42. Northern blots were performed as described in K. W. Kinzler, J. M. Ruppert, S. H. Bigner, B. Vogelstein, *Nature* **332**, 371 (1988).
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45. Supported in part by grants from the Minister of Education, Culture and Science, the Princess Takamatsu Cancer Research Fund, the Uehara Memorial Foundation, the Kato Memorial Bioscience Foundation, the Damon Runyon–Walter Winchell Cancer Research Fund, the Clayton Fund, The McAshan Fund, and NIH grants CA35494, CA06973, CA44688, CA47527, CA41183, CA47456, GM07309, and GM07184. The authors thank T. Gwiazda for expert preparation of the manuscript; J. M. Jessup, J. Willson, M. Brattain, C. Paraskeva, J. Trent, C. Reznikoff, B. Boman, and M. Schwab for providing tumors and cell lines; A. Lupas for providing his program for analyzing coiled-coil proteins; and R. White and colleagues for helpful discussions and for exchanging information prior to publication.

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Mutations of Chromosome 5q21 Genes in FAP and Colorectal Cancer Patients

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Previous studies suggested that one or more genes on chromosome 5q21 are responsible for the inheritance of familial adenomatous polyposis (FAP) and Gardner's syndrome (GS), and contribute to tumor development in patients with noninherited forms of colorectal cancer. Two genes on 5q21 that are tightly linked to FAP (MCC and APC) were found to be somatically altered in tumors from sporadic colorectal cancer patients. One of the genes (APC) was also found to be altered by point mutation in the germ line of FAP and GS patients. These data suggest that more than one gene on chromosome 5q21 may contribute to colorectal neoplasia, and that mutations of the APC gene can cause both FAP and GS. The identification of these genes should aid in understanding the pathogenesis of colorectal neoplasia and in the diagnosis and counseling of patients with inherited predispositions to colorectal cancer.

RECENT STUDIES HAVE REVEALED that the accumulation of several genetic changes is associated with colorectal tumorigenesis (*1*). Because most colorectal cancers (CRCs) arise from benign adenomatous polyps (*2*), it is of great importance to identify the genes responsible for adenoma formation. Familial adenomatous polyposis (FAP) is one of the most common autosomal-dominant diseases leading to cancer predisposition, affecting 1 in 5,000 and 1 in 17,000 of the American and Japanese populations, respectively (*3*). Affected individuals usually develop hundreds to thousands of adenomatous polyps of the colon and rectum, a small fraction of which will progress to carcinoma if not surgically treated. Gardner's syndrome (GS) is a variant of FAP in which desmoid tumors, osteomas, and other neoplasms occur together with multiple adenomas of the colon and rectum.

The gene (or genes) responsible for FAP has been assigned to chromosome 5q21 by cytogenetic and linkage analysis (*4–7*). Although FAP is a relatively rare cause of colorectal cancer (*3*), the importance of 5q21 genes has been accentuated by the finding that 5q21 alleles are often lost from the tumors of sporadic colorectal cancer patients (that is, those without obvious inherited predispositions) (*1, 8*). Moreover, losses of 5q21 alleles are the earliest genetic alterations yet identified in sporadic colorectal neoplasms, present in adenomas as small as 5 mm in diameter (*9*). Such losses are generally thought to indicate the presence of a tumor suppressor gene in the deleted region (*10, 11*).

We have used probes from within the region tightly linked to FAP as tools to search for genes expressed in normal colonic mucosa. As described in the accompanying paper (*12*), six such genes were identified by means of cosmid and YAC clones. We considered the gene FER as a candidate because of its proximity to the FAP locus as judged by physical and genetic criteria (*12, 13*), and its homology to known tyrosine kinases with oncogenic potential (*14*). Primers were designed to amplify the complete coding sequence of FER from the RNA of two colorectal cancer cell lines derived from FAP patients (*15*). The resultant 2554-bp fragments were cloned and sequenced in their entirety (*16*). Only a single conservative amino acid change (GTG → CTG, creating a valine to leucine substitution at codon 439) was observed. The region surrounding this codon was then amplified from the DNA of individuals without FAP and this substitution was found to be a common polymorphism, not specifically associated with FAP (*17*). On the basis of these results, we considered it unlikely (though still possible) that the FER gene was responsible for FAP.

