(*10*) Bondy ML, Lustbader ED, Halabi S, Ross E, Vogel VG. Validation of a breast cancer risk assessment model in women with a positive family history [*see* comment citations in Medline]. J Natl Cancer Inst 1994;86:620-5.
(*11*) Benichou J, Gail MH. Methods of inference for estimates of absolute risk derived from population-based case-control studies. Biometrics 1995;51:182-94.
(*12*) Fleiss JL. Statistical methods for rates and proportions. 2nd ed. New York: John Wiley & Sons, 1981.

Notes

Supported by the Revlon/UCLA Women's Health Research Program. K. A. McGuigan was supported by Public Health Service National Research Service Award 1T32HS00046-01 from the Agency for Health Care Policy and Research, Office of the Assistant Secretary for Health, Department of Health and Human Services, and by Public Health Service grant CA49565 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. Database development was supported by a grant from the Drown Foundation.

We thank the professional staff of the High Risk Clinic at the Revlon/UCLA Breast Center—S. Goldman, Dr. D. Wellisch, Dr. K. Duvall, Dr. A. Hoffman, and the center director Dr. S. M. Love.

Manuscript received March 14, 1996; revised May 22, 1996; accepted June 17, 1996.

Germline Mutations of hMLH1 and hMSH2 Genes in Korean Hereditary Nonpolyposis Colorectal Cancer

*Hye-Jung Han, Ying Yuan, Ja-Lok Ku, Jae-Hwan Oh, Yong-Jin Won, Koo Jeong Kang, Kwang Yun Kim, Sung Kim, Cheong Yong Kim, Jin-Pok Kim, Nahm-Gun Oh, Kee Hyung Lee, Kuk Jin Choe, Yusuke Nakamura, Jae-Gahb Park**

Hereditary nonpolyposis colorectal cancer (HNPCC) is one of the most common autosomal dominantly inherited diseases, affecting as many as one in every 200-400 individuals in the Western world. HNPCC is responsible for up to 15% of all colorectal cancers (*1*). It has recently been shown that the mismatch repair genes, hMSH2, hMLH1, hPMS1, and hPMS2, are mutated in the germline of affected members in HNPCC families (*2-6*), as well as for some apparently nonhereditary cancer patients (*7-9*). A combination of linkage and mutational analysis has indicated that hMSH2 and hMLH1 are likely to be the prevalent genes responsible for HNPCC, accounting for 80%-90% of HNPCC cases (*10-13*), while hPMS1 and hPMS2 are thought to account for only a minor fraction of cases. The identification of the causative mutations in HNPCC families is desirable, since it allows the carrier status of unaffected relatives at risk to be determined.

The Korean Hereditary Colorectal Cancer Registry was established in 1991, and we have registered HNPCC families on the basis of the minimal criteria proposed by the International Collaborative Group on HNPCC (ICG–HNPCC) (*14*). We have also registered families who do not fulfill the ICG–HNPCC criteria but where a genetic basis of colon cancer is strongly suspected (categorized as suspected HNPCC families) because of the following features: 1) vertical transmission of colorectal cancer or at least two siblings affected with colorectal cancer in a family; and 2) development of multiple colorectal tumors or at least one colorectal cancer case diagnosed before the age of 50 years. Data concerning the early-onset patients who had developed colorectal cancer before the age of 40 years without any family history of disease were also collected through the Department of Surgery, Seoul National University Hospital.

To investigate the genetic status of hMSH2 and hMLH1 genes in 25 Korean HNPCC kindreds, 17 suspected HNPCC patients, and 22 early-onset colorectal cancer patients, single-strand conformation polymorphism (SSCP) was used to screen for the mutations, followed by sequencing of the DNA fragments displaying abnormal SSCP pattern. Genomic DNAs were prepared from white blood cells as described by Blin and Stafford (*15*). The polymerase chain reaction primers and methods for amplification of each exon in hMSH2 and hMLH1 and sequence analysis have been previously described (*10,13*)

The germlime mutations detected in this study are summarized in Table 1. A total of 13 germline mutations were detected by SSCP analysis.

The frequency of mutations in hMSH2 and hMLH1 for the patients is given in Table 2. Among 25 classic HNPCC families, we found eight mutations in the hMLH1 gene (32%) but none in the hMSH2 gene. In particular, codon 586 of exon 16 in the hMLH1 gene was the frequently mutated site, accounting for four of 12 mutations

**Affiliations of authors:* H.-J. Han, J.-L. Ku, Y. Yuan, J.-H. Oh, Y.-J. Won, K. J. Kang, K. Y. Kim, S. Kim, C. Y. Kim, J.-P. Kim, N. G. Oh, K. H. Lee, K. J. Choe, J.-G. Park, Korean Hereditary Tumor Registry, Laboratory of Cell Biology, Cancer Research Center and Cancer Research Institute, Seoul National University College of Medicine, Korea; Y. Nakamura, Laboratory of Molecular Medicine, Institute of Medical Science, The University of Tokyo, Japan.

