LETTER TO JMG

Large submicroscopic genomic *APC* deletions are a common cause of typical familial adenomatous polyposis

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Familial adenomatous polyposis (FAP, OMIM #175100) is an autosomal dominant precancerous condition characterised by the appearance of hundreds to thousands of colorectal adenomas that, if not detected early and removed, invariably result in colorectal cancer (CRC). In the typical phenotype, adenomas usually occur within the second decade and become symptomatic during the third decade of life.[1] In the attenuated FAP (AFAP) phenotype, the number of colorectal adenomas is <100, with a later age at diagnosis of both polyposis and CRC than in typical FAP.[2–4]

FAP is caused by germline mutations in the tumour suppressor gene *APC*,[5 6] which encodes a protein composed of 2843 amino acids and is formed by 14 small exons, and a large exon 15 that extends over three quarters of the coding sequence. To date, more than 500 different *APC* germline mutations have been reported in FAP patients (see Human Gene Mutation Database and references therein). Most of the germline mutations reported so far are localised in the 5′ half of the gene and lead to premature truncation due to single base substitutions or small insertions/deletions, resulting in nonsense or frameshift mutations and rarely in splice site mutations. In a small number of cases, single base substitutions within exonic sequences predicted to result in missense or silent variants lead to aberrant splicing.[7 8] Most *APC* mutations are identified with conventional mutation screening methods such as heteroduplex analysis, denaturing gradient gel electrophoresis, single strand conformational analysis (SSCP), denaturing high performance liquid chromatography (DHPLC), protein truncation test (PTT), and direct DNA sequencing. Large genomic deletions cannot be detected by these methods. The mutation detection rate in FAP families ranges from 20% to 85%, depending on the patients examined and the methods used.[9–11] Large genomic deletions have been reported to account for about 2% of germline *APC* mutations,[12] but their quantitative impact remains unknown due to lack of easy screening techniques.

Deletions encompassing *APC* may occur as large, cytogenetically visible changes, or as submicroscopic rearrangements. Cytogenetically detectable interstitial deletions at the chromosomal region 5q22 have been reported in several patients exhibiting FAP and some degree of mental retardation and dysmorphism,[13–18] and were the key observation for mapping and cloning of *APC*.[19–23] Since then, a few submicroscopic deletions have been detected in FAP patients of normal intelligence and without dysmorphic features by different methods, including apparent non-parental segregation of intragenic or flanking polymorphic marker alleles, fluorescent in situ hybridisation (FISH) analysis with clones from chromosomal region 5q22, quantitative PCR, comparative genomic hybridisation, or RNA analysis.[14 24–28] All of these methods examine part of the coding sequence or flanking regions.

Multiplex ligation dependent probe amplification (MLPA)[29] is a new semiquantitative multiplex PCR approach to determine the relative copy number of DNA sequences by amplification of designed MLPA probes (for review see Sellner and Taylor[30]). These probes are specific for the desired target sequences at one end and consist of an identical sequence at the other end, permitting simultaneous PCR amplification of several probes using only one primer pair. With this technique, changes in the copy number of single exons up to complete chromosomes are detectable.

Here we present results of a systematic deletion screening with MLPA in 174 apparently unrelated FAP patients in whom no point mutation had been identified in *APC* by conventional mutation screening methods.

Key points

- We screened 174 unrelated familial adenomatous polyposis (FAP) patients in whom no point mutation in the *APC* gene was uncovered, using denaturing high performance liquid chromatography and protein truncation test for large submicroscopic genomic deletions, with amplification by the multiplex ligation dependent probe amplification (MLPA) method.
- We identified 14 different deletions in 26 patients, ranging from single exons to the whole gene including the promoter region.
- A genotype–phenotype correlation was observed; almost all deletions (22/26) were detected in the 46 patients with predominant typical FAP, whereas no deletion was found in 93 patients with attenuated FAP.
- Thus, a large deletion occurred in about half of our apparently mutation negative families with typical FAP, pointing to an overall frequency in this group of around 12%.
- The phenotype found in large deletions has implications for pathogenic hypotheses, which are discussed.