We next turned to the analysis of the four genes (MCC, TB2, SRP, and APC) in contig 3 (*12*). These genes were considered as

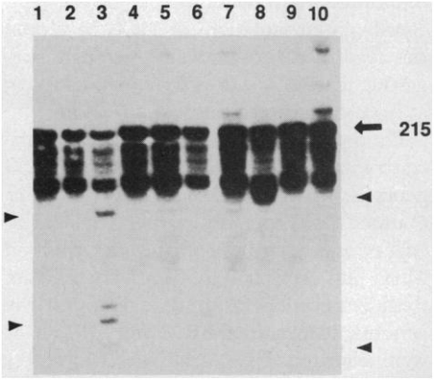

Fig. 1. PCR and RNase protection analysis of the APC gene in FAP patients. RNase protection analysis was performed on PCR products as described (*21*) and the resulting cleavage products separated by denaturing gel electrophoresis. The amplified genomic fragments containing APC exon nt 835 to 933 were 215 bp in length and contained codons 278 to 311. Lanes 1 to 10 show the results obtained from constitutional DNA of ten FAP patients. Lanes 3 (P24) and 8 (P93) show abnormal RNase cleavage products (arrowheads). Subsequent sequence analysis revealed that the abnormal patterns resulted from a C to T transition in P24 and a C to G transition in P93 (Table 1).

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Table 1. Germ line mutations of the APC gene in FAP and GS patients.

Patient	Codon	Nucleotide change*	Amino acid change	Age	Extra-colonic disease
P93	280	TCA → TGA	Ser → Stop	39	Mandibular osteoma
P24	302	CGA → TGA	Arg → Stop	46	None
P34	302	CGA → TGA	Arg → Stop	27	Desmoid tumor
P21	414	CGC → TGC	Arg → Cys	24	Mandibular osteoma
P60	713	TCA → TGA	Ser → Stop	37	Mandibular osteoma

*The mutated nucleotides are underlined.

candidates for the following reasons. First, at least three of the four genes in this contig are within a 200-kb region identified in an FAP patient (*18*). Second, allelic deletions of chromosome 5q21 in sporadic cancers appeared to be centered in this region (*19, 20*). Some tumors exhibited loss of proximal RFLP (restriction fragment length polymorphism) markers (up to and potentially including the 5′ end of MCC), but no loss of markers distal to MCC. Other tumors exhibited loss of markers distal to and perhaps including the 3′ end of MCC, but no loss of sequences proximal to MCC. This suggested either that different ends of MCC were affected by loss in all such cases, or alternatively, that two genes (one proximal to and perhaps including MCC, the other distal to MCC) were separate targets of deletion. Third, clones from FER, TB1, SRP, TB2, MCC, and APC (*12*) were used as probes on Southern blots containing tumor DNA from patients with sporadic CRC. Only two examples of somatic changes were observed in over 200 tumors studied: a rearrangement or deletion whose centrometric end was located within the MCC gene (*21*) and an 800-bp insertion within the APC gene (*22*). Finally, point mutations of MCC have been observed in two tumors (*21*), strongly suggesting that MCC was a target of mutation in at least some sporadic colorectal cancers.

On the basis of these results, we attempted to search for subtle alterations of contig 3 genes in patients with FAP. We chose to examine MCC and APC, rather than TB2 or SRP, because of the somatic mutations in MCC and APC noted above. To facilitate the identification of subtle alterations, the genomic sequences of MCC and APC exons were determined (*23, 24*). These sequences were used to design primers for polymerase chain reaction (PCR) analysis of constitutional DNA from FAP patients.

We first amplified eight exons and surrounding introns of the MCC gene in affected individuals from 90 FAP kindreds. The PCR products were analyzed by a ribonuclease (RNase) protection assay (*23*). In brief, the PCR products were hybridized to in vitro–transcribed RNA-probes representing the normal genomic sequences. The hybrids were digested with RNase A, which can cleave at single base pair mismatches within DNA-RNA hybrids, and the cleavage products were visualized after denaturing gel electrophoresis. Two separate RNase protection analyses were performed for each exon, one with the sense and one with the antisense strand. Under these conditions, approximately 35% of all mismatches are detectable (*25, 26*). No germ line mutations of MCC in FAP patients were observed.

