[CANCER RESEARCH 54, 1645–1648, April 1, 1994]

Advances in Brief

Replication Errors in Benign and Malignant Tumors from Hereditary Nonpolyposis Colorectal Cancer Patients[1]

Lauri A. Aaltonen,[2] Päivi Peltomäki, Jukka-Pekka Mecklin, Heikki Järvinen, Jeremy R. Jass, Jane S. Green, Henry T. Lynch, Patrice Watson, Gustav Tallqvist, Matti Juhola, Pertti Sistonen, Stanley R. Hamilton, Kenneth W. Kinzler, Bert Vogelstein, and Albert de la Chapelle

Department of Medical Genetics, University of Helsinki, FIN-00014 Helsinki, Finland [L. A. A., P. P., G. T., P. S., A. d. l. C.]; Departments of Surgery and Pathology, Jyväskylä Central Hospital, 40620 Jyväskylä, Finland [J-P. M., M. J.]; Second Department of Surgery, Helsinki University Central Hospital, 00290 Helsinki, Finland [H. J.]; Department of Pathology, University of Auckland School of Medicine, Auckland, New Zealand [J. R. J.]; Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1B3V6 [J. S. G.]; Department of Preventive Medicine, Creighton University School of Medicine, Omaha, Nebraska 68178 [H. T. L., P. W.]; Department of Pathology, the Johns Hopkins University Schools of Medicine, Baltimore, Maryland 21205 [S. R. H.]; and the Johns Hopkins Oncology Center, Baltimore, Maryland 21231 [K. W. K., B. V.]

Abstract

A replication error (RER) phenotype has been documented both in sporadic colorectal tumors and in tumors from patients with hereditary nonpolyposis colorectal cancer (HNPCC). In the current study 8 of 49 (16%) sporadic colorectal cancers (CRCs) and 25 of 29 (86%) CRCs from HNPCC patients were found to be RER+. All 9 (100%) CRCs from HNPCC patients with germline mutations of the mismatch repair gene *MSH2* were found to be RER+, while 16 of 20 CRCs from HNPCC kindreds unlinked or not studied for linkage to *MSH2* were RER+. Corresponding analysis in colorectal adenomas revealed that only 1 of 33 (3%) sporadic tumors but 8 of 14 (57%) HNPCC tumors were RER+. Moreover, RER was found in all 6 extracolonic cancers (endometrium, 2; kidney, 1; stomach, 1; duodenum, 1; and ovary, 1) derived from members of HNPCC families. These data suggest the involvement of mismatch repair deficiency in the premalignant stage of tumorigenesis in HNPCC cases, and suggest that mismatch repair genes (*MSH2* or others) are defective in the germline of nearly all these patients.

Introduction

Widespread alterations in simple repeated sequences have been found in several tumor types (1–6). Such alterations are most easily observed as changes in the lengths of microsatellite sequences in tumor DNA as compared with normal DNA from the same individual. The alterations have been shown to involve mono-, di-, tri-, and tetranucleotide repeats (1–3, 7), and tumors with these abnormalities have been called RER+.[3] Recently the RER phenotype was found to be associated with deficient strand-specific correction of slipped-strand and base-base mismatches, establishing it as a product of a mismatch repair defect (8).

The RER phenomenon is characteristic of colorectal cancers developing in families with HNPCC (3) but was initially noted in "sporadic" patients (1, 2, 9). This raises the possibility that it could be a result of either germline or somatic mutations. A gene on chromosome 2p (human *MSH2*) involved in mismatch repair was recently cloned (10, 11) and found to be mutated in the germline of affected members of HNPCC families (11). Approximately one-half of HNPCC is caused by mutations of *MSH2*, as inferred from linkage and sequencing analyses (3, 11, 12). The remaining cases of HNPCC are caused by other genes, at least one of which appears to be on chromosome 3p (13).

