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ORIGINAL INVESTIGATION

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Identification of a modifier gene locus on chromosome 1p35-36 in familial adenomatous polyposis

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Abstract Phenotypic variability based on nonallelic heterogeneity is a characteristic feature of the dominantly inherited disease, familial adenomatous polyposis (FAP). A modifying locus, called *Mom-1*, which strongly influences disease expression has been mapped in the mouse model of FAP to the region of murine chromosome 4, which has synteny to human chromosome 1p35-36. In the present study, this chromosomal region was investigated by using 14 microsatellite markers within a large FAP kindred in which patients harbor the same germ-line mutation but show markedly different disease characteristics. The linkage program MLINK was used to determine whether any correlation exists between these markers and the development of extracolonic symptoms in polyposis coli patients. Depending on the mode of inheritance of the affected locus, a maximum lod score was observed for markers D1S211 and D1S197, reaching 2.08 and 1.77, respectively. The observed values obtained within one large FAP family are supportive of a phenotype-modifying locus within this chromosomal region.

Introduction

Despite the observation that almost all familial adenomatous polyposis (FAP) patients harbor germ-line mutations within the same gene, viz., the adenomatous poylposis coli (APC) gene, they do not present with a uniform phenotype with respect to either colonic and/or extracolonic symptoms. Phenotypic variability has partly been correlated with different sites of mutations within the APC gene (Olschwang et al. 1993; Caspari et al. 1994, 1995; Dobbie et al. 1994, 1996a; Gayther et al. 1994; Nugent et al. 1994; Presciuttini et al. 1994; Wallis et al. 1994; Davies et al. 1995; Friedl et al. 1996). However, some cases of interfamilial variability and the occurrence of intrafamilial variability in disease expression cannot be explained by such correlations and are suggestive of non-allelic heterogeneity. In support of this, a locus that modifies the FAP phenotype has been identified in a mouse model of the disease (Moser et al. 1990, 1992; Su et al. 1992). The number of polyps in multiple intestinal neoplasia (Min) mice can be dramatically altered, depending on the genetic background within which the Min mutation is segregating. Variability of polyp number has been attributed to the Mom-1 (modifier of Min-1) locus, which maps to mouse chromosome 4 (Dietrich et al. 1993), a region of synteny with human chromosome region 1p35-36, which is frequently deleted in a variety of human cancers (reviewed by Schwab et al. 1996). A candidate gene for Mom-1 has been proposed, viz., the secretory phospholipase A_2 ($sPla_2$) gene, which is mutated on both alleles in the germ-line of the polyp-sensitive mouse strain (MacPhee et al. 1995). However, none of the studies examining the influence of the $sPla_2$ gene on FAP phenotypic variability in man with respect to colonic and/or extracolonic disease (Riggins et al. 1995; Dobbie et al. 1996b; Spirio et al. 1996) has revealed any association between the $sPla_2$ gene structure and/or expression with the patients' phenotype.

The exclusion of $sPla_2$ as an important modifier in FAP does not rule out the possibility that other modifying gene(s) exist in this chromosomal region; such genes might even interact with the $sPla_2$ pathway. In order to determine whether a locus on chromosome 1p35-36 exists that associates with the extracolonic manifestations in FAP patients, a large family (77 persons) was studied. The genotypes of 14 microsatellite markers have been determined, and a linkage analysis has been undertaken in this region.

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Subjects and methods

FAP family

A large FAP kindred, originating from Poschiavo (Graubünden) in Switzerland, has been collected since 1990. All 46 patients from this family harbor the same APC mutation, viz., an adenine deletion at codon 1987 in exon 15n, causing a frameshift and resulting in a stop codon 63 codons downstream (Scott et al. 1995). Colonic disease appears to be relatively mild in most affected persons. Interestingly, remarkable phenotypic variability among gene carriers has been documented, mainly with respect to the development of extracolonic symptoms (17 patients). These include desmoids (7 patients) diagnosed by computer tomography, osteomas (2 patients) detected during regular dental examination, lipomas (1 patient), fibromas (2 patients) diagnosed by astute medical examination, and upper gastrointestinal polyps (11 patients). The entire kindred, including information about the type of extracolonic disease, is shown in Fig. 1. Because of problems associated with the reliability of data concerning the severity of colonic polyposis (Bunyan et al. 1995), such as age-dependent penetrance, resection of the colon at an early age, and/or lack of reliable clinical information, our phenotypic description focused only on extracolonic symptoms as hallmarks of a complex disease phenotype, the diagnosis of these lesions appearing to be more reliable. Thus, patients coming from this family were divided into two groups, viz., those with only colonic disease and those with additional extracolonic symptoms.

