BRIEF COMMUNICATIONS

Mutation of an mutL Homologue in a Navajo Family With Hereditary Nonpolyposis Colorectal Cancer

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Hereditary nonpolyposis colorectal cancer (HNPCC) is the most common form of inherited colon cancer and may represent the most frequent hereditary cancer-prone disorder (*1*). The syndrome has been observed in whites, blacks, and Orientals, but only once in Native-Americans, in a large Navajo kindred (*2,3*). We believe that this Navajo family is the only known example of an American-Indian family with hereditary colorectal cancer of any type. Colorectal cancer has been observed to occur only rarely in American Indians (*4-8*).

HNPCC has recently been linked to sites on two different chromosomes: Some affected families show linkage to 2p (*9*), and others show linkage to 3p (*10*). The following four human DNA mismatch repair genes have been cloned and characterized: MSH2 (Mut S homologue 2) on chromosome 2p (*11,12*), MLH1 (mutL homologue 1) on chromosome 3p (*13,14*), and PMS1 and PMS2 on chromosomes 2 and 7 (*13*), respectively. Mutations of these genes occur in HNPCC (*12-14*).

In this brief communication, we report on an extended Navajo family (family 7) with the Lynch syndrome II variant of HNPCC that carries a mutation of MLH1 on chromosome 3p. Two visits by our cancer genetic research team during the past 14 years led to the evaluation of more than 100 members of this family. The family was thoroughly educated about the natural history of HNPCC. Detailed genealogic histories were compiled, and medical and pathology records were reviewed (Fig. 1 and Table 1). Molecular genetics methods are described in an unpublished manuscript (Nyström-Lahti M, Parsons R, Sistonen P, et al.: manuscript submitted for publication).

DNA was obtained from peripheral blood lymphocytes of family members to test for linkage. After excluding the 2p locus, we focused on 3p and used 12 microsatellite markers from this locus for pairwise linkage analysis. Linkage to the MLH1 locus was demonstrated by LOD (logarithm of the odds) 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 centimorgan [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-base-pair deletion beginning at the first nucleotide of codon 727 which predicted a frameshift addition of new amino acids to the carboxy–terminal end of the protein (*13*). The mutation was identified in two affected individuals from separate branches of the pedigree (III-14 and IV-14).

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. (*15*). It is estimated that approximately 150 000 Navajo reside in New Mexico and Arizona (*15*). 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 epidemiologic clues about host and environmental interaction in cancer etiology. Prospective studies using the MLH1 mutation could facilitate such genetic–epidemiologic research. This would allow the identification of environmental exposures in concert with knowledge of who is versus who is not inordinately predisposed to cancer.

Genetic counseling, aided by knowledge of the MLH1 status, could be of enormous benefit to this Navajo family at apparently high risk for colorectal cancer. HNPCC has no premonitory physical signs of cancer susceptibility; therefore, early diagnosis has depended on rigorous surveillance of all individuals deemed at 50% risk by pedigree analysis. Cultural differences may contribute to poor compliance. Recognition by family members of their cancer risk due to the presence of the MLH1 mutation could encourage better compliance.

Accurate cross-cultural communication requires an understanding of the perceptions of the individuals being addressed. For example, in the Navajo culture, prediction of future events, such as the possibility of developing colon cancer predicted on the basis of linkage findings, could conceivably be viewed as a curse or, at best, an omen of bad luck (Drouhard T: unpublished observations). Because of these beliefs, risk information, tempered by the availability of preventive surveillance methods and its potential for "good luck," might best be presented to the patient by someone, preferably another Navajo, who is well-

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See "Notes" section following "References."