Colorectal cancer arises as a multistep process involving mutations in oncogenes and tumor suppressor genes as normal epithelium evolves through different adenomatous stages to cancer and finally metastasis (14). The role of mismatch repair defects in this process must be clarified. In this paper we address five issues: (*a*) we hypothesize that if a RER is due to deficient mismatch repair *in vivo*, it should occur in all CRCs of patients with germline mutations of *MSH2*; (*b*) if the other tumor types characteristic of patients with *MSH2* mutations are caused by defective mismatch repair, then these tumors should manifest the RER phenotype; (*c*) if HNPCC in general is caused by defective mismatch repair genes, then CRCs occurring in HNPCC kindreds unlinked to *MSH2* should also be RER+; (*d*) if mismatch repair defects are involved in the relatively early stages of colorectal tumorigenesis, then the RER phenotype should be found in benign as well as malignant tumors; (*e*) we wished to establish whether previously observed right sided predominance of RER+-sporadic CRCs was more marked than for HNPCC tumors. If this were the case it might indicate site-specific mechanisms of pathogenesis.

Materials and Methods

Sporadic tumors (49 CRCs and 33 adenomas) were those in which no family history suggestive of HNPCC was noted at the time of diagnosis. We could not exclude that a subset of these cases was actually from HNPCC kindreds, but this is typical of those cancers encountered in routine clinical practice. The HNPCC families studied here met the stringent criteria for HNPCC set by the International Collaborative Group on HNPCC (15): (*a*) at least three relatives should have histologically verified colorectal cancer, and one of them should be a first degree relative to the other two; (*b*) at least two successive generations should be affected; and (*c*) in one of the relatives colorectal cancer should be diagnosed before the subject is 50 years of age. Eighteen tumors were from families J or C (12) in which germline mutations of *MSH2* have been described (11). The other HNPCC tumors occurred in patients from kindreds in which linkage analysis excluded *MSH2* as a causative factor ($n = 9$) or from kindreds where linkage analysis was uninformative or impossible to perform ($n = 22$) (3).[4] Ten CRCs and one colorectal adenoma have been analyzed previously for RER (3). To determine RER status of the remaining cases DNA from normal and tumor tissue was genotyped with seven microsatellite markers: D2S136, D5S404, D7S519, D8S255, D15S120, D17S787, and D20S100 (16). Additional markers (D1S216, D11S904, D13S175 and D19S197) (16) were occasionally used. When frozen tumors were available, DNA was purified from cryostat sections of tissue microdissected to remove most nonneoplastic cells, as described previously (17). DNA from normal colonic mucosa or blood

Received 1/18/94; accepted 2/22/94.

The cost of publication of the article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Part of this study was conducted at the Folkhälsan Institute of Genetics. Supported by grants from the Academy of Finland, the Finnish Cancer Society, the Sigrid Juselius Foundation, the Ida Montin Foundation, the Finnish Foundation for Gastroenterological Research, the Duodecim Foundation, the Research and Science Foundation of Farmos, the Cancer Society and Health Research Council of New Zealand, the Clayton Fund, NIH Grant CA 35494, and the Council for Tobacco Research Grant 1297-DR2.

[2] To whom requests for reprints should be addressed, at Department of Medical Genetics, University of Helsinki, P. O. Box 21, Haartmaninkatu 3, FIN-00014 Helsinki.

[3] The abbreviations used are: RER, replication error; HNPCC, hereditary nonpolyposis colorectal cancer; CRC, colorectal cancer.

[4] Unpublished data.

Table 1 *Frequency of microsatellite instability (RER) in colorectal tumors*

	Total	RER−	RER+
Sporadic carcinomas	49	41	8 (16)[a]
All HNPCC carcinomas	29	4	25 (86)
Carcinomas from *MSH2*-linked families	9	0	9 (100)
Sporadic adenomas	33	32	1 (3)
All HNPCC adenomas	14	6	8 (57)
Adenomas from *MSH2*-linked families	4	1	3 (75)

[a] Numbers in parentheses, percentage.

