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Severe Gardner Syndrome in Families with Mutations Restricted to a Specific Region of the *APC* Gene

D. Rhodri Davies,[1] John G. Armstrong,[1] Nalin Thakker,[1] Keith Horner,[2] Simon P. Guy,[1] Tara Clancy,[1] Phil Sloan,[2] Val Blair,[3] Chris Dodd,[4] Tom W. Warnes,[5] Rodney Harris,[1] and D. Gareth R. Evans[1]

[1] Department of Medical Genetics, St. Mary's Hospital, [2] University Dental Hospital, [3] Department of Cancer Epidemiology, Christie Hospital, [4] Manchester Royal Eye Hospital, and [5] Department of Gastroenterology, Manchester Royal Infirmary, Manchester

Summary

Familial adenomatous polyposis (FAP) is associated with a number of extraintestinal manifestations, which include osteomas, epidermoid cysts, and desmoid tumors, often referred to as "Gardner syndrome." Recent studies have suggested that some of the phenotypic features of FAP are dependent on the position of the mutation within the *APC* gene. In particular, the correlation between congenital hypertrophy of the retinal pigment epithelium (CHRPE) and *APC* genotype indicates that affected families may be divided into distinct groups. We have investigated the association between the dento-osseous features of GS on dental panoramic radiographs (DPRs) and *APC* genotype in a regional cohort of FAP families. DPRs were performed on 84 affected individuals from 36 families, and the dento-osseous features of FAP were quantified by a weighted scoring system. Significant DPR abnormalities were present in 69% of affected individuals. The *APC* gene mutation was identified in 27 of these families, and for statistical analysis these were subdivided into three groups. Group 1 comprised 18 affected individuals from seven families with mutations 5′ of exon 9; these families (except one) did not express CHRPE. Groups 2 comprised 38 individuals from 16 families with mutations between exon 9 and codon 1444, all of whom expressed CHRPE. Group 3 comprised 11 individuals from four families with mutations 3′ of codon 1444, none of whom expressed CHRPE. Families with mutations 3′ of codon 1444 had significantly more lesions on DPRs ($P < .001$) and appeared to have a higher incidence of desmoid tumors. These results suggest that the severity of some of the features of Gardner syndrome may correlate with genotype in FAP.

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Address for correspondence and reprints: Dr. D. Gareth R. Evans, Consultant in Clinical Genetics, St. Mary's Hospital, Hathersage Road, Manchester M13 0JH, United Kingdom.

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Introduction

Familial adenomatous polyposis (FAP) is an autosomal dominant disorder characterized by the development of multiple adenomas in the colon and rectum and a high risk of subsequent colorectal cancer. There is an association with a number of extraintestinal manifestations, which include osteomas, epidermoid cysts, and desmoid tumors. These were first described by Gardner and colleagues (Gardner and Plenk 1952; Gardner and Richards 1953; Gardner 1962), and the combination of colorectal polyposis and these other features is referred to as "Gardner syndrome" (GS). It is now also recognized that the majority of individuals with FAP have polyps of the upper gastrointestinal tract and that there is a significant risk of upper-gastrointestinal cancer (Jagelman et al. 1988). Furthermore, many have characteristic lesions of the retina, known as "congenital hypertrophy of the retinal pigment epithelium" (CHRPE) (Traboulsi et al. 1987). There is variation in the expression of the GS phenotype in families with FAP. GS was previously considered to represent a distinct disease entity, compared with colonic polyposis without apparent extraintestinal features (McKusick 1988). However, subtle abnormalities such as radio-opaque jaw lesions occur in the majority of individuals with FAP (Utsunomiya and Nakamura 1975), and, in addition, many families include affected individuals with and without extraintestinal manifestations.

Examination of the ocular fundus for CHRPE and dental panoramic radiographs (DPRs) to identify occult osseous changes have been advocated as useful for predictive testing of at-risk individuals. CHRPE is reported to occur in 58%–88% of all FAP-affected individuals (Burn et al. 1991; Giardiello et al. 1991), and there is a demonstrable family-specific effect (Hodgson et al. 1994). DPR abnormalities have been reported in up to 93% of FAP-affected individuals (Utsunomiya and Nakamura 1975; Wolf et al. 1986; Giardiello et al. 1991). The value of DPR screening for presymptomatic diagnosis of FAP has been limited by the lack of data on sensitivity and specificity of DPR changes.