MATERIALS AND METHODS

Patients

There were 1040 apparently unrelated patients with the clinical diagnosis of FAP or suspected FAP referred for *APC* mutation analysis. A pathogenic point mutation (single base substitution, small deletion/insertion) had been detected in 522 patients (50.2% overall, 74.4% in typical FAP, 16.6% in AFAP, 31.5% in index cases with unknown phenotype) by SSCP and heteroduplex analysis or by PTT of exon 15 and DHPLC analysis of exons 1–15B. Large cytogenetically detectable de novo deletions encompassing the *APC* region were detected in three patients.[31] There were 174 unrelated patients, in whom no point mutation was uncovered by PTT and DHPLC, selected for deletion screening with the MLPA method. This group included all the remaining patients with known or suspected typical FAP and a substantial number of

cases with attenuated and unknown phenotype. In four of these families (numbers 3, 40, 340, and 367), a submicroscopic deletion had been previously identified using intragenic and flanking polymorphic markers.

Phenotype classification

Clinical information on the polyposis in the patients and their families was obtained during genetic counselling sessions, and from a questionnaire, telephone interviews, and medical records. Discrimination between typical and attenuated FAP was based on the number of colorectal adenomas, age at diagnosis of FAP, and occurrence of CRC. The FAP phenotype was classified as typical when the patient presented with >100 colorectal adenomas before 35 years of age, or in the case of unavailable or unclear colonoscopic data, when clinical symptoms occurred before 35 years of age, or when CRC was diagnosed before 40 years of age and included cases with severe course. The diagnostic criterion for the attenuated phenotype (AFAP) was the occurrence of a smaller number of adenomas (10–100) at >25 years of age. An attenuated course was also assumed when >100 adenomas were diagnosed for the first time at >40 years of age. When the polyp number was unknown, AFAP was considered when first symptoms or diagnosis of CRC occurred at >45 years of age. Assumed FAP patients are first degree relatives of proven FAP patients with verified CRC, but with no confirmation of the underlying adenomatosis.

Screening for large genomic deletions by MLPA

A search for large deletions was performed using MLPA (multiplex ligation dependent probe amplification). The MLPA test kit (SALSA P043 APC exon deletion test kit; MRC Holland) contains 20 paired probes from the *APC* region to examine three fragments of the promoter region, exons 1–14, and three fragments of exon 15 (15-1, 15-2, 15-3), in addition to 11 control probes from other chromosomal regions. Deletion screening was performed according to the manufacturer's protocol.

Briefly, 100 ng genomic DNA in 5 µl TE buffer was heat denaturated and incubated with the probe set for 16 h at 60°C. Next, hybridised products were ligated, amplified by PCR and separated on an ABI 3100 capillary sequencer. DNA samples from healthy individuals and from patients with known large *APC* deletions were used as controls.

Data collection and export used Genescan and Genotyper software. Evaluation of electropherograms was performed by visual examination of peak heights of the *APC* fragments in relation to the adjacent control fragments and by calculation of dosage quotients as follows: the ratio of the peak area of each *APC* fragment and each of the two adjacent internal control fragments was calculated and normalised by the median value of the respective ratios obtained for all samples in the same run. Expected values for deletions are around 0.5. All identified deletions and all uncertain results have been confirmed in a second independent reaction. Where possible, the segregation of the deletions with the disease in the families was examined (table 1).

RESULTS

Frequency of large deletions in the *APC* gene

Using MLPA, we examined 174 unrelated FAP patients (46 with typical FAP, 93 with AFAP, 35 with unknown phenotype) in whom no point mutation had been identified. The submicroscopic deletions in the four patients identified earlier by haplotype analysis were confirmed by MLPA: a deletion of all *APC* fragments was found in the index patient of family 3, in whom a large submicroscopic alteration had been proven by FISH analysis with cosmid clones from the *APC* region.[24] A complete deletion of the gene was also found in patient 40, while partial deletions encompassing the 3' part of the gene only were observed in patient 340 (exons 8–15) and 367 (exon 14–15), respectively (table 1).