leukocytes was purified by standard methods. To study DNA extracted from paraffin-embedded formalin-fixed samples ["protocol A" described by Kallio *et al.* (18)], primers that produce fragments approximately 100 bases long were also used. These markers were D4S422, D6S294, D7S520, D9S178, D12S83, D13S158, D14S73, D18S57, D20S95, and D22S284 (16). The methodology for analyzing such microsatellite markers has been described previously (12). One sample pair consisted of a carcinoma and an adenoma because no normal tissue was available (Fig. 1, patient 1). Results were obtained with 5–14 markers in all samples. If two or more markers showed alterations in the allele sizes of the tumor DNA the tumor was categorized as RER+. Thus, the RER− category consisted of tumors with alterations detected in 0 or 1 locus.

Results

Among sporadic CRCs 8 of 49 (16%) were RERs (Table 1). In these 8 tumors 60% (SD = 23%) of the microsatellite markers tested exhibited alterations. Of the 29 CRCs derived from HNPCC patients, 25 were RERs. In these 25 tumors, 57% (SD = 26%) of the markers exhibited alterations. Of the 29 CRCs emanating from HNPCC kindreds, 9 belonged to pedigrees with germline mutations of *MSH2* [families J and C (11, 12)]. All 9 of these cancers (100%) were RER+ (Table 1). In the other 20 cancers, linkage analysis either gave evidence against germline involvement of *MSH2* (n = 7) or was uninformative or not performed (n = 13) (3).[4] Sixteen of these 20 CRCs were RER+, including 6 tumors where involvement of *MSH2* was excluded. We next studied 33 sporadic adenomas from 25 patients. Only one (3%) was RER+ (Fig. 1, Table 1). In contrast, of 14 adenomas emanating from HNPCC family members, 8 (57%) were RER+ (Fig. 1; Table 1). Four were from families C or J (with germline mutations of *MSH2*) and three of these four were RER+ (Table 1).

There were at least two differences between HNPCC patients and the sporadic patients with RER+ tumors: (*a*) the age of the 8 sporadic patients (mean age at diagnosis, 69 years; youngest patient, 56 years) was significantly higher than that of the average HNPCC patients [mean age at first CRC diagnosis, 40–45 years (19–22)]. This is consistent with previously published data (1–3, 23); (*b*) five of the eight sporadic CRCs exhibiting RER occurred in the proximal portion of the large bowel (prior to the splenic flexure). In previously reported series, 56 of 68 sporadic CRCs exhibiting RER were proximal (Table 2). After addition of the present results the figures for sporadic tumors are proximal, 61 (80%), and distal, 15 (20%), respectively (Table 2). For the hereditary RER+ CRCs, the corresponding figures are different, 14 proximal (56%) and 11 distal (44%) (P = 0.043; two-sided by χ^2 test with Yates' correction for discontinuity). Because the distribution of the tumors studied here might be biased to the left, we calculated the figures in two other ways: (*a*) for CRCs from six families linked to chromosome 2, the site distribution was 55 (65%) proximal and 30 (35%) distal (P = 0.046; two-sided by χ^2 test with Yates' correction for discontinuity) (Table 1); (*b*) for HNPCC tumors in general, the corresponding figures were assembled by combining published data (20, 24) with those described here. To avoid possible bias, only carcinomas detected prior to bowel resection were taken into account. In the Finnish HNPCC series there were 95 proximal and 60 distal tumors. The combined figures (Table 2) indicated that only 62% of the CRCs in HNPCC patients were proximal. Again, the difference between the site distributions of RER+-sporadic and HNPCC tumors proved to be statistically significant (P = 0.0033; two-sided by χ^2 test with Yates' correction for discontinuity).

Discussion

This study confirmed the hypothesis that a mutation in the *MSH2* gene causes a RER phenotype because all carcinomas from patients with germline mutations of this gene were RER+. Altogether, 25 of 29 of the studied CRCs from HNPCC kindreds were RER+, establishing a strong association between HNPCC and RER regardless of the underlying germline mutation.

