pressed as a percentage of that associated with Ras when the 30°C incubation was omitted. To monitor the association of Ras with GTP directly, we incubated 1.0 μg of Ras with 1.25 nmol of [α^{32}-P]GTP (8 Ci/mmol) and either buffer A, 2.7 μg of RasGRP (catalytic region), or 0.44 μg of p30GRF1 in 0.05 ml for 10 min at 30°C. Complexes were recovered as above. The values in this case are expressed in Fig. 2B as the percentage of maximal exchange, as observed when the control sample was adjusted to 2.0 mM EDTA and $MgCl_2$ was added to 20 mM. The values obtained with buffer represent the spontaneous dissociation-association reactions. For both guanyl nucleotide exchange assays, the results are representative of three experiments.

14. J. O. Ebinu and J. C. Stone, unpublished data.
15. E. Y. W. Chan and J. C. Stone, unpublished data.
16. *E. coli* strains were induced with isopropyl-β-D-thiogalactopyranoside to express GST-rbc7HA fusion proteins, and cells were lysed in SDS. Total cell lysates were resolved by SDS–polyacrylamide gel electrophoresis (SDS-PAGE), blotted to nitrocellulose, and probed with [^{45}Ca] as described [K. Maruyama, T. Mikawa, S. Ebashi, *J. Biochem.* **95**, 511 (1984)]. The *EF1*$^-$ allele is a quadruple substitution converting each of the calcium-binding residues 483, 485, 487, and 493 to alanine. The *EF2*$^-$ allele similarly substitutes residues 510, 512, 514, and 521 in the second EF hand. *EF1*$^-$*EF2*$^-$ contains all eight substitutions. Coomassie blue staining of a parallel gel demonstrated that equivalent amounts of fusion protein of each genotype were expressed. The experiment was duplicated.
17. [^{3}H]PDBu binding in the presence of phosphatidylserine was performed as described [Y. Tanaka, R. Miyake, U. Kikkawa, Y. Nishizuka, *J. Biochem.* **99**, 257 (1986)]. The GST-DAG protein contains residues 538 to 598 of RasGRP. Mouse brain extracts, which contain substantial amounts of PKC, were used as a positive control. The experiment was duplicated.
18. For these studies, we used a modified version of the original cDNA, rbc7HA, cloned in the retrovirus vector pBabepuro [J. P. Morgenstern and H. Land, *Nucleic Acids Res.* **18**, 3587 (1990)]. This modified sequence includes a synthetic Kozak consensus sequence and extends from the internal initiator methionine underlined in Fig. 1B to the premature stop codon. The sequence TATGATGTTCCTGATTATGCTAGCCTC was inserted immediately upstream of this stop codon. It encodes the HA ("Flu") epitope. rbc7 and rbc7HA are similar biologically.
19. Ras-GTP levels in rat2 cells and rat2 cells expressing rbc7 were compared with a ^{32}Pi-labeling method. After treatment with PMA (100 nM for 2 min), cells were lysed, and Ras-GDP and Ras-GTP were immunoprecipitated with antibody Y13-259. Ras-associated guanyl nucleotides were then separated by chromatography and quantified by phosphorimager analysis [J. C. Stone, M. Colleton, D. Bottorff, *Mol. Cell. Biol.