The genetics of inherited colon cancer

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Colorectal cancer accounts for approximately 20 000 deaths per year in the UK and neither the morbidity nor the mortality have improved significantly in the past 50 years. None of the possible screening procedures have been shown to be both effective and inexpensive as well as acceptable to patients, and none has shown any decrease in mortality. One group of patients who can, however, be targeted immediately for screening are those known to be at high risk of developing colon cancer because of a familial predisposition to the disease. There are a number of conditions which predispose to colorectal cancer (table 1). In recent years two of these, familial adenomatous polyposis (FAP) and hereditary non-polyposis colon cancer (HNPCC), have been studied in detail, the causative genes isolated and mutations identified in the germline of affected individuals. This has enabled screening of high risk individuals to be carried out and prophylactic surgery performed before the development of malignant tumours. The genes associated with these inherited conditions have also been shown to play a role in the development of sporadic colon cancer, so that many of the molecular changes associated with non-inherited disease have begun to be more clearly defined. In this leader, the current understanding of the structure and function of these genes and their role in colorectal cancer is discussed.

Table 1 Inherited conditions predisposing to colorectal cancer

<table>
<thead>
<tr>
<th>Condition</th>
<th>Details</th>
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<tbody>
<tr>
<td>Familial adenomatous polyposis</td>
<td>Autosomal dominant inheritance; multiple polyps</td>
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<tr>
<td>Hereditary non-polyposis colon cancer (HNPCC)</td>
<td>Genomic instability; sporadic polyps; Lynch syndromes (types 1 and 2)</td>
</tr>
<tr>
<td>Peutz-Jeghers syndrome</td>
<td>Genomic instability; multiple hamartomas</td>
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<tr>
<td>Juvenile polyposis</td>
<td>Genomic instability; multiple hamartomas</td>
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<tr>
<td>Gorlin syndrome</td>
<td>Genomic instability; multiple hamartomas</td>
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**Familial adenomatous polyposis**

**CLINICAL FAP**

Familial adenomatous polyposis is a dominantly inherited predisposition to colorectal cancer.\(^1\) It has an incidence of approximately 1 in 10 000\(^2\) and accounts for 1% of all cases of colorectal cancer.\(^3\) Affected individuals typically develop hundreds to thousands of adenomatous polyps in the colon and rectum usually before their 20th birthday (fig 1).\(^1\)

Histologically, the vast majority of polyps are tubular adenomas, with only occasional polyps having villous features.\(^1\) Without treatment, at least one of these adenomas will progress to carcinoma (fig 2).\(^4\) The mean life expectancy of an untreated patient with FAP is 40 years, with death usually caused by metastatic colorectal cancer.\(^4\)

Approximately 30–40% of FAP cases are caused by a new mutation\(^7\) and subsequently many already have cancer at the time of diagnosis. However, in FAP families, cancer may be prevented in siblings and first degree relatives of...
affected individuals by prophylactic colectomy immediately upon the detection of colonic disease. Until recently, the only available method for the early detection of affected individuals was to look directly for colonic disease by colonoscopy. The manifestations of FAP are not restricted to the colon and rectum and most patients exhibit both benign and malignant extracolonic features. Benign lesions most commonly include epidermoid cysts, osteomas, congenital hypertrophy of the retinal epithelium (CHRPE) (fig 3), desmoid tumours, and upper gastrointestinal polyps. Epi-
dermoid cysts, osteomas and CHRPE lesions are present many years before the onset of colonic polyposis and may be used as clinical indicators of FAP gene carriers.

In addition to colorectal cancer, patients with FAP are at an increased risk of developing other malignancies, including thyroid cancer, hepatoblastoma, gastric cancer, medullo-
blastoma, and carcinomas of the peri-ampullary area, bile duct, and duodenum.

GENETICS OF FAP
Constitutional mutations in the adenomatous polyposis coli gene (APC) are responsible for FAP. APC was localised to the long arm of chromosome 5 in 1986, when an interstitial deletion within 5q13-22 was detected cyto-
genetically in a mentally retarded patient with FAP. Genetic linkage to FAP was then confirmed using a polymorphic DNA marker known to map to 5q. In situ hybridisation using this probe narrowed the position of the APC gene to 5q21-22. Several linked polymorphic DNA markers were discovered subsequently and used to track the inheritance of the disease gene in FAP families.

