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The Genetics of Colorectal Cancer

D.T. Bishop and N.R. Hall

INHERITED SUSCEPTIBILITY TO COLORECTAL CANCER

CLINICALLY, we recognise a number of distinct syndromes which predispose to colorectal cancer. The majority of these syndromes are inherited as autosomal dominant genes, which means that a parent carrying a mutation passes on his mutation to, on average, half of his or her children. Offspring who inherit this mutation then have a high chance of developing colorectal cancer. Three broad groups of syndromes are seen (Table 1):

1. Those which predispose to large numbers of adenomatous polyps, each of which has some malignant potential and through which mechanism the increased risk of colorectal cancer is presumably attributable.
2. Syndromes which do not have associations with large numbers of adenomatous polyps, but for which the adenomas appear to have an increased malignant potential.
3. Syndromes associated with hamartomatous (non-adenomatous) polyps and which are associated with less dramatic increases in risk than those whose premalignant lesion is an adenoma.

The recognition and classification of these syndromes has followed many years of careful clinical observation, while the

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Table 1. Hereditary syndromes predisposing to colorectal cancer. All are dominantly inherited, with the exception of Turcot's syndrome where the mode of inheritance is unclear

	Colorectal features	Extracolonic manifestations
Predisposition to large numbers of adenomas		
Familial adenomatous polyposis (FAP)	Multiple adenomas (hundreds to thousands) leading to carcinoma	Duodenal polyposis and periampullary carcinoma; gastric benign hyperplastic fundal polyps, gastric adenomas; congenital hypertrophy of the retinal pigment epithelium (CHRPE); thyroid carcinoma (in females); astrocytoma, medulloblastoma; hepatoblastoma
Gardner's syndrome	As FAP	As FAP plus: epidermoid cysts; osteomas (especially mandible); desmoid tumours
Turcot's syndrome	Variable number of adenomas, may be less than 100	Astrocytoma; medulloblastoma; glioblastoma; focal nodular hyperplasia of the liver; café-au-lait patches; basal cell naevi and carcinoma; seborrheic keratoses
Predisposition to smaller numbers of adenomas		
Attenuated adenomatous polyposis coli (AAPC)	Very variable number of adenomas, from 5 to hundreds	Gastric polyps of the fundal glands
Hereditary flat adenoma syndrome	Variable number, usually less than 100	Possible association with extracolonic malignancy. CHRPE not documented
Hereditary non-polyposis colorectal cancer (HNPCC)	Few adenomas leading to carcinoma	Lynch I: none Lynch II: endometrial cancer, small bowel cancer, urinary tract transitional cell carcinomas, ovarian cancer, upper gastrointestinal cancer, pancreatic cancer and possibly others. Muir–Torre syndrome: as Lynch II plus sebaceous gland tumours, basal cell carcinomas and keratocanthomas.
Predisposition to hamartomas		
Peutz-Jeghers syndrome	Few 'Peutz–Jehgers' type hamartomas	Small bowel hamartomas; perioral and buccal pigmentation. Breast, uterine, ovarian and testicular cancer
Familial juvenile polyposis	Few 'juvenile' type hamartomas	Small bowel polyps; microcephaly, Meckel's diverticulum, mental retardation
Gorlin's syndrome	Few juvenile polyps	Basal cell carcinomas, palmar and plantar pits; broad facies, basal cell naevi, ectopic calcification of the falx, rib abnormalities
Cowden syndrome	Hamartomas (not Peutz–Jeghers type), juvenile polyps, adenomas	Facial papules, oral mucosal papillomatosis, palmoplantar keratosis; fibromas, angiomas, lipomas; benign and malignant disease of breast and thyroid; nervous system abnormalities

identification of the genes which predispose to these syndromes has been the result of the classical approach of linkage analysis. Although this will be discussed in more detail below, the main genes which cause these high penetrance adenoma-associated syndromes have now been identified (Table 2). However, evidence suggests that there may be genes which have a milder effect on risk which are yet to be found, including all of the syndromes in the third group [1, 2].

The syndromes described below each have increased risks of colorectal cancer for mutation carriers, but are rare in the general population. Although precise estimates are not currently available, it has been suggested that between one and five per cent of all colorectal cancer is attributable to these dominant syndromes [3–6]; such estimates require further verification.

FAMILIAL ADENOMATOUS POLYPOSIS

Clinically, the most easily recognisable of these syndromes is familial adenomatous polyposis (FAP). Although the date of the first identification of this syndrome is not clear, publications in the last century show apparent recognition of this entity, while its pattern of inheritance was documented early this century [7]. FAP is first manifested during the second decade of life and is characterised by the development of hundreds to thousands of adenomatous polyps in the colon and rectum. The natural progression is for the numbers of these polyps to increase, their sizes to increase, and for the development of colorectal cancer in the fourth or fifth decade of life, if the patient is left untreated [8, 9]. The current treatment is by proctocolectomy, usually with ileal pouch anal anastomosis, or subtotal colectomy with ileorectal anastomosis. The removal of the colon has the direct effect of removing the mucosa at risk of cancer development but, over time, it has become clear that susceptibility to malignancy extends to sites outside the colon and rectum. For instance, with patients now surviving into their fifties, the risk of upper gastrointestinal polyps has become apparent, and malignancy in duodenal polyps is now a major cause of death in FAP patients.

