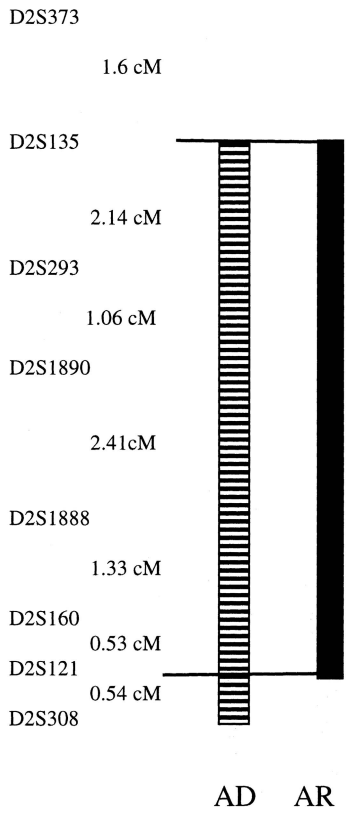

Figure 2 Comparison of the candidate regions on chromosome 2q11-q13 for autosomal dominant (AD) and recessive (AR) ectodermal dysplasia. The order of the principal markers used in the study and the distances are those of the Marshfield sex-averaged chromosome 2 linkage map. Since loci D2S135 and D2S1321 are very close to each other, only D2S135 appears in the figure.

Genome Research, http://www-genome.wi.mit.edu (for marker order in the 2q11-q13 region)

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Mosaicism and Sporadic Familial Adenomatous Polyposis

To the Editor:

Familial adenomatous polyposis (FAP [MIM 175100]) is an autosomal dominant heritable disorder caused by germ-line mutations in the APC gene (M74088 [GenBank]). There is a high new-mutation rate, with ~25% of all cases being sporadic (Bisgaard et al. 1994). Parental mosaicism can explain new mutations in genetic disorders (Hall 1988), whereas germ-line and/or somatic mosaicism has been described in genes associated with tumors such as p53 (Kovar et al. 1992), Rb1 (Greger et al. 1990; Lohmann et al. 1997), NF1 (Lázaro et al. 1995), and NF2 (Bourn et al. 1994). We were interested to determine whether APC-gene mutational mosaicism could account for some of the apparentlynew" APC mutations. Systematic studies of our registry identified five in which a germ-line mutation was established (Prosser et al. 1994), parental leukocyte DNA was available, and paternity was assured. These five patients were the subjects of detailed studies to determine the level of parental APC mutational mosaicism (table 1).

During S^{35} sequencing of parental blood-leukocyte DNA samples from the first sporadic FAP case, we noted a faint mutant allele that was reproducible on repeated analyses. The clinical history of this sporadic FAP patient (patient 17) is noteworthy. Dense distal colonic polyposis was diagnosed at age 22 years, with three distinct carcinomas and myriads of smaller polyps, many of which showed carcinomatous change. Mastectomy was required for breast carcinoma when the patient was age 37 years, and disseminated ovarian cancer resulted in death at age 44 years. Her mother (patient 16) had a mastectomy for breast carcinoma at age 46 years, was negative in a screen for colonic polyps when she was

Table 1

APC Mutations and Parental-Mosaicism Screening Strategy for Sporadic FAP Patients

Patient	Nucleotide Change	Effect on Coding Sequence	Parental-Mosaicism Screening Assay
17	1354delGT	Frameshift	S^{35} sequencing, color-selective and nonselective PCR, cloning
148	694C→T	Arg→stop at 232	Mutation-specific restriction digestion and PCR enrichment
410	4192G→T	Glu→stop at 1398	PCR cloning, single-cell analysis
893	3926delAAAAG	Frameshift	PCR cloning
1024	4135C→T	Gln→stop at 1379	Single-cell analysis

NOTE.—In all cases, a heterozygous mutation was excluded by standard analysis of parental leukocyte DNA, and paternity was assured. A description of each assay can be obtained from the corresponding author.

age 64 years, and subsequently died from ovarian cancer at age 67 years. There was an extensive family history of breast cancer due to a BRCA1 mutation (5382insC mutation [Mullen et al. 1997]), with several cases of gastric and colon cancer in the extended family. Patient 17 carried the 5382insC BRCA1 mutation, as well as an APC mutation (table 1).

