Original articles

A modifying locus for familial adenomatous polyposis may be present on chromosome 1p35-p36

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Abstract

Mutations of the APC gene cause familial adenomatous polyposis (FAP) in humans and multiple intestinal neoplasia (*Min*) in laboratory mouse strains. A dominant modifying gene (*Mom1*), which partially suppresses the *min* phenotype, has been mapped to mouse chromosome 4. This region is syntenic with human chromosome 1p35-p36. The phospholipase A2 (*Pla2s*) locus is an excellent candidate for *Mom1* and the equivalent human locus PLA2G2A is found on chromosome 1p35. It does not necessarily follow, however, than any modifier of mouse polyposis also influences human disease. In order to test whether a locus on 1p modifies FAP, subjects from 28 FAP families have been typed at microsatellite loci on this chromosome arm. The severity of their duodenal polyposis has also been assessed by endoscopy. Pedigree (lod score) linkage analysis found no evidence of a simple, dominant modifying gene, comparable with the action of *Mom1* in inbred mouse strains. Given the more complex genetic and environmental interactions likely to exist in outbred human populations, it is probably more appropriate to use tests which do not specify a mode of inheritance. Using these methods of analysis, the data suggest that a locus on chromosome 1p35-p36 may influence the severity of duodenal FAP.

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Key words: familial adenomatous polyposis; APC; modifier; 1p35-p36.

Familial adenomatous polyposis (FAP) is a disease with autosomal dominant inheritance, characterised by multiple colonic polyps, smaller numbers of adenomas elsewhere in the gastrointestinal tract, and various extracolonic features. Classical linkage analysis mapped the human APC locus to chromosome 5q21-q22,^{1,2} a region that shows frequent loss of heterozygosity in the tumours of FAP patients and in sporadic colorectal cancers.^{3,4} The APC gene was subsequently cloned^{5,6} and the mouse hom-

ologue was discovered serendipitously in the C57-BL/6 strain at about the same time.^{7,8} The phenotype of the *Min* (multiple intestinal neoplasia) mouse differs from FAP in that adenomatous polyps are fewer in number and almost always confined to the small bowel.

Although mutant APC alleles show nearly complete penetrance in mice and humans, the progeny of crosses between BL/6-*Min* mice and AKR mice had a reduced number of polyps.⁹ This observation led to the mapping of a dominant, partial suppressor of *Min*, *Mom1*, to mouse chromosome 4. The position of the *Mom1* locus is syntenic with human chromosome 1p35-p36. This region shows frequent allele loss in several human cancers, including those of the colon.

A strong candidate for *Mom1* was discovered by MacPhee *et al.*¹⁰ Null secretory phospholipase A2 (*Pla2s*) alleles were shown to correlate completely with susceptibility to greater numbers of polyps. Hence, wild type *Pla2s*, involved in arachidonic acid production and thus in cell signalling via prostaglandins and leucotrienes, may suppress polyp formation in the *Min* mouse. *Pla2s* maps to a mouse region syntenic with human chromosome 1p35 and the human homologue of *Pla2s* is located there.¹¹ It remains possible, however, that another locus close to *Pla2s* is actually *Mom1* and that alleles at the two loci are in strong linkage disequilibrium in the mice studied.

Given the genetic mapping of *Mom1* to the equivalent of human chromosome 1p35-p36, this region has become a candidate for modifying the phenotype of human FAP. It does not follow, however, that a mouse modifying locus has any effect on the equivalent disease in human. In order to determine whether there is evidence for such an effect, it is possible to search within the affected members of FAP pedigrees for an association between the severity of disease and the inheritance of marker alleles on the short arm of chromosome 1. It is preferable to study duodenal rather than colonic FAP, for the following reasons: (1) disease varies less with age; (2) disease varies little, if at all, with site of APC mutation; (3) disease is less affected by therapeutic and preventative intervention, such as colectomy;

Table 1 The Spigelman classification of the severity of duodenal FAP. Patients score points according to the number of polyps found, their size, histological type, and degree of dysplasia. Summing points scored allows severity of disease to be placed in one of the five staging categories below

Feature	Category	Points scored
Polyp number	0	0
	1–4	1
	5–20	2
	>20	3
Polyp size (max)	0 mm	0
	1–4 mm	1
	5–10 mm	2
	>10 mm	3
Histological type	None	0
	Tubular/hyperplasia/inflammation	1
	Tubulovillous	2
	Villous	3
Dysplasia	None	0
	Mild	1
	Moderate	2
	Severe	3
Severity category	Total points scored	
0	0	
1	1–4	
2	5–6	
3	7–8	
4	9–12	

(4) standardised, semiquantitative scoring of the severity of duodenal disease according to the Spigelman method¹² is available (table 1); and (5) mouse polyposis predominately occurs in the small bowel.