Genetic linkage methodology may provide accurate risk estimates of gene inheritance, especially when markers from both sides of the gene are used. In the clinical setting the risk estimate given by linkage analysis will determine the appropriate level of colonic surveillance for individual patients with FAP.

Several genes within the 5q21-22 region were investigated as potential candidates for FAP before the APC gene was eventually isolated and sequenced in 1991. APC was the only gene in which germline transmission of mutations could be identified by DNA sequencing. Since its isolation, a wide range of mutation detection systems have been used to analyse directly the APC coding region for disease-causing mutations in FAP families, giving 100% accurate presymptomatic diagnosis (fig 4). At risk individuals shown not to have a mutation by a direct test can be removed from any further colonic surveillance programmes. However, one group recommends that colon screening should be performed at three time points in mutation negative patients, to take account of the possibility of laboratory error.

MUTATION ANALYSIS OF THE APC GENE
The APC protein is coded for by approximately 8500 bases of DNA. A large number of germ-line mutations in the APC gene have been identified worldwide. Mutations from 320 patients with FAP have been reviewed (Wallis et al, unpublished data) and are
Figure 5  (A) Number and distribution of germline mutations in the APC gene taken from31,45 and Wallis et al (unpublished data). The two main “hot spots” can be seen at codons 1061 (34 cases) and codons 1309 (69 cases). (B) Number and distribution of somatic mutations in the APC gene taken from36 and43. The localization of the majority of mutations to the mutation cluster region (codons 1280–1500) can be seen.

inherent in fig 5A. Even though mutations have been detected along the whole length of the gene, 98% of them are found over the first two thirds of the coding region (fig 5A). One third of the patients have mutations at two “hotspot” regions, both 5 base pair deletions, at codons 1061 and 1309. Of the remaining 218 patients, 150 different APC mutations have been reported and of these, 80% are unique. Mutation studies have demonstrated that the predominant mode of inactivation is chain-termination. In the series of mutations shown in fig 5A, 94% produced a premature stop codon, either directly by base substitution or indirectly by frameshift. Splice site mutations predicted to produce an abnormally sized protein product accounted for a further 4% of cases. It is still not completely clear whether amino acid changes identified in a small number of patients with FAP are true disease causing alterations, although in a number of cases such alterations have been the only changes found, even after sequencing the entire gene.

The heterogeneous distribution and nature of APC mutations makes direct detection of mutation of the gene itself a difficult task. Most APC mutation screening procedures involve the analysis of DNA segments amplified by the polymerase chain reaction (PCR). A variety of methodologies have been used for this purpose, including denaturing gradient gel electrophoresis (DGGE), single stranded conformational polymorphism, heteroduplex analysis, and RNase protection. However, a different approach, the protein truncation test (PTT), which takes advantage of the predominant chain-terminating mode of inactivation, has been used recently to detect mutation.44,45 In this test amplified segments of the APC gene are transcribed into RNA, which is then translated to protein. Both steps are carried out in vitro. A germline chain-terminating mutation will produce a truncated protein product, which can be detected by polyacrylamide gel electrophoresis (fig 6). The advantages of this technique are improved sensitivity and the ability to analyse the entire coding region in five overlapping PCR reactions, instead of the 40 or so required in more conventional methods.

APC MUTATION DISTRIBUTION: GERMLINE VERSUS SOMATIC
Somatic APC gene inactivation has been shown to play an early role in sporadic colorectal carcinogenesis. Mutations are identified in approximately 65% of adenomas and carcinomas even in tumours with a diameter of less than 0-5 cm.46 Somatic inactivation of the second APC allele has also been implicated in the pathogenesis of FAP adenomas, where mutations are detected in up to 80% of these neoplasms.47,48 A summary of 367 somatic APC mutations, identified in both FAP and sporadic tumours, is presented in fig 5B. APC
Like germline mutations, the majority (99%) of somatic mutations occur within the first two thirds of the gene and more than 95% are chain-terminating.
However, a striking difference is seen between the distribution of germline and somatic mutations (figs 5A and 5B). More than 80% of somatic mutations occur between codons 1280 and 1500, compared with only 25% of germline mutations. This region has been referred to as the mutation cluster region (MCR) and it may be that a mutation within this region, in at least one APC allele, is necessary for growth advantage.

