Explaining differences in the severity of familial adenomatous polyposis and the search for modifier genes

Familial adenomatous polyposis (FAP) has become the focus of several convergent lines of scientific and medical enquiry. The year 2000 has seen the completion of the human genome project and this coincides with the next major challenge of cancer genetics, non-mendelian inheritance. Colorectal cancer remains a major source of morbidity and mortality in developed countries. Despite huge advances in understanding the pathology of the disease, its mortality has remained virtually unchanged in 50 years. The disease is curable if detected early, yet screening strategies for the general population have been largely unsuccessful. FAP is a superb model for sporadic colorectal cancer. If the genetic and other causes of variation in the FAP phenotype can be understood, this knowledge will have a dividend for the general population and may lead to new approaches for screening for colorectal cancer. This article discusses current knowledge of how the clinical features of FAP patients vary, with emphasis on the role of modifier genes, and discusses strategies for their identification.

FAP: an overview
FAP (OMIM 175100) is an autosomal dominant disorder with a population frequency of approximately 1 in 8000 and a penetrance of almost 100%. The disease is typified by florid adenomatous polyposis of the colon and rectum, although a less severe variant, attenuated adenomatous polyposis (AAPC), also exists. Patients typically present with hundreds to thousands of polyps in the colon. The minimum number for a diagnosis of classical FAP is 100. Progression of one or more of these polyps to carcinoma is almost inevitable unless prophylactic colectomy is undertaken. In addition, FAP is characterised by a number of extracolonic manifestations in some individuals: congenital hypertrophy of the retinal pigment epithelium (CHRPE); epidermoid skin cysts and benign craniofacial and long bone tumours (Gardner’s syndrome); fibrous tumours (desmoids) classically sited in the retroperitoneum and abdominal wall; and upper gastrointestinal polyposis. Upper gastrointestinal cancers, especially in the duodenum, are a serious problem in some patients who have undergone prophylactic colectomy. Other malignancies which are seen in a relatively small proportion of FAP patients include non-medullary thyroid cancer and hepatoblastoma. Desmoid disease and duodenal cancer account for the major mortality attributable to FAP in patients who have undergone colectomy.

The APC protein functions, at least some of the time, as a dimer owing to homotypic interactions between N terminal coiled coil domains. The main function of APC may be to bind to and cause the degradation of β-catenin in association with axin/conductin and glycogen synthase kinase 3β. This function is mediated through a series of seven 20 amino acid repeats in the APC protein. Three SAMP repeats, which are crucial for axin/conductin binding, lie at codons 1581, 1730, and 2043. Most truncating APC mutations seen in FAP or sporadic colorectal tumours lead to a protein with one or two remaining β-catenin binding/degradation domains and no SAMP repeats. APC also contains a series of armadillo repeats, which may bind β-catenin without degrading it, although their exact function is unknown. C terminal APC functions include domains for binding to EBI, human homologue of Drosophila discs large protein, and microtubules (via its basic domain). Although knowledge of β-catenin function—both in the normal cell and as an oncogene—has increased rapidly, the role of APC remains largely unknown. Not least of several intriguing enigmas is the special role of APC in colorectal tumorigenesis when most other epithelial tumours harbour no APC mutations but often carry β-catenin mutations.

Types of phenotypic variation in FAP and measuring the phenotype
Individuals with FAP have many clinicopathological features which are subject to variation both within and between families. As regards colonic disease, the number of colorectal adenomas clearly varies considerably, from several thousand to zero in known gene carriers. Variation among families with different APC mutations is more dramatic than within families but it is not uncommon for different members of the same FAP or AAPC family to have hundreds versus thousands, tens versus hundreds, or tens versus zero adenomas. The greater the number of colorectal adenomas, the greater is the risk of colorectal cancer. Variation in the severity of colonic disease may result either from a greater number of tumours being initiated or from faster tumour progression. It may be extremely difficult to distinguish between these possibilities using clinical or histological methods, whether using criteria of adenoma number or some alternative such as size.