Twenty-seven simplex and multiplex FAP families, comprising about 300 people in total, have been studied. Some of the pedigrees are shown in the figure, with summarised details in table 2. The genotypes of about 150 patients and other members of these pedigrees have been determined at eight microsatellite loci (table 3) extending 150 cM towards the centromere from the telomeric end of chromosome 1p, including bands p35–p36. The severity of the duodenal polyposis of the affected members of the pedigree has been determined independently of the genotyping using oesophagogastroduodenoscopy and scored on the Spigelman scale between 0 and 4. Three hypotheses have been tested: (1) that a dominant suppressor of duodenal polyposis on the short arm of chromosome 1 is segregating in all, or a proportion of, these families; (2) that a locus on 1p is involved in suppression of duodenal polyposis, with unspecified mode of inheritance and penetrance; and (3) that the degree of similarity between the phenotypes of sib pairs correlates with the coinheritance of alleles on 1p.

Methods

GENOTYPING

Samples of genomic DNA from peripheral blood, normal tissue, or archival material were extracted using standard methods. PCR reactions were performed on each DNA using the following conditions. A total of 50 to 250 ng of DNA was used as template in a final volume of 50 µl with reaction concentrations of 1 × standard PCR buffer (Promega), 1.5 mmol/l Mg²⁺, 0.5 mmol/l dNTPs, and 0.4 mmol/l of each specific oligonucleotide primer; 1 unit of *Taq* polymerase was added per reaction. An amplification protocol of 94°C (one minute)

× 1, 94°C (one minute)/55°C (one minute) × 35, 72°C (five minutes) × 1¹³ was used. PCR products were heated to 90°C for five minutes and electrophoresed on a 6% acrylamide sequencing gel (Sequagel) under denaturing conditions for two to four hours. DNA was transferred by blotting onto Hybond N+ membranes (Amersham, UK) for four to 16 hours. PCR products were detected by the enhanced chemiluminescence technique (Amersham, UK), using a randomly elongated oligonucleotide primer as a specific probe for each locus. Products were visualised by exposing membranes to Hyperfilm (Amersham, UK) for one minute to one hour.

SCORING OF DUODENAL FAP

Standard protocols were used for oesophago-duodenoscopy. Where appropriate, polyps were biopsied or removed and classified according to accepted histopathological methods. A Spigelman staging score of duodenal FAP severity was accorded to each patient on the basis of number of polyps, size, histological type, and grade (table 1).

PEDIGREE LINKAGE (LOD SCORE) ANALYSIS

For the purposes of pedigree linkage analysis, a dichotomy was introduced into the continuous distribution of duodenal disease scores. On clinical grounds, a distinction is best drawn between scores of 2 and 3 (2–3 split). Hence, in accordance with *Mom1*, patients with disease severity 2 or less were classed as affected (that is, subject to suppression of the disease phenotype) and those with severity 3 or more were classed as unaffected. In order to provide a comparison, two other classifications were used: (1) severity 1 or less as affected and severity 2 or more as unaffected (1–2 split), and (2) use of the 2–3 or 1–2 boundary as appropriate in each family to maximise segregation (“best” split). If a family showed no evidence of segregation of the phenotype (according to the particular definition of affection status being used), the family was not used for the purpose of this analysis (or for those below). Marker allele frequencies were estimated from those people marrying into the pedigrees studied. The LINKAGE package¹⁴ was used to determine two point lod scores between each of the eight markers and affection status (as defined in each of the three above ways). Penetrance of 0.9 was assumed for the dominant modifying (phenotype suppressing) locus, with a penetrance of 0.1 for the recessive non-suppressor (to take account of phenocopies). A modifying allele frequency of 0.1 was assumed. The HOMOG program was used to test whether a proportion of families showed significant evidence of linkage to chromosome 1p.