MLPA analysis revealed exonic deletions in 22 of the remaining 170 patients (fig 1). Thus in total, a deletion could be detected in 26/174 unrelated FAP patients (15%) (table 1, fig 2). In six of these 26 cases (23%), no family history of polyposis or CRC was reported, suggesting a de novo mutation. Fourteen rearrangements covered the whole *APC* gene, twelve of which included the promoter region. The other twelve different partial deletions were observed only once; five ranged from exon 8, 9, 11, 14, and the middle of exon 15, respectively, to the 3' end of the coding region, while four included exon 1, or the first 5, 7, or 10 exons of the 5' end together with the promoter region (fig 2).

Three deletions encompass only one or two exons, respectively. To exclude a variation within the hybridisation sequence in patient 380, in whom only exon 14 proved to be deleted, this exon was sequenced in both directions, and only the normal sequence was found. Long range PCR confirmed the deletion (fig 3). Exon 14 was deleted in another two patients of the family, but not in an at risk person from this family, who had not inherited the risk haplotype (data not shown). Similarly, the deletion of exons 11 and 12 in patient 113 was confirmed by long range PCR (data not shown) and by segregation with the disease in three affected individuals.

All 26 reported deletions were consistently reproduced in at least two independent MLPA reactions, and were evident by both visual examination and calculation of peak areas. In some experiments, single MLPA probes (mainly for fragment 2 of the promoter region or for exon 12 of the *APC* gene) showed non-reproducible results; the reason for this is not clear.

Clinical phenotypes in patients with large genomic deletions

The detailed clinical phenotypes of the 26 families with large submicroscopic deletions are presented in table 1. A typical FAP was present in 19 index patients. In another three index patients, a typical course was assumed on the basis of the available data and family history, although medical information was not complete. In four index patients with clinical diagnosis of FAP, the colorectal phenotype could not be assessed. Thus, a submicroscopic deletion was identified in 22/46 included index patients with a certain or an assumed typical manifestation (48%), whereas no deletion was found in the group of 93 unrelated patients with an attenuated phenotype. As AFAP is a poorly defined phenotype and presumably of heterogeneous origin, this group might encompass some cases with uncertain diagnosis. The predominant colorectal phenotype in the relatives of the index patients with submicroscopic deletions was consistent with a typical FAP, although in some affected families, relatives were diagnosed at an advanced age. A marked phenotypic variability was observed in family 3 (age at diagnosis because of symptoms 34–59 years).[24] Extracolonic manifestations (desmoids, epidermoid cysts) were reported in 3/13 families (27%) for whom sufficient clinical information was available. Patient 630, harbouring a cytogenetically visible deletion, presented with a typical colorectal manifestation and desmoids.

DISCUSSION

Deletions of individual genes or certain exons represent a frequent cause of genetic diseases and have also been reported for several hereditary tumour syndromes including breast cancer and hereditary nonpolyposis colorectal cancer (HNPCC).[32–35] To this end, a variety of different assays for the quantification of genomic sequences such as comparative

Table 1 Extent of the identified 29 large deletions (26 submicroscopic, 3 cytogenic) and the corresponding clinical phenotypes in the FAP index patients (shown in **bold**) and their relatives