We next examined three exons of the APC gene (*24*). In 90 kindreds, the RNase protection method was used to screen for mutations and, in an additional 13 kindreds, the PCR products were cloned and sequenced to search for mutations not detectable by RNase protection (*27*). Five variants were detected among the 103 kindreds analyzed (Fig. 1). Cloning and subsequent DNA sequencing of the PCR product of patient P21 indicated a C to T transition in codon 414 that resulted in a change from arginine to cysteine (Fig. 2A). This amino acid variant was not observed in any of 200 DNA samples from individuals without FAP whose DNA was examined by the same methods as used for FAP patients. P24 and P34 were from separate kindreds, but demonstrated the same abnormal RNase protection pattern (Fig. 1). Cloning and sequencing of the PCR products from those patients indicated that both had a C to T transition at codon 302 that resulted in a change from arginine (CGA) to a stop codon (TGA) (Fig. 2B). This change was not present in 200 individuals who did not have FAP. As this point mutation resulted in the predicted loss of the recognition site for the enzyme Taq I, appropriate PCR products could be digested with Taq I to detect the mutation. This allowed us to determine that the stop codon precisely co-segregated with disease phenotype in 21 members of the family of P24, including 12 patients with FAP (examples in Fig. 3). The inheritance of this change in affected members of the pedigree provided additional evidence for the importance of the mutation.

Cloning and sequencing of the PCR product from FAP patient P93 indicated a C to G transversion at codon 280, also resulting in a stop codon (change from TCA to TGA) (Fig. 2C). This mutation was not present in 200 individuals without FAP. Finally, one additional mutation resulting in a serine (TCA) to stop codon (TGA) at codon 713 was detected in a single patient with FAP (patient P60).

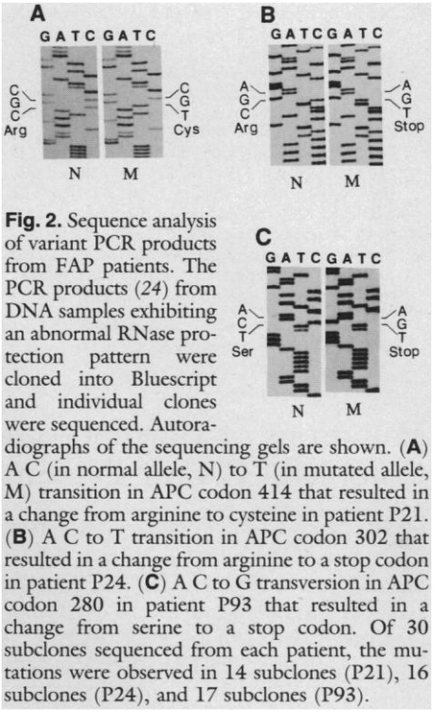

Fig. 2. Sequence analysis of variant PCR products from FAP patients. The PCR products (*24*) from DNA samples exhibiting an abnormal RNase protection pattern were cloned into Bluescript and individual clones were sequenced. Autoradiographs of the sequencing gels are shown. (**A**) A C (in normal allele, N) to T (in mutated allele, M) transition in APC codon 414 that resulted in a change from arginine to cysteine in patient P21. (**B**) A C to T transition in APC codon 302 that resulted in a change from arginine to a stop codon in patient P24. (**C**) A C to G transversion in APC codon 280 in patient P93 that resulted in a change from serine to a stop codon. Of 30 subclones sequenced from each patient, the mutations were observed in 14 subclones (P21), 16 subclones (P24), and 17 subclones (P93).