Correspondence to: Jae-Gahb Park, M.D., Laboratory of Cell Biology, Cancer Research Institute, Seoul National University College of Medicine, 28, Yongon-dong, Chongno-gu, Seoul 110-744, Korea.

See "Notes" section following "References."

Table 1. Summary of mutations found in hMSH2 and hMLH1

Sample	Exon	Codon	Mutation	Base change*
hMSH2				
SNU-YC13†	10	506	Missense	GAC(Asp)→TAC(Tyr)
hMLH1				
SNU-H1‡	15	574	Missense	CTC(Leu)→CCC(Pro)
SNU-H2‡	14	542	Missense	CAG(Gln)→CTG(Leu)
SNU-H4‡	11	336	Frame-shift	1-bp (G) deletion
SNU-H5‡	16	586	Frame-shift	1-bp (C) insertion
SNU-H7‡	9	Splicing donor	Abnormal splicing	ACC]gtaa→ACC]gaaa
SNU-H14	14	549	Missense	CTT(Leu)→CCT(Pro)
KRU-H1‡	16	586	Frame-shift	1-bp (C) insertion
KHU-H1	16	586	Frame-shift	1-bp (C) insertion
SNU-H1006	8	217	Missense	CGC(Arg)→TGC(Cys)
SNU-H1009	19	751	Frame-shift	2-bp (AA) deletion
SNU-H1010	9	231	Frame-shift	1-bp (T) deletion
SNU-H1017	16	586	Frame-shift	1-bp (C) insertion

*bp = base pair; Asp = aspartic acid; Gln = glutamine; Leu = leucine; Arg = arginine; AA = amino acid; Pro = proline; Cys = cysteine; Tyr = tyrosine.
†YC = early-onset colorectal cancer.
‡Genomic DNA change previously reported (*13*).

(33%). The mutation in the four families is likely the result of a common origin rather than the biological hot spot. Recent reports (*16,17*) from other regions also describe frequent mutations in exon 16 of hMLH1. These, together with our results, suggest that exon 16 is likely to be the clustered site for mutations in the hMLH1 gene.

In suspected HNPCC families, one missense and three frame-shift mutations were detected in the hMLH1 gene (24%); no mutations were detected in the hMSH2 gene. The criteria (*14*) by which HNPCC was defined have underestimated the prevalence of HNPCC because it excludes cases of small nuclear families and extracolonic cancers associated with HNPCC. Although such criteria have greatly contributed to the diagnosis of HNPCC, the recently described (*2-6*) genetic defects in mismatch repair genes related to HNPCC may obviate reliance on such strict criteria. The 24% incidence of mutations in suspected HNPCC families suggests that our criteria are useful for the diagnosis of HNPCC and can be applied when devising strategies for the surveillance of at-risk members in these suspected HNPCC families.

In 22 patients with early-onset sporadic colorectal cancer, only one missense mutation in hMSH2 was identified. The relatively low incidence of mutations may have resulted from the fact that most of these patients (17 of 22) had cancers located in the rectum instead of the colon.

Since we screened the mutation only in the hMSH2 and hMLH1 genes, we cannot rule out the possibility of a higher frequency of genetic alteration in other mismatch repair genes. Interestingly, mutations in the hMLH1 gene are much more frequent than in the hMSH2 gene in Korean HNPCC patients. As a whole, only one hMSH2 mutation was detected among 13 mutations in both of the genes. This result is different from those of previous reports (*10,18,19*). The role of the hMSH2 gene in predisposition to HNPCC has been reported to be more significant than that of hMLH1 (*2-6,10,19*). Recently, however, more frequent mutations in hMLH1 gene in Dutch (35%) and Finnish HNPCC kindreds (58%) have been reported (*17,20*). Some of these proved to be founder mutations. Although we cannot completely rule out the founder effect for the higher mutation rate in hMLH1, our results reveal that the hMLH1 gene has a relatively significant role in the predisposition of Korean HNPCC. It is important to note that the significant ratio of mutations in hMLH1 and hMSH2 genes is based on the identical screening approach and condition, so it is unlikely that the low proportion of hMSH2 mutations is due to biased selection or technical shortcomings.

The results of accurate presymptomatic diagnosis can be used to screen family members and assess their risk of developing colorectal cancer. The criteria for suspected HNPCC in this study, as well as those of the ICG–HNPCC will, when combined with genetic diagnosis, have a great impact on the management of families affected by HNPCC. The information on the mutations derived from this study will contribute to the surveillance of those at risk in HNPCC families.

Table 2. Frequency of mutations in hMSH2 and hMLH1

	hMSH2: No. of frequencies/ No. of genes (%)	hMLH1: No. of frequencies/ No. of genes (%)
ICG–HNPCC*	0/25 (0)	8/25 (32)
Suspected HNPCC*	0/17 (0)	4/17 (24)
Early-onset colorectal cancer	1/22 (5)	0/22 (0)

*International Collaborative Group on Hereditary Nonpolyposis Colorectal Cancer (HNPCC).