All patients with FAP share the common feature of multiple colorectal adenomas. In some families, this is the only manifestation of FAP while, in others, family members also have various extra-colonic features. This latter group of families was classified as Gardner syndrome after the late Dr Eldon Gardner [10]. The extra-colonic features include epidermoid cysts, osteomas and

Table 2. Genes involved in hereditary predisposition to colorectal cancer and carcinogenesis

Gene	Chromosomal location	Protein function	Usual type of mutation	Possible role in colorectal cancer
Genes involved in hereditary predisposition and carcinogenesis				
APC	5q21	Cell adhesion?	Deletion/mutation	Initiation of adenomas
hMSH2	2p16	DNA mismatch repair	Mutation	Allows further mutations
hMLH1	3p21	DNA mismatch repair	Mutation	Allows further mutations
hPMS1	2q31-q33	DNA mismatch repair	Mutation	Allows further mutations
hPMS2	7p22	DNA mismatch repair	Mutation	Allows further mutations
Genes involved in progression of neoplasia				
TP53	17p13	Transcription factor	Deletion/mutation	Progression of adenomas
DCC	18q21	Cell adhesion	Deletion/mutation	Progression of intramucosal to invasive carcinoma
RAS family		GTP binding proteins	Mutation	Progression of adenomas
KRAS	12p	Signal transduction		Metastasis
NRAS	1p			
HRAS	11p			
CMYC	8q24	DNA synthesis/apoptosis	Over-expression	May act at all stages
Genes with putative role in carcinogenesis:				
MCC	5q21	Unknown	Mutation/deletion	Uncertain
DRA	7q22-q31	?Transcription factor	Uncertain (LOH rare)	Adenoma formation
RB1	13q14	?Transcription factor	Amplification	Uncertain
NM23	17q22	NDP kinase activity	Allele loss/mutation	Metastasis

LOH, loss of heterozygosity.

desmoid tumours which, although benign, are locally invasive and difficult to remove surgically [9]. However, as with most dominant conditions, there is variable phenotypic expression exhibited in related family members (despite them, by necessity, carrying the same mutation in the polyposis gene) [2].

Although precise estimates of population frequency are always difficult to obtain, one of the most detailed studies estimated that at birth one in 8000 had the familial polyposis mutation [11]. This high frequency and the ready identification of families made the syndrome a natural candidate for linkage studies. A number of groups were involved in this study searching throughout the genome. The important lead came in 1986 when Herrera and colleagues [12] treated a patient with Gardner's syndrome and mental retardation, who was subsequently found to have a deletion of a portion of chromosome 5q. Direct investigation of the FAP families with DNA markers which mapped in this region demonstrated linkage. Interestingly, families that had extra-colonic manifestations, as well as those without, appeared to map to the same region [13, 14]. Although there has been the occasional suggestion of a family with FAP not due to this gene (for instance, Stella and colleagues [15], and the subsequent debate of Burn and Chapman [16]), the overall level of heterogeneity is very low.

In 1991, the gene was cloned and sequenced [17, 18]; the gene is now termed *APC* (for adenomatous polyposis coli) and consists of 15 exons, the last of which is 6578 nucleotides in length. The protein product consists of 2843 amino acids. The majority of the germline mutations are distributed over the first half of the gene, with few families showing the same mutation. The most notable exception is a five base-pair deletion at codon 1309 which has been found to be associated with younger development of colonic adenomas and correspondingly earlier malignancy [19]. Almost all of the mutations in the *APC* gene result in nonsense mutations, deletions causing frame shifts or other inactivating mutations resulting in a truncated protein [19–23]. Because of this, it has been possible to use the detection of a truncated protein as the basis for a mutation detection system [24]. One such assay was successful in detecting a shortened protein product in 82% cases [25]. Although the DNA sequence of the *APC* gene gives little information with respect to its function, it has been observed that the *APC* gene product associates with β-catenin and perhaps α-catenin, suggesting a function in cell adhesion [26, 27].

A number of features relating to mutations have become apparent with subsequent research (Figure 1). First, as mentioned above, families that were classified as Gardner's syndrome and those which were classified as FAP are now shown to be due to mutations in the same gene; in fact, there are examples of particular mutations which have been identified both in patients with FAP and with Gardner's syndrome [28] so that we shall use the term FAP to include both sets of patients for the rest of this discussion. Other factors (presumably environmental or genetic) must, therefore, explain the considerable variable expression in phenotype both within and between families. The correlation of mutations with phenotype has also shown that there is a very clear association between site of mutation (and hence size of protein product) and the presence of CHRPEs (areas of congenital hypertrophy of the retinal pigment epithelium which is only visible by ophthalmoscopy). In one study, 60% of FAP patients have one or more CHRPE [29]. It has been shown that most patients with CHRPE have mutations in exons 9 to 15, while only rarely do patients with mutations in earlier exons have these eye lesions [30]. The number of polyps in the colon also seems to relate to the site of *APC* mutation. For instance, Nagase and colleagues [22] found that families which have profuse polyp covering (over 5000 polyps) have mutations between codons