FAP no.	Deleted exons	Deleted promoter	Relationship	Colorectal phenotype	Mode of diagnosis¶	Age at diagnosis (years)	Colorectal polyps	Colorectal polyp distribution	Colectomy (age in years)	CRC (age in years)
3‡ §	1–15-3	X	Index	Typical	By chance	34	Profuse	Mainly distal	34	34
			Brother	(Typical)	Symptoms	36	Multiple	Mainly distal	36	36
			Brother	(Attenuated)	Call up	43	Some hundred	Whole colon	Yes	No
			Nephew	Typical	Call up	16	20	Throughout the colon		
			Mother	Attenuated	Symptoms	59				59
15	1–5	X	Index	Typical	Symptoms	31	Multiple	Mainly distal	31	36
			Father	Attenuated	Symptoms	64	Numerous	Whole colon, not specified	No	No
			Daughter	Typical	Call up	11	>100	Mainly distal	No	No
			Cousin	Typical	Symptoms	33				Yes
40‡	1–15-3	X	Index	(Typical)		28	Multiple	Whole colon, not specified	28	38
113	11–12		Index	Typical	Symptoms	17	Multiple	Less in rectosigmoid	20	No
340‡	8–15-3		Index	Typical	Symptoms	29	>100	Whole colon, not specified	29	
			Mother	(Typical)	Symptoms	41				42
			Son	Typical		19	Profuse	Mainly distal		
367‡	14–15-3		Index	Typical		15			16	
			Mother	Typical	Symptoms	28	Multiple		29	
			Uncle	Typical	Symptoms	31				31
380	14		Index	Typical		26			26	
			Brother	(Typical)	Symptoms	40	Profuse			
			Brother	Typical		30			30	
			Brother	(Typical)		24			24	
			Cousin	(Attenuated)	Call up	33	50–100		35	No
630†	1–15-3	X	Index	Typical	Symptoms	18	>100	Mainly distal	18	No
657	14–15-2		Index	Typical	Symptoms	28	>100	Mainly distal	No	No
663	1	X	Index	Typical	Symptoms	25	Several		25	No
			Mother	(Typical)		25			26	No
			Grandfather	(Typical)		<35				<35
671†	1–15-3	X	Index	–						
695	1–15-3	X	Index	Typical		19	Numerous	Whole colon, not specified	36	36
			Mother	(Typical)	Symptoms	35				36
750	1–15-3	X	Index	Typical	By chance	32	>100	Whole colon, not specified	32	No
			Mother	(Typical)	Symptoms	44	Not known	Not known	44	44
766	15-2–15-3		Index		Symptoms	40	Numerous		41	No
			Brother	(Typical)		33			33	
			Son	(Typical)		26			26	
801	1–15-3		Index	Typical	Symptoms	31	Multiple	Whole colon, not specified	31	31
			Father	(Typical)	Symptoms	44	Multiple	Whole colon, not specified	44	44
838	1–15-3	X	Index		Symptoms	43	Numerous	Whole colon, not specified		No
870	1–15-3	X	Index	(Typical)					28	No
903	9–15-3		Index	Typical	Symptoms	29	>100	Whole colon, not specified	29	29
943	11–15-3		Index	Typical	Symptoms	25	Profuse			
			Mother	(Typical)	Symptoms					40
			Grandmother	(Typical)	Symptoms					38
949	1–15-3	X	Index		Symptoms	42	Multiple		43	42
974†	1–15-3	X	Index	–						
1067	1–15-3	X	Index	Typical	Symptoms	24				
			Mother	(Typical)						42
1084	1–15-3	X	Index	Typical	Symptoms	29	1500	Whole colon, not specified	29	
1096	1–15-3	X	Index	Typical	Call up	14	Multiple			
			Mother	(Typical)	Symptoms	29				29
			Grandfather	(Typical)	Symptoms					39
1098	1–7	X	Index	Typical	Symptoms	27	>100	Mainly distal		
			Father	(Typical)	Call up	30	Multiple		31	49
1121	1–15-3		Index		Symptoms	46	Multiple	Mainly distal		
1132	1–10	X	Index	Typical	Symptoms	26			26	
			Daughter	Typical	Call up	16	Few			
			Mother	(Typical)						41
1164	1–15-3	X	Index	(Typical)	Symptoms	24	>30	Mainly distal		No
1203	1–15-3	X	Index	Typical	Symptoms	<33	Numerous		<33	

Words in brackets indicate cases with uncertain phenotype. †Three patients with cytogenetically detectable deletions; patient 630 published in Raedle *et al*; patients 671 and 974 were too young to have developed polyposis; ‡four patients with large submicroscopic deletions previously detected by polymorphic markers; §patient published in Mandl *et al*. ¶Symptoms: patients diagnosed because of bowel symptoms of FAP; call ups: FAP patients diagnosed in an asymptomatic state by prophylactic endoscopic bowel screening (family screening of subjects at risk).

