the Navajo is an orderly system of interrelated elements, in which good and evil are maintained in harmony. If universal harmony is disturbed, illness, death, or other disasters may occur. When an individual feels physically ill, he/she will look for an underlying spiritual cause. It is clear that in the family, especially the older people, under discussion still believe that a spiritual cause, a curse, or failure to observe a taboo (e.g., "peeing on a spider") is responsible for the disease in the family and not a deleterious gene. This may explain the low compliance with screening in some of them. Another important factor is that most Native Americans believe in the power of thought. Negative thoughts or words are like "poisoned arrows that can pierce the heart." One of the family members explained that avoiding negative thoughts (being at high risk of developing cancer) "may be one of the most important reasons for the low response rate."

The Native American values are more present oriented in contrast with the Anglo-American values, which are more future oriented. This may interfere with the discussion about genetic risks in the process of genetic counseling. Someone who predicts the future may be regarded as a witch. A way around this is to explain it in the form of a story or anecdote. Describe other families who have had similar circumstances. Tell about the life of members of a family who are under close surveillance and contrast their lives with a family that does not comply with the recommendations. We are convinced that more effective genetic counseling of this Navajo family might best be undertaken by another Navajo who has resided on the reservation and who is thoroughly familiar with the culture. The information could then be reinforced by a physician.

This HNPCC family will be followed, and particular attention will be paid to compliance with the surveillance and management recommendations provided to those individuals who were harbingers of the deleterious MLH1 gene. This will clearly require a decade or more to assess behavioral changes of germ-line carriers. Changes might be reflected by evaluating their participation in colonoscopy, possibly prophylactic subtotal colectomy, and prophylactic total abdominal hysterectomy and bilateral salpingo oophorectomy. Meanwhile, the study of this family is being expanded to include additional branches of the family who reside on reservations in eastern Arizona and New Mexico and who may also be at risk for HNPCC cancers.

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