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ARTICLE

Exclusion of an extracolonic disease modifier locus on chromosome 1p33–36 in a large Swiss familial adenomatous polyposis kindred

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Familial adenomatous polyposis (FAP), an autosomal dominantly inherited colorectal cancer predisposition syndrome, displays considerable inter- and intrafamilial phenotypic heterogeneity, which represents a major problem in genetic counselling of *APC* mutation carriers. The *Min* mouse model indicated a putative disease modifier locus on chromosome 4, which is syntenic to human chromosome 1p35–36. This finding was subsequently supported by parametric and nonparametric linkage analyses in FAP families, however, without identifying functional variants in candidate genes. Recently, germline mutations in the base-excision repair gene *MYH* (1p33–34) have been described in patients with multiple adenomas, pointing to a possible role as disease modifier in FAP. Here, we present critical reassessment of one of the largest FAP kindreds published, which was previously used in linkage mapping of 1p35–36. In this family, all affected members harbour the same *APC* germline mutation (5945delA), but display marked phenotypic variability, in particular regarding the occurrence of extracolonic disease that segregates in several branches of the family tree. Using updated clinical information, additional mutation carriers and polymorphic markers, fine mapping of the critical region as well as mutation analysis of the *MYH* gene were performed. These investigations allowed us to significantly exclude (i) the 1p33–36 region as a modifier locus and (ii) *MYH* as a modifier gene for extracolonic disease in this FAP kindred. Our results do not eliminate 1p33–36 from suspicion in other families, but clearly indicate that in our family linkage analysis of further putative candidate regions is necessary to identify a disease modifier locus in FAP.
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Introduction

Familial adenomatous polyposis (FAP) is an autosomal dominantly inherited predisposition to colorectal cancer caused by germline mutations in the adenomatous polyposis coli (*APC*) gene.[1] It is a clinically heterogenous disease in which patients develop hundreds to thousands of adenomas throughout the large intestine. Extracolonic disease may also occur, for example upper gastrointestinal (GI) polyposis, soft tissue tumours (in particular desmoid tumours), osteomas and congenital hypertrophy of the retinal pigment epithelium (CHRPE).[2–4] Despite established genotype–phenotype correlations, considerable phenotypic variation with respect to colonic and extracolonic disease has been reported in patients harbour-

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ing identical *APC* mutations,[5,6–8] indicating that other genetic factors (modifier genes) are likely to play important roles in disease development.[3,5,9,10]

In support of this, loci that modify polyp multiplicity have been identified in different mouse models of FAP (*Min* and *Apc1638N*).[11–13] The *Mom1* and *Mom2* loci were found to affect tumour number in *Min* mice, and their synteny regions on the human chromosomes 1p35–36,[11] 18q21 and 18q23[14,15] are known to be frequently deleted/lost in a variety of human cancers, including colon tumours.[16–19] In *Apc1638N* mice, additional (X-ray responsive) modifier loci have been suggested.[20,21]

In view of the significant differences between the mouse and human FAP phenotype, the observations in the mouse do not necessarily apply to the human situation and mouse candidate modifiers should be evaluated with respect to the occurrence of both colonic and extracolonic disease manifestations.[11,12,22,23] For the *Mom1* locus, the secretory phospholipase A2 (*Pla2g2a*) gene has been suggested to be a strong candidate for suppression of the *Min* phenotype.[24,25] So far, no functional variants of *Pla2G2A* have been detected in humans,[26–28] and the possibility that other genes in the region can function as modifiers lead our group and others to further investigations of 1p35–36. However, these studies neither significantly excluded nor confirmed a human FAP modifier locus for extracolonic disease at 1p35–36.[22,29]

Recently, germline mutations in the base-excision repair gene *MYH* (region 1p33–34) have been described in patients with multiple adenomas, some of which had extracolonic disease (duodenal polyps).[30,31] This may implicate *MYH* as a possible FAP modifier, as *MYH* mutations/variants in combination with germline *APC* mutations could be expected to enhance the FAP disease phenotype.