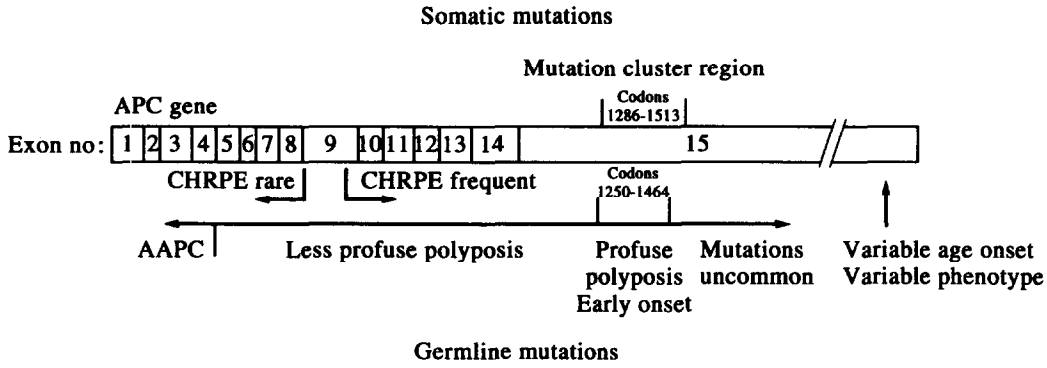

Figure 1. The *APC* gene: diagrammatic representation of the 15 exons which comprise the gene. The sites of mutations occurring in the germline of patients with FAP and related syndromes are shown in the lower half, along with their phenotypic consequences. In the upper half, the common region for somatic mutations occurring both in FAP and sporadic colorectal cancer is shown.

1250 and 1464, while families with less than 2000 polyps have mutations in other parts of the gene (Figure 1). Only one family with a mutation beyond the middle of the gene has been identified, and this family shows a milder, more highly variable phenotype [31].

While there are many families with the clear cut phenotype of FAP, there are a small number of families in which there is an apparent high risk of colorectal cancer and a variable number of colonic polyps. Within these families, some individuals have tens of polyps while other gene-carrying family members have only a few (although still in excess of what is normally seen in the general population). Leppert and associates identified one such family, and showed that the family was linked to the *APC* locus [32]. Further families with a similar phenotype are now classified as having attenuated adenomatous polyposis coli (AAPC) [33]. These and other families have been found to have mutations in the 5′ end of the *APC* gene, all within the first four exons [34].

Lynch and colleagues [35] also identified another syndrome, the hereditary flat adenoma syndrome, in which small tubular adenomas are observed in family members; these adenomas are frequently dysplastic. Owing to the relative paucity of polyps and yet strong colorectal cancer risk, these families were initially thought to belong to the hereditary nonpolyposis colorectal cancer syndrome (described below). The families also have features of AAPC and their true genetic cause has been clarified following demonstration of linkage to the *APC* gene in two families [36].

HEREDITARY NON-POLYPOSIS COLORECTAL CANCER (HNPCC)

The HNPCC syndromes are mainly associated with a predisposition to colorectal cancer, but there is no evidence of any increased numbers of adenomas in the bowel. Awareness of this syndrome is largely due to Dr Henry Lynch [6, 37] who has published extensively on families with this syndrome. He credits the first identification of such a syndrome to the family identified by Warthin in 1913 [38]. This family contained individuals with cancers in the uterus, stomach and intestine. Through extensive analysis of other families, Lynch classified the families into two categories:

1. Hereditary site-specific colorectal cancer or Lynch syndrome I. In this syndrome, affected family members have only colorectal cancer.
2. Cancer family syndrome or Lynch syndrome II. Affected family members develop extra-colonic tumours as well as their colonic tumours. The most common of the extra-colonic tumours is that of the endometrium, although the risk appears to extend to the stomach, ovary, upper renal tract, small bowel and possibly other sites [39].

An example of an HNPCC family is shown in Figure 2 [40]. A third category called the Muir–Torre syndrome is sometimes included separately, although this may just be a variant of Lynch syndrome II [41, 42].

3. Muir–Torre syndrome. Affected family members may develop tumours which are at the same sites as in Lynch syndrome II, although some family members also develop benign and malignant skin lesions, characteristically sebaceous gland tumours (Table 1) [43–45].

Family studies suggest that those classified as Lynch I are rare as compared to the Lynch II families. Within each of these families, it is clear that a single dominant gene is sufficient to explain the inheritance of overall risk, although there is a wide variation in the sites of malignancy observed in different family members. One of the difficulties of these syndromes is that, in general, there is no hint of gene carrier status to act as a clue to the clinician for those at increased risk. This contrasts with FAP where early development of polyps with or without observation of CHRPE is diagnostic. Certain features, apart from a dominant inheritance of cancer susceptibility, are a valuable guide in the diagnosis of HNPCC, although none alone is a distinguishing characteristic [46–48]. The peak age of cancer development is young, being around 45 years, and in the colorectum this is 20 or 30 years before the peak age of onset of the sporadic form. Two thirds of the colorectal tumours are located proximal to the splenic flexure, again in contrast to sporadic colorectal cancers which are predominantly distally sited. There is also an increased incidence of metachronous disease and of multiple primary cancers in different organs. A detailed review of the hereditary syndromes has recently been published [49].