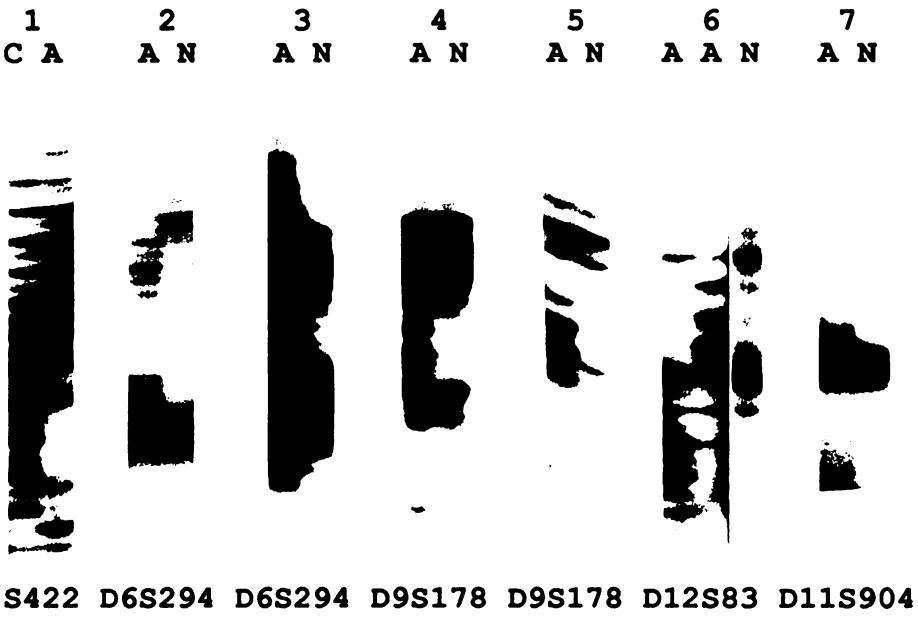

Fig. 1. RER adenomas identified in this study. *Lanes 1–6*, HNPCC patients, *Lane 7*, sporadic patient. *N*, normal DNA; *A*, adenoma DNA; *C*, carcinoma DNA.

Table 2 *Site distribution of hereditary (HNPCC) and unselected (mostly sporadic) CRCs in the colorectum*

		Proximal		Distal		
	N	No.	%	No.	%	Refs.
HNPCC						
Total	407	252	62	155	38	This study; Refs. 20, 24[a]
2p-linked kindreds	85	55	65	30	35	This study
Studied for RER	29	17	59	12	41	This study
RER+	25 (86)[b]	14	56	11	44	This study
RER−	4 (14)	3	75	1	25	This study
Unselected CRC						
Total			~34		~66	25
Studied for RER	659	232	35	427	65	This study; Refs. 1–3, 23, 26
RER+	76 (12)	61	80	15	20	This study; Refs. 1–3, 23, 26
RER−	583 (88)	171	29	412	71	This study, Refs. 1–3, 23, 26

[a] In Refs. 20 and 24, results are reported as the percentage of a total number of tumors studied, and the numbers are calculated from that information.
[b] Numbers in parentheses, percentage.

We show here for the first time that RER is common in adenomas derived from patients with HNPCC. The fact that all adenomas were not RER+ is understandable, as all adenomas found in members of HNPCC kindreds are not likely to be related to the susceptibility gene. In fact, in six kindreds linked to the *MSH2* locus, only five of nine individuals with adenoma as the only sign of HNPCC had a haplotype associated with the cancer phenotype (data not shown). Our results support the hypothesis that the RER phenotype reflects the basic defect in HNPCC tumors. The findings provide strong evidence that adenomatous polyps are precursors for CRCs in HNPCC patients, setting a rationale for the efficiency and desirability of screening of susceptible individuals by colonoscopy. Furthermore, studying adenomas for RER+ could prove to be useful in the diagnosis of HNPCC, especially in kindreds without known germline mutations. However, it is important to note that at least one adenoma from an individual with a germline mutation of *MSH2* (as inferred from extensive haplotype analysis) was RER−. Another adenoma from this same individual was RER+. This suggests that the RER phenotype is not necessary for adenoma formation in HNPCC patients. It is more likely that the genetic instability created by RER accelerates the adenoma-carcinoma transition once adenomas are formed.