AFFECTED PEDIGREE MEMBER (APM) LINKAGE ANALYSIS

This method,¹⁵ comparing the ibs (identity by state) genotypes of affected pedigree members,

Pedigrees informative for APM analysis showing chromosome 1p genotypes of people classed as "affected". Subjects shown are those whose allele sharing was analysed by the APM method according to the 1-2 split or 2-3 split or "best" split or all of these, for defining affection status by Spigelman scores. Spigelman scores in these pedigrees are shown as blocked in quadrants of the pedigree symbol. Hence, totally blocked in represents a score of 4 and O a score of zero. Other subjects, whether without FAP or with FAP but not having had endoscopy, are denoted by a ?. For the sake of brevity, the pedigrees do not show the full family structure or the genotypes of any subjects not classed as "affected" for the purposes of APM analysis, although these subjects do provide useful information when calculating the APM statistic. Loci are shown in descending order: D1S243, D1S214, D1S228 and D1S199.

was used to search for non-random segregation of markers on chromosome 1p in affected subjects. Affection statuses with respect to the

severity of duodenal FAP were defined in the same three ways as above, within the group of FAP patients who had undergone endoscopy.

Table 2 Summary of number of FAP patients and their Spigelman scores in the families studied

Family	No with FAP	No with OGD & DNA	No. with Spigelman score				
			0	1	2	3	4
1	17	9	0	3	2	3	1
2	3	3	0	3	0	0	0
3	3	3	0	1	1	1	0
4	11	6	0	1	1	2	2
5	2	2	0	1	1	0	0
6	2	2	0	0	2	0	0
7	3	3	0	0	1	1	1
8	7	2	0	1	0	1	0
9	4	4	0	0	2	2	0
10	3	2	0	1	1	0	0
11	3	3	0	0	0	2	1
12	5	4	0	0	3	1	0
13	2	2	0	0	1	1	0
14	2	2	0	1	0	1	0
15	10	5	1	2	2	0	0
16	3	3	0	0	0	2	1
17	5	3	0	2	1	0	0
18	2	2	0	1	0	1	0
19	6	3	0	0	2	1	0
20	8	5	0	0	2	1	2
21	3	3	0	1	0	2	0
22	4	3	1	0	1	0	1
23	4	2	0	1	1	0	0
24	5	3	0	1	0	2	0
25	4	3	0	0	2	1	0
26	4	3	0	1	1	1	0
27	4	3	0	0	3	0	0
28	2	2	0	0	1	1	0

Table 3 The markers used in the study, their genetic map positions, and their approximate cytogenetic map positions

Marker	Cytogenetic map position
tel	
17cM	D1S243 1p36
16cM	D1S214 1p36
17cM	D1S228 1p35
20cM	D1S199 1p34
17cM	D1S255 1p31
27cM	D1S200 1p31
36cM	D1S216 1p22
cen	D1S248 1p13

Pedigrees with more than one affected member (excluding parent–child pairs) were included in the analysis. In practice, about 10 of the 27 families were included in the APM analysis. This method has the advantage of not requiring a specific mode of inheritance to be specified, which may be advantageous when studying modifier loci with uncertain penetrance and mode of action. The single locus APM program was used, with the $1/\sqrt{p}$ weighting function favoured, as recommended. The SIM program was used to provide simulated distributions of the APM statistic for each marker. APM was

used in preference to affected sib pair methods of analysis, because the structure of the families and the available blood/tissue samples meant that few pairs of affected sibs could be studied, but that several pairs of affected non-sib relatives existed.