The first strong evidence for linkage for an HNPCC gene came from the study of Peltomäki and colleagues [50], who showed that in two extensive families there was linkage to the short arm of chromosome 2. This finding followed an extensive search of the genome using several hundred microsatellite markers and investigation of a large portion of the genome before linkage was observed. Detailed analysis of these families showed that the gene lay in a region approximately 5 cM in size. Subsequently a joint analysis of families typed for markers in

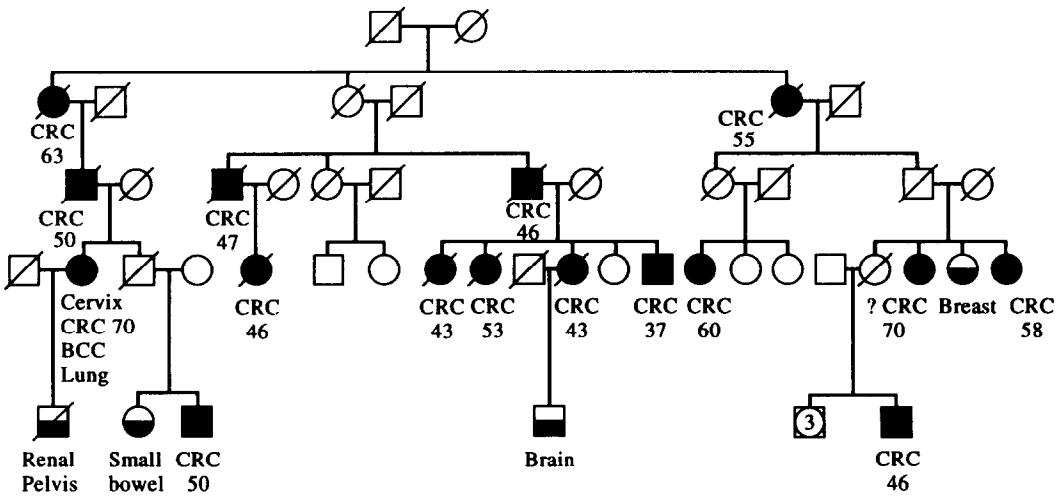

Figure 2. A typical HNPCC pedigree: this family was originally reported by Dunstone and Knaggs in 1972 [40]. It now stretches over six generations with over fifty members having cancer, and only part is shown here. It displays the typical features of dominant inheritance of cancer susceptibility at young age. Most of the cancers are of the colorectum (CRC) but there are a few cancers in other sites, notably the renal pelvis and small bowel. It is not clear whether this should be classified as a Lynch I or Lynch II family and the distinction between the two no longer appears to have a genetic basis. Key: ○ Female; □ male; ⊘ ⊡ deceased; ● ■ Colorectal cancer; ○ □ Other cancer; BCC, basal cell carcinoma. Age of cancer diagnosis is shown beneath the symbol.

this region and reported to the EUROFAP HNPCC Linkage Consortium suggested that this gene explained approximately 60% of HNPCC families [51]. There was strong evidence that at least one other gene had to be involved since there were a number of extensive families which were clearly due to inherited predisposition but which were not due to this chromosome 2 gene. However, much of this heterogeneity was found to be explained by the observation of linkage to chromosome 3p in a set of families not linked to the 2p locus [52].

The majority of the genes related to inherited susceptibility have been shown to be tumour suppressor genes [53]. These genes presumably control cellular differentiation and repression of proliferation; failure of the system means that cells can go into a less restrained growth phase enabling the clonal expansion of these cells. Such clonal expansions may develop into a malignancy should other requisite genetic events occur within the same clone. The usual method of identifying tumour suppressor genes is to examine the loss of heterozygosity (LOH) of DNA sequences surrounding the location of the familial gene (and implying therefore that if these flanking sequences are lost, then so is a copy of the familial gene). For instance, for the breast cancer gene, *BRCA1*, analysis of tumours obtained from obligate gene carriers in *BRCA1*-linked families showed that the wild-type (i.e. the chromosome inherited from the spouse without the mutation) was lost during the development of the malignancy [54].

Similar studies of the HNPCC gene, however, produced rather startling findings. Instead of finding evidence for loss of the wild type chromosome in family members carrying the chromosome 2 mutation, Aaltonen and colleagues [55] showed that, in the majority of these tumours, alleles of sizes distinct from those in the germline were also observed; these markers are technically termed 'microsatellite' or 'dinucleotide repeat' markers since their DNA sequence contains a number of cytosine (C) and adenine (A) nucleotides adjacent to each other. Such tracts are highly polymorphic in humans (i.e. there is wide variation in the precise number of CA units in unrelated individuals). In some cases, the number of CA repeats had increased over that seen in the germline, while in others the number had decreased; the original germline bands could also be seen (Figure 3). This modification in allele sizes could be shown in microsatellite markers from all over the genome, and suggested that the genes involved in inherited susceptibility to colorectal cancer were also involved in the faithful replication of DNA. This facet of the tumours was noted for both the chromosome 2 locus and the chromosome 3 locus through the examination of tumours from families linked to these regions [52, 55].