To assess the role of the 1p32–36 region as a candidate modifier locus for extracolonic disease, we reinvestigated a large Swiss FAP kindred (No. 1460), part of which was previously used in linkage analysis, and where a lod score of 2.08 was obtained for an autosomal recessive model.[29] In the 7 years since this analysis has been performed, 13 additional members were identified and updated clinical information on the known mutation carriers was gathered, which enabled us to perform an extended linkage analysis of the 1p32–36 region as well as mutation analysis of the new candidate modifier gene *MYH*.

Methods

Patient data

The study was performed on a large, genetically isolated Swiss FAP kindred ($n > 200$), whereof all affected members ($n = 63$) share the same *APC* germline mutation, at codon 1982 in exon 15n, 5945delA (confirmed by sequencing and protein truncation test).[29,32] In 50 members (Figure 1; Table 1) belonging to the pedigree branches with extracolonic manifestations, histopathological data and reports from colonoscopies, gastro-duodenal endoscopies, computer tomographies, surgery, autopsies, as well as information from regular dental examinations, were collected and re-evaluated for the present study. Only patients with verified data from clinical and histopathological reports were used for linkage analysis. Written informed consent was obtained from all individuals.

Genotyping of polymorphic markers

Genotyping was performed using fluorescently labelled primers from the ABI Prism Linkage mapping Set-MD10 (PE Applied Biosystems;[33–35]) and primers of additional microsatellite markers from the 1p32–36, selected according to their map location and their heterozygosity status (http://www.ucsc.genome.org,[36]ftp://bioinformatics.weizmann.ac.il/pub/databases/genethon/Gmap/Nature-1995/data/[35]). PCR reactions were performed according to the manufacture's protocol and analysed on an ABI PRISM® 3700 DNA Analyzer (Applied Biosystems). Genotyping was exclusively performed in *APC* mutation carriers and their married-in members.

Linkage analysis

Microsatellite data was checked for genotyping errors using the PEDCHECK program,[37] two-point parametric lod score linkage analysis was performed using the MLINK program from the LINKAGE package[38] for both autosomal dominant (disease allele frequency 0.0781) and autosomal recessive (disease allele frequency 0.1000) models. Marker allele frequencies were set to be equal. Penetrance of 1.00 and 0.95 was used for the recessive model, and of 0.90 and 0.85, and age-dependent penetrance for the dominant model. Liability classes for age-dependent penetrance were estimated from our pedigree data and defined as a function of age at diagnosis.[39] Following liability classes were used: 0.157 ($\leqslant$20 years), 0.368 ($\leqslant$30 years), 0.684 ($\leqslant$40 years), 0.895 ($\leqslant$50 years), 0.947 ($\leqslant$60 years) and, 0.999 ($\leqslant$70 years). Penetrance of phenocopies was set to be 0.001. Only verified *APC* mutation carriers (and their married-in members) were included in the analysis, as only in these members extracolonic manifestations are expected to result from both *APC* and modifier gene mutations. Polyposis patients with colonic disease only were classified as having an 'unaffected' affection status, married-in members as having an 'unknown' phenotype. Patients presenting with extracolonic disease manifestation(s) were evaluated applying two different sets of criteria: (a) stringent criteria: only patients displaying at least adenomatous polyps in the upper GI tract and/or desmoids were classified as 'affected' with the others being classified as 'unknown'; (b) loose criteria: all patients with confirmed extracolonic manifestation(s) were scored as 'affected'

Table 1 Phenotypic characteristics in 50 *APC* mutation carriers from FAP family no. 1460