FAP is caused by germ-line mutation of the adenomatous polyposis coli gene (*APC*) on chromosome 5q21-

22. The *APC* gene recently has been identified (Groden et al. 1991; Joslyn et al. 1991; Kinzler et al. 1991; Nishisho et al. 1991), and ~200 constitutional mutations have now been reported (Nagase and Nakamura 1993). The position of the mutation along the coding sequence of the *APC* gene influences the phenotypic expression of FAP. A common mutation at codon 1309 is associated with early onset of polyposis and cancer (Caspari et al. 1994), whereas mutations at the extreme 5′ end of the gene are associated with an attenuated phenotype (Spirio et al. 1993). The presence of CHRPE has been shown to be dependent on the position of the mutation within the *APC* gene. Families with mutations upstream of exon 9 do not express CHRPE (Olschwang et al. 1994), and it recently has been reported that families with mutations downstream of codon 1444 in exon 15 of the *APC* gene also do not express this retinal sign (Caspari et al. 1995; Scott et al. 1995).

Phenotype-genotype correlation has not previously been reported for the presence of dento-osseous manifestations in FAP. In the present study, we confirm the existence of three distinct groups in FAP, groups based on the correlation of CHRPE with the genotype, as reported recently by Caspari et al. (1995). In order to investigate whether there is any association between dento-osseous features of FAP and *APC* genotype, we have studied the DPRs of affected individuals in these groups; and we present here the first evidence of correlation between the severity of the dento-osseous phenotype and position of the *APC* gene mutation.

Subjects, Material, and Methods

Phenotypic Markers

A regional register was established in the northwest of England, for families with FAP. Affected and at-risk individuals were offered ophthalmic examination and dental radiography as part of a presymptomatic screening program.

Indirect ophthalmoscopy was performed by an ophthalmologist (blinded to results of mutation analysis) using both slit-lamp microscopy and a head-mounted instrument. The number and type of CHRPE were recorded and quantified by calculating the CHRPE coefficient for each patient, as described elsewhere (Olschwang et al. 1993). CHRPE were classified as follows: (1) typical large CHRPE (greater than one quarter of the optic-disk diameter) and (2) small pigmented spots. The CHRPE coefficient was calculated by multiplying the number of large CHRPEs by 3 and adding this to the sum of all the small pigmented spots in both eyes. Atypical lesions were excluded, since their occurrence was uncommon and their significance uncertain.

DPRs were examined blind by three independent observers (a dental radiologist and two consultants in oral medicine), for osseous and dental abnormalities. Radiodense lesions were classified as osteomas, dense bone islands, or hazy sclerosis; and the dental abnormalities were considered to be a feature of FAP were supernumerary teeth, unerupted teeth, and odontomes. A weighted scoring system was devised to quantify the extent of abnormality on DPR, and a DPR score was calculated for each individual. The score given to each abnormality was based on the latter's frequency in FAP, compared with that found in the general population. A DPR score of ≤ 2 was considered normal, a score of 3–4 as minimal but insignificant change, a score of 5–6 as equivocal, and a score of >6 as significantly abnormal. With these criteria, a DPR score of >6 identified FAP-affected individuals with a specificity and positive predictive rate of 100% and a sensitivity of 69%. The scoring system and its application in the presymptomatic diagnosis of FAP are described in detail elsewhere (Thakker et al. 1995).

Molecular Analysis

Genomic DNA was extracted from venous blood, for mutation analysis. Exons 1–14 and regions A–K of exon 15 of the *APC* gene (nucleotides 1–5323) were amplified by PCR using flanking primers, as described elsewhere (Groden et al. 1991). PCR products were subjected to SSCP analysis on nondenaturing 8% polyacrylamide gels at 4°C, and the bands were visualized by means of silver staining. SSCP variants were sequenced in both the forward and reverse directions by the Prism® Ready Reaction DyeDeoxy Terminator Sequencing Kit on the ABI 373A DNA sequencer (Applied Biosystems). All mutations were confirmed either by sequencing the products of a second independent PCR reaction or, where appropriate, by detection of mutation-specific RFLP. Putative mutations were confirmed by demonstrating that the change segregated with the FAP phenotype in each family.