The APC mutation identified in patient 17 was within a simple repeat region, a GT deletion in a run of four GT dinucleotides in exon 10 (table 1). On observing the faint mutant allele in the maternal leukocyte-DNA sample (patient 16), we were first concerned that it might represent sample contamination by leukocyte DNA of patient 17. However, sequencing of two other samples from patient 16 that had been taken several years apart confirmed the faint mutant allele, and we never saw this sequence in paternal or control leukocyte DNAs. To exclude the possibility that the mutant sequence was due to PCR error, we devised a PCR cloning strategy that reduced the number of PCR cycles and allowed easy identification of bacterial clones carrying the 1354delGT allele. We designed PCR primers for cloning into pBluescript that were out of frame for wild-type sequence and in frame for β-galactosidase in the presence of the patient 17 APC mutation (details are available from the corresponding author, on request). This gave blue colonies for mutant alleles and white colonies for the wild type.

Plating of PCR clones derived from patient 17 gave the expected 50% blue colonies. Ten blue colonies were picked at random and were sequenced, confirming the 1354delGT allele in every clone. All 10 white colonies picked were wild-type sequence, as expected. The blue:white colony ratio was substantially lower in clones derived either from patient 16 or from control DNAs, typically <10% blue colonies in replicate PCR cloning experiments. Approximately 60% of blue clones derived from patient 16 and from control DNA were due to plasmid rearrangements. However, sequencing of 37 blue clones with the correct-size PCR insert from patient 16 DNA identified eight alleles that were identical to the 1354delGT mutation in patient 17 (fig. 1), demonstrating the utility of our PCR cloning–enrichment strategy. Although some blue colonies were due to plasmid rearrangements, mutant sequence was never identified in any clones derived from control DNA, in multiple experiments.

To formally quantify the level of mosaicism in blood leukocytes, PCR amplification products from patients 16 and 17 and from control leukocyte-DNA templates were cloned without selection for color. Mutation screening using heteroduplex analysis of 41 clones that contained the correct-size insert derived from patient 17 DNA templates gave 21 mutant and 20 wild-type alleles. For patient 16's leukocyte DNA, 310 colonies were picked, and 113 contained the correct-size APC fragment, 4 (3.5%) of which were 1354delGT mutant alleles. Screening of 300 colonies derived from control DNA identified 160 clones containing correct APC amplification products, and none were mutant alleles (0/160 vs. 4/113; $P < .03$, by Fisher's exact test). These data establish that patient 16 DNA contained a small proportion of alleles from somatic DNA that are identical to the mutant allele that was responsible for FAP in her daughter. We were unable to determine the tissue distribution of mosaicism, because of the death of patient 16 prior to these studies and because archival paraffin-embedded material had been infected with mold and was destroyed.

Tailored assays were then designed to detect the levels of the respective APC mutation (table 1) in the other four sporadic cases. Preliminary "spiking" experiments demonstrated that an ~0.1% mutant-allele presence was detectable (data not shown). None of the assays detected any other cases of parental mosaicism. Parental blood-leukocyte DNA for patient 148 was analyzed by mutation-specific PCR enrichment; for patient 893, 200 verified colonies for each sample of parental leukocyte DNA were screened by single-strand conformation polymorphism; for patient 1024, PCR products from single-cell preparations of 350 paternal lymphocytes and 120 maternal lymphocytes were analyzed by restriction digestion.

We were surprised to identify a further case of APC mutational mosaicism, in a proband with FAP. Patient

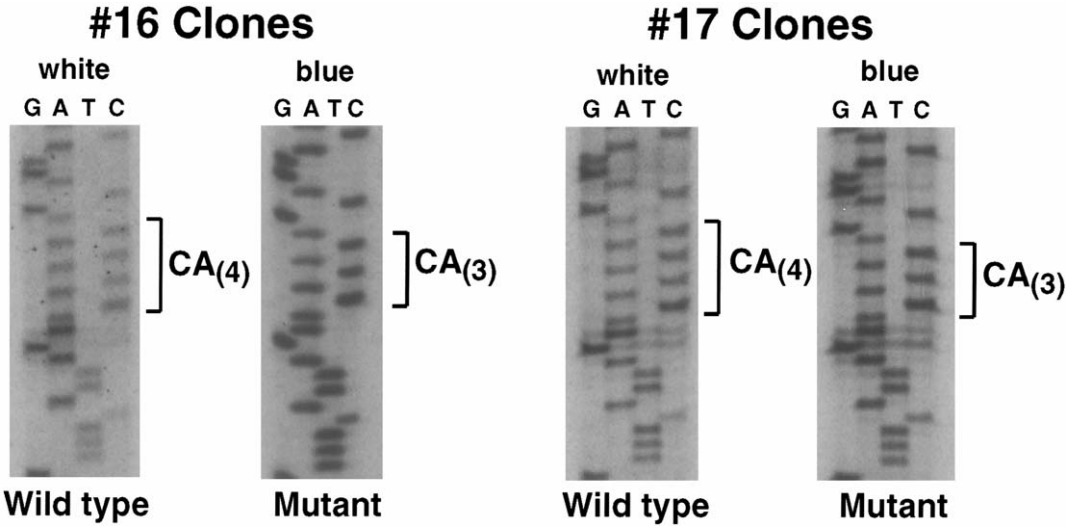

Figure 1 S^{35} sequencing of PCR products from blue and white colonies from patients 16 and 17, showing the presence of mutant (three GT/CA) and wild-type (four GT/CA) alleles, respectively (reverse PCR primer was used to sequence).