QUANTITATIVE SIB PAIR ANALYSIS

The Haseman-Elston method¹⁶ was used to determine the regression of phenotype differences on the number of alleles shared by pairs of sibs. This method may help to minimise confounding effects, such as environmental differences, that may occur between more distantly related subjects. Affection status in this method is not defined in the way used for the pedigree linkage and APM methods; rather, the Spigelman score is used as a quantitative variable. In order to prevent the results of the analysis from depending on a few outlying data points, the absolute phenotypic difference between sibs was used in preference to the square of this difference in the regression calculations. The families provided 38 pairs of sibs and, where identity by descent was impossible to determine, expected allele sharing i bs was calculated; t tests were used to determine the significance of any correlation obtained (after correcting degrees of freedom for the few multiple sibships in the data). A non-parametric trend test was run alongside the regression analysis as a check of consistency.

Results

The pedigree linkage (lod score) analysis provided no good evidence for the existence of a dominant modifier (suppressor) of duodenal FAP on the short arm of chromosome 1 (table 4). In one case (D1S255, 2–3 split), a maximum lod score of +0.82 was obtained at $\theta = 0.0$, but inspection of haplotypes shows this to result from low information of this marker

Table 4 Summary of maximum lod scores (Z_{max}) obtained from pedigree linkage analysis at recombination fraction θ under the three types of affected status classifications used

Locus	1–2 split		2–3 split		"Best" split	
	Z_{max}	θ	Z_{max}	θ	Z_{max}	θ
D1S199	0.00	0.50	0.02	0.25	0.00	0.50
D1S200	0.00	0.50	0.04	0.28	0.02	0.35
D1S214	0.00	0.50	0.00	0.50	0.00	0.50
D1S216	0.00	0.50	0.00	0.50	0.00	0.50
D1S228	0.00	0.50	0.00	0.50	0.00	0.50
D1S243	0.06	0.15	0.00	0.50	0.05	0.22
D1S248	0.00	0.50	0.00	0.50	0.00	0.50
D1S255	0.00	0.50	0.82	0.00	0.08	0.22

Table 5 Results of APM analysis¹⁵ corrected using the SIM program. Only statistically significant results are shown (using a corrected threshold of $p < 0.01$ for statistical significance). The $1/\sqrt{p}$ weighting function is used, but results under the 1 and $1/p$ weightings are very similar. SIM was run for 100 replicates

Split	Locus	APM statistic	Probability
2-3	D1S228	4.50	<0.001
2-3	D1S243	3.92	<0.002
"Best"	D1S228	4.65	<0.002
"Best"	D1S243	3.30	<0.005
"Best"	D1S199	2.39	<0.007

Table 6 Results of Haseman-Elston regression analysis on sib pairs. The regression equation is shown for each locus, together with correlation coefficient ρ , and probability value appertaining to the t statistic calculated from the regression analysis (degrees of freedom = 35). In the equations, y = absolute phenotypic difference between sib values (varies from 0 to 4) and x = allele sharing at chromosome 1p between those sibs (varies from 0 to 2, corrected for ibs where appropriate)

Locus	Regression equation	ρ^2	p
D1S243	$y = 1.56 - 0.41x$	0.086	<0.075
D1S214	$y = 1.32 - 0.22x$	0.029	<0.316
D1S228	$y = 1.19 - 0.12x$	0.007	<0.624
D1S199	$y = 1.06 - 0.00x$	0.000	<0.981
D1S255	$y = 1.26 - 0.17x$	0.019	<0.421
D1S200	$y = 1.03 + 0.03x$	0.001	<0.895
D1S216	$y = 0.94 + 0.10x$	0.006	<0.654
D1S248	$y = 0.93 + 0.11x$	0.004	<0.724

in families were it would be expected not to segregate with disease. Almost all other lod scores reached a maximum value of barely above 0.0, usually at $\theta = 0.5$. Even if non-stringent significance thresholds (for example, $Z_{\max} > 2.0$) are used to assess lod scores for evidence of linkage, therefore, the results from these families do not suggest that a gene with an effect analogous to *Mom1* acts in human FAP families. However, under the parameters used, no lod score falls below the -2.0 required to exclude genetic linkage of a FAP modifier locus to chromosome 1p. HOMOG analysis provided no evidence in support of genetic heterogeneity when testing the possibility that some families were linked to chromosome 1p and others to an unknown locus elsewhere (details not shown).