Statistical Analysis

Families and individuals for whom no *APC* gene mutation could be identified were excluded from all statistical analyses. Analysis of variance was used to calculate the intrafamily correlation for DPR score and number of DPR lesions (Cochran and Cox 1957). Mixed models were fitted, incorporating a fixed effect for group (identified on the basis of both the location of the mutation within the *APC* gene and the presence or absence of CHRPE) and random effects for family and individuals. F-tests were used to test the hypotheses that the variation between families within groups and differences between groups were zero (Cochran and Cox 1957).

Results

Phenotypic Markers

DPRs were performed on 84 affected individuals from 36 families. At least one individual from each of these

families was examined for the presence of CHRPE. Affected individuals in 17 families expressed CHRPE (had a CHRPE coefficient >3), and in a further 17 families no affected individuals expressed CHRPE. In the remaining two families there was discordance between family members, for the presence of CHRPE. Significant changes on DPR (DPR score >6) were identified in 58 (69%) of the 84 affected individuals examined.

APC Gene Mutation Analysis

Mutations in the *APC* gene were identified in 27 of the 36 families (authors' unpublished data). Two families had splice-site mutations; three had point mutations leading to stop codons; one had a large insertion; and the remainder had a 1–5-bp deletion or insertion mutation resulting in frameshift and a premature downstream stop codon. A further individual had a previously identified de novo deletion of the entire *APC* gene.

Phenotype-Genotype Correlations

Three groups were identified on the basis of both the location of the mutation within the *APC* gene and the presence or absence of CHRPE. The correlation between CHRPE coefficient and the position of the mutation within the *APC* gene is shown in figure 1*A*. Group 1 had early mutations (5′ of exon 9 of *APC*) and no CHRPE. Group 2 had mutations between exon 9 (codon 311) and codon 1444 in exon 15 of the *APC* gene. All affected individuals with mutations in this region had CHRPE. Group 3 had mutations between codon 1444 and codon 1560 of the *APC* gene and had no CHRPE. A fourth group comprised individuals from families where no *APC* gene mutation was identified. One family in group 1 had CHRPE (small pigmented spots only); this family had a mutation in the splice donor consensus sequence of exon 4 of the *APC* gene. The individual with a deletion of the *APC* gene was also included in group 1.

Data on the families in each group are given in table 1. The correlation between the DPR score for each individual and the position of the mutation in the *APC* gene is shown in figure 1*B*. DPRs were performed on 18 affected individuals from 7 families in group 1, 38 affected individuals from 16 families in group 2, and 11 individuals from 4 families in group 3. DPRs were also performed on 17 individuals from nine families where no *APC* gene mutation was identified (group 4), and these data are also shown in table 1. This latter group was excluded from the statistical analysis.

The DPR data for each group are shown in table 2. The intrafamily correlation for DPR score was .70, and that for number of DPR lesions was .54; both correlations are significant ($P < .001$). The variation in DPR scores and number of DPR lesions, after allowance was made for differences between groups, was nonsignificant

