Susceptibility markers in colorectal cancer

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Many susceptibility factors contribute to an individual's risk of developing colorectal cancer. Family history of colorectal cancer (particularly with early age of onset), maleness and increasing age are all factors associated with increasing risk. About three quarters of colorectal cancers are thought to be due to somatic mutations, and both high- and low-penetrance predisposing genes contribute to the remaining quarter of cases. Many of the highly penetrant dominant genes are known, but others remain to be identified. Describing the contribution of individual genes is likely to be very complex, as some modify the impact of other genes and other environmental factors rather than incurring a direct, easily attributable effect. The two dominant predisposing syndromes are familial adenomatous polyposis and Lynch syndrome, the first due to a mutant tumour-suppressor gene *APC*, and the second due to mutations in a number of genes responsible for mismatch repair in DNA at cell division.

Establishing genetic susceptibility for colorectal cancer will soon be possible, and could save lives by allowing targetting of screening and the encouragement of preventive behaviours. However, there will always be a risk of making healthy people "sick" through the identification of predisposing genes, and there are many potential ways by which a gene carrier may be stigmatized by society, insurance companies and employers.

Introduction

Susceptibility to colorectal cancer can be predicted on the basis of a family history of the disease, particularly when this involves early age of onset. Other factors of relevance are age and sex, increasing age and maleness being associated with increasing risk (Figure 1). Features of rare syndromes such as Gorlin's syndrome and Peutz-Jeghers syndrome are predictive of elevated risk, as is a personal history of a resected adenomatous polyp or colorectal cancer. Clinical features of familial adenomatous polyposis and Lynch syndrome (hereditary nonpolyposis colorectal cancer) are the most important predictors. In addition to clearly pathological mutations in the *APC* and mismatch repair genes, allelic variants at these loci are likely to be of importance. Interactive loci such as the 'Modifier of Min' (*MOM1*) gene discovered in the mouse are of growing interest, as are genes which interact with environmental factors to increase mutagenicity or to diminish availability of protective substances such as folic acid.

Understanding genetic susceptibility markers will bring clinical benefits and increase the possibilities for further research into the etiology of colorectal cancer *in vivo*, much as the study of genetic muscle disorders has contributed to the knowledge of the genetics, biochemistry and physiology of normal muscle function.

Family history

Population studies consistently demonstrate a twofold increase in colorectal cancer in first-degree relatives of an individual with colorectal cancer (Brown *et al.*, 1988). The cancers are seen at a comparable age to those in the general population, and have a similar location and age of onset (Lynch & Lynch, 1998). The causes of this familial risk are largely unknown, but presumably include a contribution from partially penetrant susceptibility genes to colonic neoplasia, common environmental exposures (which are risk factors for colorectal cancer and which aggregate in families) and interactions between genetic and
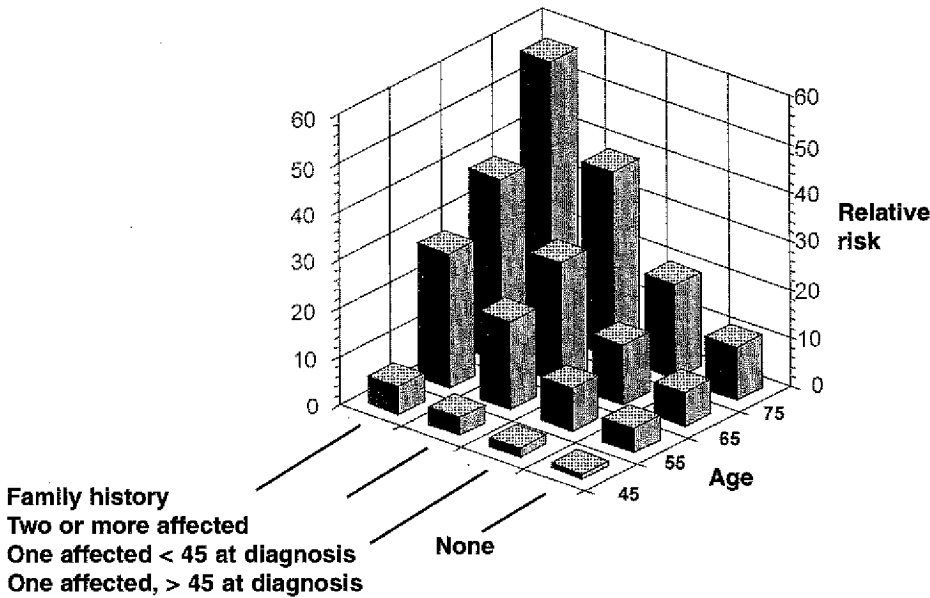
Figure 1. Risk of colorectal cancer by age and extent of family history, relative to risk of a 45-year-old with no family history
The data for this figure are taken from a study of colorectal cancer in families in Melbourne, Australia conducted by Dr J. St John (personal communication)