GENOTYPE–PHENOTYPE CORRELATIONS IN FAP
In keeping with the theory that mutations in the MCR result in a greater propensity for tumour formation, three separate studies have correlated mutations within this region with severe colon cancer in FAP. A study from Japan52 correlated profuse polyposis (>5000 polyps) with the occurrence of germline mutations between codons 1250 and 1464, whereas sparse polyposis (<2000 polyps) was seen in patients with mutations outside this region. Also, in 1994, Caspari et al53 and Gayther et al54 correlated a severe FAP colonic phenotype (poly number, age of onset and age of death due to colorectal cancer) with the common 5 base pair deletion at codon 1309.
At the other end of the spectrum, APC mutations in patients with an attenuated form of FAP, who develop fewer colonic polyps at a later age, have been shown to occur at the extreme proximal end of the gene.55
GENOTYPE–PHENOTYPE CORRELATIONS FOR EXTRACOLONIC MANIFESTATIONS

Both intra- and interfamilial differences in the expression of extracolonic manifestations (ECM) are seen in FAP. With the accumulation of mutation data researchers have strived to determine whether the APC mutation position may also be a factor in the variability of ECM expression.

A significant correlation has been made between the position of APC mutations and the expression of CHRPE, the retinal pigment-pigmentation present in up to 90% of patients with FAP which exhibits minimal intrafamilial variation. Mutation studies have shown that retinal lesions are always present in patients with mutations between codons 413 and 1387, whereas they are absent in patients carrying germline mutations outside this region. For newly diagnosed CHRPE positive patients, this correlation has reduced the region to screen for mutations to just 35% of the coding region, effectively halving the workload.

A further correlation between the position of the mutation and “desmoid tumours” has been determined in a recent study, in which 90% of patients with FAP with a mutation distal to codon 1445 were shown to possess these tumours.

Human and murine studies suggest that the FAP phenotype is modified by other unlinked loci. In 1993, Paul et al described a wide spectrum of phenotypes in patients with FAP sharing identical APC mutations. In addition, the expression of the FAP-like phenotype in the Min mouse model, produced by a germline mutation in the murine APC homologue, is affected by different genetic backgrounds. The modifying effect has been linked to the MOM-1 (Modifier Of Min) locus on mouse chromosome 4, a region corresponding to human chromosome 1p35. The gene for secretory type II phospholipase A2 (Pla2s) has recently been proposed as a candidate for MOM-1, in which high concentrations of the gene product correlate with a resistance to tumour formation.

It can only be a matter of time before modifying loci are identified for human FAP.

The expression of different splice forms of the APC gene may also explain the variable phenotype seen in FAP. In particular, one splice variant has been identified that lacks exon 1, a region known to be essential for homodimerisation and interactions with other proteins.

APC PROTEIN STRUCTURE–FUNCTION RELATIONS

The cellular function of the APC protein remains elusive mainly because it has little sequence similarity to other proteins. Antibody studies have identified it as a cytoplasmic protein, expressed in the upper portions of epithelial colonic crypts, suggesting that it is functional in the mature colocyte.

Several structural features have been identified along the 2843 amino acid length. It is the aim of current research programmes to assign specific functions to these regions, to clarify both its normal cellular function as well as the mechanism of action of mutant truncated protein forms.