With the possible exception of the caecum, the variation in adenoma distribution along the colorectum in any FAP

Abbreviations used in this paper: FAP, familial adenomatous polyposis; AAPC, attenuated adenomatous polyposis; CHRPE, congenital hypertrophy of the retinal pigment epithelium; DRP, discordant relative pair; APC, adenomatous polyposis gene.

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patient is essentially random (Crabtree et al, unpublished). It is likely that the ratio of adenomas to colonic crypts is approximately constant for a given individual, within the limits of a random distribution, regardless of location within the colon. The simplest way to measure the severity of colonic polyposis is to count the number of polyps seen on the fixed (and therefore washed) colectomy specimen. Most polyposis centres use an estimate of total polyp number based on counting the number of polyps within a sample area of fixed dimension and then correcting for total colonic mucosal area. This is often performed on a regional basis, the final count being the sum of counts from the individual regions of the colorectum.

It could be argued that macroscopic polyp counts are inaccurate and potentially confounded by three types of error. Firstly, the sensitivity of naked eye examination is such that there is a minimum size which can be detected by this methodology. Two individuals with different macroscopic counts may have the same absolute number of adenomas; therefore what is being observed could be a difference in polyp size—a different type of phenotypic variation—rather than number. Secondly, colonic dimensions may account for differences in macroscopic count—that is, the larger the colon the larger the polyp count—although this should not vary if the same area is counted and scaled by the same factor in different individuals. Thirdly, colonic polyposis is a slowly progressive disease and therefore comparison of individuals should take into account the age at which polypectomy is performed.

A simple approach to deal with these variables is to examine the severity of the polyposis microscopically. The severity of polyposis can be graded by estimating the ratio of adenomas to colonic crypts. This has been done retrospectively and the approach shows a good correlation between microscopic appearances and macroscopic count (Crabtree et al, unpublished). Similarly, using this approach it is possible to show that colonic size is not a major confounding variable. Age has a small effect on the severity of colonic polyposis. We have estimated the absolute increase in polyp number to be of the order of 5% per annum after the early teens.

In addition to variation in the number of colonic adenomas, a number of other features of FAP are subject to phenotypic variability, including: age of onset of colorectal cancer; upper gastrointestinal disease; desmoid disease; CHRPE; features associated with Gardner's syndrome; and extracolonic neoplasia. Variation in some of these features, such as the development of malignancy,7 is relatively well described, although probably subject to stochastic or environmental influences. For other features, however, it is difficult to be confident about the degree of inter- and intrafamilial variation, owing to the different methods used by clinicians. Some features, such as CHRPE,18 show unequivocal consistent interfamilial variation (see below). For other traits, even the existence of consistent variability is uncertain: for upper gastrointestinal disease, for example, a standardised Spigelman score exists19 but some centres do not use this score, and others do not routinely undertake screening of duodenal polyposis.

Sources of differences in disease severity

Perhaps the most striking source of phenotypic variability in FAP families is the site and/or type of the germline APC mutation. Severe colorectal disease—typically over 1000 colorectal adenomas and earlier onset of cancer—is associated with germline mutations between codons 1250 and 1460.23 The most severe disease is associated with germline mutations around codon 1300, including the most common germline mutation at codon 1309. Mild disease is associated with truncating germline mutations in the 5′ and 3′ regions of the APC gene (so called attenuated FAP or AAPC). Although some mutations, such as those in the alternatively spliced region of exon 9 of APC,15–17 consistently produce a mild colonic phenotype, the precise spectrum of mutations which cause AAPC is not known. AAPC patients may, moreover, develop severe upper gastrointestinal disease.23–25 Mild colonic disease is also associated with missense APC variants, such as I1307K26 and E1317Q.27 The I1307K variant is said to increase tumour risk by creating a hypermutable site. E1317Q, however, presumably exerts an influence through some functional effect and it may be premature to exclude a functional effect of the nearby I1307K variant.