Microsatellite markers (CA-repeats) analysis

The genotypes of 46 mutation carriers and 31 non-carriers from this kindred have been determined at 14 highly polymorphic microsatellite loci (D1S468, D1S214, D1S244, D1S436, D1S496, D1S193, D1S211, D1S463, D1S447, D1S451, D1S197, D1S427, D1S417, D1S200) spread accross 79 cM of chromosome 1p35-36 (Gyapay et al. 1994). Genomic DNA was obtained from blood lymphocytes as previously described (Miller et al. 1988). The total polymerase chain reaction (PCR) volume was 25 µl: 2.5 µl 10 × PCR buffer (Gibco BRL), 200 ng DNA, 50 pmol PCR primers (Gyapay et al. 1994), 250 µM each dNTP, 3 mM $MgCl_2$, 0.1 µl 3000 Ci [α-^{32}P] dCTP/mmol (Dupont), and 0.2 U *Taq* polymerase (Gibco). The PCR conditions were as follows: denaturation at 94°C for 1 min, annealing at 55°C for 30s, and elongation at 72°C for 1 min, repeated over 25 cycles. Products were loaded onto a 5% denaturing polyacrylamide gel and run in 0.5 × TBE buffer (1 × TBE buffer = 0.09 TRIS-borate, 0.002 M EDTA, pH 8.3) at 50 W constant power for 150–300 min. The gels were fixed in 10% methanol and 10% acetic acid for 10 min, briefly rinsed in water, and dried. The dried gels were exposed to X-ray film (Kodak Biomax MR) for 1–4 days.

Linkage analysis

The accumulated data were analyzed by the LINKAGE package sub-routine MLINK for two-point linkage. Disease status was defined as follows: FAP patients presenting with any extracolonic symptom were classified as affected, polyposis patients over 26 years of age with only colonic disease as unaffected, and all other persons in the pedigree as having an unknown phenotype. Both dominant and recessive modes of action on the affected locus were evaluated. An allele frequency of 0.33 was used for the modifying allele that enhances the disease complexity. Marker allele frequencies were used as defined by Gyapay et al. (1994). Two-point lod scores between each of ten markers and affected status were determined.

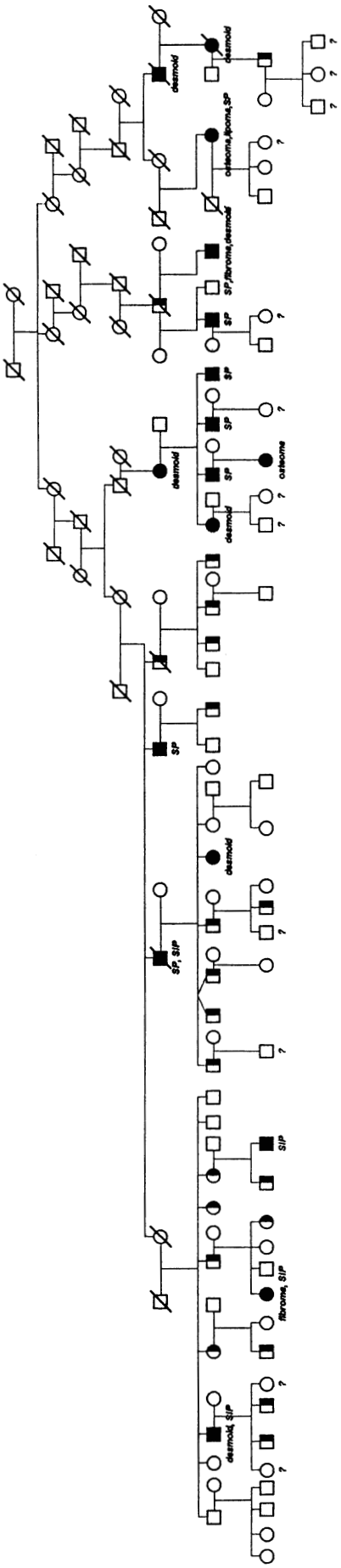

Fig. 1 Entire FAP kindred. *SIP* Small intestinal polyps, *SP* stomach polyps, ? unknown phenotype in a mutation carrier; *black symbol* affected with colonic and extracolonic disease, *halfblack symbol* affected only with colonic disease, *open symbol* unaffected