ID	*Colorectal polyps*	*Stomach polyps*	*Duodenum polyps*	*Desmoids or fibromas*	*Other*	*Included in LA*	*AS*
1460-1	<100					Yes	1
1460-4	<100			Yes		Yes	2
1460-6	>100			Yes		Yes	2
1460-7	<100	Yes		Yes		Yes	2
1460-8	Unknown			Yes		Yes	2
1460-9	>100					Yes	1
1460-10	<100	Yes		Yes		Yes	2
1460-11	<100					Yes	1
1460-16	>100	Yes			Osteoma	Yes	0/2
1460-19[a]	>100			Yes		Yes	2
1460-21	Unknown				Osteoma	Yes	0/2
1460-24	<100		Yes		Salivary gland adeno-carcinoma, prostate tumour	Yes	2
1460-26	>100	Yes	Yes	Yes		Yes	2
1460-28	<100					Yes	1
1460-33	<100					Yes	1
1460-42	>100					Yes	1
1460-44	<100					Yes	1
1460-46[a]	<100			Yes		Yes	2
1460-47	>100				Bronchial-Ca	Yes	0
1460-48[a]	>100					Yes	1
1460-55	>100					Yes	1
1460-86	<100					Yes	1
1460-88	<100					Yes	1
1460-89	<100					Yes	1
1460-91	<100	Yes		Yes		Yes	2
1460-93	>100					Yes	1
1460-12	<100					Yes	1
1460-106	<100					Yes	1
1469-1	<100	Yes	Yes	Yes	Osteomas, lipoma	Yes	2
1469-4	<100			Yes	Osteoma	No	2
1489-B[a]	>100			Yes		Yes	2
1489-E	Unknown					Yes	1
1501-1[a]	< 100	Yes		Yes	Osteomas	Yes	2
1501-2	>100	Yes	Yes			Yes	2
1501-4	<100			Yes		Yes	2
1501-5	Unknown					Yes	1
1747-1	<100					Yes	1
1779-1[a]	>100	Yes			Osteoma	Yes	0/2
1489-C	Unknown					Yes	1
1489-D	Unknown					Yes	1
1489-F	Unknown					Yes	1
1460-112	<100	Yes				No	0/2
1779-2	>100	Yes				Yes	0/2
1460-116	<100	Yes	Yes			No	2
1460-105	<100					Yes	1
1460-122	Unknown			Yes	Leukemia	No	2
1460-5	Unknown					Yes	1
1489-no	<100		Yes	Yes		No	2
1489-no	Unknown		Yes			Yes	2
1624-4	<100	Yes	Yes	Yes		No	2

LA = linkage analysis; AS = affection status used in linkage analysis.
[a]Patients included in *MYH* mutation screening.

(Table 1). One patient with bronchial carcinoma was classified as 'unknown' in all analyses.

MYH mutational analysis

Coding regions and exon–intron boundaries of *MYH* (GenBank accession number NM_012222) were screened by dHPLC using the 3500HT WAVE nucleic acid fragment analysis system (Transgenomic). Samples with different elution profiles, in comparison to control samples run in parallel, were then directly sequenced in forward and reverse orientations. The sequencing reactions were perfomed using the Big Dye Teminator Cycle Sequencing kit

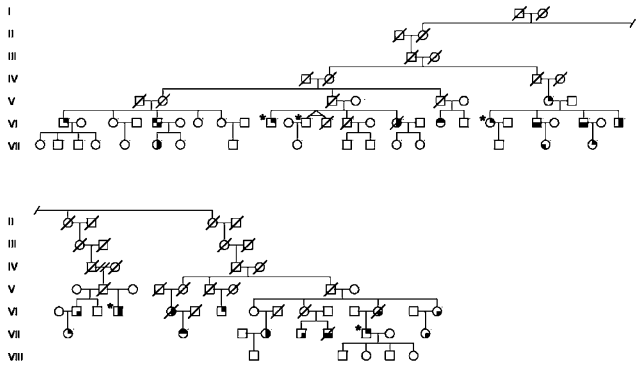

Figure 1 Extract from FAP kindred no. 1460 displaying branches with extracolonic disease manifestations. Only *APC* mutation carriers and their married-in members are included. Symbol description: (**a**) upper right quadrant, presence of desmoids and fibromas; (**b**) lower right quadrant, upper gastrointestinal polyps; (**c**) lower left quadrant, osteomas; (**d**) upper left quadrant, other extracolonic manifestations. Patients included in *MYH* gene mutation analysis are marked by asterisk.