* **13**, 7311 (1993)]. The bars in Fig. 3A represent the standard deviation of the mean of three separate experimental values. The amounts of Ras-GTP were compared in rat2 cells expressing the empty vector and rat cells expressing full-length RasGRP with a method that takes advantage of the ability of Ras-GTP to bind the Ras-binding domain of Raf [S. Taylor and D. Shalloway, *Curr. Biol.* **6**, 1621 (1996)]. Cells were treated with endothelin-1 (100 nM for 10 min) and then lysed. The amount of Ras that associated with either GST-Raf (Raf-binding domain) or GST (negative control) was determined by immunoblotting with an antibody to Ras. Lysates contained very similar amounts of Ras.
20. H. Daub, F. U. Weiss, C. Wallasch, A. Ullrich, *Nature* **379**, 557 (1996).
21. I. Ambar and M. Sokolovsky, *Eur. J. Pharmacol.* **245**, 31 (1993).
22. W. Kolch *et al.*, *Nature* **364**, 249 (1993).
23. RNA was extracted from adult rat tissues with the Trizol method. Total RNA (10 μg) was resolved by electrophoresis in 1% agarose in borate buffer, blotted onto Hybond nylon membrane (Amersham), hybridized with RasGRP cDNA probe, and washed at high stringency.
24. In situ hybridization was done on 10-μm sections of adult rat brain [D. M. Simmons *et al.*, *J. Histotechnol.* **12**, 169 (1989)]. A ^{35}S-labeled RNA probe consisting of bases 1831 to 2014 of RasGRP was used for hybridization, and the sections were dipped in NTB2 emulsion and exposed for 15 days. Control hybridizations to adjacent sections with a sense probe showed a low amount of diffuse, uniform labeling.
25. A. B. Vojtek, S. M. Hollenberg, J. A. Cooper, *Cell* **74**, 205 (1993); L. Van Aelst, M. Barr, S. Marcus, A. Polverino, M. Wigler, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 6213 (1993); X. Zhang *et al.*, *Nature* **364**, 308 (1993); P. H. Warne, P. Rodriguez-Viciana, J. Downward, *ibid.*, p. 352; P. Rodriguez-Viciana *et al.*, *ibid.* **370**, 527 (1994); P. Rodriguez-Viciana *et al.*, *Cell* **89**, 527 (1997).
26. G. D. Borasio *et al.*, *Neuron* **2**, 1087 (1989); G. D. Borasio *et al.*, *J. Cell Biol.* **121**, 665 (1993); M. Lohof, N. Y. Ip, M. Poo, *Nature* **363**, 350 (1993); S. Cowley, H. Paterson, P. Kemp, C. J. Marshall, *Cell* **77**, 841 (1994); H. Kang and E. M. Schuman, *Science* **267**, 1658 (1995); C. D. Nobes, J. B. Reppas, A. Markus, A. M. Tolkovsky, *Neuroscience* **70**, 1067 (1996); H. N. Marsh and H. C. Palfrey, *J. Neurochem.* **67**, 952 (1996); K. C. Martin *et al.*, *Neuron* **18**, 899 (1997); R. Brambilla *et al.*, *Nature* **390**, 281 (1997).
27. H. Dudek *et al.*, *Science* **275**, 661 (1997).
28. M. J. Berridge, *Nature* **361**, 315 (1993).
29. M. S. Boguski and F. McCormick, *ibid.* **366**, 643 (1993).
30. A. Aitken, C. B. Klee, P. Cohen, *Eur. J. Biochem.* **139**, 663 (1984).
31. J. P. Aris *et al.*, *Biochim. Biophys. Acta* **1174**, 171 (1993).
32. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
33. We thank R. Kay for advice on cDNA cloning and L. Agellon, D. Brindley, N. Dower, M. James, D. Lowy, H. Ostergaard, E. Shibuya, and B. Sykes for useful discussions. Supported by grants to J.C.S. from the National Cancer Institute, Canada.