410 has no family history worthy of note but, during the teenage years, developed orbital rhabdomyosarcoma, a tumor very rarely associated with FAP (Lynch et al. 1982; Armstrong et al. 1991). The FAP syndrome followed an aggressive course, with dense polyposis and severe desmoid disease. When testing the primers designed for rapid screening of parental PCR clones, by introduction of a new *Dra*I restriction site into the 4192G→T mutant alleles, we noted a deviation from the expected heterozygote allele ratio. Replicate experiments performed on leukocyte DNA after repeated blood sampling consistently showed that the intensity of the mutant allele was less than expected, even after allowance had been made for the staining intensity of the respective restriction fragments (fig. 2). PCR products from both parents and the patient were cloned, and no mutants were detected in 335 maternal and 210 paternal alleles. However, there were 98 wild-type and only 38 mutant alleles identified on analysis of colonies from patient 410, a result significantly different ($P < .0003$) from the expected 1:1 ratio but in accordance with the dosimetry studies using patient 410's blood-leukocyte DNA templates (table 2). Twenty-eight undigested alleles were sequenced, and all confirmed the presence of a wild-type TTTAAA *Dra*I restriction site, excluding PCR error as the explanation for these findings. Sequencing of three of the clones that did digest with *Dra* I confirmed the presence of the mutant allele, as expected. Further confirmation that a proportion of circulating leukocytes do not carry the mutant APC allele was obtained by sequencing of PCR products from 18 fresh single-cell lymphocyte preparations. Twelve lymphocytes contained exclusively wild-type alleles, whereas the other six showed mutant and wild-type sequence. These studies show that, despite the FAP phenotype, patient 410 is mosaic and does not carry mutant APC in all peripheral blood cells.

We were interested to investigate the tissue distribution of the mutant and wild-type alleles. Determination of the level of 4192G→T mutation in a variety of tissues from patient 410 was possible because surgically resected colon, colonic adenomas, kidney, and orbital rhabdomyosarcoma were available in paraffin-embedded blocks. When the *Dra*I restriction assay was used, substantial mutation-level variation between tissues was

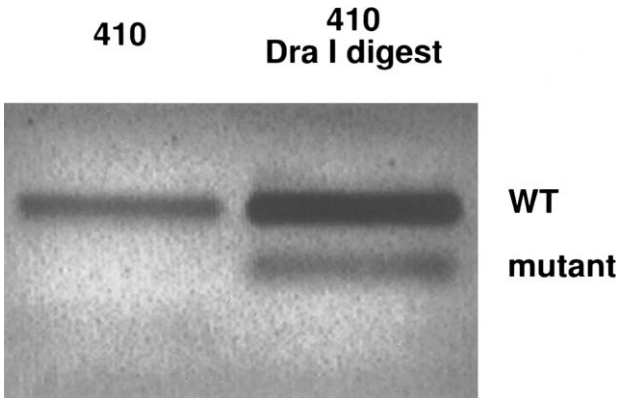

Figure 2 PCR products of patient 410's peripheral blood-leukocyte DNA templates digested with *Dra*I, showing readily visible differences in between wild-type (134-bp) and mutant (112-bp) allele intensities.

Table 2

Quantitation of Mosaicism for the 4192G→T Mutant APC Allele in Patient 410

Tissue	Mutant:Wild-Type Volume Ratio[a]
Peripheral blood leukocyte	.22
Kidney	.08
Normal colonic mucosa	.44
Rhabdomyosarcoma	1.44
Adenoma 1	.42
Adenoma 2	.28

[a] Aggregates of three separate assays for each tissue.

noted (fig. 3), with complete absence of the mutant allele in renal tissue. Dosimetry of bands derived from leukocyte DNA agreed well with the results of prior cloning studies (table 2). The mutant:normal volume ratio in rhabdomyosarcoma suggests loss of wild-type allele but with normal-tissue contamination contributing the faint normal allele. The microdissected adenomas have mutant:normal volume ratios that are similar to those for leukocyte DNA, supporting the notion that FAP polyps can be polyclonal in origin (Novelli et al. 1996).