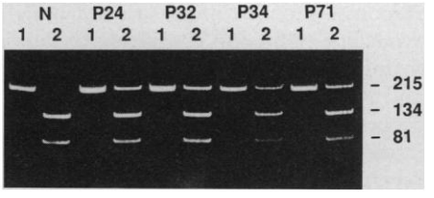

Fig. 3. Taq I digestion of variant PCR products of the APC gene. PCR products (*24*) before (lane 1) or after (lane 2) digestion with Taq I were separated by electrophoresis on a 10% polyacrylamide gel. Taq I cleaves the normal 215-bp PCR product containing APC codons 278 to 311 into 134- and 81-bp subfragments. A mutation at codon 302 results in loss of the Taq I site. PCR products were derived from the DNA of: (N) normal individual; (P24, P32, and P71) DNA from three affected members of a single FAP kindred; (P34) DNA from an affected member of a second FAP kindred.

In addition to these germ line mutations (summarized in Table 1), we identified several somatic mutations of MCC and APC in sporadic CRCs. Seventeen MCC exons (*23*) were examined in 90 sporadic CRCs by RNase protection analysis. In each case where an abnormal RNase protection pattern was observed, the corresponding PCR products were cloned and sequenced. This led to the identification of six point mutations [two described previously (*21*)], each of which was not found in the germ line of these patients (Table 2).

Four of the mutations resulted in amino acid substitutions and two resulted in the alteration of splice site consensus elements. Mutations at analogous splice site positions in other genes have been shown to alter RNA processing in vivo and in vitro (*28*).

Three exons of APC were also evaluated in sporadic tumors. A total of 60 tumors were screened by RNase protection, and an additional 98 tumors were evaluated by sequencing of pooled clones (*29*). Three mutations were identified, each of which proved to be somatic. Tumor T27 contained a somatic mutation of CGA (arginine) to TGA (stop codon) at codon 332 (Fig. 4A). Tumor T135 contained a GT to GC change at a splice donor site (Fig. 5A). Tumor T34 contained a 5-bp insertion (CAGCC between codons 289 and 290) resulting in a stop at codon 292 due to a frameshift (Fig. 4B).

We serendipitously discovered one additional somatic mutation in a colorectal cancer. During our attempt to define the sequences and splice patterns of the MCC and APC gene products in colorectal epithelial cells, we cloned cDNA from the colorectal cancer cell line SW480. The amino acid sequence of the MCC gene from SW480 (*30*) was identical to that previously found in clones from human brain (*21*). The sequence of APC in SW480 cells, however, differed significantly, in that a transition at codon 1338 resulted in a change from glutamine (CAG) to a stop codon (TAG). To determine if this mutation was somatic, we recovered DNA from archival paraffin blocks of the original surgical specimen (T201) from which the tumor cell line was derived 28 years ago (*31*). A PCR product containing codon 1338 was amplified from the archival DNA and used to show that the stop codon represented a somatic mutation present in the original primary tumor and in cell lines derived from the primary and metastatic tumor sites, but not from normal tissue of the patient (Fig. 6).

The ten point mutations in the MCC and APC genes so far discovered in sporadic CRCs are summarized in Table 2. Analysis of the number of mutant and wild-type PCR clones obtained from each of these tumors showed that, in eight of the ten cases, the wild-type sequence was present in approximately equal proportions to the mutant. This was confirmed by analysis of RFLP with flanking markers from chromosome 5q (*7, 12*) which demonstrated that only two of the ten tumors (T135 and T201) exhibited an allelic deletion on chromosome 5q (example in Fig. 5B). These results are consistent with previous observations showing that 20 to 40% of sporadic colorectal tumors had allelic deletions of chromosome 5q (*8, 9*). Moreover, these data suggest that mutations of 5q21 genes are not limited to those colorectal tumors which contain allelic deletions of this chromosome.

The results described above document that the APC gene is mutated in the germ line of FAP and GS patients and in some sporadic cancers. The prevalence of such mutations cannot yet be fully assessed, because only a small portion of the gene product has been examined in detail. However, the fact that several of the observed mutations have profound effects on the predicted gene products (creating stop codons or destroying splice site recognition elements) provides strong evidence for the involvement of the APC gene in colorectal tumorigenesis. A second gene, MCC, located close to the APC gene (*12*), has so far been found to be mutant only in sporadic CRCs.