Table 1 Maximum LOD score (Z_{max}) for marker loci

Marker	Affected locus			
	Dominant		Recessive	
	Z_{max}	θ	Z_{max}	θ
D1S468	–0.06	0.4	–0.02	0.5
D1S214	–0.06	0.4	–0.01	0.3
D1S244	–0.05	0.4	0.14	0.5
D1S436	0.02	0.4	–0.01	0.4
D1S496	1.13	0.2	1.17	0.0
D1S193	1.15	0.0	0.07	0.4
D1S463	1.59	0.0	1.33	0.0
D1S211	1.05	0.0	**2.08**	0.0
D1S447	1.46	0.0	1.31	0.0
D1S451	0.44	0.2	0.86	0.0
D1S197	**1.77**	0.1	0.03	0.4
D1S427	0.01	0.1	0.00	0.5
D1S417	0.00	0.2	0.00	0.5
D1S200	0.83	0.2	0.01	0.2

Results and discussion

A set of 14 microsatellite markers spanning a distance of 79 cM of chromosome 1p35-36 was employed to determine whether a FAP modifier locus existed in this region. All these markers represent loci with a variable number of CA-repeats and are highly polymorphic in the general population. Our large FAP kindred provides an excellent model for this type of analysis as all the patients, presenting with different disease symptoms, harbor the same APC mutation, excluding the influence of different mutation sites on disease expression. The haplotypes of the markers were determined in 77 persons (including 46 mutation carriers) from this kindred, and the obtained data were analyzed by the MLINK program.

The mode of action of the modifier locus is unknown. In the studied FAP pedigree, the ratio of the number of children with extracolonic symptoms to the number of all children carrying a mutation in those family branches where the complex disease occurs is 0.45, which suggests