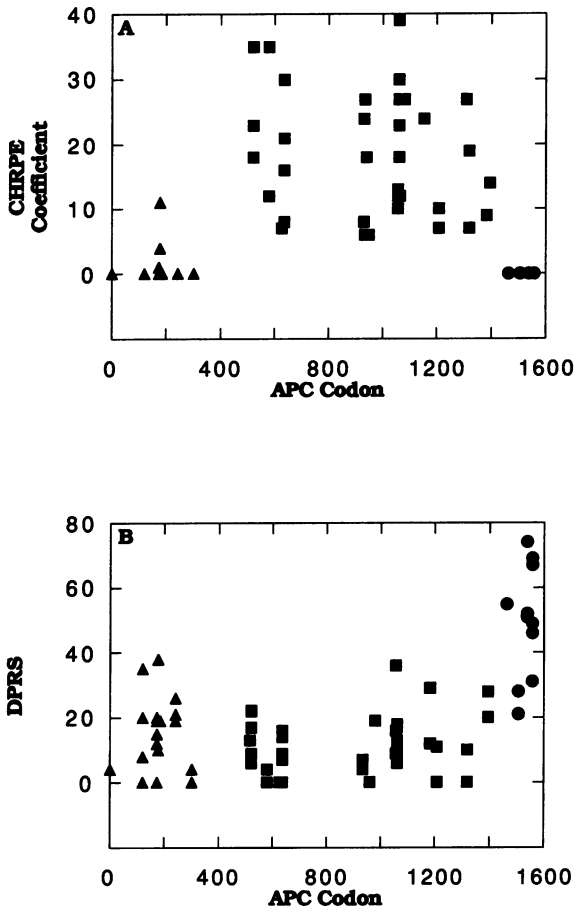

Figure 1 *Graph A,* Correlation of mean CHRPE coefficients for FAP-affected individuals, and position of the mutation within the *APC* gene. *Graph B,* Correlation of DPR scores for each affected individual, with the position of the mutation within the *APC* gene. Group 1 individuals are represented by triangles, group 2 individuals by squares, and group 3 individuals by circles.

($P > .05$). The mean number of DPR lesions was 3.78 for group 1, 3.32 for group 2, and 15.73 for group 3. The mean DPR score was 15.00 for group 1, 10.66 for group 2, and 49.36 for group 3. The difference between groups was significant for both DPR score and number of lesions ($P < .001$). Estimates of the group effects, together with 95% confidence intervals, are shown in table 2. The mean number of DPR abnormalities in the fourth group (where no *APC* gene mutation was identified) was 2.23, and the mean DPR score 8.71.

Other Extraintestinal Features

Analysis of the pedigrees of the 36 families in this cohort revealed a total of 163 dead and living FAP-affected individuals. Of these, 13 (8%) had desmoid tumors and include 1 (2.8%) of 36 individuals from the 7 families in group 1, 2 (2.9%) of 69 individuals from the 16 families in group 2, 8 (38.0%) of 21 individuals

Table 1

Phenotypic Features of FAP in the 36 Families, and Position of *APG* Gene Mutation

Family	N[a]	Exonic Position of *APC* Mutation[b]	CHRPE[c]	DPR Score(s)[d]	Desmoid[e]
Group 1:					
FAPO8	1	Del 5q15-q22	–	4	0
FAPO2	6	3	–	35, 20, 8, 0	0
FAP44	11	4	–	20, 19, 15, 12, 0	0
FAP19	2	Splice 4–5	+	38, 10	0
FAP57	2	5	–	19	0
FAP38	9	6	–	26, 21, 19	0
FAP06	5	8	–	4, 0	1
Group 2:					
FAP37	5	Splice 11–12	+	13	0
FAP04	7	12	+	22, 17, 9, 6	0
FAP14	3	13	+	4, 0	0
FAP36	2	14	+	0	0
FAP30	8	14	+	16, 14, 9, 7, 0, 0	1
FAP20	5	15C	+	7	0
FAP32	2	15D	+	4	1
FAP26	2	15D	+	0	0
FAP59	3	15D	+	18	0
FAP43	3	15E	+	36, 16, 9	0
FAP50	10	15E	+	17, 13, 11, 10. 7, 6	0
FAP45	2	15E	+	18, 6	0
FAP17	2	15F	+	29, 12	0
FAP28	3	15F	+	11, 0	0
FAP33	5	15G	+	10, 0, 0	0
FAP15	7	15G	+	28, 20	0
Group 3:					
FAP39	1	15H	–	55	0
FAP22	5	15I	–	28, 21	5
FAP25	5	15I	–	74, 52, 51	2
FAP21	10	15I	–	69, 67, 49, 46, 31	1
Group 4:					
FAP10	3	...	–	4, 0	0
FAP13	7	...	+/–	19, 16, 8, 7, 0	0
FAP16	5	...	+/–	21, 8	0
FAP23	4	...	–	0	0
FAP34	1	...	–	4	0
FAP35	4	...	–	0	0
FAP55	11	...	–	28, 17, 8	2
FAP58	1	...	–	4	0
FAP62	1	...	–	4	0

[a] Total no. of living and dead affected individuals identified in each kindred.