Our results on the RER status of sporadic adenomas confirm the finding of Young *et al.* (26) that RER+ is rare in such tumors. We found only 1 RER+ adenoma of 33 tested. Interestingly the patient with this adenoma had three other colorectal tumors (two adenomas and one carcinoma), all of which were RER−. These data could be interpreted to suggest that a mismatch repair gene was somatically mutated only in the RER+ adenoma from this patient, and that she had no germline mutation. In contrast, another patient with four colorectal tumors has been described in which all (two adenomas and two carcinomas) were RER+, suggesting a germline component (1). It will be of great interest in the future to determine what fraction of patients with sporadic RER+ tumors have germline, as opposed to strictly somatic, mutations of mismatch repair genes.

It was important that all six extracolonic cancers (from endometrium, stomach, ovary, kidney, and duodenum) from HNPCC patients were RER+. This suggests that the entire spectrum of tumorigenesis in HNPCC patients (Lynch syndrome tumor spectrum) is related to an underlying defect in mismatch repair. Our results are consistent with those of Risinger *et al.* (5) who found that endometrial cancers from HNPCC kindreds were usually RER+. The human *MSH2* gene is a "housekeeping" gene that is expressed in all tissues thus far analyzed (10, 11). Why tumors from patients with germline mutations of this gene should be limited to a few tissue types is enigmatic and provocative.

Our finding that colorectal tumors from HNPCC kindreds unlinked to the *MSH2* locus are still RER+ is consistent with the findings of Lindblom *et al.* (13) and Aaltonen *et al.* (3). Lindblom *et al.* showed that three tumors from HNPCC kindreds not linked to chromosome 2 were RER+. These investigators also found that at least one kindred exhibited significant linkage to markers on chromosome 3p. In our study, 16 of 20 carcinomas and 5 of 10 adenomas from non-*MSH2*-linked or uninformative kindreds were RER+. Taken together, the results strongly argue that mismatch repair genes are generally responsible for HNPCC, and that mismatch repair genes other than *MSH2* will be found to be altered in the germline of HNPCC kindreds unlinked to chromosome 2.

Of previously reported sporadic RER+ CRCs (1–3, 23, 26), only 12 of 68 were distal. Relatively low age at onset, family history positive for cancer, or metachronous tumors were reported in 5 of these 12 cases (1, 23, 26). Of the eight sporadic RER+ CRCs studied here, three were distal. One of these patients had a mother who had died at age 73 years of uterine cancer, a tumor characteristic of HNPCC (20–22). Thus, altogether at least 6 of the 15 distal sporadic RER+ cases studied thus far are suggestive of a hereditary component. One of the proximal RER+-sporadic cases detected in this study turned out to have a family history of intraabdominal tumors. Altogether, of the 61 proximal CRCs exhibiting RER from sporadic patients, only 5 had any reported evidence of a familial basis (1–3, 23, 26, and this study).

The combined results suggest that tumors in the distal part of the colorectum which exhibit RER are often indicative of a germline mutation in a mismatch repair gene. To explain the more preponderant rightsidedness of RER+ tumors in sporadic than in HNPCC patients we hypothesize that in addition to inherent site-specific differences between cells, environmental factors such as the occurrence of carcinogens might be involved. More studies are obviously needed to better define the role of environmental and genetic factors in RER+ colorectal tumors, regardless of their location in the bowel, and in RER+ tumors in other organs.

Acknowledgments

We thank Lea Pylkkänen, Kirsi Hopponen, Tuula Lehtinen, Sinikka Lindh, Sharon M. Stewart, Susan Booker, Jennifer Cavalieri, Jane Lynch, Sue Slominski-Caster, Tom Smyrk, and Steve Landspa for assistance with samples and information.