These findings raise several interesting questions. The first concerns the relationship between GS and FAP. GS is thought to be a variant of FAP, as the inheritance of both is linked to the same region of 5q21 (*7*), and some individuals within classic GS kindreds have no evidence of extra-colonic manifestations (*3*). The results of Table 1 extend these observations by demonstrating that patients with the identical mutation (C to T transition at codon 302) can either have GS symptoms (patient P34) or no evidence of extra-colonic disease even at 46 years of age (patient P24). Thus, the specific mutation does not completely specify the extra-colonic manifestations of FAP and GS, and the exact phenotype is likely to be the result of other genetic or environmental influences.

A second question concerns the relation between MCC, APC, and colorectal neoplasia. One possibility is that only one of the two genes is important in sporadic cancers, and mutations in the other are inconsequential. The only direct evidence indicating that either MCC or APC are important in sporadic neoplasia is provided by the mutations. However, it is possible, as noted previously (*21*), that some somatic mutations in tumors represent coincidental epiphenomena. One argument against this is that clonal mutations (that is, mutations present in virtually every neoplastic cell within a tumor) have previously been described only in genes suspected of being important in neoplasia (*1, 32*). In addition, the rate at which functional point mutations occur in tumor cells (*33*), including those of the colon (*34*), is quite low, similar to that of normal cells

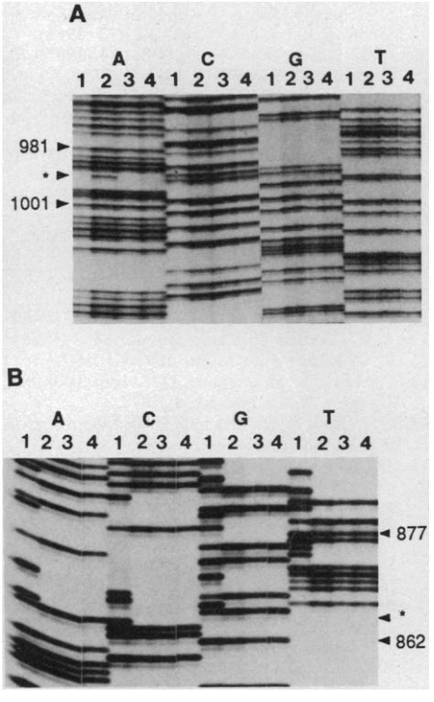

Fig. 4. Screening for somatic mutations in sporadic CRC by DNA sequencing. PCR products encompassing APC nt 934 to 1312 (**A**) and nt 835 to 933 (**B**) were cloned and at least 100 independent clones were pooled for sequencing (*27*). Autoradiographs of sequencing gels representing four different tumors are shown in each panel, with the lanes grouped so that mutations could easily be observed through visual comparison of the band patterns (*39*). Tumor T27 [lane 2 in (A)] contained a C to T transition at the first base of codon 332 (nt 994) creating an "A" in the antisense sequence illustrated [* in (A)]. Tumor T34 [lane 1 in (B)] contained a 5-bp insertion (CAGGC) beginning at codon 290 [* in (B)]. Nucleotide numbers of the sequences, shown to the right and left, correspond to the APC cDNA (*12*).

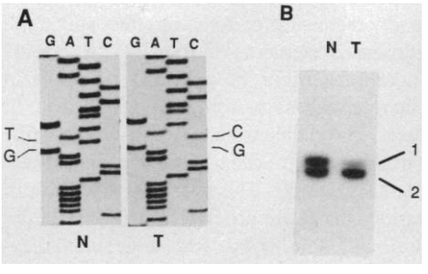

Fig. 5. Sequence and RFLP analysis of sporadic CRC T135. (**A**) Sequence analysis of the T135 PCR product shows a T (in normal allele) to C (in mutated allele) transition in the splice donor site. Exon nt 934 to 1312 were amplified (*24*), and individual clones were sequenced after cloning of the PCR product into Bluescript plasmid vector. Sixteen of twenty clones contained the mutant sequence illustrated. (**B**) RFLP analysis of tumor T135. DNA from normal colonic mucosa (N) or carcinoma (T) were digested with Msp I. Southern blots containing the DNA were hybridized with a ^{32}P-labeled probe from within the MCC gene (pL5.71-3). Two polymorphic alleles of 4.4 kb (allele 1) and 4.3 kb (allele 2) are detected by this probe (*19*). The intensity of the signal corresponding to allele 1 in the carcinoma was greatly diminished compared to that of allele 2, indicating that an allelic loss had occurred.