6 May 1997; accepted 2 April 1998

Mutations in the *SMAD4/DPC4* Gene in Juvenile Polyposis

James R. Howe,* Stina Roth, John C. Ringold, Robert W. Summers, Heikki J. Järvinen, Pertti Sistonen, Ian P. M. Tomlinson, Richard S. Houlston, Steve Bevan, Frank A. Mitros, Edwin M. Stone, Lauri A. Aaltonen

Familial juvenile polyposis is an autosomal dominant disease characterized by a predisposition to hamartomatous polyps and gastrointestinal cancer. Here it is shown that a subset of juvenile polyposis families carry germ line mutations in the gene *SMAD4* (also known as *DPC4*), located on chromosome 18q21.1, that encodes a critical cytoplasmic mediator in the transforming growth factor–β signaling pathway. The mutant SMAD4 proteins are predicted to be truncated at the carboxyl-terminus and lack sequences required for normal function. These results confirm an important role for *SMAD4* in the development of gastrointestinal tumors.

Familial juvenile polyposis (JP) is an autosomal dominant disease in which individuals are predisposed to hamartomatous polyps and gastrointestinal cancer. Gastrointestinal malignancy develops in 9 to 68% of JP patients (*1*). Two groups have reported that a subset of JP patients harbor mutations in the protein phosphatase gene *PTEN*, located on chromosome 10q23 (*2*). *PTEN* is somatically mutated in many human tumor types and is the gene responsible for Cowden disease and Bannayan-Ruvalcaba-Riley syndrome (*3*). Other groups have found no evidence of linkage to markers on 10q or *PTEN* mutations in JP families (*4*). These results suggest that there is genetic heterogeneity in JP families, or that JP patients previously described with 10q abnormalities may have actually been Cowden disease or Bannayan-Ruvalcaba-Riley syndrome patients (*5*).

We recently mapped a gene predisposing to JP to chromosome 18q21.1, between markers D18S1118 and D18S487 (*6*), an interval that contains the two putative tumor suppressor genes *DCC* and *SMAD4* (*7*). The high incidence of colorectal can-

J. R. Howe and J. C. Ringold, Department of Surgery, University of Iowa College of Medicine, Iowa City, IA 52242, USA.
S. Roth and L. A. Aaltonen, Department of Medical Genetics, Haartman Institute, FIN-00014, University of Helsinki, Finland.
R. W. Summers, Department of Medicine, University of Iowa College of Medicine, Iowa City, IA 52242, USA.
H. J. Järvinen, Second Department of Surgery, Helsinki University Central Hospital, Haartmaninkatu 4, 00290 Helsinki, Finland.
P. Sistonen, Finnish Red Cross Blood Transfusion Service, Kivihaantie 7, 00130 Helsinki, Finland.
I. P. M. Tomlinson, Molecular and Population Genetics Laboratory, Imperial Cancer Research Fund, 44, Lincoln's Inn Fields, London, WC2A 3PX, UK.
R. S. Houlston and S. Bevan, Section of Cancer Genetics, Institute of Cancer Research, Sutton, Surrey SM2 5NG, UK.
F. A. Mitros, Department of Pathology, University of Iowa College of Medicine, Iowa City, IA 52242, USA.
E. M. Stone, Department of Ophthalmology, University of Iowa College of Medicine, Iowa City, IA 52242, USA.
*To whom correspondences should be addressed. E-mail: james-howe@uiowa.edu

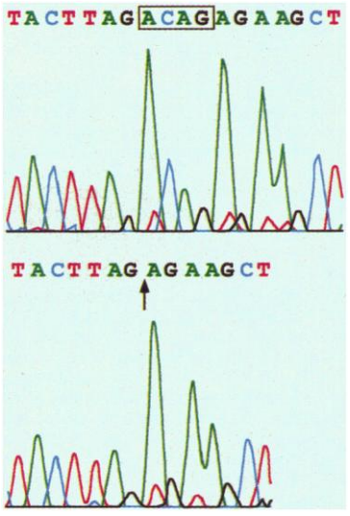

Fig. 1. Sequences of the wild-type (upper) and mutant (lower) alleles of *SMAD4* exon 9 (nucleotides 1365 to 1382) from an affected member of the Iowa JP kindred. The rectangle indicates the 4 bp deleted in the mutant allele (arrow).

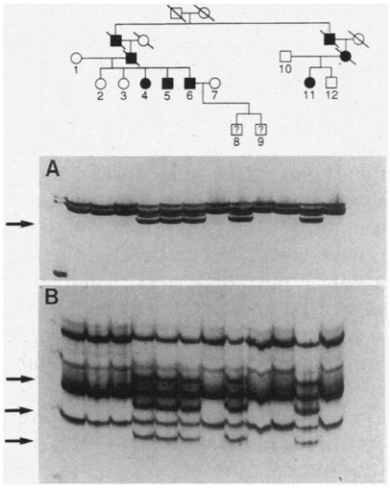

Fig. 2. (**A**) Denaturing and (**B**) nondenaturing gels of Iowa JP kindred family members showing the *SMAD4* exon 9 PCR product. Affected individuals 4, 5, 6, and 11, as well as one at risk (*8*), all have an extra band [arrow in (A)] on denaturing gels that is produced by the 4-bp deletion. The mutant allele is also seen as a shift by SSCP analysis [arrows in (B)].