The reasons for the association between germline mutation and the severity of colonic FAP are unknown. One possibility is that the probability of tumour initiation and/or progression is related to optimal retention of N terminal and loss of C terminal functions in the APC protein; β-catenin binding and degradation repeats may be of particular importance in this regard. Another possible underlying mechanism is the stability of the truncated APC protein. The common codon 1309 5 bp deletion is reported to produce a very stable truncated protein, whereas mutations in the 5′ and 3′ regions of the APC gene may produce unstable protein28 and/or act in a dominant negative fashion.29 A broader spectrum of “second hits” may also be selected in FAP patients with germline mutations near codon 1300, effectively raising the somatic mutation rate.30 The tendency to develop desmoids and CHRPE in FAP families also depends on the site of the germline mutation. While exceptions have been reported, desmoids are associated with germline APC mutations—and indeed somatic mutations—between codons 1403 and 1578.31 CHRPE is associated with mutations between codons 463 and 1387.32 Presumably, these associations reflect the different roles of the APC protein and the importance of each of the functional domains in the cells of origin of these lesions.

In addition to allelic heterogeneity at APC, some families with a phenotype like FAP or, more commonly, like AAPC, do not result from germline APC mutations and/or are not linked to 5q21.12–14 Thus locus heterogeneity may also determine disease severity in a small number of cases. Generally, these non-APC, FAP-like families have few or none of the extracolonic features associated with FAP or AAPC proper.

The position and type of the germline APC mutation cannot explain variation in disease severity within or between families with the same germline mutation. Some of this residual variation results from chance somatic mutation—particularly for extracolonic features—or inconsistent clinical practice. There is good evidence, for example, that the use of dye spray at colonoscopy can lead to identification of over 1000 polyps in patients reported to have fewer than 10 tumours.34 Differences in the micro- or macroenvironment may also account for some differences in disease severity between individuals carrying the same germline APC mutation. Desmoids are associated with pregnancy35 and intra-abdominal surgery.36 It has been proposed that carcinogens such as bile affect the severity of upper gastrointestinal and colonic FAP.37 Other ingested and/or circulating carcinogens could, in theory, affect tumorigenesis in FAP just as they could in sporadic tumours.

Modifier genes and FAP

despite potential differences caused by environment, clinical practice, or chance, same sex siblings in their late teens or early twenties often show large phenotypic differences in FAP which cannot easily be explained except by invoking the action of modifier genes.38–41 The idea of modifier genes
is an old one. Classical genetic theory is founded on two competing theories, mendelian (single gene) inheritance and polygenic inheritance. Mendelian inheritance assumes that a single locus gives rise to a phenotypic parameter based on the inheritance of alleles with either negative or dominant properties, such as eye colour. Polygenic theories of inheritance flow from the biometric work of Galton, and later Fisher, and explain the inheritance of quantitative traits, such as height and intelligence, as the cumulative effect of many alleles working at different loci. The reality is that these inheritance patterns represent opposing ends of an inheritance spectrum which allows inheritance possibilities intermediate between these two dichotomies. One such intermediate state is that of essentially discontinuous traits controlled by single loci, which are rendered pseudo-continuous by modifier genes acting to vary the expression of traits resulting from one gene. Modifier genes were originally discussed in the particular context of genetic dominance and natural selection of butterfly mimics. In the context of human disease, modifier genes have been defined as inherited genetic variation (distinct from disease locus) that leads to a quantitative or qualitative difference in any aspect of disease phenotype.\(^{42}\) Strictly, modifiers do not determine whether or not a disease develops, but this definition is likely to be relaxed under certain circumstances in practice (for example, failure of a BRC41/2 mutation carrier to develop breast cancer in her lifetime).