The observation of microsatellite instability in HNPCC tumours provided a short cut to the identification of the responsible genes. Unlike the situation for breast cancer, where there was no known function to the *BRCA1* gene, and hence only positional cloning was available to those attempting to identify the precise gene, knowledge of this phenotype suggested that genes involved directly in DNA replication or repair could also be responsible for HNPCC. In 1993, Strand and colleagues [56] showed that, in yeast, mutations in any one of three DNA mismatch repair enzymes led to several hundred-fold increases in the stability of such tracts of CA repeats. In yeast, the genes are known as *PMS1*, *MLH1* and *MSH2* [57], and they are homologues of the well-characterised mutHLS system in *E. coli* [58–60]. The search for human homologues of these yeast genes, as candidates for the HNPCC genes, immediately proved successful when the human homologue of *MSH2*, termed *hMSH2*, was shown to be precisely at the chromosome 2p HNPCC locus [61, 62]. Mutation analysis of the *hMSH2* showed mutations which were transmitted within HNPCC families and shared by affected relatives. Similarly, the homologue of *MLH1* (*hMLH1*) was identified, and this mapped to the region of chromosome 3 encoding that HNPCC gene. Again, mutations in this gene correlated with inherited susceptibility [63, 64]. All of the mutations currently characterised lead to aberrant or truncated products (some of which involve deletions of whole exons) which provides convincing evidence that these are the genes involved in susceptibility to HNPCC [65, 66]. Subsequent to this analysis, two other genes *hPMS1* and *hPMS2*, which

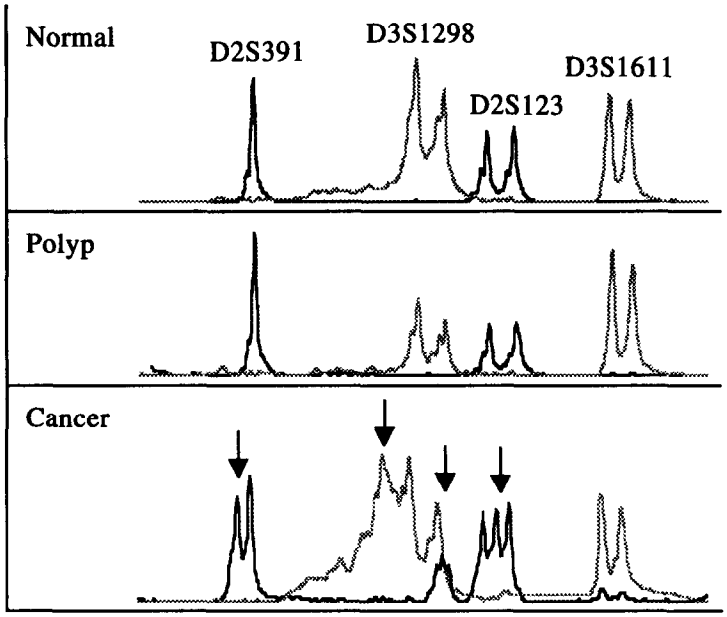

Figure 3. Microsatellite instability: the three panels represent alleles from normal, adenomatous and malignant tissue extracted from a member of a family with a germline mutation of *hMSH2*. Four microsatellite markers have been amplified using the polymerase chain reaction and the products detected by fluorescence. In the figure, the location of the peaks indicates the sizes of the alleles for the particular marker. The marker D2S391 is homozygous, showing only one peak, whereas the other three are heterozygous (two peaks). The pattern seen in tissue from the polyp is the same as that of normal mucosa, whereas in the cancer, four new alleles are present (arrowed). Microsatellite instability is evident in all markers except D3S1611. The markers examined here happen to be close to the locations of *hMSH2* and *hMLH1*, although similar evidence of instability in the cancer would be seen for any other markers chosen.

are homologues of *PMS1*, have been implicated in HNPCC susceptibility and may, indeed, explain a small percentage of all HNPCC families [67].

Although this is an early stage in analysis, there is little evidence to suggest that the phenotypes encoded by mutations in these various genes produce distinct cancer phenotypes. For instance, Hall and colleagues [68] showed that two families classified as Muir–Torre were due to mutations in *hMSH2* as were families classified as Lynch syndrome II [50]. It may therefore be that mutations in either of these genes could produce the same phenotype (i.e. Lynch syndrome I, Lynch syndrome II and Muir–Torre syndrome). This finding is not unexpected, since all these genes probably act in the same pathway. Inactivation of any one is, therefore, likely to produce the same overall effect on cancer susceptibility.