(Applied Biosystems) and analysed on an ABI PRISM 310 Genetic Analyser (Applied Biosystems).

Results

Clinical data

In previous studies,[29,32] we reported a large FAP kindred (no. 1460) whose affected members present with a highly variable phenotype, on the level of both colonic as well as extracolonic disease manifestations. A total of 50 family members of this kindred, belonging to subbranches displaying extracolonic disease were clinically re-evaluated for this study (Table 1). In general, the polyposis phenotype among *APC* mutation carriers was found to be relatively mild, as could be expected by the site of the germline mutation, with 26 (65%) patients displaying attenuated polyposis (less than 100 polyps). The polyposis phenotype was very variable, ranging from severe forms with more than 1000 polyps (two patients) to the very mild form, where no polyps (three patients at age 22, 29, and 47) or less then 10 polyps (three patients at age 32, 33, and 47) were present.

In 26 patients, extracolonic tumours developed, the majority of these being desmoids (16/26; 61.5%) and upper gastrointestinal polyps (16/26; 61.5%). Adenomatous origin of the polyps was confirmed in nine patients, other were fundus gland polyps, which developed to a great extent in three patients.

Linkage analysis

Simulation linkage analysis, previously performed in family no. 1460, using the same diagnostic criteria and

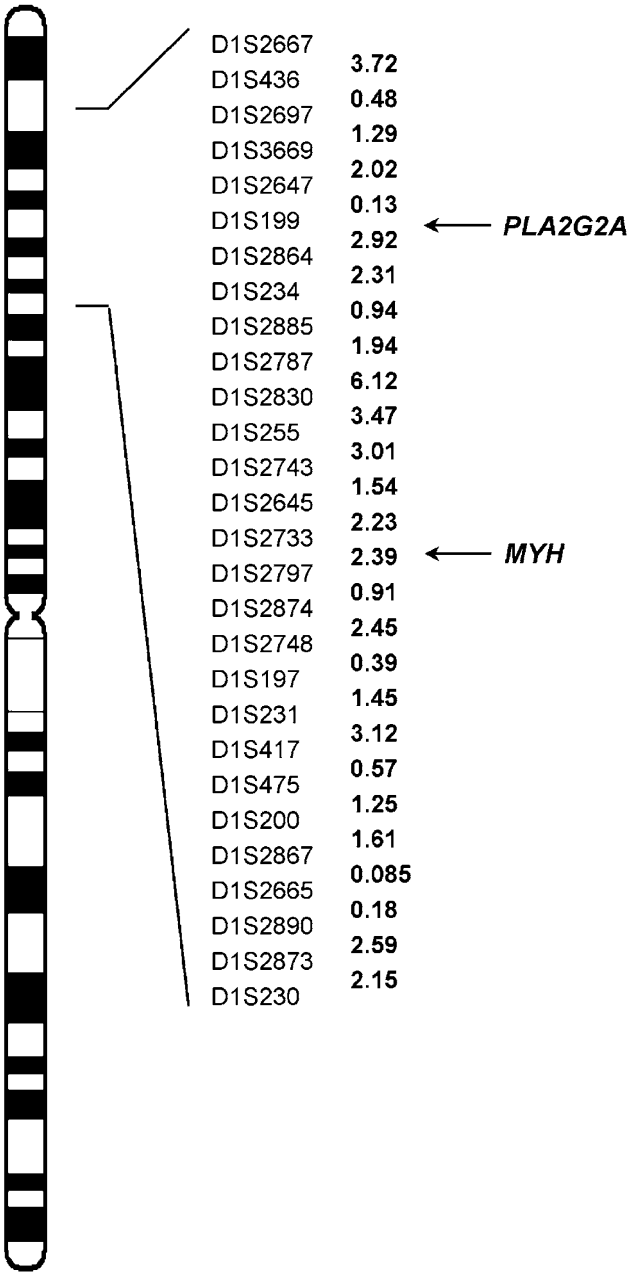

Figure 2 Physical map of the 1p32–36 region. Marker order, physical distance (Mb) and position of the secretory phospholipase A2 gene (*Pla2g2a*) and base-excision repair gene (*MYH*) were determined according to the UCSC genome bioinformatics site.[36]