[b] The position of the mutation is the exon number where the mutation was found. 15C–15I refer to regions of exon 15 of the *APC* gene as described by Groden et al. (1991).

[c] + = All affected individuals examined expressed CHRPE; – = none of the affected individuals examined expressed CHRPE; and +/– = discordance between affected individuals.

[d] Each score represents the DPR score for an FAP-affected individual in each family.

[e] Data are no. of individuals in each family diagnosed with desmoid tumor.

from the 4 families in group 3, and 2 (5.4%) of 37 individuals from the 9 families in the group where no *APC* gene mutation was identified. Although the numbers are small, this result suggests that group 3 also had an excess of desmoid tumors. Many FAP-affected individuals had cutaneous lesions; however, few were removed and examined histologically, and therefore the data are inadequate to allow direct comparisons between each group. All affected individuals in group 3 were, however, noted to have cutaneous lesions.

Four families were identified in group 3. All affected individuals in these families had extensive DPR abnormality (DPR score >20) and had no CHRPE. There was, however, a striking preponderance of other features of

Table 2

FAP-Related Lesions Seen on DPR

Group	No. of Affected Individuals[a]	No. of Families	Mean No. of DPR Abnormalities (95% Confidence Interval)	Mean DPR Score (95% Confidence Interval)
1	18	7	3.78 (1.83–5.73)	15.00 (9.83–20.17)
2	38	16	3.32 (1.98–4.66)	10.66 (7.10–14.22)
3	11	4	15.73 (13.24–18.22)	49.36 (42.75–55.97)

[a] As determined by DPR.

GS. As shown in table 1, all affected individuals in family FAP22 had desmoid disease. This family included an individual who developed a desmoid tumor of his neck at the age of 2 years, which, despite surgery, progressed to an extensive infiltrative tumor of his head and neck, invading the oral cavity and resulting in the individual's death at the age of 10 years. His mother also had a desmoid tumor in the neck region during early childhood, and both she and an affected sister developed extensive multifocal inoperable desmoid disease in the abdomen and abdominal wall. The *APC* gene mutations identified in these four families are as follows: FAP39, 4-bp (AGAG) deletion at codon 1463; FAP22, 4-bp deletion (TCCA) at codon 1505; FAP25, 2-bp deletion (AG) at codon 1538; and FAP21, a 1-bp (T) insertion at codon 1557. Representative DPRs from an affected individual in each of these families are illustrated in figure 2.

Discussion

Extraintestinal manifestations are common in patients with FAP, and ~70% of all affected individuals have significant abnormalities detectable on a dental panoramic radiograph. Evidence is now emerging that specific mutations are associated with certain phenotypic patterns, and our study has suggested that families with the most severe extraintestinal involvement may have mutations in a specific region of the *APC* gene.

Our findings confirm recent observations that CHRPE tend not to occur if the mutation in the *APC* gene is located 5′ of exon 9. One family in this group did express CHRPE; the *APC* gene mutation found in this family was in the splice donor consensus sequence and therefore has a less predictable effect on the resultant protein product than does a truncating mutation. In addition to this, CHRPE are absent in families with mutations downstream of codon 1444, and CHRPE therefore appear to be restricted to families with mutations between exon 9 and codon 1444, in exon 15. Four families with significantly more severe dento-osseous changes on DPR had mutations restricted to a small region of the most 3′ exon of the *APC* gene, between codons 1444 and 1560.