The first third of the APC protein contains a series of heptad repeat sequences predicted to form α-helical structures, known to facilitate protein dimerisation. Protein–protein interactions may be a prerequisite for the normal function of this protein. In addition, the potential for dominant interference by the truncated mutant form has been demonstrated in vivo by wild-type–mutant associations. Two repeat regions within the middle third of the APC protein have recently been shown to associate with an adherens junction protein called β-catenin. Adherens junctions are required for cell adhesion and cytoskeletal integrity, thereby regulating normal cell growth and behaviour. The implications of this interaction are not exactly known. However, both a downstream targeting role for signalling by β-catenin has been proposed, as well as a regulatory role for APC, as interaction with the distal binding domain has been shown to down-regulate the cytoplasmic β-catenin pool.

Finally, the wild-type APC protein has recently been shown to associate with cytoplasmic microtubules. This activity is localised to a basic domain contained in the carboxy-terminal region, and is therefore absent in mutant truncated molecules. The precise implications of this association are unclear, but microtubule polymerisation is known to be induced by APC molecules. It may be that the APC protein controls epithelial cell growth by serving as a link between the catenin molecules and the microtubule cytoskeleton.

Hereditary non-polyposis colon cancer

Hereditary non-polyposis colon cancer accounts for about 5–10% of all colorectal cancer. Like FAP, it is an autosomal, dominantly inherited condition. Tumours develop slightly later in life than in FAP, with a mean age of diagnosis of 40–45 years, and are found predominantly in the proximal, rather than the
distal, colon. Although adenomas are found in the colons of patients with HNPCC, the numbers are similar to those seen in the general population. Synchronous and metachronous tumours are seen in 20–30% of patients. Unlike FAP, the diagnosis cannot be made on an isolated affected individual but is based instead on family history. The “Amsterdam Criteria” are used to diagnose HNPCC and include: (1) at least three relatives must have histologically proven colorectal cancer; (2) one relative must be a first degree relative of another; (3) at least two generations should be affected; and (4) cancer should be diagnosed under 50 years of age. FAP should be excluded.73

HNPCC can be divided into three related syndromes. Colon cancer is the only malignancy seen in Lynch Type 1 or site specific colon cancer. Lynch type 2 disease includes colon cancer along with endometrial, ovarian, small bowel, stomach, hepatobiliary, or transitional cell tumours of the urinary tract.74 Recently, it has been shown that Muir–Torre syndrome is also part of the HNPCC phenotype.75 In this condition, patients have sebaceous gland tumours and other skin cancers such as keratoacanthomas, as well as the spectrum of tumours seen in Lynch type 2 disease.

Until 1993, monitoring of individuals at risk of developing HNPCC depended on regular surveillance by colonoscopy or ultrasound where appropriate. Over the past two years, the localisation and subsequent isolation of the causative genes for the disorder has meant that molecular methods can be used to identify those at high risk so that they can be closely monitored whereas those at low risk could be removed from the screening programme completely or at least have less frequent investigations.

### Mapping of the HNPCC Genes

The initial chromosomal localisation of a gene for HNPCC was achieved by studying two large pedigrees with 345 different microsatellite markers scattered throughout the genome. Co-segregation of the disease was eventually obtained with a marker which mapped to the short arm of chromosome 2 at 2p15.76 Fourteen other families were also studied and at least eight of these showed no evidence of linkage, suggesting the presence of genetic heterogeneity in HNPCC. This was confirmed when a second locus was localised on 3p21.77 Preliminary evidence from linkage studies suggested that these two genes accounted for the majority, but not all cases, of HNPCC.78

### Genomic Instability in HNPCC

An interesting observation was made when the tumours of HNPCC patients were studied.79 A form of genomic instability was noted when DNA from normal tissues was compared with DNA extracted from the tumour of the same patient. Tumour DNA showed widespread alterations in short repeated sequences (microsatellites) distributed throughout the genome. These were seen as additional bands over and above the usual one or two alleles identified in the normal tissue DNA. The finding suggested that replication errors, caused by slippage of DNA polymerase, had occurred during tumour development and had not been repaired. This phenotype, termed RER+ (for replication error positive), was found irrespective of the causative gene in a family.