environmental factors (Kim, 1997). Colon cancer in these families is not linked to high-penetrance genes (described below), implying that these genes are not the major cause of familial colorectal cancer.

About three quarters of all colorectal cancers are thought to result from somatic mutations. At the present time, there is no certain way of picking out from the crowd those subjects with a predisposition who will make up the remaining quarter (NHS Executive, 1997). About 3 to 5% of colorectal cancer cases have a known, dominantly inherited predisposition and another 5% of families appear to have highly penetrant predisposing genes which have not yet been identified.

The risk of colorectal cancer in relatives of cases has been shown, in a number of studies, to be related to the age of onset in any close relative and to the number of affected relatives. Figure 1 shows the relationship between the risk of colorectal cancer by age and extent of family history. We use as a baseline the risk of colorectal cancer in a 45-year-old who has no family history. As this person gets older his or her risk increases simply on the basis of their own ageing process (at age 55 years, the risk of developing colorectal cancer in the next year is five times that at age 45, at age 65 it is seven times the risk of a 45-year-old and at age 75 it is 11 times the risk at 45 years). The risk also increases with the number of affected relatives. Thus, a 45-year-old with one affected relative diagnosed after 45 years of age has a risk 1.8 times that of a 45-year-old without a family history. The relative risk increases to 3.7 if the diagnosis was before 45 years and to 5.7 if there are two affected first-degree relatives. The risk increases across all levels of family history and at all ages, so that a 75-year-old with two affected first-degree relatives has a risk of colorectal cancer in the next year over 50 times that of a 45-year-old with no family history.

Lynch syndrome (hereditary nonpolyposis colon cancer) is due to a mutation in a highly

penetrant susceptibility gene, and was referred to in the past as the cancer family syndrome. Patients are at risk of many extracolonic malignancies, including cancer of the endometrium, stomach, small bowel, hepatobiliary and urinary tract. For example, gene-carrying women have a 42% risk (to age 70 years) of developing endometrial cancer, which exceeds colorectal cancer risk in some age groups (Dunlop *et al.*, 1997). An individual with a family history including any of this spectrum of cancers, not just colorectal, could be considered to be likely to have an underlying genetic susceptibility. Lynch syndrome is most often suspected on the basis of family history. The modified Amsterdam criteria (Table 1) make use of the pattern of disease in families without access to direct mutation searching (Vasen *et al.*, 1994). Family history alone is not sufficiently sensitive for use in assessing risk for an individual. When screening strategies are being planned for someone with a Lynch syndrome family history, additional factors such as local screening availability, advice given to other family members, and the possibility of non-penetrance, early death or non-paternity should be considered. The Amsterdam criteria are useful in research to ensure inclusion of high-risk individuals in studies, whereas in clinical use they will only identify a proportion of high-risk people. An indirect application of the Amsterdam criteria is in selecting the families most likely to yield positive results when searching for mismatch repair gene defects (Wijnen *et al.*, 1998).