Results from APM analysis suggest, however, that there is a tendency for non-random segregation of alleles at certain of the loci studied with respect to those affected (that is, with low severity of disease). APM data are summarised in table 5. Using a non-stringent threshold for significance ($p < 0.01$, table 4), alleles at loci D1S199 (1p34), D1S228 (1p35), and D1S243 (1p36) (table 2) were shared more often than expected by affected pedigree members. This sharing was strongest under the 2-3 severity split, which is the most plausible biologically for defining affection status, and under the "best" split. The probability values obtained were similar, irrespective of the APM weighting function used (details not shown). For the 1-2 split, the same loci showed the strongest evidence of allele sharing, but typically at a level of significance a factor of 10 lower.

The Haseman-Elston quantitative sib pair method showed a negative correlation between the number of alleles shared and the absolute difference between Spigelman scores at the D1S243, D1S214, and D1S228 loci (table 6). These correlations were not statistically significant, although that at D1S243 approached

significance at the 5% level. The non-parametric trend tests were in agreement with the regression analysis results in all cases (details not shown). The Haseman-Elston results therefore provide support, albeit limited, for the results of the APM analysis and supply further evidence for a modifier of FAP on chromosome 1p35-p36.

Discussion

There is evidence that a locus on chromosome 1p modifies the phenotype of duodenal FAP in humans. Alleles at this locus probably do not act as simple, dominant suppressors of duodenal disease, since the lod scores derived from pedigree linkage analysis are almost uniformly negative. Any human FAP modifier on 1p does not, therefore, act in the same way as *Mom1* in inbred mouse strains. This is unsurprising, given the otherwise relatively uniform genetic background of these strains compared with an outbred population of mice or humans. A modifying effect on FAP of alleles on 1p is, however, suggested by the results from APM analysis. These results are corroborated to some extent by quantitative sib pair analysis. There is no multipoint version of the APM program, but results suggest that any human FAP modifier is likely to map to band 1p35 or 1p36. These bands are syntenic with the location of *Mom1* and 1p35, in particular, is the corresponding position of *pla2s*, the *Mom1* candidate locus.

Some caution must be applied to the interpretation of these results. Several tests, albeit non-independent, have been performed on the data and the stringency used in assessing possible linkage ($p < 0.01$) is lower than that used for classical linkage analysis. The possibility of false positive (type I) errors must therefore be borne in mind. APM analysis, moreover, is sensitive to allele frequencies and necessarily only analyses a subset of families in this case, since many families do not provide more than one subject with mild duodenal disease, and some of those that do only provide parent-offspring pairs, which are of no value to APM. Nevertheless, the consistency between APM under different weighting functions and under different definitions of affection status (1-2, 2-3, and "best" splits) both provide some confidence that the results are evidence of a genuine effect of a chromosome 1p locus.

If, indeed, there exists a modifier of FAP on chromosome 1p35-p36, it remains to be characterised. Clearly, the human PLA2G2A (mouse *Pla2s* homologue) should be the first candidate to be studied in duodenal FAP. It would be especially interesting to determine whether the colonic phenotype of FAP patients were influenced by PLA2G2A or another locus on 1p; for reasons given above, this would be problematical, although not impossible. Given that most FAP patients undergo prophylactic colectomy for colorectal cancer, duodenal adenomas and carcinomas remain one of the main causes of mortality in these people. The characterisation of a modifier of duodenal disease might therefore be of greater clinical sig-

nificance than a modifier of colonic polyposis. For similar reasons, the identification of a locus affecting the formation of intra-abdominal desmoids in FAP patients would also be important.

The significance of loss of heterozygosity (LOH) on chromosome 1p in sporadic human colorectal carcinomas also remains to be investigated. MacPhee *et al.*¹⁰ suggested that LOH would not be expected at PLA2G2A, owing to its non-cell autonomous action. Mapping of a minimum region of allele loss would allow this reasoning to be tested, ideally using polymorphisms yet to be identified within the PLA2G2A gene. It is possible, like BRCA1,¹⁷ that LOH on chromosome 1p affects a different locus, even if a human *Mom1* homologue does exist there. The high frequency of LOH in cancers other than those of the colon^{18,19} is consistent with this possibility.