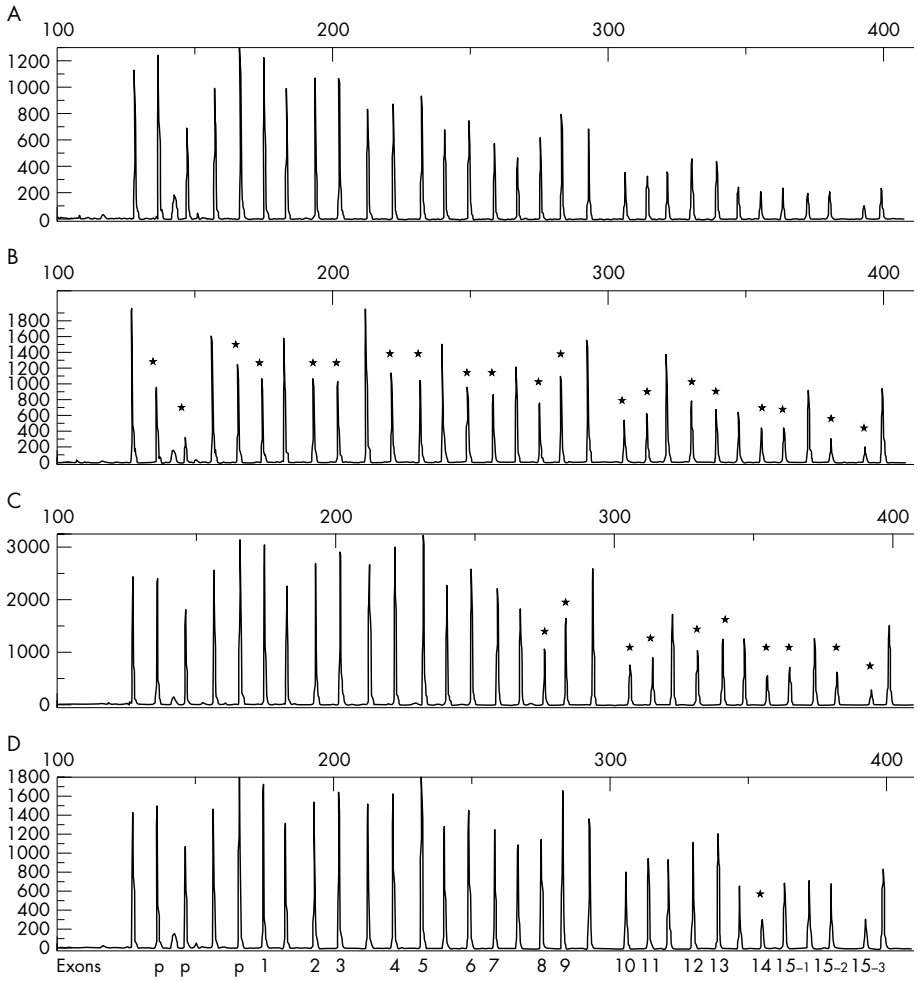

Figure 1 Electropherograms of MLPA products showing: (A) a normal control; (B) a deletion of the whole *APC* gene (patient 1084); (C) a deletion of exons 8–15 (patient 340), and (D) a deletion of exon 14 (patient 380). *Deleted exons. P, promoter region.

genomic hybridisation, FISH, Southern blotting, or quantitative PCR protocols were applied. However, most of these methods are either unable to detect changes of single exons, are time consuming, are not applicable as a multiplex approach, or require large amounts of DNA. Moreover, analysis at the cDNA level is not sufficient for identification of deletions when the altered allele is not expressed or the mutant mRNA is unstable.

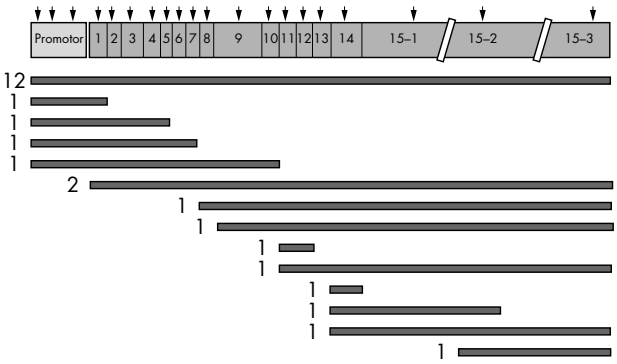

Figure 2 Distribution of *APC* deletions identified by MLPA in 26 FAP patients. The arrows represent the site of probe hybridisation in the different exons. The numbers at the left side of the bars indicate how often the deletion was identified in our sample.