Where there is a family history of colorectal cancer, early age of onset in an affected relative and two or more affected generations are the most predictive factors for risk for an individual (Gaglia *et al.*, 1995). However, other independent risk factors, such as age and male sex, greatly modify an individual's risk. For example, Guillem *et al.* (1992) found that, at screening colonoscopy, those at greatest risk for harbouring an asymptomatic adenoma were males over the age of 50 years having at least one first-degree relative with colorectal cancer.

Personal history
Previous colorectal adenomas or cancer, without regular follow-up, indicate an overall increase in susceptibility to colorectal cancer. Such clinical observations can be further refined by pathological and genetic analysis of the neoplasm, and also by the age and follow-up history of the individual. Because there is variability in the normal adenoma–carcinoma sequence, some types of adenoma confer a greater risk than others. Sessile villous lesions behave differently to pedunculated adenomas, and flat adenomas are associated with an increased potential for malignant change (Jass, 1995). Microadenomata, the pathologically detectable precursors of adenomas, are very common, but only a fraction of them ever progress to malignancy, depending on the genetic status of the individual. For example, the risk is very low for an individual microadenoma in familial

Table 1. Revised criteria for recognition of Lynch syndrome (modified Amsterdam criteria)

There should be at least three relatives with a Lynch syndrome-related cancer (colorectal cancer, cancer of endometrium, small bowel, ureter or renal pelvis)

- One should be the first-degree relative of the other two.
- At least two generations should be so affected.
- At least one cancer should be diagnosed before 50 years of age.
- Familial adenomatous polyposis should be excluded.
- Tumours should be verified by pathological examination

From Vasen *et al.* (1999)

adenomatous polyposis, but high in Lynch syndrome. Similarly with adenomas; in familial adenomatous polyposis the risk of an individual adenoma becoming malignant is very small, whereas the risk is higher in Lynch syndrome.

Adenomas have much greater malignant potential than metaplastic polyps (Winawer, 1993a). Removal of polyps reduces the subsequent rate of colorectal cancer, confirming their premalignant potential (Winawer, 1993b).

Microsatellite instability in a colorectal tumour is predictive of a high risk of recurrence for an individual, regardless of family history (Brown *et al.*, 1999). Inflammatory bowel disease, and in particular ulcerative colitis, carries an increased risk for colorectal cancer. Those at highest risk have ulcerative colitis throughout the colon, rather than localized disease, are over 40 years of age, and have had ulcerative colitis for more than 10 years. Interestingly, this does not depend on continuous manifestation of the disease; those who have a short episode are at the same risk of colorectal cancer 10 years later as those who have 10 years without remission. Patients with ulcerative colitis have a small but increasing risk which is equivalent to about 0.5–1% chance of colorectal cancer per year of follow-up (Ekbom, 1998). For this reason, many gastroenterology units follow up patients for many years, despite lack of definitive evidence that this prevents colorectal cancer. Recent case–control studies have shown that the patients with ulcerative colitis who are at highest risk of colorectal cancer are those with a family history of colorectal cancer, whereas those with no family history have a risk which may not be significantly different from the average person without colitis (Nuako *et al.*, 1998). This finding implies that any inherited genetic risk is not associated with both the development of colitis and colon cancer risk, but that they are separate, discrete risk factors, and that inflammation is not a sufficient risk factor in isolation to have a clinically significant impact in most people.

Pathological susceptibility markers
Microsatellite instability is seen in about 15% of all colorectal cancers (Bodmer *et al.*, 1994), and at a much higher rate in Lynch syndrome colorectal cancers. The latter association probably explains why it is commonly seen in the younger age group. Functional assays for binding of the mismatch recognition genes can be used to test cells for a mismatch repair defect (Aquilina *et al.*, 1994).

This phenotype is likely to be recessive, reflecting total loss of function of the binding protein that recognizes DNA mismatches, for example at CA and CT repeats. This explains the observation that normal cells from Lynch syndrome gene carriers do not exhibit a microsatellite instability phenotype, as the cells in a heterozygous individual have one normal, working copy of the mutated, inherited gene.