Genetic instability appears to influence the APC mutation spectrum in colorectal tumors (Huang et al. 1996), and so microsatellite analysis (Farrington et al. 1998) was performed on DNA purified from archival paraffin-embedded tissues from patients 17 and 410. Patient 17's colon tumor showed instability at three of the four CA-repeat markers analyzed but at none of three poly-A markers (fig. 4). Although breast cancers rarely show microsatellite instability (Wooster et al. 1994), we are not aware of any report describing microsatellite analysis in colon-tumor tissue from a BRCA1-mutation carrier. Patient 410's tumors showed low-level instability for three CA-repeat markers that gave sufficient data for analysis (different markers in each tumor), but there was no poly-A instability. Genomic sequencing of hMSH2 (U03911/U04045 [GenBank]) and hMLH1 (U07343/U07418 [GenBank]) (Farrington et al. 1998) in patients 16, 17, and 410 did not identify any pathological variants, although hPMS1, hPMS2, and hMSH6 were not analyzed.

In this study, we have established that combined somatic/gonadal mosaicism for an APC mutation can arise rarely and that a clinical phenotype attributable to that mutation is not always apparent. In addition, we have shown germ-line transmission of the mutant allele to offspring. There may be a phenotypic threshold that is likely related to both the overall contribution and the tissue distribution of the mutant allele, as was found in patient 410. Presumably, the patch size in patient 410 was critically distributed, to allow expression of the FAP phenotype. There is one other report of a mosaic APC-gene variant in a patient with FAP (Mandl et al. 1994); however, this variant was not pathogenic, since it was incidental to a frameshift APC mutation segregating with FAP in that family.

Our findings have clinical relevance to counseling regarding recurrence risk to siblings of "sporadic" FAP patients with phenotypically normal parents who do not appear to carry the APC mutation as assessed by conventional methods. We have shown that gonadal mosaicism is a possibility and that therefore the risk to siblings of an index sporadic case is higher than the population risk. Somatic mosaicism has been suggested as a common cause of classic neurofibromatosis 2 (Evans et al. 1998), and isolated gonadal mosaicism has recently been demonstrated in tuberous sclerosis (Yates et al. 1997), emphasizing the broader clinical relevance of our findings. In addition to the implications with regard to germ-line transmission, somatic mutational mosaicism of the APC gene has relevance to cancer risk, since APC plays a pivotal role in neoplastic transformation (Kinzler and Vogelstein 1996). Individuals may carry, in a small proportion of epithelial stem cells, a mutant APC allele that confers an increased cancer risk, even though there is no obvious evidence of FAP.

We hypothesize that APC mutational mosaicism can be due to genetic instability and that passage through the germ line of instability-prone individuals represents a mechanism by which new mutations might arise. In this context, the predominance of CA:GT dinucleotide-repeat instability is noteworthy; and the new germ-line mutation in patient 17 also arose within a short CA:GT repeat in APC. Although we did not identify any germ-line hMSH2 or hMLH1 mutations in patients 16, 17,

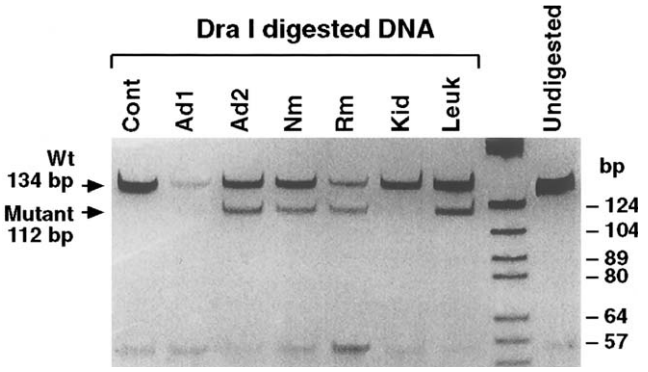

Figure 3 Investigation of dosage of mutant APC allele (4192G→T) in various tissues from patient 410, with *Dra*I digestion of PCR products. Control (i.e., nonmutant) leukocyte DNA lacks the *Dra*I site and so gives a full-length 134-bp fragment on *Dra* I digestion. Results for two adenomatous colonic polyps (lanes Ad1 and Ad2), normal colonic mucosa (lane Nm), rhabdomyosarcoma (lane Rm), renal tissue (lane Kid), and peripheral blood-leukocyte DNA (lane Leuk) are shown.