References

(*1*) Vasen HF. What is hereditary nonpolyposis colorectal cancer (HNPCC)? Anticancer Res 1994;14:1613-6.

(*2*) Fishel R, Lescoe MK, Rao MR, Copeland NG, Jenkins NA, Garber J, et al. The human mutator gene homolog MSH2 and its as-

sociation with hereditary nonpolyposis colon cancer [published erratum appears in Cell 1994;77:167]. Cell 1993;75:1027-38.
(3) Leach FS, Nicolaides NC, Papadopoulos N, Liu B, Jen J, Parsons R, et al. Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. Cell 1993;75:1215-25.
(4) Bronner CE, Baker SM, Morrison PT, Warren G, Smith LG, Lescoe MK, et al. Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. Nature 1994;368:258-61.
(5) Papadopoulos N, Nicolaides NC, Wei YF, Ruben SM, Carter KC, Rosen CA, et al. Mutation of a mutL homolog in hereditary colon cancer [*see* comment citation in Medline]. Science 1994;263:1625-9.
(6) Nicolaides NC, Papadopoulos N, Liu B, Wei YF, Carter KC, Ruben SM, et al. Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. Nature 1994;371:75-80.
(7) Han HJ, Yanagisawa A, Kato Y, Park JG, Nakamura Y. Genetic instability in pancreatic cancer and poorly differentiated type of gastric cancer. Cancer Res 1993;53:5087-9.
(8) Loeb LA. Microsatellite instability: marker of a mutator phenotype in cancer. Cancer Res 1994;54:5059-63.
(9) Aaltonen LA, Peltomaki P, Mecklin JP, Jarvinen H, Jass JR, Green JS, et al. Replication errors in benign and malignant tumors from hereditary nonpolyposis colorectal cancer patients. Cancer Res 1994;54:1645-8.
(10) Liu B, Parsons RE, Hamilton SR, Petersen GM, Lynch HT, Watson P, et al. hMSH2 mutations in hereditary nonpolyposis colorectal cancer kindreds. Cancer Res 1994;54:4590-4.
(11) Nystrom-Lahti M, Parsons R, Sistonen P, Pylkkanen L, Aaltonen LA, Leach FS, et al. Mismatch repair genes on chromosomes 2p and 3p account for a major share of hereditary nonpolyposis colorectal cancer families evaluable by linkage. Am J Hum Genet 1994;55:659-65.
(12) Lindblom A, Tannergard P, Werelius B, Nordenskjold M. Genetic mapping of a second locus predisposing to hereditary non-polyposis colon cancer. Nat Genet 1993;5:279-82.
(13) Han HJ, Maruyama M, Baba S, Park JG, Nakamura Y. Genomic structure of human mismatch repair gene, hMLH1, and its mutation analysis in patients with hereditary nonpolyposis colorectal cancer (HNPCC). Hum Mol Genet 1995;4:237-42.
(14) Vasen HF, Mecklin JP, Khan PM, Lynch HT. The International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer (ICG-HNPCC). Dis Colon Rectum 1991;34:424-5.
(15) Blin N, Stafford DM. A general method for isolation of high molecular weight DNA from eukaryotes. Nucleic Acids Res, 1976; 3:2303-8.
(16) Nystrom-Lahti M, Kristo P, Nicolaides NC, Chang SY, Aaltonen LA, Moisio AL, et al. Founding mutations and Alu-mediated recombination in hereditary colon cancer. Nat Med 1995;1:1203-6.
(17) Wijnen J, Khan PM, Vasen H, Menko F, van der Klift H, van den Broek M, et al. Majority of hMLH1 mutations responsible for hereditary nonpolyposis colorectal cancer cluster at the exonic region 15-16. Am J Hum Genet 1996;58:300-7.
(18) Miyaki M, Konishi M, Muraoka M, Kikuchi-Yanoshita R, Tanaka K, Iwama T, et al. Germ line mutations of hMSH2 and hMLH1 genes in Japanese families with hereditary nonpolyposis colorectal cancer (HNPCC): usefulness of DNA analysis for screening and diagnosis of HNPCC patients. J Mol Med 1995;73:515-20.
(19) Fishel R, Kolodner RD. Identification of mismatch repair genes and their role in the development of cancer. Cur Opin Genet Dev 1995;5:382-95.
(20) Nystrom-Lahti M, Moisio AL, Hofstra RM, Osinga J, Mecklin JP, Jarvinen HJ, et al. DNA mismatch repair gene mutations in 55 kindreds with verified or putative hereditary non-polyposis colorectal cancer. Hum Mol Genet 1996;5:763-9.

Notes

Supported by grants from the 1996 Good Health R & D Project, the Ministry of Health and Welfare of the Republic of Korea, and from the Korea Science and Engineering Foundation (KOSEF-CRC94K2-0402-04-00-3).

We are grateful to the patients who participated in the study. Also, we greatly appreciate the work of doctors who have contributed data and carried out follow-up investigations of HNPCC and suspected HNPCC patients and their family members.

Manuscript received April 17, 1996; revised June 18, 1996; accepted June 25, 1996.