Genetic factors predisposing to colorectal cancer
Colorectal cancer provides an excellent system for the study of genetic changes occurring during the development of a common human cancer. Most colorectal cancers arise from benign adenomas, which means that the developing carcinoma can be observed, removed and studied at all stages from an aberrant crypt focus to metastatic carcinoma. Adenomas normally arise from a single stem cell, which is demonstrated by the usually monoclonal nature of all adenomas from the smallest visible lesion, in contrast to the polyclonal composition of colorectal epithelium.

The study of the stochastic genetic events leading from early adenoma to colorectal cancer has led to the identification of three major classes of genes involved in familial risk: oncogenes which actively confer a direct growth-promoting effect, tumour-suppressor genes which normally restrain proliferation, and DNA mismatch repair (MMR) genes which identify and correct DNA replication errors. Mutations in MMR genes lead to genetic instability, as defects in somatic cell DNA replication are not corrected, resulting in mutations in other genes such as type-II TGF-β receptor (Yagi *et al.*, 1997).

Since colorectal cancer development is a multistep process, involving mutations in at least seven genes, many genotypes are likely to be involved in susceptibility to this disease. Most but not all of these genes exert a biological effect only when both alleles are mutated; in other words, they are recessive at the cellular level. However, some defects in tumour-suppressor genes such as *APC* can exert a phenotypic effect even in the heterozygous state. Many genes are known to

be part of the multistep process and some have been shown to be important predictors of colorectal cancer when a germline mutation is present.

Although the genetic changes which give rise to colorectal tumours often occur in a particular sequence (Figure 2), it is the accumulation of mutations rather than the temporal sequence which determines the malignant potential of a tumour (Fearon & Vogelstein, 1990). In multipotent stem cells, the accumulation of mutations occurs in a stochastic, stepwise manner, with each mutation providing a selective advantage for subsequent cell generations, leading to an expanded population of daughter cells (Bodmer *et al.*, 1994). *APC* mutations occur early (Powell *et al.*, 1992) and are important for initiation. This explains the severe, young colorectal cancer phenotype of familial adenomatous polyposis. In contrast, *ras* oncogene mutations usually occur in larger adenomas, being present only in 10% of those smaller than 1 cm. It is thought that such mutations are responsible for the development of a small adenoma into a larger

lesion. Similarly, allelic losses on chromosomes 5q, 17p and 18q and others are frequently observed in malignant tumours, but rarely in adenomas. Such mutations occurring later in the cascade of mutational events are unlikely to have major relevance in the search for cancer susceptibility, as they are largely somatic mutational events commencing in a single cell. Nevertheless, it is conceivable that variations which predispose to mutational change in any of these genes could influence the nature and rate of cancer development.

There is continuing debate about whether loss of a single *APC* allele can increase proliferation. Whereas significant changes in proliferation have not been seen in *Apc* knock-out mice, our own work in humans with familial adenomatous polyposis has shown a significant increase in the number of mitoses per crypt (Mills *et al.*, 2000). In contrast, Wasan *et al.* (1998) reported an increase in crypt fission rather than proliferation. DNA hypomethylation is probably not a pivotal event and the role of mismatch repair deficiency early in the process is also equivocal.

Pathological stage	Genetic event
Normal epithelium	
	APC mutation (in familial adenomatous polyposis)
Hyperproliferative epithelium	*APC* mutation
Early adenoma	K-*ras* mutation
Intermediate adenoma	Mismatch repair deficiency DCC (18q) loss DPC4 JV18
Late adenoma	*p53* loss
Carcinoma	
	Other changes
Metastasis	

Figure 2. A genetic model for colorectal tumorigenesis
Based on Kinzer & Vogelstein (1996) and Kim (1997)

Mouse models

Mice bred to have defective copies of the major genes involved in colorectal cancer can be of great value in the evaluation of susceptibility factors (Kim *et al.*, 1993). The Min mouse, *Apc*1638N and *Apc*1638T and the *Apc*$^{\text{delta }716}$ are the four mouse models of defective Apc function. The *Apc*1638T mouse is interesting as it involves a mutation near the end of the coding sequence, leaving the critical catenin-binding function intact. These mice do not develop significant intestinal tumorigenesis and homozygotes can survive to term.