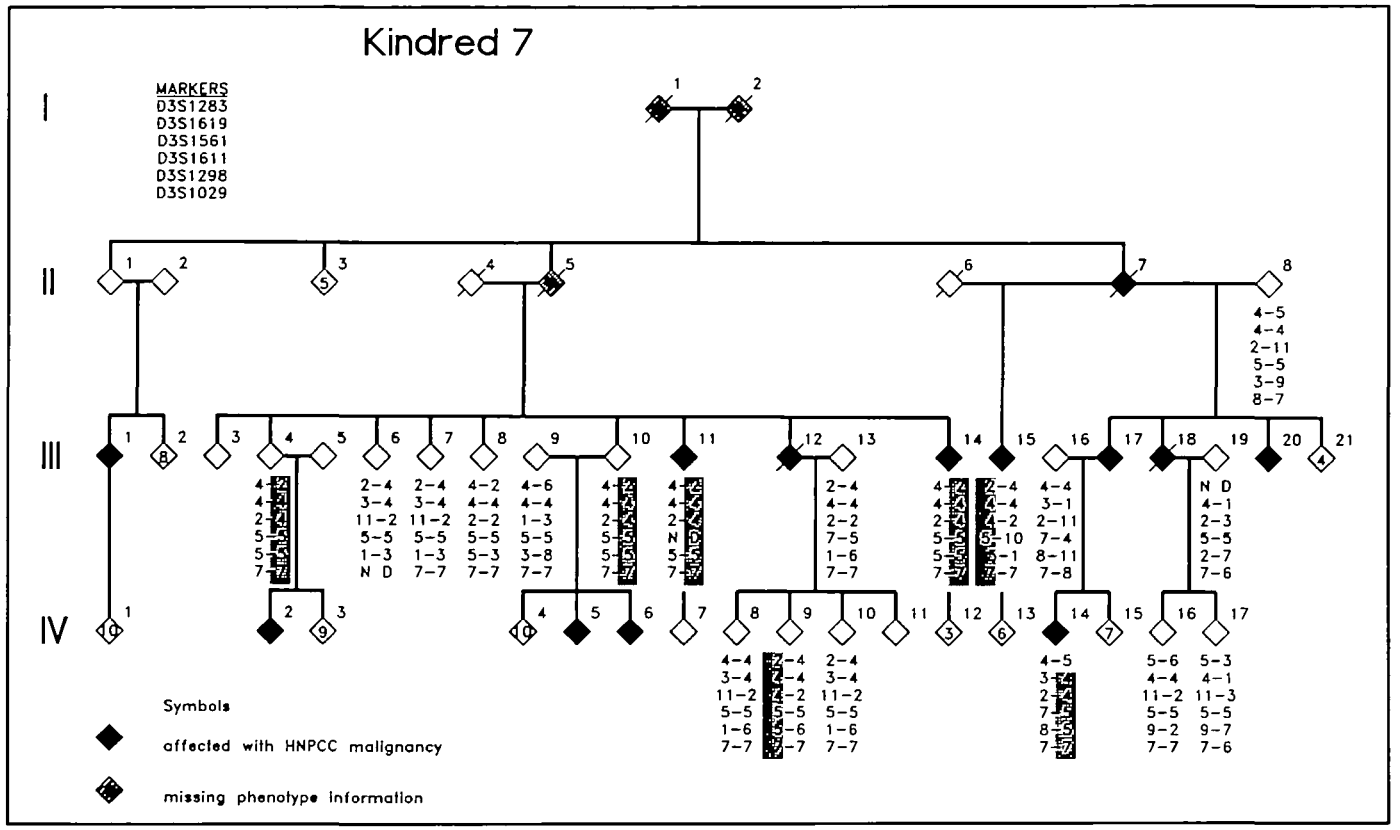

Fig. 1. Pedigree of family 7 (encrypted to protect confidentiality of family members). Alleles of six markers linked to the MLH1 gene are shown (Nyström-Lahti M, Parsons R, Sistonen P, et al.: manuscript submitted for publication). The disease haplotype segregating in this family is shaded.

Table 1. Tumor registry of family 7

Pedigree No.	Age, y	Basis of diagnosis*	Diagnosis
II-7	41	DC	Carcinoma of stomach
III-1	52	PR	Adenocarcinoma of stomach
III-11	40	PR	Adenocarcinoma of ascending colon
	43	PR	Adenocarcinoma of sigmoid colon
III-12	34	PR	Adenocarcinoma of splenic flexure
III-14	29	PR	Adenocarcinoma of hepatic flexure
III-15	45	PR	Adenocarcinoma of splenic flexure
III-17	54	PR	Adenocarcinoma of descending colon
III-18	33	PR	Adenocarcinoma of transverse colon
III-20	39	PR	Adenocarcinoma of transverse colon
IV-2	23	PR	Adenocarcinoma of ascending colon
IV-5	40	PR	Cystadenocarcinoma of ovary
	41	PR	Adenocarcinoma of cecum
IV-6	41	PR	Adenocarcinoma of sigmoid colon
IV-14	31	PR	Adenocarcinoma of sigmoid colon
	31	PR	Adenocarcinoma of sigmoid colon

*DC = death certificate confirmation; PR = pathology report verification.

grounded in cultural behavior, and the information could then be reinforced by a physician.