FAMILIAL CANCER

While the observation of families with dominant inheritance of colorectal cancer provides overwhelming evidence that a powerful genetic susceptibility is important in some families, epidemiological studies provide equally strong evidence to indicate that other weaker genetic or environmental factors must also be involved in susceptibility to bowel cancer, even where there is no obvious hereditary component. These studies are conducted by interviewing a large number of cases with colorectal cancer and a similar number of controls (individuals of the same age and sex as cases but without a diagnosis of colorectal cancer), and obtaining a family history of each of these individuals. All studies conducted to date have shown that families of colorectal cancer cases are more likely to have a history of colorectal cancer than families of controls [69]. This increased risk of cancer in the relatives has been estimated to be between a two- and four-fold increase over that of the general population. Similar enquiries performed using individuals with adenomatous polyps (but without malignancy) have shown that their family history of colorectal cancer is similar to that of colorectal cancer cases (for a review, see Bishop and Thomas [69]). This provides further evidence of the importance of the adenoma in the development of colorectal cancer. Family studies have also shown that the risk of colorectal cancer in the relatives of colorectal cancer cases is higher if the cancer is diagnosed at a particularly young age [5, 70]. For instance, relatives of an individual with colorectal cancer, diagnosed before the age of 45 years, have a 12% risk of developing bowel cancer by the age of 80 years, while relatives of cases diagnosed between 45 and 54 years have a risk of 7.5% by the age of 80 years and relatives of older onset cases have a risk of 4% by the age of 80 years [5]. The risk is also increased if there are multiple affected close relatives with colorectal cancer [71].

Segregation analysis, which attempts to explain the family aggregation of colorectal cancer in terms of the most likely genetic explanation, has, in each analysis conducted to date, suggested a dominant mode of inheritance [72–74]. However, these studies have obtained quite distinct estimates depending on whether the disease of interest is considered to be the phenotype of adenoma presence in family members as in the study of Cannon-Albright and associates [73], or if the phenotype is the actual occurrence of colorectal cancer as in studies by Houlston and colleagues [72] and Bishop and colleagues [74]. For the phenotype of colorectal cancer, the latter two studies have suggested a rare, dominant gene with a lifetime risk of the order of 50% to gene carriers, while the analysis of polyps suggested that a more common genetic susceptibility was acting and that the majority of adenomas developed in those who were genetically predisposed. The difference in these results is not easy to interpret as the analyses involved different assumptions and were based on quite distinctly collected family groups. The one general conclusion is that an inherited susceptibility could

explain a greater proportion of colorectal cancer than that observed in the high risk HNPCC families.

To this end, and given the uncertainties of segregation analysis, a number of groups have attempted to identify the specific genes involved in determining the risk of colorectal cancer. Natural candidates for these genes would be mutations in the genes already known to predispose to colorectal cancer, but in which the mutations were less destructive. For instance, a mutation in the *APC* gene which does not lead to a truncated protein might lead to a protein which had a modified, less effective behaviour. Currently, no such mutations have been identified. A candidate mutation in the *hMSH2* gene was found to be equally prevalent in individuals without colorectal cancer, those with colorectal cancer at any age or those with colorectal cancer at a particularly young age [75], and hence this particular mutation is presumably no more than a polymorphism which has no effect on colorectal cancer risk.

Epidemiological evidence strongly suggests that environmental factors and, in particular, diet play a significant role in colorectal cancer formation [76]. For instance, the majority of studies which have examined protein intake and its association with colorectal cancer risk have produced significant findings. Genes which modify possible dietary determinants of risk may therefore affect susceptibility. The main candidate genes so far investigated are those coding for enzyme systems which detoxify carcinogenic and other substances and this area is reviewed in detail by Smith and colleagues in this issue. It has been known for many years that an individual with a particular phenotype, which is known to be determined by the acetylator locus (a genetically determined system for the detoxification of arylamines, which are present in large amounts of cooked protein), is at increased risk of colorectal cancer [77, 78]. This increased risk for the "fast" acetylator phenotype over the "slow" phenotype (the two principal phenotypic variants) is of the order of 2-fold. More recently, others have shown that a null glutathione S-transferase M1 phenotype (a system which involves catalysing the conjugation of glutathione in a variety of carcinogens and cytotoxic drugs) is also associated with a moderate increased risk of colorectal cancer [79]. Similarly, an association between various alleles at the *HRAS* locus and various malignancies, including colorectal cancer, has been documented [80]. Detailed analysis by Kadlubar and associates [81] has suggested that individuals who were both rapid acetylators and had a particular CYP1A2 type (a cytochrome p450 protein involved in catalysing the N-oxidation of aromatic amines) had a 2.5-fold increased risk over the general population of developing colorectal cancer. This difference is highly statistically significant. However, this research area has been complicated by large numbers of both negative and positive findings, and it is not yet clear whether these results imply that there are many different genes, each of which will contribute to this evidence for family aggregation or, alternatively, if the most important genetic mechanism has yet to be identified.

GENES INVOLVED IN CARCINOGENESIS

Current opinion favours the development of a colorectal cancer from a pre-existing adenoma, which itself arose from normal mucosa, as the most common mechanism of cancer development [82]. Indeed, the adenoma-carcinoma sequence is a tenet on which most of our understanding of colorectal carcinogenesis is founded. While these changes are visible in terms of the physical structure of the developing neoplasia, the increase in size and degree of dysplasia of these tumours is also associated with a changing prevalence of genetic abnormalities in the developing clone. In the first class of genes to be considered here, all three of the *RAS* oncogenes have been shown to have involvement in this process with *KRAS* playing the predominant role (Table 2). *KRAS* mutations are found in approximately 40% of colorectal carcinomas and also in larger adenomas [83]. The majority of mutations are activating and involve codon 12 [84, 85].Mutations are also identified in metastases, some of which are not found in the primary tumour, suggesting that the *RAS* genes promote carcinoma formation from adenomas, and may also increase the likelihood of metastasis [86]. *CMYC* has also been shown to be overexpressed in colorectal cancer, and again the degree of expression increases with the developing malignancy [87].