While the phenotypes of the mouse models show important differences from the human, with predominance of small gut tumours, these models have been of great value in studies of the biology of colorectal cancer and in investigations of chemopreventive agents. Mouse mutants with defective mismatch repair genes have been less valuable, as the phenotype does not include gastrointestinal tumours.

Inherited predisposition to colorectal cancer

Mendelian syndromes and genes of major effect

Although the molecular genetics of most colorectal cancers remain unclear, some cases can be designated as having a genetic predisposition on the basis of their family history, clinical findings, pathology or molecular genetic features. Clinical overlap between the syndromes sometimes makes diagnosis difficult, but this is being clarified as genetic and functional histopathological analysis becomes available. Figure 3 shows the syndromes currently known to genetically predispose to colorectal cancer.

The two major, dominantly inherited forms of colorectal cancer are familial adenomatous polyposis and Lynch syndrome. This nomenclature describes the phenotype of these two conditions at the histological level, but the range of mutations in causative genes is much more disparate. Almost all cases of familial adenomatous polyposis result from a pathological mutation in a single gene on chromosome 5, *APC*. However, Lynch syndrome results from loss of function of one of at least five separate genes, each one of which encodes part of a protein complex which is responsible for mismatch repair during DNA replication.

Familial adenomatous polyposis

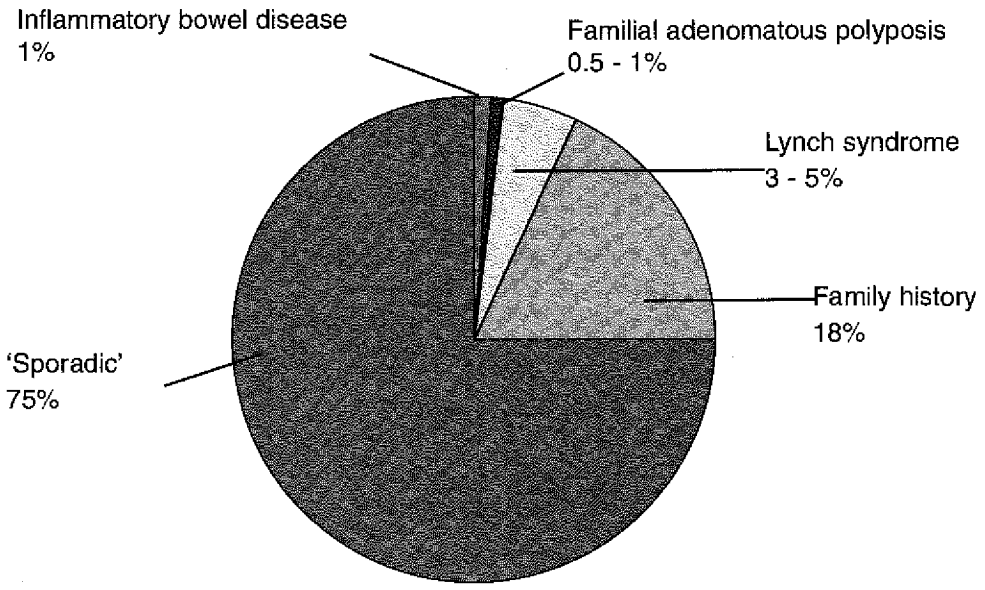Familial adenomatous polyposis is the most genetically determined of all inherited cancer-

Figure 3. Predisposition to colorectal cancer

predisposing syndromes. A mutation in *APC* causes multiple colorectal adenomatous polyps to develop during the teens and early adulthood. In the absence of prophylactic colectomy, the large number of adenomas leads to the almost certain development of colorectal cancer at a young age. Much has been learned about the *APC* gene since its localization and cloning in the early 1990s (Bodmer *et al.*, 1987; Nishisho *et al.*, 1991), and the relationship of germline mutations to individual phenotype (Figure 4) has been described in more detail than for any other inherited cancer predisposition.