With the MLPA assay, semiquantitative PCR techniques have been improved and are available for routine diagnosis of large deletions, including HNPCC[36 37] and FAP. The detection rate of point mutations in FAP is up to 85% in patients with a typical phenotype and 20–30% in attenuated cases.[9–11] The proportion of large genomic rearrangements has not been systematically investigated to date. Using MLPA, we examined 174 apparently unrelated FAP patients of normal intelligence and without known dysmorphic features, in whom no point mutation had been identified by routine mutation screening methods. In 26 families, we identified 14 different large submicroscopic genomic deletions. The size of deletions ranges from single exons to the whole gene, including the promoter region.

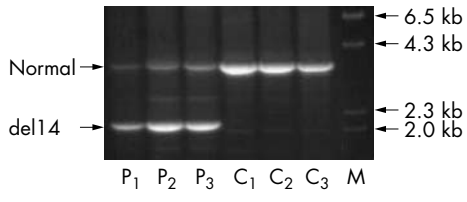

Figure 3 Agarose gel showing PCR products obtained with primers localised in *APC* intron 13 (forward) and exon 15A (reverse). In three controls (C_1–C_3) only the normal fragment of 3952 bp is present, whereas in the three patients of family 380 (P_1–P_3), an additional fragment of about 2000 bp is visible. M is the size marker.

Table 2 Reported large submicroscopic deletions of the *APC* gene and the corresponding phenotypes

Author	Year	Method	No. of FAP families	Mutation negative	No. of deletions (% of unselected FAP families)*	No. of deletions (% of mutation negative FAP families)*	No. of deletions (% of mutation negative typical FAP families)*	Size of deletions in the *APC* gene	Age at diagnosis (years)	Colorectal polyps (CRC, age at diagnosis)	Extraintestinal manifestation (number of affected families)
Gismondi	1998	PCR, sequencing			1			310 bp, stop codon 1575	26		Desmoid, osteomas, no CHRPE
deRosa	1999	Quantitative PCR, polymorphic markers	18	9	3 (17%)	33%		Whole gene		>100 (CRC 36–42)	Desmoids, osteomas, CHRPE
								Whole gene	28–35	Hundreds (CRC 30–42)	CHRPE
								Whole gene	29	Profuse	Osteomas, CHRPE
Cao	2000	cDNA analysis (PTT)	28	8	2* (7%)	25%		Exon 11	15–47	Multiple->1000	CHRPE
								Exon 14	43–50		
Su	2000	cDNA analysis	49	22	3* (6%)	14%		Exon 11–12, exon 14			
Flintoff	2001	Quantitative PCR	68	22	4 (6%)	18%		At least 8-3', at least 8–15F, 15A–F	62 (one patient)		
Su	2002	cDNA analysis			1			Exon 15	21	Typical (innumerable polyps)	Epidermoid cysts
					1			Exon 15	42	Attenuated (70–90)	
Sieber	2002	Real-time PCR, polymorphic markers		203	7	3.5%	12%	6 whole gene	26.5 (median)	>100 colorectal polyps	29%
This study	2004	MLPA		174	26	15%	48%	14 whole gene, 12 partial deletions	16–64	Predominant typical	23% (desmoids, epidermoid cysts)

*Without proven splice site mutations.

The deletions were found almost exclusively in the group of patients with typical polyposis. No deletion could be detected in 93 unrelated individuals with attenuated FAP. Thus, patients with submicroscopic exon spanning deletions presented predominantly with a typical colorectal polyposis (numerous or >100 colorectal adenomas, symptoms and CRC at an early age) regardless of whether the deletion spanned the whole gene or just a few exons (table 1). The proportion of index patients without a family history of polyposis or CRC, suggesting a de novo mutation, was similar to that for point mutations. The predominant colorectal phenotype in the relatives of the index patients was consistent with a typical FAP, although in some families, affected members were diagnosed at an advanced age (40–64 years), pointing to a somewhat milder manifestation.[24] One reason for an apparent intrafamilial variability of disease manifestation could be delayed diagnosis, especially in those cases where FAP was diagnosed for the first time in the index patient and afterwards in siblings and parents.