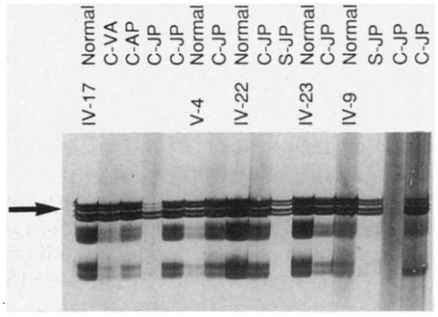

Fig. 3. PCR amplification of *SMAD4* exon 9 from microdissected polyps. Pedigree numbers correspond to affected individuals as described (*6*). Loss of the wild-type allele (arrow) is seen in a juvenile polyp from patient IV-17 (fourth lane from the left). DNA was extracted from paraffin-embedded polyps after microdissection (*28*). Amplification of exon 9 was performed with the primers 5′-TAGGCAAAGGTGTGCAGTTG-3′ and 5′-TGCACTTGGGTAGATCTTATGAA-3′, which generate a 152-bp product from within the exon. C, colon; S, stomach; VA, villous adenoma; AP, adenomatous polyp; JP, juvenile polyp.

cer (as well as one case of pancreatic cancer) in affected members of the JP kindred displaying 18q21 linkage (the Iowa JP kindred) (*8*) led us to hypothesize that one of these tumor suppressor genes could be the gene predisposing to JP. Because of the complexity of *DCC* [29 exons spanning 1.4 Mb (*9*)], we initially searched for germ line mutations by single-strand conformation polymorphism (SSCP) analysis of five family members (three affected, two unaffected) (*10*). Shifts were detected in exons 1, 8, and 16, but these did not cosegregate with the disease. We then changed our mutation screening strategy and began sequencing genomic polymerase chain reaction (PCR) products generated from one affected individual for each exon of *DCC* and *SMAD4* (*11*). After sequencing 14 *DCC* exons and all 11 *SMAD4* exons, we detected a 4–base pair (bp) deletion in exon 9 of *SMAD4*. The patient's affected brother had the same heterozygous deletion and his unaffected mother had the wild-type sequence for exon 9. To confirm this mutation, we subcloned the exon 9 PCR product from this patient into a plasmid vector and sequenced the individual alleles (*12*). One allele was the wild type and the other had a 4-bp deletion (Fig. 1) between nucleotides 1372 and 1375 (codons 414 to 416) of the cDNA sequence [GenBank accession number U44378 (*13*)]. This deletion causes a frameshift that creates a new stop codon at the end of exon 9 (nucleotides 1432 to 1434 of the wild-type sequence, codon 434).

We next analyzed exon 9 of *SMAD4* from all 46 members of the Iowa JP kindred by PCR amplification and denaturing polyacrylamide gel electrophoresis. The altered allele was present in all 13 affected individuals, none of 7 spouses, and 4 of 26 individuals at risk [two-point lod score of 5.79, $\theta = 0$ (the lod score is the logarithm of the odds favoring linkage and θ is the recombination fraction)]. This altered allele was also readily observed on SSCP gels (Fig. 2). To exclude the possibility that this alteration represented a polymorphism, we amplified exon 9 from 242 unrelated individuals (484 chromosomes). The altered allele was not observed in this population. DNA extracted from gastrointestinal polyps was also used to amplify *SMAD4* exon 9. This analysis revealed loss of the wild-type allele in 1 of 11 tumors derived from five affected individuals (Fig. 3).