About 80% of familial adenomatous polyposis families have a different, distinct mutation of *APC*, although almost all of the disease-causing mutations so far found inactivate *APC* and result in protein truncation. *APC* codes for a large 2843-amino-acid protein which is involved in cell fate determination, adhesion and cytoskeleton function, and is an integral part of the Wnt signalling pathway by complex formation with glycogen synthase kinase 3 beta (GSK3 beta) (Brown *et al.*, 1999), β-catenin and other proteins including axin and conductin. There are 15 exons in *APC*, of which exons 1 to 14 are short and exon 15 encodes three quarters of the protein. A higher density of polyps occurs in families with a muta-tion near the centre of the gene in exon 15, whereas a sparse pattern of adenomas, known as attenuated familial adenomatous polyposis, is associated with mutations at the extreme 5′(proximal) end of the gene. It is likely that the most severe phenotype is a consequence of mutations which disrupt the mediation of β-catenin degradation in the Wnt signal transduction pathway. Events leading to oncogenic activation of β-catenin, which promotes tumour progression via interaction with a downstream target, can result from inactivation of tumour-suppressor activity of a mutated *APC* gene, from activation of Wnt receptors, or from direct mutation of the β-catenin gene itself (Polakis, 1999). In the nucleus, β-catenin upregulates the oncogene *c-myc* (He *et al.*, 1998), among other oncogenes.

Recognition of families and individuals at risk of developing familial adenomatous polyposis relies on careful pedigree analysis aided by a multidisciplinary approach including genetics, surgery, gastroenterology and pathology. This approach has been adopted in the Northern Region of England, and has been shown to be effective in reducing the burden of colorectal cancer in such families (Burn *et al.*, 1991). However, the new mutation rate for germline *APC* mutations is 20–30% and, unfortunately, new
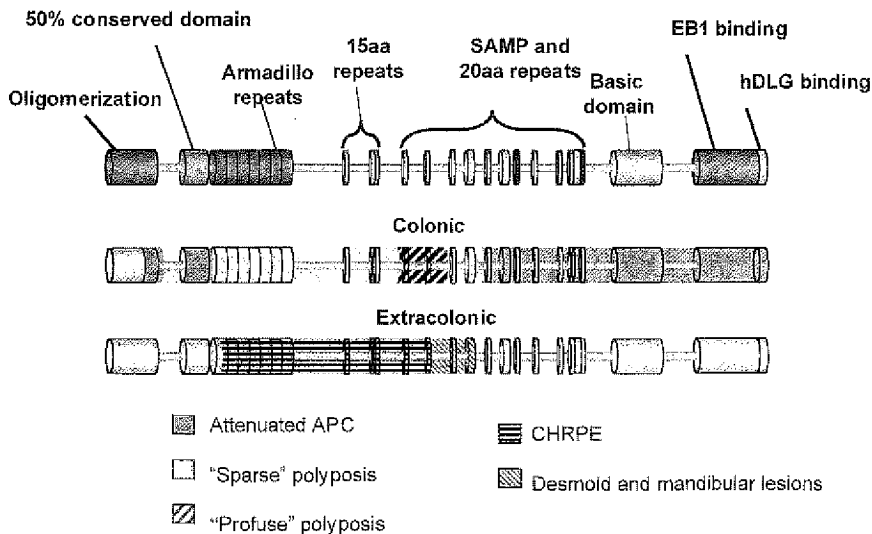

Figure 4. Structure of APC protein and genotype/phenotype correlation

mutation cases appear to exhibit a more severe phenotype than familial cases, with mutations more common at codon 1309. It is therefore possible that a high new mutation rate combined with improving survival rates into or beyond reproductive years will lead to an increase in incidence of familial adenomatous polyposis (Gayther et al., 1994).