Osteomas, epidermoid cysts, and desmoid tumors occurred in many of our families with FAP and appeared to occur regardless of the location of the mutation within the *APC* gene. However, the features were relatively mild or isolated to a limited number of individuals in the family. Individuals in the four families described with severe dento-osseous changes on DPR appeared to have more extensive features of GS with more osteomas, more epidermoid cysts, and more extensive and often multifocal desmoid disease (especially families FAP22 and FAP25). A recent report has also suggested that desmoid tumors may be more common in families with mutations in this region (Caspari et al. 1995). Other studies have shown no significant association between the presence of extraintestinal manifestations and specific *APC* mutations (Gurbuz et al. 1994), and one study has suggested that desmoid tumors are more common in families with a 5-bp deletion at codon 1309 (Nugent et al. 1994). It is not clear, however, whether many families with mutations between codons 1444 and 1560 of the *APC* gene were identified in these other reports.

The identification of mutations in three-quarters of the families included in this study compares favorably with the findings of other groups. The spectrum of *APC* gene mutations is similar to that reported elsewhere (Nagase and Nakamura 1993); however, fewer point mutations were identified. This may be a property of our cohort of patients, or it may be due to the method of screening. The sensitivity of SSCP declines with increasing size of PCR fragment (Sheffield et al. 1993), and it is possible that some of the families where no *APC* gene mutation was found may have mutations within the coding regions screened. Further work, using the in vitro–synthesized protein assay (Powell et al. 1993), is being undertaken on these families, to further exclude this possibility. Alternatively, the other families may have mutations either 3′ of the region screened or elsewhere in the *APC* gene that are not identified by the SSCP analysis. No families in this group had consistently severe DPR changes or GS, and therefore it is likely that these phenotypic features are confined to families with germ-line mutations in the small region of the *APC* gene described.

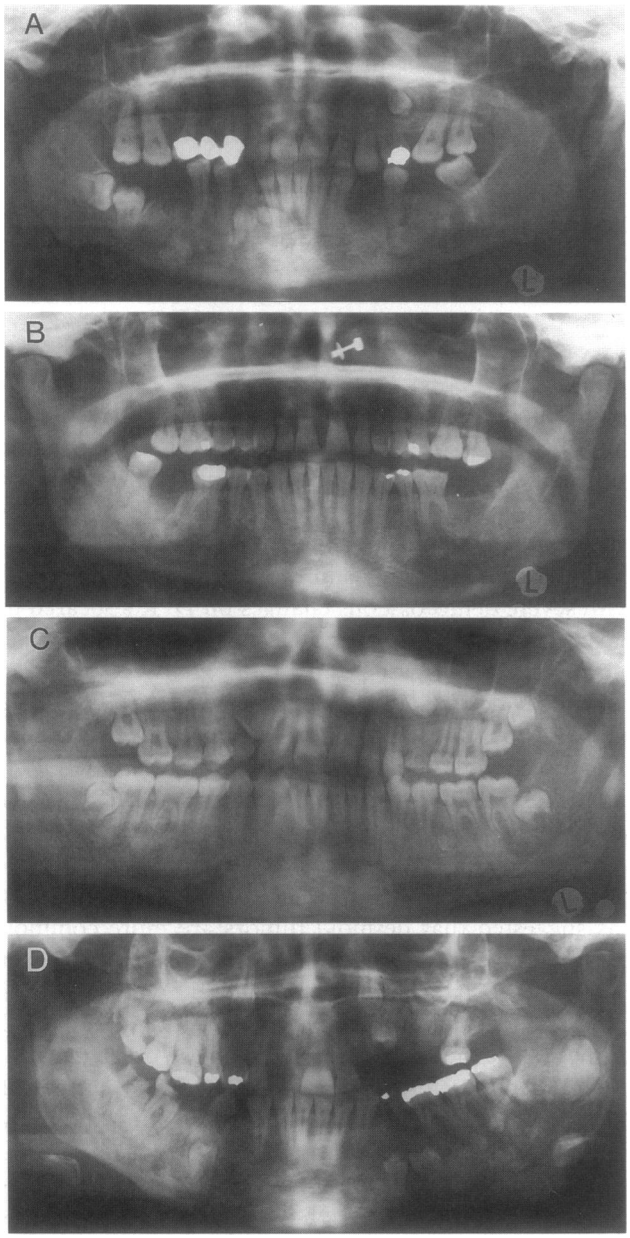

Figure 2 Representative DPRs from each family in group 3. Panel *A* shows numerous osteomas and areas of mandibular sclerosis, and dental abnormalities are also present (DPR score under our criteria was 55); panel *B* shows several mandibular dense bone islands and osteomas (DPR score 28); panel *C* shows extensive maxillary and mandibular sclerosis and supernumerary teeth (DPR score 52); and panel *D* shows patchy sclerosis throughout the maxilla and mandible, with several discrete osteomas (DPR score 69).