Eight additional unrelated JP patients were subsequently analyzed for mutations of all exons of *SMAD4* by SSCP and genomic sequencing (Table 1). Two JP kindreds were found that segregated a similar 4-bp deletion in exon 9. Because of the nature of the sequence in this region, these deletions can begin at any of four consecutive nucleotides and result in the same mutant sequence and new stop codon. The three kindreds segregating these deletions were all Caucasian and originated from Iowa, Mississippi, and Finland. There was no common ancestral haplotype, as assessed by analysis of microsatellite markers close to *SMAD4*. Sequencing did not reveal any intragenic polymorphisms that would be useful in evaluating common ancestry, and it is unclear whether this defect is an ancestral founder mutation or a mutational hotspot. A patient with colonic and gastric JP (whose father has a history of gastroin-

Table 1. Analysis of *SMAD4* mutations in nine unrelated JP patients. Under "Type," F refers to familial and S to sporadic JP. wt, wild type.

Patient	Type	Codon (exon)	Nucleotide change	Predicted effect	Controls
I-13*	F	414–416 (9)	4-bp deletion	Frameshift, stop at codon 434	0/242
M-1*	F	414–416 (9)	4-bp deletion	Frameshift, stop at codon 434	0/242
JP 5/1*	F	414–416 (9)	4-bp deletion	Frameshift, stop at codon 434	0/242
JP 11/1	S	348 (8)	2-bp deletion	Frameshift, stop at codon 350	0/101
JP 10/1	S	229–231 (5)	1-bp insertion	Frameshift, stop at codon 235	0/107
JP 6/1	S	wt	–	–	
JP 4/1	F	wt	–	–	
JP 1/1	S†	wt	–	–	
JP 2/13‡	F	wt	–	–	

*Sequence variant segregates with JP phenotype in respective family (13, 5, and 2 affected individuals with the mutation in the Iowa, Mississippi, and JP 5 Finnish kindreds, respectively). †JP 1/1 has a brother with colon cancer but no family members with documented JP. ‡Multipoint lod score of 1.00 with chromosome 18q21 markers in this family (6 affected and 11 normal individuals).

testinal symptoms but has not been evaluated clinically) was found to have a 2-bp deletion in exon 8 of *SMAD4*, at nucleotides 1170 to 1171 (codon 348). This deletion causes a frameshift that creates a stop codon at nucleotides 1178 to 1180 (codon 350). Another patient diagnosed with 30 to 40 colonic juvenile polyps at age 6 but with no family history of JP (four siblings and both parents unaffected) was found to have a 1-bp insertion between nucleotides 815 and 820 of exon 5; this change added a guanine to a stretch of six sequential guanines in the wild-type sequence and created a frameshift and a new stop codon at nucleotides 830 to 832 (codon 235). No *SMAD4* mutations were found in four other unrelated JP patients.

Somatic mutations in *SMAD4* have been reported in up to 50% of human pancreatic tumors (*13*, *14*) and 15% of colorectal tumors (*15*). The occasional development of pancreatic cancer and the high incidence of colorectal cancer (40% in the Iowa JP kindred) in JP families is consistent with these findings in sporadic tumors. It remains to be determined whether the locus on 18q21 involved in the development of sporadic colorectal cancers is *SMAD4*, *DCC*, another closely linked gene, or a combination of these genes.

SMAD4 is a member of the *SMAD* family of genes, which code for cytoplasmic mediators in the transforming growth factor–β (TGF-β) signaling pathway (*16*). This pathway mediates growth inhibitory signals from the cell surface to the nucleus. Upon activation by TGF-β or related ligands, serine-threonine kinase receptors phosphorylate various SMAD proteins, which then form heteromeric complexes with SMAD4 in the cytoplasm (*17*). These complexes then migrate to the nucleus, where they are thought to regulate transcription through association with various DNA binding proteins (*18*). The growth inhibitory effect of TGF-β on pancreatic cancer cell lines requires functional SMAD4 (*19*).

