Explaining variation in familial adenomatous polyposis: relationship between genotype and phenotype and evidence for modifier genes


Background: Familial adenomatous polyposis (FAP) is characterised by variable phenotypic expression. Part of this is attributable to a relationship between APC genotype and phenotype but there remains significant intrafamilial variation. In the Min mouse model of FAP, differences in the severity of gastrointestinal polyposis result from the action of modifier genes.

Aims: To determine whether phenotypic variation in human FAP has an inherited component consistent with the action of modifier genes.

Method: We systematically examined polyp numbers in colectomy specimens from patients with classical FAP. Variation both between and within families was analysed. Formal modelling of the segregation of disease severity in families was performed.

Results: There was strong evidence for a relationship between site of mutation and the number of colorectal polyps, with germline mutations in the “cluster region” causing the most severe disease and those with mutations between codons 1020 and 1169 having the mildest disease. In addition to this genotype-phenotype relationship, we found evidence for non-APC linked genetic modifiers of disease expression. First degree relatives had more similar polyp counts than more distant relatives. Formal modelling of the segregation of disease severity in families revealed further evidence for the action of modifier genes, with a best fit to a mixed model of inheritance.

Conclusion: Our data provide good evidence to support the hypothesis that modifier genes influence the severity of FAP in humans.

COLORECTAL CANCER

Familial adenomatous polyposis (FAP) is a disease characterised by multiple colorectal adenomas, specific extracolonic features, and a greatly increased risk of gastrointestinal cancer. Most of the clinical features of FAP are characterised by variable expression. For example, the number of adenomatous polyps in the large bowel can vary considerably, independently of age. Furthermore, many extracolonic features, such as intra-abdominal desmoids, are seen in only a minority of cases. Some of the variable expression of FAP reflects the influence of different germline APC mutations. It has been shown, for example, that mutations in exon 9 and towards the 5' and 3' ends of the APC gene are associated with an attenuated phenotype. With respect to classical FAP, the greatest numbers of colorectal polyps are probably produced by germline mutations close to codon 1309. However, apart from these specific sites within the APC gene, the dependence of polyp number on genotype is unknown.

Variation observed within families or groups of individuals with the same germline mutation suggests that factors other than APC genotype can affect disease expression in FAP. Part of the unexplained phenotypic variation may result from the action of modifier genes not linked to APC. Studies of the Min (multiple intestinal neoplasia) mouse model of FAP have shown that the numbers of gastrointestinal polyps vary among strains. Linkage studies have mapped modifier loci to chromosome 4, with secretory phospholipase A2, a prime candidate for a modifier of Min. Linkage studies in humans have however revealed only weak evidence for a modifier gene on chromosome 1p35-p36, the region syntenic to that containing Momt. No functional variants in secretory phospholipase A2 have been identified in humans. Thus there is no direct evidence that the unexplained variation in FAP severity results from inherited factors rather than from alternative mechanisms such as different environments or simply by chance.

Our aims in this study on variation in the severity of colonic FAP as assessed by number of polyps at prophylactic colectomy, were twofold. Firstly, we analysed genotype-phenotype associations in classical FAP according to functional domains of the APC protein. Secondly, we examined the evidence that modifier loci other than APC act to determine the severity of colonic polyposis in humans, and investigated which genetic model, if any, is most parsimonious with respect to the observed pattern of familial aggregation.

METHODS

Data collection

In total, 221 patients (124 male, 97 female) with classical FAP (>100 colorectal adenomas) who had undergone prophylactic colectomies were entered into this study, having been systematically ascertained through the St Mark's Hospital Polyposis Registry, Harrow, UK. The germline APC mutation was known for 175 patients who were therefore employed for the analysis of genotype-phenotype associations. A total of 166 individuals from 55 families were available for intrafamilial analysis of modifying genes; in 128 of these patients, an APC mutation had been identified. Median age of patients at colectomy was 21.1 years and 45% were aged 14–21 years at the time of colectomy (interquartile range 17.8–29.7 years). Polyp counts on colectomy specimens were performed routinely, as described previously, and corrected for differences in colon...
length. Although adenoma number appears to depend on patient age—for example, very young children with APC mutations rarely have macroscopic polyps—we have previously shown that age is unlikely to be a major determinant of severity in the patients analysed in this study because polyp counts change little over time once patients are in their teens and older. Nevertheless, we reassessed the influence of age on the number of polyps using regression analysis in the group of patients studied here, some of whom had been studied in the previous analysis.

**Relationship between genotype and phenotype**

Germline APC mutations were denoted by their nucleotide and/or codon position using the APC cDNA reference sequence (Genbank NM_000038). To analyse the relationship between genotype and phenotype, APC mutations were grouped according to their position within the functional domains of the gene (fig 1). The presumption was that truncating mutations in the same region of the gene are likely to result in similar phenotypic effects. The domains defined were: (i) pre-armadillo region (codons 168–453); (ii) armadillo repeat region plus (codons 454–1019); (iii) β-catenin binding region (codons 1020–1168); (iv) post-β-catenin binding region (codons 1169–1250); (v) central mutation cluster region (MCR, codons 1250–1400); and (vi) post-MCR (codons 1400–1580). Affected members of the same family were all assumed to have an identical APC mutation, whether or not the mutation had been established within all affected family members. Data were log transformed on the basis of the observed distribution, thereby improving the normality of the different groups and reducing the differences in regional variances (details not shown). Differences in the severity of polyposis (number of adenomas at colectomy) between regions were assessed using F and t tests. In order to adjust for multiple testing, the Bonferroni correction was applied to significance levels.