References

1. Ionov, Y., Peinado, M. A., Malkhosyan, S., Shibata, D., and Perucho, M. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. Nature (Lond.), *363:* 558–561, 1993.
2. Thibodeau, S. N., Bren, G., and Schaid, D. Microsatellite instability in cancer of the proximal colon. Science (Washington DC), *260:* 816–819, 1993.
3. Aaltonen, L. A., Peltomäki, P., Leach, F., Sistonen, P., Pylkkänen, L., Mecklin, J-P., Järvinen, H., Powell, S., Jen, J., Hamilton, S. R., Petersen, G. M., Kinzler, K. W.,

Vogelstein, B., and de la Chapelle, A. Clues to the pathogenesis of familial colorectal cancer. Science (Washington DC), *260:* 812–816, 1993.

4. Han, H-J., Yanagisawa, A., Kato, Y., Park, J-G., and Nakamura, Y. Genetic instability in pancreatic cancer and poorly differentiated type of gastric cancer. Cancer Res., *53:* 5087–5089, 1993.
5. Risinger, J. I., Berchuck, A., Kohler, M. F., Watson, P., Lynch, H. T., and Boyd, J. Genetic instability of microsatellites in endometrial carcinoma. Cancer Res., *53:* 5100–5103, 1993.
6. Peltomäki, P., Lothe, R. A., Aaltonen, L. A., Pylkkänen, L., Nyström-Lahti, M., Seruca, R., David, L., Holm, R., Ryberg, D., Haugen, A., Brögger, A., Börresen, A-L., and de la Chapelle, A. Microsatellite instability is associated with tumors that characterize the hereditary nonpolyposis colorectal carcinoma syndrome. Cancer Res., *53:* 5853–5855, 1993.
7. Peltomäki, P., Aaltonen, L. A., Mecklin, J-P., and de la Chapelle, A. Läpimurto paksusuolisyövän geneettisen taustan selvittämisessä. Duodecim, *16:* 1367–1369, 1993.
8. Parsons, R., Li, G-M., Longley, M. J., Fang, W-H., Papadopoulos, N., Jen, J., de la Chapelle, A., Kinzler, K. W., Vogelstein, B., and Modrich, P. Hypermutability and mismatch repair deficiency in RER+ tumor cells. Cell, *75:* 1227–1236, 1993.
9. Peinado, M. A., Malkhosyan, S., Velazquez, A., and Perucho, M. Isolation and characterization of allelic losses and gains in colorectal tumors by arbitrarily primed polymerase chain reaction. Proc. Natl. Acad. Sci. USA, *89:* 10065–10069, 1992.
10. Fishel, R., Lascoe, M. K., Rao, M. R. S., Copeland, N. G., Jenkins, N. A., Garber, J., Kane, M., and Kolodner, R. The human mutator gene homolog *MSH2* and its association with hereditary nonpolyposis colon cancer. Cell, *75:* 1027–1038, 1993.
11. Leach, F. S., Nicolaides, N. C., Papadopoulos, N., Liu, B., Jen, J., Parsons, R., Peltomäki, P., Sistonen, P., Aaltonen, L. A., Nyström-Lahti, M., Guan, X-Y., Fournier, R. E. K., Todd, S., Lewis, T., Leach, R. J., Naylor, S. L., Weissenbach, J., Mecklin, J-P., Järvinen, H., Petersen, G. M., Hamilton, S. R., Green, J., Jass, J., Watson, P., Lynch, H. T., Trent, J. M., de la Chapelle, A., Kinzler, K. W., and Vogelstein, B. Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. Cell, *75:* 1215–1225, 1993.
12. Peltomäki, P., Aaltonen, L. A., Sistonen, P., Pylkkänen, L., Mecklin, J-P., Järvinen, H., Green, J. S., Jass, J. R., Weber, J. L., Leach, F. S., Petersen, G. M., Hamilton, S. R., de la Chapelle, A., and Vogelstein, B. Genetic mapping of a locus predisposing to human colorectal cancer. Science (Washington DC), *260:* 810–812, 1993.