SMAD4 is a 552–amino acid protein (*13*). Its COOH-terminus appears to be important for the formation of SMAD4 homotrimers, which then complex with other SMAD proteins. Mutations that disrupt homotrimer formation lead to loss of TBF-β signaling (*20*). A SMAD4 mutant lacking 38 COOH-terminal amino acids has a dominant negative effect on SMAD2-mediated mesoderm induction in *Xenopus* embryos and forms oligomers with wild-type SMAD4 that may be responsible for this loss of activity (*17*). The majority of somatic mutations described in *SMAD4* map to the COOH-terminus between codons 330 and 526 (*13*, *14*, *21*, *22*) within several highly conserved domains. The 4-bp deletion detected in three JP families is predicted to produce a COOH-terminally truncated protein of 433 amino acids, with loss of regions critical for normal function. The 1-bp insertion and 2-bp deletion seen in two other patients are predicted to result in truncated proteins of 234 and 349 amino acids, respectively. Although deletion of the wild-type allele was seen in only one of 11 polyps, some of these may have been contaminated with normal cells during microdissection. Alternatively, other somatic *SMAD4* mutations may have been present in these samples, or germ line mutation of *SMAD4* may induce tumors through a dominant negative effect.

One of the features of the gastrointestinal polyps seen in compound *Apc-Smad4* mutant heterozygote mice is the increased proliferation of stromal cells (*23*), which is one of the characteristic features of juvenile polyps seen in humans. It has also been shown in *Xenopus* embryos that wild-type *SMAD4* induces mesodermal markers and that mixtures of mutant and wild-type SMAD4 inhibit this response (*17*). In JP patients, it would appear that germ line *SMAD4* mutations predispose to focal abnormalities of mesenchymal development (hamartomas) and cancer through disruption of the TGF-β signaling pathway. JP may be a genetically heterogeneous condition, as evidenced by the fact that not all families are linked to 18q markers (*24*) and not all families studied had germ line *SMAD4* mutations. It is possible that germ line mutations in genes encoding different components of the TGF-β signaling pathway may be present in these other JP kindreds. The roles of the Cowden disease gene (*PTEN*) and Peutz-Jeghers syndrome gene [*LKB1* (*25*)] in cell growth control remain unclear, although *PTEN* may be down-regulated by TGF-β (*26*). Further studies on the components of the TGF-β pathway may add to our understanding of these hamartomatous polyposis syndromes.