Table 2. Somatic mutations in sporadic CRC patients.

Patient	Codon*	Nucleotide change†	Amino acid change
T35	MCC 12	GAG/gtaaga → GAG/gtaaaa	Splice donor
T16	MCC 145	ctcag/GGA → atcag/GGA	Splice acceptor
T47	MCC 267	CGG → CTG	Arg → Leu
T81	MCC 490	TCG → TTG	Ser → Leu
T35	MCC 506	CGG → CAG	Arg → Gln‡
T91	MCC 698	GCT → GTT	Ala → Val‡
T34	APC 289	CCAGT → CCCAGCCAGT	Insertion
T27	APC 332	CGA → TGA	Arg → Stop
T135	APC 438	CAA/gtaa → CAA/gcaa	Splice donor
T201	APC 1338	CAG → TAG	Gln → Stop

*For splice site mutations, the codon nearest to the mutation is listed. lowercase letters represent introns, uppercase letters represent exons. †The underlined nucleotides were mutant; ‡Previously reported (*21*).

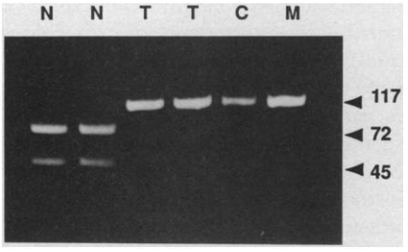

Fig. 6. Somatic mutation in cell line SW480. PCR products containing APC codons 1323 to 1362 (nt 3970 to 4086) were digested with Pst I (*31*). Pst I cleaves the normal 117-bp product into subfragments of 72 and 45 bp. A mutation at codon 1338 resulted in loss of the Pst I site (Table 2). The DNA samples were derived from (C) cell line SW480, originating from the primary colorectal tumor of patient T201; (T) the original colorectal tumor, preserved in paraffin, of patient T201; (N) paraffin-preserved normal colon epithelium from patient T201; (M) cell line SW620, derived from a lymph node metastasis of patient T201. DNA was purified from paraffin embedded samples as described (*31*).

(approximately 10^{-10} per base pair per cell generation). Accordingly, the prevalence of nonfunctional point mutations in human cancer cells, including CRC, is also low (*35*). The prevalence of point mutations in APC and MCC genes is much greater (*36*), presumably because such mutations provide a selective growth advantage to the cells in which they occur. Finally, all of the ten somatic point mutations thus far observed in MCC and APC alter the encoded amino acid, create stop codons, or affect splice site consensus elements (Table 2). "Random" mutations might be expected to result in silent changes in a higher proportion of cases. Nevertheless, it is impossible to rule out that some of the observed mutations are inconsequential. This issue has wide implications, as many other tumor-related genes are being sought by "reverse-genetic" techniques. The demonstration of somatic mutations in such genes generally provides, at least initially, the most cogent evidence in favor of their involvement. If a substantial portion of such mutations turn out to be inconsequential, the search for such genes will be considerably complicated.

Another possibility is that both MCC and APC are involved in colorectal neoplasia. This possibility is supported by the fact that some sporadic tumors have allelic deletions that remove the proximal part of 5q21 (up to and possibly including MCC) but leave the region containing APC intact, while other tumors have the reverse pattern (*19, 20*). This suggests that two separate loci on 5q21 may each be involved in different tumors. Moreover, both MCC and APC are mutated relatively commonly. We estimate that a minimum of 15% of colorectal cancers contain gross structural alterations or point mutations of MCC, and the frequency of APC mutation also appears appreciable (though not enough of the APC gene has been examined to get a quantitative estimate).

A unifying hypothesis is that both MCC and APC encode proteins that operate within the same biochemical pathway, perhaps even together in a complex. This is consistent with the sequence similarities shared between the two genes (*12*). If true, one might expect to observe that mutations in either MCC or APC would have similar phenotypic effects. This two-gene hypothesis might also explain the phenotypic variations observed within individual FAP kindreds (*3*); a co-segregating gene (such as MCC) could influence the effect of an inherited mutant gene (APC) if it varied among the population. Although these suggestions are conjectural, they are testable through the examination of other sporadic tumors and FAP patients for MCC and APC gene mutants and variants, and through the biochemical analysis of MCC and APC gene products.