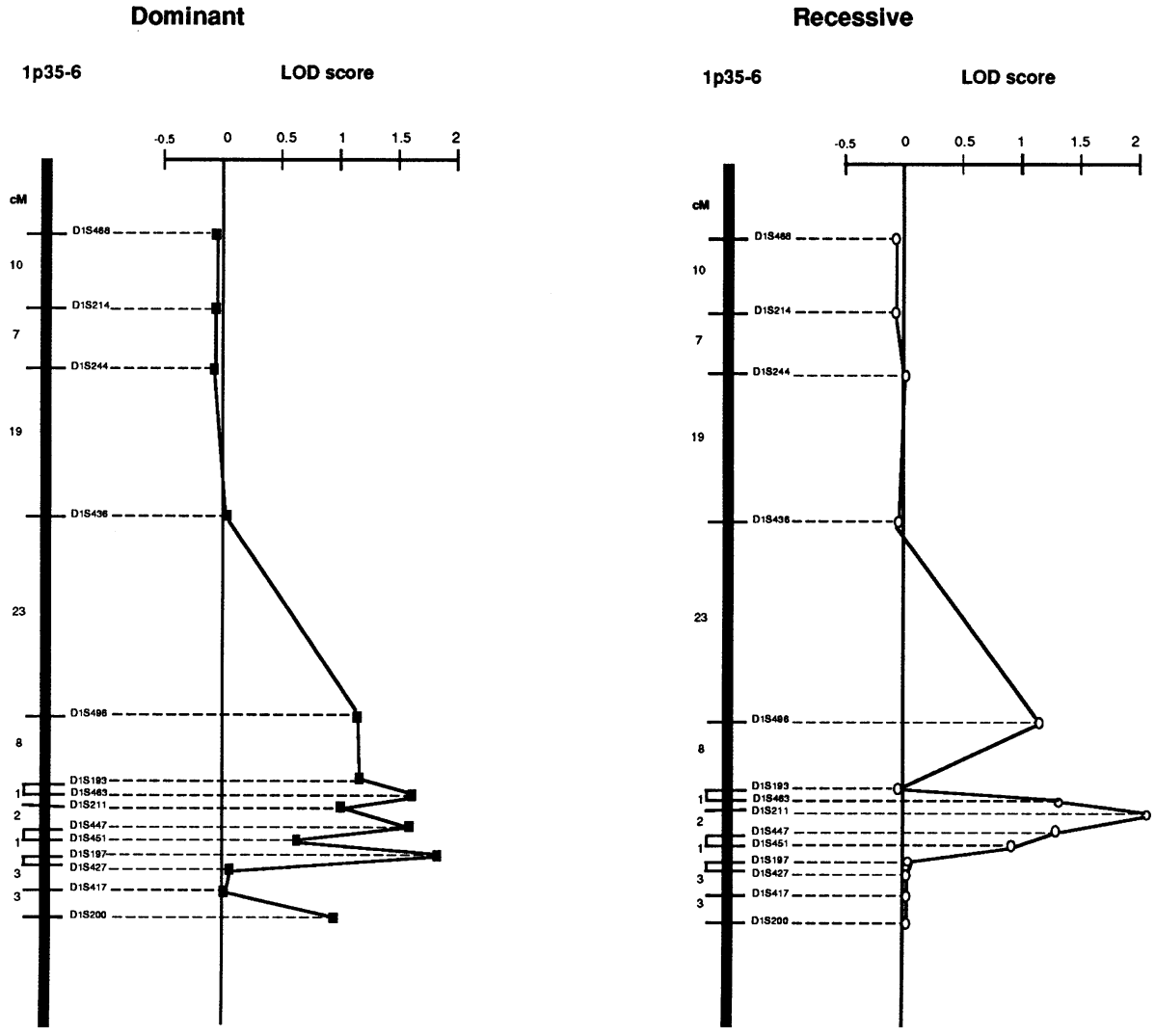

Fig. 2 Maximal LOD scores for markers on 1p35-36

that the dominant mode of inheritance is more likely. However, both dominant and recessive modes were evaluated. The frequency of the potential allele leading to more complex disease expression was derived from the number of patients presenting with extracolonic disease compared with the overall frequency of FAP patients. These patients represent about one third of all FAP-affected persons; thus, allele frequencies 0.66 and 0.33 for the affected locus were used. Age-dependent penetrance with respect to the development of extracolonic manifestations exists, although no precise data have so far been defined. Therefore, the analysis was performed without liability classes; however, polyposis patients under 26 years of age were evaluated as having an unknown phenotype, even if they had not yet developed extracolonic symptoms.

By using a combination of these parameters, maximum lod scores (Z_{max}) for the marker loci were observed as shown in Table 1 (with information about θ) and Fig. 2 (with a chromosomal map of markers). The highest values of Z_{max} were obtained for markers D1S211 and D1S197, viz., 2.08 and 1.77 at $\theta = 0.0$ and $\theta = 0.1$, respectively. The marker with the highest maximum lod score (D1S211 or D1S197) varied depending on whether the mode of inheritance was recessive or dominant. The map distance between these two loci is 3 cM.

The largest Z_{max}, 2.08, observed in this study appears to be relatively high within one family and is a strong indication that a modifier gene is located in the region bordered by microsatellite markers D1S496 and D1S427, which might cosegregate with the complex phenotype of FAP patients. We are aware of the necessity of further tests, including more families, to confirm these results. This, however, presents difficulties, because of a paucity of appropriate kindreds with sufficient family members to enable accurate linkage analysis and with patients having different disease characteristics.

Tomlison et al. (1996) report a similar type of analysis on 28 small FAP families by using 8 microsatellite markers spanning a region of 150 cM between positions 1p13 and 1p36. The association of genotype at these marker loci with the severity of duodenal polyposis in FAP patients has been analyzed by parametric (LINKAGE) and non-parametric analysis, viz., affected pedigree member linkage analysis (APM) and quantitative sib-pair analysis (ASP). Results of the APM analysis suggest a tendency for non-random segregation of alleles among those affected with low disease severity. Regarding the report of Tomlison et al. (1996), the marker with the highest lod score, D1S255 (0.82), is localized, with respect to the markers used in our study, 1 cM proximal from D1S496 and 7 cM telomeric from D1S211 (our highest lod score). As the markers analyzed by Tomlison et al. (1996) cover the region of 1p35-36 with much lower density, they completely by-pass the region representing the most candidate region in our study.

Changes on chromosome 1p are frequent in most forms of human cancer, and the region between markers D1S496 and D1S197 is especially affected in neuroblastoma, colorectal cancer, and MEN2 (Schwab et al. 1996). Recently, a region between two loci, D1S201 and D1S197, which includes our marker loci with the highest lod score, has been shown to be commonly deleted in gastric tumors, indicating the presence of the tumor suppressor gene for gastric cancer within this region (Ezaki et al. 1996).

Interestingly, the $sPla_2$ gene is located closest to the marker D1S199, by a distance not larger than 440 kb (Praml et al. 1995). This marker lies as far as 27 cM distal from marker D1S211 for which the highest Z_{max} has been observed in our study, adding further evidence for the exclusion of $sPla_2$ as a candidate modifier, at least with respect to extracolonic disease.

Variable FAP expressivity of even identical mutations is a difficult problem in genetic counseling, both within and between families. Allelic heterogeneity does not appear to account for all the variation and other gene(s) are probably involved in the phenotypic expression of this "monogenic" disease. Although further studies into this region are required to identify this gene, the evidence presented in this report indicates that a modifying locus exists in chromosomal region 1p35-36 and that it appears to influence extracolonic disease expression.

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