REFERENCES AND NOTES

1. H. J. Järvinen and K. O. Franssila, *Gut* **25**, 792 (1984); J. R. Jass, in *Familial Adenomatous Polyposis and Other Polyposis Syndromes*, R. K. S. Phillips, A. D. Spigelman, J. P. S. Thomson, Eds. (Edward Arnold, London, 1994), pp. 203–214; J. R. Howe, F. A. Mitros, R. W. Summers, in preparation. Estimates of gastrointestinal cancer vary widely because there have been few studies in large families with long-term follow-up.
2. E. D. Lynch *et al.*, *Am. J. Hum. Genet.* **61**, 1254 (1997); S. Olschwang *et al.*, *Nature Genet.* **18**, 12 (1998).
3. D. Liaw *et al.*, *Nature Genet.* **16**, 64 (1997); D. J. Marsh *et al.*, *ibid.*, p. 333.
4. D. J. Marsh *et al.*, *Cancer Res.* **57**, 5017 (1997); G. J. Riggins, S. R. Hamilton, K. W. Kinzler, B. Vogelstein, "Normal PTEN Gene in Juvenile Polyposis," *J. Neg. Obs. Gen. Oncol.* [online] **1**, 1 (1997). Available at http://128.220.85.41:5002/NOGO.
5. C. Eng and H. Ji, *Am. J. Hum. Genet.* **62**, 1020 (1998).
6. J. R. Howe *et al.*, *ibid.*, p. 1129.
7. K. Eppert *et al.*, *Cell* **86**, 543 (1996).
8. T. J. Stemper, T. H. Kent, R. W. Summers, *Ann. Int. Med.* **83**, 639 (1975).
9. K. R. Cho *et al.*, *Genomics* **19**, 525 (1994).
10. Primers were designed for exons 1 to 29 of *DCC* with the Primer3 server (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3.cgi) and the published intron-exon boundaries (*14*). Primers for amplification of the *SMAD4* gene have been described (*27*). PCR was performed in a 10-μl volume with 25 ng of DNA, 200 μM each of the deoxynucleotide triphosphates dGTP, dATP, dTTP, and dCTP, 1 μl of 10× buffer [100 mM tris-HCl (pH 8.3), 500 mM KCl, 15 mM $MgCl_2$, 0.01% w/v gelatin], 2 pmol of each primer, and 0.25 units of Taq DNA polymerase. PCR was performed for 1 min at 94°C, 1 min at 55°C (or optimal annealing temperature), and 1 min at 72°C for 30 cycles. After amplification, 5 μl of stop solution (95% formamide, 10 mM NaOH, 0.05% bromophenol blue, 0.05% xylene cyanol) was added, and samples were heated to 95°C for 3 min, then loaded onto 6% nondenaturing polyacrylamide gels (with and without 10% glycerol). DNA was detected by silver staining. Informed consent for DNA studies was obtained from family members with the approval of the Institutional Review Board at the University of Iowa.
11. PCR products were subjected to electrophoresis through 2% agarose gels and stained with ethidium bromide to confirm the presence of a single band of the expected size. The products were isolated with the Qiaquick PCR purification kit (Qiagen, Santa Clarita, CA) and then sequenced with the ABI Prism Dye Terminator Cycle Sequencing kit (PE Applied Biosystems, Foster City, CA). Cycle sequencing included 1 cycle at 98°C for 5 min, followed by 30 cycles at 94°C for 10 s, 50°C for 5 s, and 60°C for 4 min. Individual PCR primers were used for sequencing the sense and antisense strands for each exon. Reactions were analyzed with an ABI model 373XL stretch fluorescent automated sequencer.
12. Exon 9 was PCR amplified and the gel products purified and ligated into the p-GEM-T Easy plasmid vector (Promega). JM109 cells were transformed with the vector and plated onto LB-ampicillin plates containing 0.5 mM isopropylthio-β-D-galactoside and 5-bromo-4-chloro-3-indolyl-β-D-galactoside (80 μg/ml). Recombinant clones were grown overnight at 37°C in LB-ampicillin (100 μg/ml) medium. Cells were harvested and lysed, and cycle sequencing was performed with DPC4S9 and AS9 primers.
13. S. A. Hahn *et al.*, *Science* **271**, 350 (1996).
14. M. Schutte *et al.*, *Cancer Res.* **56**, 2527 (1996).
15. S. Thiagalingam *et al.*, *Nature Genet.* **13**, 343 (1996).
16. J. L. Wrana and L. Attisano, *Trends Genet.* **12**, 493 (1996).
17. G. Lagna *et al.*, *Nature* **383**, 832 (1996).
18. J. Wrana and T. Pawson, *ibid.* **388**, 28 (1997).
19. A. M. Grau *et al.*, *Cancer Res.* **57**, 3929 (1997).
20. Y. Shi *et al.*, *Nature* **388**, 87 (1997).
21. Y. Takagi *et al.*, *Gastroenterology* **111**, 1369 (1996).
22. S. K. Kim *et al.*, *Cancer Res.* **56**, 2519 (1996).
23. K. Takaku *et al.*, *Cell* **92**, 645 (1998).
24. L. A. Aaltonen, R. S. Houlston, S. Bevan, I. P. M. Tomlinson, unpublished data.
25. A. Hemminki *et al.*, *Nature* **391**, 184 (1998).
26. D.-M. Li and H. Sun, *Cancer Res.* **57**, 2124 (1997).
27. C. A. Moskaluk *et al.*, *Diagn. Mol. Pathol.* **6**, 85 (1997).
28. J. R. Howe, D. S. Klimstra, C. Cordon-Cardo, *Histol. Histopathol.* **12**, 595 (1997).

29. We thank A. Hemminki, P. Kristo, A. Loukola, E. Avizienyte, R. Salovaara, K. Saastamoinen, S. Lindh, T. Lehtinen, and T. Kosonen for assistance. Supported in part by the Owen H. Wangensteen Faculty Research Fellowship of the American College of Surgeons, the Carver Trust Medical Research Initiative Grant, the Clinical Cancer Center at the University of Iowa, the Academy of Finland, the Helsinki University Central Hospital, the Finnish Cancer Society, the Research and Science Foundation of Farmos, the European Commission, the Sigrid Juselius Foundation, and the Coeliac Disease Society.

17 March 1998; accepted 7 April 1998