13. Lindblom, A., Tannergård, P., Werelius, B., and Nordenskjöld, M. Genetic mapping of a second locus predisposing to hereditary non-polyposis colorectal cancer. Nature Genet., *5:* 279–282, 1993.
14. Fearon, E. R., and Vogelstein, B. A genetic model for colorectal tumorigenesis. Cell, *61:* 759–767, 1990.
15. Vasen, H. F. A., Mecklin, J-P., Meera Khan, P., and Lynch, H. T. The international collaborative group on hereditary non-polyposis colorectal cancer (ICG-HNPCC). Dis. Colon Rectum, *34:* 424–425, 1991.
16. Weissenbach, J., Gyapay, G., Dib, C., Vignal, A., Morissette, J., Millasseau, P., Vaysseix, G., and Lathrop, M. A second generation linkage map of the human genome. Nature (Lond.), *359:* 794–801, 1992.
17. Vogelstein, B., Fearon, E. R., Hamilton, S. R., Kern, S. E., Preisinger, A. C., Leppert, M., Nakamura, Y., White, R., Smits, A. M. M., and Bos, J. L. Genetic alterations during colorectal tumor development. N. Engl. J. Med., *319:* 525–532, 1988.
18. Kallio, P., Syrjänen, S., Tervahauta, A., and Syrjänen, K. A simple method for isolation of DNA from formalin-fixed paraffin-embedded samples for PCR. J. Virol. Methods, *35:* 39–47, 1991.
19. Mecklin, J-P. Frequency of hereditary colorectal cancer. Gastroenterology, *93:* 1021–1025, 1987.
20. Vasen, H. F. A., Johan, G., Offerhaus, A. Hartog Jager, F. C., Menko, F. H., Nagengast, F. M., Griffioen, G., Hogezand, R. B., and Heinz, A. P. The tumour spectrum in hereditary non-polyposis colorectal cancer: a study of 24 kindreds in the Netherlands. Int. J. Cancer, *46:* 31–34, 1990.
21. Mecklin, J-P., and Järvinen, H. J. Tumor spectrum in cancer family syndrome (hereditary nonpolyposis colorectal cancer). Cancer (Phila.), *68:* 1109–1112, 1991.
22. Lynch, H. T., Lanspa, S., Smyrk, T., Boman, B., Watson, P., and Lynch, J. Hereditary nonpolyposis colorectal cancer (Lynch syndromes I and II). Genetics, pathology, natural history, and cancer control, part I. Cancer Genet. Cytogenet., *53:* 43–160, 1991.
23. Lothe, R. A., Peltomäki, P., Meling, G. I., Aaltonen, L. A., Nyström-Lahti, M., Pylkkänen, L., Heimdal, K., Andersen, T. I., Möller, P., Rognum, T. O., Fosså, S. D., Haldorsen, T., Langmark, F., Brögger, A., de la Chapelle, A., and Börresen, A-L. Genomic instability in colorectal cancer; relationship to clinicopathological variables and family history. Cancer Res., *53:* 5849–5852, 1993.
24. Lynch, H. T., Watson, P., Lanspa, S. J., Marcus, J., Smyrk, T., Fitzgibbons, R. J. Jr., Kreigler, M., and Lynch, J. F. Natural history of colorectal cancer in hereditary nonpolyposis colorectal cancer. Dis. Colon Rectum, *31:* 439–444, 1988.
25. Gordon, N. L. M., Dawson, A. A., Bennett, B., Innes, G., Eremin, O., and Jones, P. F. Outcome in colorectal adenocarcinoma: two seven-year studies of a population. Br. Med. J., *307:* 707–710, 1993.
26. Young, J., Leggett, B., Gustafson, C., Ward, M., Searle, J., Thomas, L., Buttenshaw, R., and Chenevix-Trench, G. Genomic instability occurs in colorectal carcinomas but not in adenomas. Hum. Mutat., *2:* 351–354, 1993.