Submicroscopic large deletions were found in 48% (22/46) of our mutation negative FAP families with predominant typical FAP. When considering a detection rate for point mutations of about 75% in patients with typical manifestation (own data), the overall frequency of large deletions would be around 12% in typical FAP.

There are only few reports of submicroscopic exon spanning deletions in the *APC* gene. Phenotypes were reported in 19/22 unrelated families published so far (table 2). Taken together, whole gene alterations tend to present as typical FAP, whereas partial deletions seem to show more intrafamilial and interfamilial heterogeneity, ranging from typical to attenuated phenotypes.[12 24 26 27 38] Consistent with our results, Sieber *et al* identified six whole gene spanning deletions exclusively in 60 unrelated mutation negative patients with classical FAP, but none in 143 patients with AAPC.[28] Su *et al* observed a different phenotype in two unrelated patients harbouring the same deletion of the entire exon 15.[27] Similar to our observations, extracolonic manifestations (desmoids, osteomas) were reported in 5/19 families (26%).[25 28] The methods used for systematic screening procedures comprise cDNA analysis, polymorphic markers, and quantitative PCR. Mutation detection rates range from 6% to 17% (10–33% in the mutation negative families)[25 26 28 38 39] (table 2). Assuming a high frequency of large deletions in typical cases, this variation might be explained by an ascertainment bias due to the definition and distribution of the phenotypes in the different patient samples. The methods used for deletion analysis and the *APC* region examined might also contribute to the differences[28] (table 2).

Until now, phenotype descriptions of 16 adult FAP patients (13 unrelated families) harbouring large cytogenetically visible chromosomal deletions around 5q21/5q22 have been reported (table 3). The clinical spectrum ranges from unambiguously typical cases to patients who tend to have a later onset of the disease. A clear attenuated course was reported by Pilarski *et al*.[13] In general, the polyps were concentrated mainly in the proximal parts of the colon in most of the patients and there was a high frequency of extraintestinal FAP related manifestations (62%) and congenital hypertrophy of the retinal pigment epithelium (CHRPE).

Table 3 Reported cytogenetically detected deletions of the *APC* gene and the corresponding phenotypes

No. of families	Author	Year	Deletion chromosome 5	Mode of diagnosis	Age at diagnosis	Colorectal polyps	Colorectal polyp distribution	Colectomy (age in years)	CRC (age in years)	Extraintestinal
1	Herrera	1986	q15–q22	Symptoms	42	>100			42	Desmoid tumour
1	Hockey	1989	q15–q22		27			27		Dental anomalies, epidermoid cysts
			q15–q22		31			31		Dental anomalies, epidermoid cysts
			q15–q22			Extensive			42	
1	Kobayashi	1991	q22.1–q31.1	Symptoms	15	>500	Throughout			Osteomas, dental anomalies, epidermoid cysts, CHRPE
2	Lindgren	1992	q15–q23.2	Symptoms	20	300 (30y)		30		
			q15q22	Call up	13	1				Epidermoid cysts
1	Cross	1992	q22–q23.2	Symptoms	25	>100	Mainly proximal			Epidermoid cysts, ostemas, CHRPE
			q22–q23.2	Symptoms	54	Multiple				CHRPE
2	Hodgson	1993	q21.3–q23.1	Symptoms	38	>100	Proximal	38	No	Epidermoid cysts, desmoid tumour, duodenal polyps
			q21.3–q23.1	Symptoms	20	>100	Mainly proximal			CHRPE, dental anomalies
2	Olschwang	1993	*APC* region							CHRPE
1	Barber	1994	q15–q22.3	Symptoms	43	Hundreds			43	
1	Pilarski	1999	q22–q23.2	No	39	50–60	Mainly proximal+distal			No CHRPE
1	Raedle *	2001	q21–q22	Symptoms	18	>100	Mainly distal	18	no	Desmoid tumour, no osteomas, no CHRPE

*Patient 630, already mentioned in table 1.