parameters as employed for linkage analysis (see Methods) revealed a maximum expected lod score of 3.8–5.3 for autosomal dominant models, and 1.9–2.7 for autosomal recessive models. In all, 28 polymorphic markers spanning 58.7 cM (50.2 Mb) [36] of the 1p32–36 region (Figure 2) were

used for two-point linkage analysis under an autosomal dominant model with age-dependent penetrance. No evidence for the existence of a dominant modifier locus for extracolonic FAP disease was found. The lod scores throughout the region 1p33–36 were below −2 (except for three markers, D1S3669, D1S255 and D1S2733, with lod scores of −1.6, −1.9 and −0.6, respectively), thus excluding this region that includes both *Pla2G2A* and *MYH*, as a possible modifier locus (Table 2). To exclude the possibility that our negative results were due to choosing the wrong mode of inheritance, the analysis was also performed using an autosomal recessive model at penetrance 0.95 and 1.00, as well as an autosomal dominant model at reduced penetrance of 0.90 and 0.85, respectively (data not shown). All analyses, under both stringent and loose diagnostic criteria, resulted in negative lod scores below −2, except for the markers D1S3669, D1S417 and D1S231 (lod scores below −1.5, 0.1, and 0.8, respectively), hence excluding the 1p32–36 region as a modifier locus of extracolonic disease in our FAP kindred.

MYH mutation analysis

For *MYH* gene mutation analysis, six patients (i) coming from different pedigree branches and (ii) displaying different extracolonic disease manifestations were selected (Figure 1; Table 1). No DNA variants could be detected in all but one of them. Patient 1501-1 with multiple desmoids, osteomas, stomach and duodenal adenomas, but only one colonic polyp was found to harbour a heterozygous V22M variant of the *MYH* gene (exon 2). Subsequent mutation analysis on all family members identified only one more patient (1460-16) and his nonaffected father as V22M carriers. The variant represents an already described polymorphism, which was previously reported at a population frequency of 9–10%.[30,31]

Discussion

The present study excludes the 1p33–36 region as a modifier gene locus for extracolonic disease in our large Swiss FAP kindred no. 1460. The analysis was performed on updated family information, and used both more affected family members and more microsatellite markers. Since our initial investigation, restricted to the 1p35–36 region in 1996, 13 additional patients either developed extracolonic tumours or were newly referred to our department. Out of these, four were classified as having an 'affected' or 'unknown' diagnosis, depending on the stringency of the affection criteria used. Four patients previously classified as 'affected' were for the present analysis scored as

Table 2 Lod scores for autosomal dominant model with age-dependent penetrance using markers from the 1p32–36 region