**Evidence for modifying genes**

In general, closer relatives from a FAP kindred would tend to share alleles at a modifier locus and thus have more similar phenotypes. To examine intrafamilial effects independent of germline APC mutation, we first computed correlation coefficients between different types of relatives within families (siblings, parent-offspring, and second degree relatives) using log transformed data which had been corrected for germline mutations. All standard statistical analyses were undertaken using the program STATA (version 7.0; Stata Corporation, 702 University Drive East, College Station, Texas 77840, USA). To assess the evidence for a major gene component against polygenic effects, we performed a segregation analysis under a mixed model using the program PAP version 4.0. Evidently, as only individuals with FAP could be scored for number of polyps, unaffected individuals were treated as unknown. Significance tests were based on standard likelihood ratio tests (that is, to test competing hypotheses, minus twice the log likelihood calculated under the general model was subtracted from the likelihood when one or more parameters were held constant, the difference being distributed as a χ² with a number of degrees of freedom equal to the number of free parameters).

**RESULTS**

There was no significant relationship between age and number of adenomas (r²=0.09, p>0.3) after patients were stratified by mutation, implying that polyp number changes little around the time that colectomies are undertaken. These data are therefore in agreement with our previous analysis using paired data which showed no significant effect of age on polyp count in patients having prophylactic colectomy.

Therefore, for the purpose of this specific analysis, age correction was not applied. Figure 1 shows the severity of colonic polyposis according to mutations in each of the six functional domains of APC. The most severe disease was associated with mutations within the MCR (mean polyp count 3459). Mutations within the pre-armadillo region and 3′ to the MCR were associated with moderate polyposis (mean polyp counts 1087 (937.4) and 1454 (1551), respectively), while mutations in the remaining domains were associated with mild colonic polyposis ( arma (mean 646 (766.3); β-catenin, mean 703 (1024); post-β-catenin, mean 550 (870.6)).

Twenty two of the affected individuals harboured mutations within the MCR of APC. Fourteen of these were carriers of the 1309 del5bp mutation. There was no difference in the number of colorectal adenomas in these patients compared with those who possessed other MCR mutations (p=0.66). Similarly, there was no difference between the number of adenomas in the 29 patients with the common mutation at codon 1061 in the β-catenin binding domain compared with individuals with other mutations in this domain (p=0.98).

To compute correlations between different groups of relatives and combine data across the different APC domain groups, trait values were Z transformed:

\[ z = (x - \mu) / s \]

where \( x \) is log transformed polyp count; \( \mu \) is the mean for the APC domain group; and \( s \) is the standard deviate for the APC domain group.

There was a highly significant correlation (r=0.42) in the severity of disease in sibling pairs (table 1). The correlation between parent-offspring pairs (r=0.29) was also significant but weaker whereas the correlations between more distantly...
related relatives were much weaker and non-significant (table 1). This pattern of familial correlation is consistent with a genetic component influencing FAP severity but not related to APC mutation.

Table 2 shows the results of segregation analysis. Single gene models fitted the data better than a simple polygenic model. Of the two single gene models, the dominant model fitted the observed data better than the recessive model. There was however evidence from the segregation analysis to suggest that a single gene genetic model is not adequate to explain the distribution of polyps in families and that a more complex model is required. Both single gene models fitted the data less well than the mixed model ($\chi^2 = 7.7$, comparing a dominant model with a mixed model; $\chi^2 = 9.2$, comparing a recessive model with the mixed model).

**DISCUSSION**

There are a number of possible sources of variation in the expression of the FAP phenotype. In our study of classical FAP, we have found clear evidence of a relationship between the site of the germline APC mutation and the severity of the colonic phenotype, as measured by the number of adenomatous polyps at prophylactic colectomy. While previous data emphasised the different mutation spectrum in classical and attenuated FAP, functional polymorphisms such as MTHFR may provide a genetic explanation for a higher correlation between siblings than among parent-offspring pairs. There is evidence for such a mechanism in some mendelian diseases. While to date there is no direct evidence for such an effect in FAP, functional polymorphisms such as APC1307K do exist.

Since the identification of Mmt1 in the Min mouse, the existence of modifier genes for FAP has been the subject of intense speculation. We have provided evidence that the clinical phenotype in human colonic FAP is consistent with the action of modifier genes. Direct confirmation of the hypothesis will require identification of the responsible genes. In the absence of a clear model of inheritance of FAP severity in our data, it is likely that identification of FAP modifier genes will rely on testing candidate loci. These candidates include genes implicated in colorectal carcinogenesis a priori such as CDX2, MTHFR, and DNA repair genes, and genes located within chromosomal regions syntenic to susceptibility loci in mouse models. Although there may be some limited benefits for clinical practice in FAP, identification of FAP modifier genes is most likely to be useful in providing insight into mechanisms of colorectal tumorigenesis, indicating possible therapeutic targets and suggesting excellent candidates for colon cancer susceptibility genes in general.

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