Finally, the demonstration that APC is mutated in the germ line of FAP and GS patients has obvious clinical implications. Members of kindreds with FAP can now be directly tested for mutations of this gene, pre- or postnatally. Those individuals who have not inherited the gene will be spared the discomfort of repeated medical evaluations and colonoscopies as well as the anxiety associated with disease expectation. Because adenomas do not generally develop until the second or third decade of life in FAP patients, attempts to prevent this development in pre-symptomatic individuals with suitable drugs may prove worthwhile (*37*). As the phenotypic manifestations of FAP can vary widely (*3, 38*), it is likely that some patients with CRC, but without polyposis, have inherited mutations of APC. Thus, the continued analysis of patients with CRC may provide important insights into inherited colorectal cancer risk in the general population.

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15. The cDNA was generated from RNA and used as a template for PCR as described (*21*). The primers used were 5′-AGAAGGATCCCTTGTGCAGTGTGGA-3′ and 5′-GACAGGATCCTGAAGCTGAGTTTG-3′. The underlined nucleotides were altered from the true FER sequence to create Bam HI sites. The cell lines used were JW and Difi, both derived from colorectal cancers of FAP patients [C. Paraskeva, B. G. Buckle, D. Sheer, C. B. Wigley, *Int. J. Cancer* **34**, 49 (1984); M. E. Gross *et al.*, *Cancer Res.* **51**, 1452 (1991)].
16. The PCR products were cloned in the Bam HI site of Bluescript SK (Stratagene) and pools of at least 50 clones were sequenced together by means of T7 polymerase, as described in J. M. Nigro *et al.*, *Nature* **342**, 705 (1989).
17. To amplify the regions surrounding codon 439, the following primers were used; 5′-TCAGAAAGTGCTGAAGAG-3′ and 5′-GGAATAATTAGGTCTCCAA-3′. PCR products were digested with Pst I, which yields a 50-bp fragment if codon 439 is leucine, but 26- and 24-bp fragments if it is valine. The primers used for sequencing were chosen from the FER cDNA sequence (*14*).
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20. Y. Miki *et al.*, *Jpn. J. Cancer Res.*, in press.
21. K. W. Kinzler *et al.*, *Science* **251**, 1366 (1991).
22. An 800-bp insertion was observed with a probe encompassing nt 4424–5584 of the APC gene. It was observed in tumor DNA digested with Msp I,

Taq I, Eco RI, Bgl II, and Pst I, but not in DNA of normal colon from the same patient.
23. Sequences of the intron-exon borders of the 17 exons containing the coding region of MCC, and the primers used for amplifying these sequences, are available from the authors upon request or electronically from the NCBI data repository by anonymous ftp from the pub/apc directory at ncbi.nlm.nih.gov (numerical address 130.14.20.1). The eight exons examined in FAP patients included those containing MCC nt 392 to 1195, 1576 to 1863, and 2306 to 2406. PCR and RNase protection analysis were performed as described (*21*).
24. Sequences of the intron-exon borders of the eight exons containing APC nucleotides (nt) 835 to 8960, and the primers used for amplifying these sequences, are available from the authors upon request or electronically from the NCBI data repository by anonymous ftp from the pub/apc directory at ncbi.nlm.nih.gov (numerical address 130.14.20.1). The entire 3′ end of the APC cDNA (nt 1959 to 8955) was encoded in one exon, as indicated by restriction endonuclease mapping and sequencing of cloned genomic DNA. The three exons examined in FAP patients included those containing nt 835 to 933, nt 934 to 1312, and the first 300 nt of the large 3′ exon (nt 1959 to 2259).
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35. Analysis of colorectal cancers with anonymously derived probes has demonstrated that point mutations resulting in the creation of new restriction sites or in the ablation of existing sites occur at $<5 \times 10^{-6}$ per base pair [S. J. Baker *et al.*, *Science* **244**, 217 (1989); B. Vogelstein *et al.*, *ibid.*, p. 207]. The prevalence of "random" point mutations can also be estimated from the frequency of tumors that contain two separate point mutations within the same allele. It can be assumed that even if one such point mutation is functional, the other is more likely to be inconsequential. Sequencing studies of the p53 gene in colorectal and other human cancers [reviewed in M. Hollstein, D. Sidransky, B. Vogelstein, C. C. Harris, *Science* **253**, 49 (1991)] have revealed only one case in which two such mutations occurred, and in this case the second "mutation" may have been a germ line variant rather than a somatic mutation [S. J. Baker *et al.*, *Cancer Res.* **50**, 7717 (1990)]. Because the aggregate number of base pairs sequenced in these tumors was $>2 \times 10^5$, the frequency of such mutations was $<5 \times 10^{-6}$ per base pair.
36. From the number of base pairs examined, corrected for the sensitivity of mutation detection (35% for RNase protection analysis and 100% for sequencing of pooled clones), the prevalence of point mutations was estimated to be 6×10^{-5} per base pair and 4×10^{-5} per base pair in the exons and flanking intronic sequences of MCC and APC, respectively.
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Genes for Epilepsy Mapped in the Mouse