		Lod score at recombination fraction (θ)												
Marker	*Region*	*0*	*0.01*	*0.01*	*0.02*	*0.03*	*0.04*	*0.05*	*0.06*	*0.07*	*0.1*	*0.2*	*0.3*	*0.4*
D1S2667	1p36.22	−4.94	−2.67	−2.34	−1.99	−1.77	−1.60	−1.46	−1.35	−1.25	−1.01	−0.53	−0.25	−0.08
D1S436	1p36.13	−3.95	−1.72	−1.41	−1.11	−0.93	−0.81	−0.72	−0.65	−0.59	−0.47	−0.28	−0.18	−0.09
D1S2697	1p36.13	−3.45	−2.03	−1.70	−1.33	−1.11	−0.94	−0.81	−0.70	−0.61	−0.40	−0.10	−0.03	−0.01
D1S3669	1p36.13	−1.63	−1.55	−1.49	−1.38	−1.28	−1.19	−1.11	−1.04	−0.97	−0.79	−0.40	−0.17	−0.04
D1S2647	1p36.13	−2.37	−2.18	−2.03	−1.80	−1.62	−1.47	−1.35	−1.24	−1.15	−0.93	−0.49	−0.26	−0.11
D1S199	1p36.13	−5.86	−4.18	−3.79	−3.32	−3.01	−2.75	−2.54	−2.36	−2.20	−1.81	−0.97	−0.46	−0.15
D1S2864	1p36.12	−4.71	−4.07	−3.70	−3.19	−2.82	−2.53	−2.28	−2.06	−1.88	−1.42	−0.56	−0.18	−0.03
D1S234	1p36.11	−6.23	−5.10	−4.62	−4.01	−3.59	−3.25	−2.98	−2.74	−2.53	−2.03	−1.01	−0.46	−0.14
D1S2885	1p36.11	−4.59	−3.63	−3.15	−2.56	−2.16	−1.86	−1.62	−1.42	−1.25	−0.86	−0.24	−0.06	−0.03
D1S2787	1p35.3	−2.38	−2.26	−2.15	−1.97	−1.81	−1.68	−1.56	−1.45	−1.36	−1.11	−0.56	−0.25	−0.07
D1S2830	1p35.1	−2.87	−2.19	−1.88	−1.50	−1.24	−1.04	−0.88	−0.74	−0.62	−0.34	0.11	0.18	0.07
D1S255	1p34.3	−1.98	−1.48	−1.23	−0.92	−0.71	−0.56	−0.43	−0.33	−0.24	−0.04	0.23	0.24	0.12
D1S2743	1p34.2	−2.77	−1.86	−1.56	−1.22	−1.00	−0.83	−0.69	−0.58	−0.48	−0.26	0.10	0.17	0.10
D1S2645	1p34.2	−2.50	−2.14	−1.91	−1.57	−1.33	−1.14	−0.98	−0.84	−0.72	−0.44	0.02	0.13	0.08
D1S2733	1p34.1	−0.66	−0.63	−0.60	−0.55	−0.50	−0.45	−0.41	−0.37	−0.33	−0.23	−0.04	0.00	−0.01
D1S2797	1p33	−2.66	−1.76	−1.46	−1.12	−0.91	−0.75	−0.62	−0.51	−0.42	−0.22	0.07	0.08	0.01
D1S2874	1p33	−2.74	−2.41	−2.17	−1.82	−1.57	−1.37	−1.20	−1.06	−0.94	−0.65	−0.12	0.07	0.07
D1S2748	1p33	−3.99	−3.06	−2.72	−2.32	−2.04	−1.82	−1.63	−1.48	−1.34	−1.00	−0.37	−0.10	0.00
D1S197	1p33	−0.78	−0.75	−0.71	−0.65	−0.59	−0.54	−0.49	−0.45	−0.41	−0.31	−0.11	−0.04	−0.04
D1S231	1p32.3	−0.08	−0.07	−0.06	−0.05	−0.04	−0.03	−0.03	−0.02	−0.02	−0.01	−0.01	−0.01	−0.01
D1S417	1p32.3	0.77	0.76	0.74	0.72	0.70	0.67	0.65	0.63	0.61	0.54	0.36	0.22	0.10
D1S475	1p32.3	−1.94	−1.60	−1.39	−1.10	−0.91	−0.76	−0.63	−0.53	−0.44	−0.25	0.03	0.06	0.02
D1S200	1p32.3	−1.73	−1.25	−0.99	−0.68	−0.47	−0.31	−0.17	−0.06	0.03	0.24	0.51	0.46	0.24
D1S2867	1p32.2	−2.21	−1.78	−1.52	−1.15	−0.90	−0.71	−0.55	−0.42	−0.31	−0.07	0.25	0.22	0.06
D1S2665	1p32.2	−2.24	−1.92	−1.71	−1.43	−1.22	−1.06	−0.93	−0.82	−0.72	−0.48	−0.09	−0.01	−0.06
D1S2890	1p32.2	−2.14	−2.01	−1.89	−1.69	−1.51	−1.36	−1.22	−1.09	−0.98	−0.69	−0.13	0.07	0.06
D1S2873	1p32.1	−0.95	−0.74	−0.59	−0.37	−0.22	−0.10	−0.01	0.07	0.13	0.26	0.38	0.28	0.11
D1S230	1p31.3	−2.57	−2.37	−2.22	−1.98	−1.80	−1.65	−1.51	−1.39	−1.28	−1.00	−0.41	−0.13	−0.01