One explanation for the genotype/phenotype correlation in familial adenomatous polyposis is that some mutations, such as a truncating mutation at codon 1309, result in a dominant negative effect at the protein level. There is experimental evidence that wild-type APC activity is strongly inhibited by a mutant allele with this codon 1309 mutation, and this results in a severe phenotype. In contrast, a mutation associated with a mild phenotype (attenuated APC, or AAPC) produces a gene product which associates only weakly with the wild-type product (Dihlmann et al., 1999). In AAPC, colorectal cancer occurs at a later age and extracolonic manifestations are less common (Lynch et al., 1995). It is possible to attribute this mild phenotype to mutations in three distinct regions of the gene; at the 5′ end, within exon 9, and at the 3′ distal end of APC. When such a mutation is known in a family seeking genetic counselling, it is possible to modify risks according to the known genotype/phenotype relationship, and bowel examination may be less frequent than in a family with a mutation such as APC^{1309}.

Variation in the familial adenomatous polyposis phenotype even in those with identical mutations presents difficulties in counselling, both within and between families. However, in all cases of familial adenomatous polyposis, colorectal cancer susceptibility remains high, and regular screening and prophylactic surgery are essential components of care in such families (see section below on genetic modifiers).

Lynch syndrome

Lynch syndrome is the preferred term for the form of hereditary nonpolyposis colorectal cancer associated with an MMR gene defect. This relatively common syndrome is characterized by the development of neoplastic lesions in a variety of tissues (gastrointestinal, endometrial, ovarian, uroepithelial) and, most prominently, the colorectum (Aarnio et al., 1995; Marra & Boland, 1995; Vasen

et al., 1995; Watson & Lynch, 1993). Clinically, the colorectal neoplastic process in hereditary non-polyposis colorectal cancer appears to follow an adenoma-to-carcinoma progression similar to that described in familial adenomatous polyposis or other colorectal cancer settings, though several aspects of the clinical manifestations, as well as the molecular pathophysiologies underlying them, may be distinctive (Kinzler & Vogelstein, 1996). The disease was traditionally recognized by the familial clustering of colorectal cancers in persons without obvious polyposis.

Classical Lynch syndrome is caused by inherited mutations in one of the Mut-related family of MMR genes, including hMLH1, hMSH2 and hMSH6 (Fishel et al., 1993; Kolodner et al., 1994, 1995; Nicolaides et al., 1994). Three other genes involved in the MMR complex, PMS1, PMS2 and MLH3, are rarely or never associated with a Mendelian phenotype. This has been attributed to redundancy between them (Lipkin et al., 2000) (see below). These genes encode protein products that are responsible for recognizing and correcting errors that arise when DNA is replicated (Dunlop et al., 1997; Leach et al., 1993). An early manifestation of this defect in vivo is the appearance of microsatellite instability. A second mutation is required in colorectal cells to inactivate the MMR function. Microsatellite instability contributes to the progressive accumulation of secondary mutations throughout the genome and thereby affects crucial growth-regulatory genes, ultimately leading to cancer.

More than 100 different germline mutations have been identified in the MMR genes known to be associated with the Lynch syndrome. Mutations in hMSH2 and hMLH1 account for roughly equal proportions of Lynch kindreds, and are together responsible for a majority of colorectal cancer in these families (Aaltonen et al., 1998). However, germline disease-associated mutations are found in only about 40–70% of probable Lynch syndrome families. Other germline mutations and/or different classes of genes may be discovered which play an etiological role in susceptibility to hereditary nonpolyposis colorectal cancer. One family has been described in which a probable pathological mutation has occurred in the TGF-β receptor II gene (Yagi et al., 1997), a gene known to show altered expression in colorectal cancer. This, and

the attenuated form of familial adenomatous polyposis, might be regarded as falling into the broader category of hereditary nonpolyposis colorectal cancer but not Lynch syndrome.