Modifier genes for mendelian diseases such as FAP are also likely to be low penetrance risk alleles for colorectal cancer and modifiers of other related mendelian diseases.\(^{42}\) Therefore, if modifier genes can be found for FAP they may offer insight into and new management strategies for sporadic colorectal cancer. Modifier genes have been shown to act, or have a very high probability of existence, in a number of other disease states. For example, Easton and colleagues\(^{32}\) have shown that variation in the severity of neurofibromatosis is consistent with the action of modifier alleles. Modifier alleles are almost certainly common polymorphisms; otherwise, their effects would simply go unnoticed. Commensurate with the theory that modifier genes are acting on FAP is the expectation that variation between individuals increases as their genetic risk decreases: for example, cousins would tend to differ more in their disease severity than siblings because the latter would be more likely to carry the same modifier alleles.

Accurate measurement of clinical features will allow this theory to be tested using statistical analysis of variance.

Important evidence for the existence of modifier genes in FAP comes from work on the Min mouse model of polyposis. This has demonstrated the existence of a modifier locus (Mom1) on mouse chromosome 4 in the region syntenic with human chromosome 1p35-p36.\(^{44}\) Linkage studies detected some evidence for a human FAP modifier gene on 1p.\(^{41,44}\) Soon after the linkage studies were completed, the secretory phospholipase A2 (PLA2S) gene was identified as a strong candidate for Mom1 in the mouse (although it still remains possible that another gene nearby is the true modifier). No functional variants of PLA2S were however identified in humans and the existence of a modifier of human FAP on 1p therefore remains unproven.\(^{39,40}\)

### Searching for FAP modifier genes in humans

Identification of cancer modifier genes will have three benefits. Firstly, the management of patients with the mendelian tumour syndrome will improve: patients who carry modifier alleles predisposing to severe disease would, for example, receive more intensive or radical therapy or screening. Secondly, identifying modifier genes is a crucial initial step in understanding fundamental cancer biology as regards how mutations in different genes interact in genetic pathways of tumorigenesis. Thirdly, modifier genes for mendelian disease are excellent candidates as common low penetrance genes which have a very important effect on increasing cancer risk in the general population: a gene which, say, causes a benign inherited lesion to progress rapidly to cancer is highly likely to have the same effect on sporadic forms of the same benign lesion. The study of modifier genes for mendelian disease may well be a more efficient way of identifying some low penetrance cancer predisposition genes than the alternative methods, such as large scale association studies.

FAP is well suited to modifier gene identification compared with other mendelian tumour syndromes, particularly as regards variation in the number of colonic polypos, for the following reasons. (1) FAP is a disease of multiple tumours thus minimising the variation between individuals caused by chance. (2) It is an early onset disease thus reducing environmental effects but usually without immediate mortality. (3) FAP tumours probably only require one somatic mutation to occur for a tumour to grow in contrast with hereditary non-polyposis colon cancer in which cancers must acquire several somatic mutations and are therefore more likely to be subject to chance and environmental influences. (4) Phenocopies and misdiagnoses are rare and there is very little evidence for locus heterogeneity, at least in classical FAP. (5) Most FAP families are under the care of specialist registries and therefore are well recorded. (6) International collaborative groups for FAP already exist.

(7) The APC gene itself is extremely well characterised. (8) FAP has well described histology and natural history. (9) Lastly and very importantly, there are very strong genetic and histological parallels between the pathogenesis of FAP tumours and the equivalent sporadic cancer, carcinoma of the colorectum.\(^{39}\) The consistency of available clinicopathological data and the above considerations strongly suggest that the identification of FAP modifier genes should concentrate on the number of colonic polyps as the phenotypic variable of choice.

Power calculations have shown that “discordant relative pairs” (DRPs) are probably best for identifying FAP modifier genes by linkage analysis (and possibly for association analysis also). Each DRP should come from the same family and both should be affected by FAP; one must have severe disease and one must have mild disease. Definitions of “severe” and “mild” must, to some extent, be heuristic, but rational criteria can be set up. The difference between individuals probably exists as a continuum: some relative pairs show little difference, some show extreme differences. The minimum measurable difference between individuals for microscopic adenoma crypt ratios is dependent on the density of polyposis (that is, the probability that an individual crypt is an adenoma) and the number of crypts sampled. The limits of confidence for this methodology can be determined with probability theory using the binomial or Poisson distributions. For macroscopic data, a difference of greater than the upper limit of a confidence interval generated from a random sampling approach can be used. This approximates to an individual having twice as many polyps as his affected relative, once minor effects such as polynics and age have been compensated for. Using the above criteria, approximately 30% of sibling pairs are discordant for colonic polyposis severity. Initial data (Crabtree et al, unpublished) suggest that a greater proportion of cousins are discordant using this methodology, consistent with the existence of modifier genes.