'unknown', because original data provided by the patient's record could not be confirmed from histopathological records. Furthermore, unlike the previous analysis, only *APC* mutation carriers and their spouses were used for linkage analysis. These differences may explain why the lod score for the autosomal recessive model dropped from a previously observed 2.08 (D1S211) to below −2 (instead of marker D1S211, markers D1S2645 and D1S2733 were used), and for the autosomal dominant model from 1.77 (D1S197) to below −0.7 (Table 2).

The rationale for considering upper GI polyps and desmoids together in linkage analysis was three-fold: (i) in *all* parent–child pairs, where both presented with extracolonic disease, upper GI polyps *and* desmoids were observed (Figure 1); (ii) overall, 50% of patients with desmoid disease also displayed upper GI polyps (and *vice versa*); (iii) the occurrence of upper GI polyps together with desmoids in FAP has been well documented in the literature.[40–43]

Although our linkage results for an autosomal recessive mode of inheritance resulted in significant exclusion of the 1p32–36 region, we put our emphasis on autosomal dominant models which seem to be more appropriate in our FAP kindred for several reasons. Firstly, the ratio of *APC* mutation carriers with, compared to those without, extracolonic disease varied between 0.42 and 0.52, depending on the affection criteria applied. Secondly, in some of the subbranches of family no. 1460, extracolonic manifestations are clearly transmitted through generations (Figure 1). When comparing 12 parent–child pairs with extracolonic disease present, transmission of extracolonic disease through the generations could be observed in 100% of informative pairs, suggesting an autosomal dominant mode of inheritance. Using the stringent phenotype criteria, that is, only including patients with at least upper GI adenomatous polyps or desmoids, transmission was seen in eight out of nine pairs (88%).

Phenotype analysis revealed the same clinical heterogeneity as previously reported.[32] Furthermore, when comparing the group of patients with <100 and those with >100 colorectal polyps, no statistically significant relationship could be found between polyp number and the occurrence of extracolonic disease in general ($\chi^2=0.44$, $P=0.50$). The same was true if only desmoids ($\chi^2=1.20$, $P=0.27$) or only upper GI polyps were taken into account ($\chi^2=0.10$, $P=0.75$). This indicates that the severity of colonic polyposis does not correlate with the presence of extracolonic disease manifestations; hence, polyp number and extracolonic disease may represent two genetically related but distinct entities. We cannot, however, exclude an underestimation of the actual frequency of extracolonic disease which, in particular, may be true for patients screened before the event of cross-sectional imaging (for the detection of desmoid tumours) and side-viewing endoscopy (duodenal polyps).

Our linkage analysis data are in agreement with the results from the mutation screening in *PLA2G2A*[27] and *MYH*, where, except for the heterozygous V22M variant present in two patients belonging to different branches of the family tree (and observed in 2% of Swiss control population, unpublished results), no other DNA alterations could be identified in the coding region of the *MYH* gene.

In conclusion, our data on this large Swiss FAP kindred significantly exclude the 1p33–36 region as a modifier locus and *MYH* as a modifier gene for extracolonic disease. Since simulation linkage analysis revealed a maximum expected lod score of 3.8–5.3 for autosomal dominant and 1.9–2.7 for autosomal recessive models, future work will concentrate on performing a genome-wide linkage analysis in this FAP kindred, which should help in the identification of a modifier locus in FAP.

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