The prodigious advances in the molecular genetics of HNPCC, culminating in the cloning of the MSH2 and the MLH1 genes, offer unique opportunities for targeted genetic counseling, surveillance, management, and genetic–epidemiologic studies of these cancer-prone families. In the Navajo kindred reported on here, a host of culturally related phenomena has contributed to limited compliance by these family members with our surveillance recommendations. However, we are hopeful that the ability to determine who is versus who is not at increased risk for cancer of specific anatomic sites by identifying the MLH1 mutation will help facilitate our cancer control objectives in this kindred.

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Notes

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Modulation of Serum and Breast Ductal Fluid Lipids by a Very-Low-Fat, High-Fiber Diet in Premenopausal Women

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Breast ductal fluid is the result of apocrine secretion from the cells lining the breast ducts. In about one third of normal premenopausal women, this fluid can be obtained by simple negative-pressure nipple aspiration using a breast pump (*1,2*). Since breast ductal fluid bathes the breast ductal cell and the majority of breast cancers develop in the intraductal environment, the effects of diet on breast cancer development and breast cell growth may be mediated by biochemical changes in breast ductal fluid. A previous study (*3*) has shown that this fluid contains increased concentrations of cholesterol, triglycerides, and fatty acids compared with the levels found in plasma. A low-fat, high-fiber diet is known to modulate plasma cholesterol, triglycerides, and fatty acids. The present study examined the effects of such a diet on the levels of lipids in breast ductal fluid to demonstrate the effects of diet on the local environment of the breast ductal cell.

Premenopausal women aged 25-45 years were recruited for this controlled feeding study conducted at the University of California, Los Angeles (UCLA), General Clinical Research Center. All women were healthy and weighed between 90% and 110% of ideal body weight. Twelve of 22 women screened were found to have obtainable amounts of breast ductal fluid that averaged 40 μL per aspiration. Seven of the 12 women were nulliparous, and all parous women reported breast-feeding at least 2 years prior to entry into the study. Written informed consent was obtained from all women, and all procedures were approved by the UCLA Human Subject Protection Committee.

The duration of the study was 2 months. During the first month, the women were instructed to consume a diet that met American Heart Association recommendations and that contained 30% of calories as fat. During the second month, all the women were provided with all major meals within a very-low-fat, high-fiber diet that provided 10% of calories as fat, 100 mg of cholesterol, and 35-45 g of dietary fiber, using usual American foods. Levels of total cholesterol and triglycerides were determined in plasma and in breast ductal fluid by using standard enzymatic reagents and methods (Abbott Diagnostics, Pasadena, Calif.). Total fatty acids were extracted from plasma and breast ductal fluid and were converted to methyl esters by the direct transesterification method of Lepage and Roy (*4*). The results were expressed as absolute concentrations (μmol/L) of total fatty acids.

There was a small, but statistically significant, decrease (61.72 ± 8.66 versus 60.73 ± 8.72; $P<.05$) in body weight at the end of the 1 month of consuming the very-low-fat, high-fiber diet ad libitum. The average weight loss was 1 kg for a period of 1 month, with a range of 0.5-4.0 kg. Total plasma cholesterol decreased significantly from 174 ± 24 to 152 ± 25 mg/dL (−12%), whereas plasma triglycerides increased by 16% ($P<.05$) at the end of 1 month of the very-low-fat, high-fiber diet period as shown in Table 1. There were no significant changes in cholesterol or triglyceride concentrations in breast ductal fluid after 1 month of diet intervention. Furthermore, the concentrations of total cholesterol and triglycerides were fivefold higher in the breast ductal fluid than in plasma.

The dietary intervention had differential effects on the concentrations of plasma and breast ductal fluid fatty acids, as shown in Table 2. There was a significant increase ($P<.05$) in the plasma concentrations of C14:0, C16:0, C16:1, and C18:1 fatty acids, whereas there were significant reductions ($P<.05$) in the plasma concentrations of C18:0, C18:2, and C24:0 fatty acids at the end of 1 month of the very-low-fat, high-fiber diet. Arachidonic acid (C20:4) concentration did not change significantly. However, there was a tendency for decreased concentrations of all the fatty acids studied in the breast ductal fluid. The concentrations of C16:0, C18:0, C18:1, C18:2, C18:3, C20:1, and C20:2 fatty acids all showed a significant decrease ($P<.05$) at the end of 1 month of intervention with a very-low-fat, high-fiber diet. The concentrations of

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