The mechanism underlying this observation was suggested by previous studies of bacteria and yeast, where it had been shown that defects in mismatch repair genes resulted in instability of short repeated sequences. The best studied mismatch repair system is the DNA adenosine methylase (DAM)-instructed MutHLS pathway in Escherichia coli.80 This pathway is responsible for repair of nucleotide mispairing and occurs by recognition of mismatches by the mutS, mutL and mutH proteins, excision of the region of DNA containing the mismatch and subsequent resynthesis of the DNA. In Saccharomyces cerevisiae, an increase in destabilisation of repetitive DNA of up to 70-fold is caused by mutations in genes of the yeast mismatch repair pathway.81 The bacterial and yeast genes involved in mismatch repair are shown in table 2.

The association between mismatch repair and instability in bacterial and yeast systems suggested that a similar defect in a repair mechanism may be the cause of the instability seen in HNPCC and led to cloning of the genes for HNPCC.

### HNPCC Genes

The strong homology between the human and yeast genes was used by one group to isolate the first of the HNPCC genes. Fishel et al.82 mapped the human homologue of MSH2, called hMSH2, to chromosome 2p15, the region previously implicated by linkage analysis to contain a HNPCC gene. Leach et al.83 used a more traditional positional cloning approach and also identified the same gene. In order to confirm that hMSH2 was indeed the causative gene, both groups were able to show mutations within conserved regions of this gene in HNPCC families. A second human mismatch repair gene, hMLH1, was shown to map to 3p21 implicating it as the second gene involved in HNPCC.84 Again, mutations in this gene were found in HNPCC families.85 Two further human homologues of the mutL gene—hPMS1 and hPMS2—were also found to be mutated in HNPCC families,86 thereby implicating four genes in the pathogenesis of HNPCC (table 1). The hMSH2 gene causes around 60% of cases of HNPCC, hMLH1 causes around 30% and the other two together are the causative genes in the remainder (unpublished data,

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Yeast</th>
<th>Human</th>
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<tr>
<td>MutS</td>
<td>MSH2</td>
<td>hMSH2</td>
</tr>
<tr>
<td>MutL</td>
<td>MSH1</td>
<td>hMLH1</td>
</tr>
<tr>
<td>MutL</td>
<td>PMS1</td>
<td>hPMS1</td>
</tr>
<tr>
<td>MutL</td>
<td>PMS2</td>
<td>hPMS2</td>
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</table>
Table 3  Human mismatch repair genes which cause HNPCC

<table>
<thead>
<tr>
<th>Gene</th>
<th>Location</th>
<th>Size (base pairs)</th>
<th>Protein size (number of amino acids)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hMSH2</td>
<td>2p21</td>
<td>2727</td>
<td>909</td>
</tr>
<tr>
<td>hMLH1</td>
<td>3p21</td>
<td>2268</td>
<td>756</td>
</tr>
<tr>
<td>hPMS1</td>
<td>2q31−33</td>
<td>2795</td>
<td>932</td>
</tr>
<tr>
<td>hPMS2</td>
<td>7p22</td>
<td>2586</td>
<td>862</td>
</tr>
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Eurofap meeting, Budapest 1994). However, the exact figures may vary from one centre to another and are still based on relatively small numbers of families.

The structure and size of the four genes are now known (table 3) and germline mutations have been identified.\(^{85-89}\) Mutation detection in the HNPCC genes has the same problems associated with detection of mutations in APC, further compounded by the fact that four genes rather than one are involved. As with APC, the mutations are to be found widely distributed throughout the genes, with the majority being either point mutations or small insertions or deletions. Most mutations are truncating, that is to say introduce premature stop codons, so that the PTT technique, used to detect mutation in APC, is a useful initial screening method for HNPCC.\(^ {85-86}\) Methodologies similar to those used to screen APC are also useful for a more exhaustive search (fig 7), particularly to identify missense mutations which would be missed by PTT. The majority of mutations are different in each family. Papadopoulos et al\(^ {85}\) identified a 165 base pair deletion in seven kindreds of Finnish origin, but at least five of these could be traced back to a common ancestor. An exon 5 splice site mutation has been found in four of 33 British families and is therefore the commonest mutation so far.\(^ {90}\)

Environmental as well as genetic factors, such as modifier genes, are likely to be involved in determining the phenotype. For example, two mutations in exon 12 of MSH2 have been identified in two families with Muir-Torre syndrome but exon 12 mutations have also been found in classic HNPCC\(^ {91}\) without the association of skin tumours. To date, no genotype-phenotype correlations have been made and it is not even possible to identify which gene might be causative in a particular family from the clinical information.