A possible hypothesis to explain the variation in the desmoid disease in FAP is that it may arise as a result of a two-hit mechanism at the APC locus. Mutations between codons 1444 and 1560 could have a greater tendency to cause a GS-related lesion than do other inactivating *APC* gene mutations. Individuals who inherit a germ-line mutation between codons 1444 and 1560 of the *APC* gene may develop an extraintestinal lesion if they develop a second somatic inactivating *APC* mutation. If, however, the germ-line mutation causing FAP is not in this region but is elsewhere in the *APC* gene, then the somatic event constituting the second hit may be between codons 1444 and 1560. Since this represents ~4% of the entire *APC* coding region, this is a much less likely event; and therefore individuals with other inactivating germ-line mutations have many fewer GS manifestations. Some credence to this is given by two reports of somatic mutations in desmoid tumors. One study identified seven somatic mutations and one somatic allele loss in eight desmoid tumors from seven patients with FAP. All seven somatic mutations were found between codons 1399 and 1581 of the *APC* gene; the desmoid tumor displaying loss of the normal allele occurred in an individual with a germ-line mutation at codon 1462 (Miyaki et al. 1993). A further report describes a somatic *APC* gene mutation in a desmoid tumor from a patient who had a constitutional deletion of the entire *APC* gene. This was a 107-bp deletion between codons 1438 and 1473 (Sen-Gupta et al. 1993). The location of these somatic mutations in desmoid tumors corresponds to the region of the *APC* gene involved in our four families with severe GS. Proof of such a "site-specific" second-hit mutation hypothesis would depend on the finding of such mutations at the somatic level in other tumors associated with GS.

A possible corollary to this is the mechanism of adenoma formation in FAP. Adenomas occur largely independently of the *APC* mutation; however, they are more profuse when associated with a germ-line mutation in a specific region of the *APC* gene (Caspari et al. 1994). Two-thirds of somatic *APC* mutations observed in colorectal tumors occur near this region (the mutation-cluster region), which represents ~8% of the *APC* coding sequence (Nagase and Nakamura 1993). Mutations in specific regions of the *APC* gene therefore may promote either adenoma formation or GS-related tumors. Individuals with germ-line mutations in these regions may be more likely to develop profuse polyposis or severe GS, respectively.

Recent reports have stressed that the phenotypic features of FAP are variable among individuals with identical *APC* mutations (Paul et al. 1993), and work done on the Min (multiple intestinal neoplasia) mouse has demonstrated the importance of the effect of a modifying locus (Dietrich et al. 1993). We cannot exclude the possibility that the severe GS phenotype seen in these families is due to the effect of modifying genes or even environmental factors. The consistency of severe changes among the individuals in these families, however, argues in favor of an intrinsic property of specific mutations.

The APC gene product is a 2,843-amino-acid poly-

peptide of unknown function. A feature of the second 1,000 residues of the APC protein is a 20-amino-acid imperfect repeat sequence (Groden et al. 1991). This sequence is iterated seven times, and all four mutations seen in our families with severe GS occur between the second and fourth of these repeats. To date, no function has been ascribed to these repeats.

Specific mutations within genes have recently been shown to be associated with variable phenotypes, notably the *RET* proto-oncogene (van Heyningen 1994). Similarly, the spectrum of clinical presentation in FAP is reflected by the location of the mutation within the *APC* gene. Our study has demonstrated both a further possible phenotype-genotype correlation and that a specific subgroup of mutations may give rise to a distinctive phenotype with extensive osseous and dental abnormalities on DPR, lack of CHRPE, and a preponderance of other features of GS.

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