The second class of genes involved in colorectal carcinogenesis are the tumour suppressor genes (Table 2). The first to be recognised was the *APC* gene. Germline mutations in this gene produce FAP with the defining phenotype of large numbers of colorectal adenomas. Analysis of adenomas from FAP patients suggests that mutations or deletions of the wild-type allele are present in approximately half the adenomas (e.g. Ichii and colleagues [88]); this may, of course, be an underestimate because of technical limitations, and hence there is some speculation that all adenomas may have undergone a second event. The *APC* gene is also frequently altered in sporadic colorectal neoplasia. In one study, 31% of adenomas and 36% of the carcinomas had two alterations, while 68% of all tumours examined had undergone at least one inactivating event [89]. Somatic mutations are, like their germline counterparts, truncating or nonsense mutations, and mainly fall in a small part of the gene called the "mutation cluster region" [90] (Figure 1).

The *MCC* (mutated in colorectal cancer) gene lies adjacent to the *APC* gene, and was originally a candidate for the FAP gene [91]. No germline mutations have ever been found in FAP patients in this gene, although 15% of tumours have inactivating mutations suggesting a possible role [28]; this is countered, however, by the failure to identify mutations in the remaining allele of hemizygous tumours as would be expected for a tumour suppressor gene [92].

The *DCC* (deleted in colorectal cancer) gene was initially recognised because over 70% of colon cancers were found to be hemizygous in this region, while some tumours showed homozygous deletions [93]. It is thought that *DCC* expression maintains cell adhesion [94] and that *DCC* loss is involved in progression from intramucosal to invasive carcinoma [95].

The majority of colorectal cancers are hemizygous for the *TP53* gene [96], while the retained allele is almost always mutated [97]. The mutations are commonly missense and cluster in four conserved coding regions. Allele loss or mutations are rare in adenomas with moderate dysplasia, but are more common in later stages of progression [98]. The observation on mutation in *TP53* correlates with Dukes' staging and patient survival [99]. Other genes, whose role has been discussed but without any strong implicating conclusions, are the *DRA* (down-regulated in adenomas) gene [100], the retinoblastoma *RB1* gene [101, 102] and the *NM23* gene [103–105] (Table 2).

MULTISTEP CARCINOGENESIS

The detailed knowledge of adenoma-carcinoma development led Vogelstein and colleagues to investigate the genetic changes occurring during this process. While longitudinal observation of these changes is not practicable, cross-sectional analyses, involving the careful examination of large numbers of adenomas

Table 3. Genetic alterations in adenomas and carcinomas, adapted from Vogelstein and associates, 1988 [83]

	Tumours with specific alterations (%)			
	RAS mutation	5q deletion	18q deletion	17p deletion
Adenomas from FAP patients	12	0	13	6
Adenomas from non-FAP patients	42	29	11	6
Adenomas with invasive carcinoma	57	29	47	24
Carcinoma	47	36	73	75

and carcinomas for their changes, can suggest the most common sequence of genetic events and their timing. The findings are summarised in Table 3. While *APC* deletions are found in similar proportions of FAP adenomas, non-FAP adenomas and carcinomas, 17p and 18q deletions are found predominantly in carcinomas; this discrepancy in the pattern of findings suggests that *APC* deletions may be required as early events in the process, while 17p and 18q deletions are associated with the later stages of carcinoma development [83]. In addition, the absolute number of genetic changes increased from stage to stage, again consistent with the concept that increasing size and degree of dysplasia would be associated with increasing numbers of mutations. These observations led to a model of carcinoma development [106] which is depicted in Figure 4.

MICROSATELLITE INSTABILITY

Increasing evidence has now accumulated that microsatellite instability is a feature not only of HNPCC tumours, but also of sporadic colorectal and extra-intestinal malignancies. Two patterns seem to be emerging. Firstly, in HNPCC families, it appears that most tumours in gene carriers show microsatellite instability. For example, approximately 75% of colorectal and endometrial tumours in HNPCC family members show instability, whereas only approximately 15% of sporadic malignancies appear to display this phenomenon [55, 107–109]. Small numbers of other tumours in HNPCC families have also been shown to demonstrate microsatellite instability, including stomach, ovary, duodenum, kidney and colorectal adenoma [107].

Secondly, the incidence of instability in the context of 'sporadic' cancer is not random. Rather, tumours at sites which are seen as part of the spectrum of HNPCC seem to be more prone to instability than at other sites. Certain tumours, such as lung, testis, brain and possibly breast [110–112], do not exhibit instability and these are tumours rarely seen in HNPCC gene carriers. Microsatellite instability is very rare in bladder cancer [113], but has been demonstrated in two of four transitional cell carcinomas of the upper renal tract (one of which was in a Lynch II family member) [114].