MODE OF ACTION OF THE HNPCC GENES

Many of genes involved in carcinogenesis are tumour suppressors characterised by loss of heterozygosity (LOH) in tumours as classically described by Knudson for retinoblastoma.\(^ {91}\) Initially, there was no evidence of LOH at either 2p15 or 3p21, suggesting that the mismatch repair genes act via a different mechanism. However, it is now clear that HNPCC does in fact follow a version of the Knudson two hit hypothesis from a number of lines of evidence. Analysis of a limited number of tumours from individuals with germline mutations in MSH2, MLH1 or PMS1 demonstrated the presence of a somatic mutation in the second copy of the gene.\(^ {83-86,91}\) Cell lines with a mutation in only one copy of MSH2 were shown to be proficient in mismatch repair,\(^ {92}\) whereas those lines with deficiencies in mismatch repair have been shown to be hemizygous for a mutation in MSH2 or MLH1.\(^ {94}\) Recently, loss of the wild-type allele was found in up to 25% of tumours from patients with germline mutations in MLH1.\(^ {95}\) All of these studies suggest that inactivation of both alleles is necessary for tumorigenesis.

Two studies have shown how mutations in the mismatch repair genes might lead to the development of cancer by demonstrating that loss of these genes leads to a general mutator phenotype. In other words, mutations in the mismatch repair genes result in a higher than normal mutation rate allowing the accumulation of mutations in other genes such as tumour suppressor genes or oncogenes.\(^ {96-97}\)

MISMATCH REPAIR GENES IN SPORADIC COLON CANCER

Replication errors have also been identified in sporadic colon cancer as well as other tumours including endometrial, breast, prostatic, pancreatic, stomach, and lung tumours. Between 12 and 25% of colorectal tumours are MSK+ and these tumours showed many of the features of HNPCC lesions in that they were frequently diploid or near diploid and occurred in the right side of the colon. Patients with replication errors at more than one microsatellite marker also showed a significant increase in survival.\(^ {98} \) Preliminary results on very small numbers of tumours have suggested that replication errors become evident early in the
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development of colorectal cancer.\(^9\)\(^8\)\(^1\) Young et al.\(^1\) studied a much larger series of 46 adenomas but could find no evidence of instability. Also, it was shown recently that the only adenomas with replication errors were those with foci of carcinoma in situ, suggesting that the errors are manifest at the adenoma–carcinoma transition.\(^1\)

In general, patients with RER+ sporadic colorectal tumours do not have germline mutations in the mismatch repair genes. A recent study has demonstrated that patients with colorectal cancers diagnosed over 35 years of age showed instability in only 12% of tumours. In contrast, 58% of patients with cancer under 35 years of age had RER+ tumours. Close to 50% of these young patients have been shown to have germline mutations in mismatch repair genes,\(^8\) a result which has important implications for genetic testing and subsequent management of their children.

**Summary**

Over the past five years, considerable progress has been made in the study of the genes involved in inherited colon cancer syndromes. These advances have also resulted in important findings for sporadic colon cancer. The identification of the causative genes for FAP and HNPCC has enabled presymptomatic diagnosis to be carried out routinely. High-risk individuals are therefore targeted for screening preventing the unnecessary development of cancers and early death in these families. The identification of genes associated with HNPCC means that it will now become possible to look at a broader group of patients, apart from those who fulfil strict diagnostic classifications, to determine whether this syndrome is more common than previously thought. It also enables us to look for low penetrance mutations which may be responsible for apparently sporadic disease.

Dietary intervention studies are already underway in those patients at high risk of developing FAP and gene therapy studies can now be considered. A similar situation should soon become possible for HNPCC. The next five years should therefore be even more exciting than the past.


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