A genotype–phenotype correlation is well established in FAP patients.[1 3 9 10 40–43] The predominant typical phenotype, observed in our patients with large submicroscopic deletions and in most other studies, can be expected when the predicted truncation of the protein is compared with those resulting from point mutations. The predicted site of truncation resulting from partial deletions starting from exon 7 up to the middle of exon 15, and then continuing until the 3′ end of the coding region corresponds to truncating nonsense or frameshift mutations within these exons, which are known to most often cause typical FAP.[9 44 45] The same applies to the two intragenic deletions spanning exon 14 and exon 11–12, respectively, which lead to out of frame mutations and subsequent truncated proteins. Exon 14 skipping usually leads to a typical phenotype, as we demonstrated recently.[7] The deletion of exon 14 to the middle of exon 15 deletes the β-catenin binding domain and is therefore predicted to cause typical FAP. The four deletions at the 5′ end of the gene affecting exon 1, or the first 5, 7, or 10 exons, should be functionally equivalent to whole gene deletions, as the start codon and the promoter region are affected.

The intrafamilial variation of the predominant typical phenotype in large submicroscopic deletions is comparable to that observed in families with point mutations of the *APC* gene, where different expression of the disease among patients carrying the same mutation, even within the same family, is well known, pointing to the possible influence of modifying genes, and stochastic and environmental factors.[46 47]

The typical presentation observed in most of the submicroscopic whole gene deletions has implications for pathogenic hypotheses. Various molecular mechanisms underlying the phenotypic effect of germline mutations in the *APC* gene have been proposed. A common hypothesis suggests a recessive tumour suppressor model, in which the inactivation of both alleles is necessary for tumour development. Others have hypothesised that the truncated APC protein could possibly interfere with the normal protein in a dominant negative fashion,[2 16 25 48 49] or have speculated FAP to be caused by haploinsufficiency.[19 25]

With respect to the phenotypic consequences, whole gene deletions seem to be equivalent to point mutations between codon 168 and 1250 or 1450 and 1600. This is supported by studies in mice and FAP patients showing that the truncated APC protein is unstable and therefore acts like a null allele.[50–52] Moreover, the presence of CHRPE, which has been described in most families with large deletions, is not apparent in AFAP.[53 54] Hence, the typical phenotype observed in patients bearing truncating point mutations cannot be explained by a dominant negative mechanism.[54]

It has been suggested that mutations at the 5′ and 3′ end of the *APC* gene, causing AFAP, result in null alleles due to instability of the truncated protein or mRNA, respectively.[3 4] However, the observed phenotypes in whole gene deletions argue strongly against this explanation. In a recent work, Heppner Goss *et al* demonstrated that AFAP mutations at the 5′ end of the gene facilitate translation initiation at the internal start codon 184, producing a putative functional protein that may explain the mild phenotype.[55] Chain terminating mutations at the 3′ end of the gene are predicted to result in relatively large truncated proteins with enough residual activity to lead to an attenuated manifestation.[2] The same mechanism would be expected in submicroscopic deletions exclusively spanning this region; however, these have not been detected to date. Our results provide an additional clue to the assumption that the underlying genetic basis of AFAP and multiple colorectal adenomas is more heterogeneous than previously suspected.

We present the first systematic evaluation of large submicroscopic genomic deletions in the *APC* gene using the MLPA technique. We identified deletions in approximately half of the mutation negative families with a predominant typical FAP phenotype, suggesting an overall frequency of around 12% in unselected FAP patients with typical manifestation of the disease. A genotype–phenotype

correlation within this group could not be observed. Thus, screening for large submicroscopic genomic deletions should substantially increase the mutation detection rate in FAP and should be implemented in routine mutation detection protocols, possibly restricted to patients with a typical course.

MLPA has proven to be an easy, rapid, and reliable screening method for the identification of large exon spanning deletions in the *APC* gene, and was superior to other techniques. However, in some cases, a non-reproducible variation of individual peak areas was observed, and a possible inter-assay variability has to be considered. Deletions of single exons should be confirmed by long range PCR because false positive results due to variants in the target sequence of the probes are possible.

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ELECTRONIC-DATABASE INFORMATION

OMIM 175100 (FAP); NT_034772 (genomic), NM_000038.2 (mRNA)
Human Gene Mutation Database (HGMD): http://uwcmml1s.uwcm.ac.uk/uwcm/mg/search/119682.html

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