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The neurological mutant mouse strain El is a model for complex partial seizures in humans. The inheritance of epileptic seizures with seven conventional chromosomal markers and over 60 endogenous proviral markers was studied by means of backcrosses of El with two seizure-resistant strains, DBA/2J and ABP/LeJ. The major gene responsible for this epileptic phenotype (*El-1*) was localized to a region distal with respect to the centromere on chromosome 9. At least one other gene, *El-2*, linked to proviral markers on chromosome 2, also influences the seizure phenotype. In addition, a potential modifier of seizures was detected in the DBA/2J background. The location of *El-1* on distal chromosome 9 may allow identification of an epilepsy candidate gene in humans on the basis of conserved synteny with human chromosome 3.

EPILEPSY IS A SIGNIFICANT HEALTH problem in humans and, with the exception of stroke, it is the most common human neurological affliction. The epilepsies have been divided into two broad etiological categories: (i) idiopathic epilepsy, which includes those convulsive disorders that are genetically determined but cannot be associated with observable structural or biochemical pathology, and (ii) symptomatic epilepsy, which includes those disorders arising from definable injury, disease, or neurostructural abnormality (*1–3*). The phenomenon of genetic heterogeneity has hindered attempts to understand the modes of inheritance (single gene or polygenic) of various idiopathic epilepsies in humans (*4, 5*).

The El (epilepsy) mouse, discovered in 1954 (*6*), is considered a genetic model for human temporal lobe epilepsies or complex partial seizures with secondary generalization (*7*). El seizures can occur spontaneously at 80 to 100 days of age or can be induced at earlier ages by vestibular stimulation (*8–11*). The seizures in El mice appear to originate in the hippocampus or deep temporal lobe structures and then spread to other brain regions (*9*). El seizures are accompanied by electroencephalographic abnormalities, loss of postural equilibrium, urinary incontinence, excessive salivation, and head, limb, and chewing automatisms (*8, 10, 11*) resembling temporal lobe seizures in humans (*12*). El mice also display a seizure-associated hippocampal gliosis that is not associated with an observable neuronal loss (*13*). Phenytoin and phenobarbitol, the anticonvulsant drugs of choice for the treatment of human partial epilepsies, can also inhibit seizure activity in El mice (*14, 15*). The genetic basis of the El seizures has not been clearly established (*16*).

We outcrossed El mice to either ABP/LeJ (ABP) or DBA/2J (D2) mice (*17*) to study the segregation of El seizure genes. ABP and D2 are suitable controls because they do not seize spontaneously and have low susceptibilities to seizures induced by vestibular stimulation (Fig. 1). At 30 days of age, all parental, F1, and backcross mice were tested by vestibular stimulation twice each week for 10 weeks (for a total of 20 tests). The relative frequency of seizures was used for assessment of seizure susceptibility. The F1 mice were reciprocally backcrossed to the respective ABP or D2 parents. The progeny of both backcrosses showed a somewhat continuous distribution of seizure susceptibility (Fig. 1), indicating that multiple genetic factors influence the manifestation of

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