The existence of modifier loci means that all diseases are potentially "complex". The study of truly complex human genetic disease, such as diabetes mellitus, asthma, hypertension, and rheumatoid arthritis, provides many problems, but these diseases are quite common, at least, and the supply of samples for study does not provide an insurmountable challenge. By contrast, the study of modifier loci in rare mendelian diseases is severely limited by the number of families suitable for study. This is true even for a relatively "common" rare disease such as familial adenomatous polyposis. Mouse models are one way of identifying candidate modifier loci for human mendelian disease by overcoming this problem. This study has provided evidence to suggest that the mouse modifier of the *Min* phenotype may have a human homologue that affects familial adenomatous polyposis.

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- 1 Bodmer WF, Bailey CJ, Bodmer J, *et al.* Localization of the gene for familial adenomatous polyposis on chromosome 5. *Nature* 1987;328:614-16.
- 2 Leppert M, Dobbs M, Scambler P, *et al.* The gene for familial polyposis coli maps to the long arm of chromosome 5. *Science* 1987;238:1411-13.
- 3 Solomon E, Voss R, Hall V, *et al.* Chromosome 5 allele loss in human colorectal carcinomas. *Nature* 1987;328:616-19.
- 4 Nakamura Y. The role of the adenomatous polyposis coli (APC) gene in human cancers. *Adv Cancer Res* 1993;62:65-87.
- 5 Groden J, Thliveris A, Samowitz W, *et al.* Identification and characterization of the familial adenomatous polyposis coli gene. *Cell* 1991;66:589-600.
- 6 Kinzler KW, Nilbert MC, Su LK, *et al.* Identification of FAP locus genes from chromosome 5q21. *Science* 1991;253:661-5.
- 7 Moser AR, Pitit HC, Dove WF. A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. *Science* 1990;247:322-4.
- 8 Su LK, Kinzler KW, Vogelstein B, *et al.* Multiple intestinal neoplasia caused by a mutation in the murine homologue of the APC gene. *Science* 1992;256:668-70.
- 9 Dietrich WF, Lander ES, Smith JS, *et al.* Genetic identification of *Mom-1*, a major modifier locus affecting *Min*-induced intestinal neoplasia in the mouse. *Cell* 1993;75:631-9.
- 10 MacPhee M, Chepenik KP, Liddell RA, Nelson KK, Siracusa LD, Buchberg AM. The secretory phospholipase A2 gene is a candidate for the *Mom1* locus, a major modifier of *Apc*(*Min*)-induced intestinal neoplasia. *Cell* 1995;81:957-66.
- 11 Johnson LK, Frank S, Vades P, Pruzanski W, Lusi AJ, Seilhamer JJ. Localization and evolution of two human phospholipase A2 genes and two related genetic elements. In: Wong PYK, Dennis EA, eds. *Phospholipase A2*. New York: Plenum Press, 1990.
- 12 Spigelman AD, Williams CB, Talbot IC, Domizio P, Phillips RK. Upper gastrointestinal cancer in patients with familial adenomatous polyposis. *Lancet* 1989;ii:783-85.
- 13 Gyapay G, Morissette J, Vignal A, *et al.* The 1993-94 Genethon human genetic-linkage map. *Nature Genet* 1994;7:246-339.
- 14 Terwilliger J, Ott J. *Handbook of human genetic linkage*. Baltimore: Johns Hopkins University Press, 1994.
- 15 Weeks DE, Lange K. The affected pedigree member method of linkage analysis. *Am J Hum Genet* 1988;42:315-26.
- 16 Haseman JK, Elston RC. The investigation of linkage between a quantitative trait and a marker locus. *Behav Genet* 1972;2:3-19.
- 17 Futreal PA, Liu Q, Shattuck-Eidens D, *et al.* BRCA1 mutations in primary breast and ovarian carcinomas. *Science* 1994;266:120-2.
- 18 Martinsson T, Weith A, Cziepluch C, Schwab M. Chromosome 1 deletions in human neuroblastomas: generation and fine mapping of microclones from the distal 1p region. *Genes Chromosomes Cancer* 1989;1:67-78.
- 19 Dracopoli NC, Harnett P, Bale SJ, *et al.* Loss of alleles from the distal short arm of chromosome 1 occurs late in melanoma tumor progression. *Proc Natl Acad Sci USA* 1989;86:4614-18.