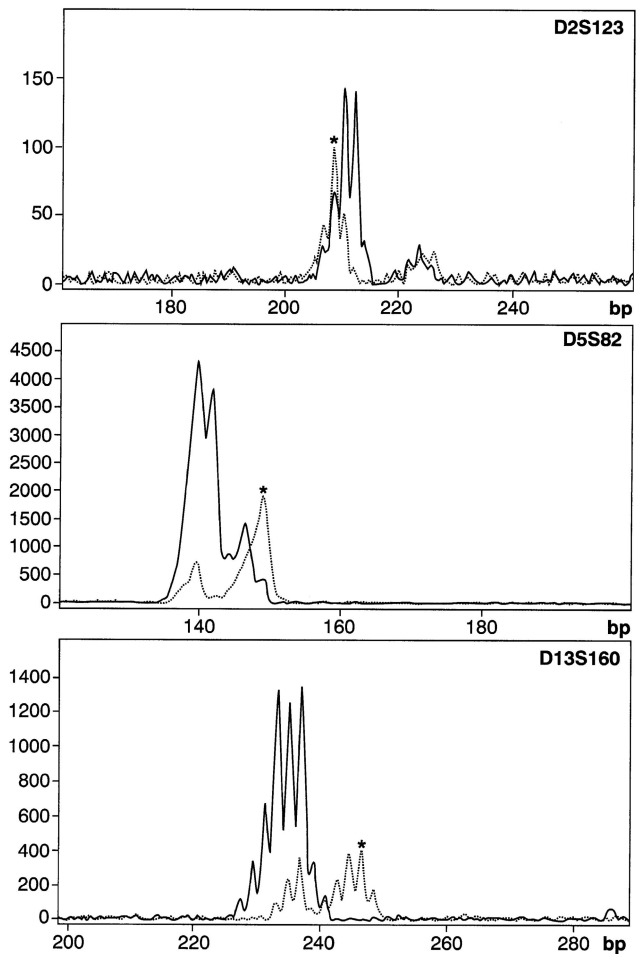

Figure 4 Traces from ABI 310 genetic analyzer with GeneScan software, showing band shifts between patient 17's normal-mucosa DNA template (*solid line*) and colon-cancer DNA templates (*dotted line*), for three CA-repeat markers analyzed. The asterisks (*) indicate the band shifts observed in the tumor tissue.

or 410, the presence of tumor instability associated with mosaicism is intriguing. A constitutional mutator phenotype due to mismatch repair–gene mutations has been reported in association with increased mutation rate in normal tissues (Parsons et al. 1995; Hackman et al. 1997). Indeed, one of these reported patients had clinical FAP without a detectable heterozygous APC mutation. Of further interest, a defined MLH1 mutation has been reported in association with transmission of instability at the FRAXA locus (Sharrock et al. 1997).

Both the atypical phenotype in these patients and the association with other molecular events—namely, BRCA1 mutation and microsatellite instability—is intriguing and may reflect an underlying process leading to genetic instability. Mutation of BRCA1 would not be expected to induce tumor microsatellite instability, but we estimate that the probability of the association of mutations in BRCA1 and APC is 1/10 million (Bisgaard et al. 1994; Ford et al. 1995), and so a chance occurrence seems remote in the case reported here. It will be of interest to determine whether mosaicism for mutations in other genes is a more general phenomenon than has been previously recognized.

Acknowledgments

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Web/Genbank (for APC cDNA [M74088], hMSH2 cDNA [U03911 and U04045], and hMLH1 cDNA [U07343 and U07418])
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for colorectal cancer [MIM 114500] and FAP [MIM 175100])

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HPS Gene Mutations in Hermansky-Pudlak Syndrome

To the Editor:

We recently reported a series of mutations of the *HPS* gene in non–Puerto Rican patients with Hermansky-Pudlak syndrome (MIM 203300) (Oh et al. 1998). One of these mutations was designated incorrectly in some places in the article; a frameshift in codon Q397 was incorrectly designated "E397" in the text (this mutation is now designated "c1189delC," in the new nomenclature; Antonarakis 1998). In addition, subsequent to publication we determined that patient 20 is homozygous for a novel frameshift due to a single-base deletion in codon G96, designated "c288delT," with the first-cousin parents both being heterozygous for this mutation. Thus, *HPS* gene mutations have now been identified in all patients and families who show apparent linkage to 10q23. With our original description of the gene (Oh et al. 1996) and the recent report of Shotelersuk et al. (1998), this brings the number of reported *HPS* gene mutations to 11 and further underscores the lack of missense mutations identified in patients with this disorder.

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Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for Hermansky-Pudlak syndrome [MIM 203300])