The predisposition to HNPCC is inherited as an autosomal dominant so that the germline of affected individuals should be heterozygous for the relevant mismatch repair gene. The presumption is, therefore, that a single functional copy is sufficient for normal development. In addition, as the normal cells do not show a mutator phenotype [62], this suggests that the action of the mutator genes is recessive. Following Knudson's hypothesis, therefore, a second event is required in the somatic cells of HNPCC individuals to knock out normal function. How then do tumours develop? The explanation for *APC* in colorectal cancers and for *BRCA1* in breast cancers is that loss of heterozygosity (revealing non-disjunction or mitotic recombination) is observed for the "wild-type" chromosome; at least for *hMSH2* in colorectal tumours, this has not been found. One possibility is that the action is not completely recessive so that heterozygotes have a marginally poorer repair capability and, hence, are slightly more likely to allow the development of a second mutation in *hMSH2*. Tumours from HNPCC should therefore be heterozygous for two distinct *hMSH2* mutations (the second of which should completely knock out the function of *MSH2* [115]), creating the basis for the mutator phenotype and implying that rates of allele loss should be lower throughout the tumour genome than in tumours without instability. Indeed, this is consistent with previous observations [55]. Another possibility suggested by Jiricny [116] is that a second "hit" is required elsewhere in the genome, and that this second event brings about the development of neoplasia. During the period of uncontrolled growth, the quantitative effect of only a single copy of *hMSH2* might then not be sufficient to retain complete integrity resulting in the development of numerous mutations all over the genome (including of course in *hMSH2*).

The confusion with respect to HNPCC tumours does not

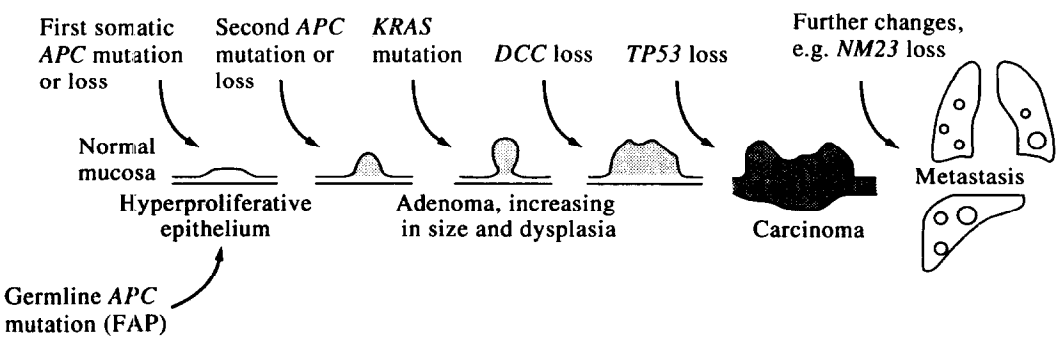

Figure 4. Colorectal carcinogenesis: this model is adapted from Fearon and Vogelstein [106] and illustrates a possible genetic pathway for the development of colorectal carcinoma from normal mucosa.

help the understanding of sporadic colorectal tumours with microsatellite instability. If the effect is entirely recessive or almost recessive, then microsatellite instability should be rare because the selection within the tumours for the mutator phenotype would be indirect, requiring at least two hits in the same gene. One explanation of this could be that the majority of tumours with microsatellite instability do, indeed, have one inherited mutation (which would only be possible if there were common mutations which predisposed to colorectal cancer; such mutations would seem to be implausibly common). A second possibility [13] is that there is a dominant selective advantage as a result of the first mutation. This would, of course, imply that the spectrum of somatic mutations would be different from that of germline mutations. Clearly, more research is required before the process is completely unravelled.

FINAL THOUGHTS

In the last 18 months, dramatic advances in our understanding of the hereditary non-polyposis colorectal cancer syndromes have revealed a novel mechanism for genetic mutation mediated by defective repair of DNA replication errors. Diagnostic molecular genetics has already made a big impact on the management of FAP, and these new developments may have even more far reaching consequences due to the greater prevalance of HNPCC. However, the frequency of germline mutations in the DNA mismatch repair genes and their age related penetrance have yet to be determined. In the large kindreds where linkage to one of these genes is confirmed, unaffected family members at risk of inheriting the mutated gene can now be offered presymptomatic diagnosis. Those without the mutation can be spared lifelong screening, whereas those inheriting the mutation need close and regular evaluation. In the latter group, colonoscopy is required frequently (annually or 2 yearly) because of the possibility that there is an accelerated adenoma-carcinoma pathway in HNPCC [117]; prophylactic surgery is likely to become an acceptable alternative. Screening for potential extra-colorectal malignancies is usually recommended, although its value is unproven, and tumours may be advanced by the time a diagnosis is made [118]. Most HNPCC families are small and not suitable for linkage studies; here demonstration of the precise mutation will be required to provide accurate diagnosis. Finding mutations in even one of these genes is a major undertaking and, with there being up to four genes to analyse, the difficulties are further magnified.

Despite these and other advances, there are perhaps three major challenges ahead. The first is to explain how individuals inheriting mutations in the same gene (sometimes even the same mutation) can have such widely differing phenotypic expressions or even none at all (if the mutation is not fully penetrant). At this stage, we do not know whether such variation is genetically or environmentally mediated. Secondly, clarification of the contribution of genetic susceptibility to apparently sporadic cancer, which has been predicted for many years, still remains elusive. Finally, we have yet to translate a molecular genetic understanding of carcinogenesis into preventative or therapeutic measures. With the advent of gene therapy, this may become the first of the three challenges to be realised.

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