Candidate genes with possible effects on the severity of colonic FAP include the following: the putative human homologue of Mom1 (1p35-p36); APC 11070K (in...
Ashkenazim); APC E1317Q: carcinoma metabolism genes such as members of the NAT family; SMAD3; COX2; and Har-1 VNTR. These and other candidate polymorphisms—wherever possible with putative functional effects—can be tested for associations with severe or mild disease in DRPs; a sample size of about 100 would be required. If alleles at candidate loci are found to be associated with disease severity, other variations in the same gene or nearby (within 0.5 cM approximately) must be searched for and tested for separately for association with disease severity.

To identify new FAP modifier genes, about 150 DRPs (generally siblings) would be required if a genome wide screen using highly polymorphic markers were to be undertaken at a map density of about 10 cM. DRPs would be analysed for significant lack of allele sharing at each marker. Regions with evidence of linkage would be fine mapped by typing all additional markers in the region.

A APC may be subject to different modifier genes than classical FAP. Although AAPC is relatively poorly described, it is known to be associated with protein truncating germline APC mutations in exons 4 and 9 which undergo physiological alternative splicing to leave an infarne mRNA missing that exon 9. Other exons—such as 1, 2, 3, 10a, 11, 12, 13 and 14—may also undergo physiological or pathological splicing out from APC mRNA. Alternative splicing has also been proposed for the other early exons of APC whether using cryptic splice sites or in combination with other exons. It is possible that mild disease of AAPC results because many patients can produce a near fully functional APC protein by splicing out the exon containing their germline mutation from the APC mRNA. One study has found evidence that the severity of disease in an AAPC family with exon 9 mutations was associated with the levels of alternative mRNA splice variants of this exon 9; in other words, the relative levels of the truncated (and possibly unstable) protein and the splice variant protein determined disease severity. Candidate genes for AAPC modifiers must act in trans; APC itself is the best candidate for effects on mRNAs splicing but disease severity would not then segregate independently of disease. Other candidate genes include the SR proteins and hnRNPA1.

Translating results into clinical practice

Predictive testing for modifier genes may allow patients to be given information about their disease and encourage intervention (such as prophylactic colectomy) at a later or earlier age, based on APC and modifier genotypes. In practice, AAPC patients (and patients with missense APC variants or hereditary non-polyposis colorectal cancer if modifier genes are found to be important for these diseases) may actually benefit more than FAP patients because modifier genotypes may influence the often difficult clinical decision as to whether or not to manage these patients conservatively (by regular colonoscopy) or by prophylactic colectomy. Testing of modifier genes would assist greatly in making these decisions, in association with clinical data and data on the specific germline APC mutation.

Conclusions

Variability in the FAP/AAPC phenotype is very well characterised compared with other mendelian diseases. The causes of this variation are partly established. In particular, there are convincing associations between the site or type of the germline mutation and the number of colorectal adenomas, the age specific risk of colorectal cancer, CHRPE, and desmoid disease. There is also good evidence—from humans and, particularly, from mouse models—for the involvement of modifier genes which influence the severity of FAP. Several of the features which comprise the FAP phenotype may be subject to the influence of modifier genes. Currently, however, it is only practical to analyse the effects of modifiers on the number of colonic polyps developed by individuals with the same germline APC mutation. Identification of modifier genes by association and linkage analysis—for example, using relative pairs with discordant severities of disease—can provide insights into disease processes, affect the clinical management of FAP/AAPC, and provide important information regarding the risk of colorectal cancer in the general population.


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