One of the recently identified MMR genes, *MLH3*, associates with *MLH1*, *MSH2* and *MSH3* to form a complex involved with repair of insertion–deletion loops of single-stranded DNA. Its role in human cancer predisposition is uncertain but it is thought to show functional redundancy with *Pms1* and *Pms2*. This would explain why *PMS1* and *PMS2* mutations are only rarely found in Lynch syndrome families (Lipkin *et al.*, 2000).

Other syndromes
Juvenile polyposis
Juvenile polyps arise from the lamina propria, rather than the epithelium, which is the source of hyperplasia in adenomas. Solitary juvenile polyps are diagnosed in approximately 1% of children and account for the majority of gastrointestinal polyps found in childhood. Juvenile polyposis coli is a rare autosomal dominant syndrome which is characterized by multiple polyps in the colon and occasionally elsewhere in the gastrointestinal tract. This inherited condition is associated with a high risk of colorectal cancer, probably due to the development of foci of adenomatous change which progresses to dysplasia and adenocarcinoma. In some cases of juvenile polyps without a family history and of polyps arising in the juvenile polyposis syndrome, the predisposing factor is a mutation in one of two genes; *PTEN* on chromosome 10 (Jacoby *et al.*, 1997) or *SMAD4* on chromosome 18. It is likely that removal of juvenile polyps will be preventive for colorectal cancer.

There have been reports of families with atypical juvenile polyps, adenomas and colorectal cancers as well as inflammatory and metaplastic polyps. In one such family, cases of mixed polyps follows an autosomal dominant inheritance pattern, and the putative gene has been localized to the long arm of chromosome 6 (Thomas *et al.*, 1996).

Basal cell naevus syndrome (Gorlin's syndrome)
Hamartomatous intestinal polyps have been reported in Gorlin's syndrome (Schwartz, 1978), in which there is an association with broad facies, basal cell naevi, ectopic calcification of the falx and bony abnormalities of sites including the ribs,

mandible and maxilla. Malignancies seen in this condition include medulloblastoma, malignant naevi and less commonly, colorectal cancer (Murday & Slack, 1989). Premature termination of the *patched* protein resulting from germline mutations in one of two tumour-suppressor genes, Patched 1 and 2, are thought to be responsible for this syndrome, but no genotype/phenotype correlation has yet been described (Wicking *et al.*, 1997).

Peutz–Jeghers syndrome
The predisposing polyps in this rare syndrome are pathologically discrete from other polyps and are called Peutz–Jeghers polyps. They exhibit some adenomatous features and have an increased malignant potential, appearing in the stomach, small bowel and colon. The distinguishing feature of this syndrome is the mucocutaneous melanin pigment seen around and inside the mouth and between the fingers. At least some cases of this disorder are due to mutations in the serine/threonine (*STK11*) tumour-suppressor gene on chromosome 19 (Jenne *et al.*, 1998).

Turcot's syndrome
In this condition, multiple colorectal adenomas are associated with central nervous system tumours, particularly of the brain. This phenotype has been seen with variants of both Lynch syndrome and familial adenomatous polyposis genotypes. With an *APC* variant, the brain tumours are cerebellar and medulloblastomas, whereas with *MLH1* or *MSH2* variants, glioblastoma multiforme is more frequently seen.

Modifiers and genes of minor effect
Allelic variants
A polymorphism at codon 1307 in *APC* is relatively common in the Ashkenazi Jewish population, and increases adenoma formation in this group (Gryfe *et al.*, 1999). This mutation causes a change of isoleucine to lysine (shown as I1307K). The underlying mutation changes a thymine to an adenine, resulting in a sequence of eight adenines which is more mutable than the wildtype. This leads to mutational susceptibility in somatic colon cells which in turn confers a higher risk of neoplastic development. Because the gene variant is dominantly inherited, but the

penetrance for the phenotype is low, this form of predisposition could be described as familial rather than dominant. The lifetime risk of colorectal cancer for an individual with the polymorphism is about 10%, but because it is common (6% in New York Ashkenazim and 28% in those with a family history), it is thought to underlie 3–4% of colo-rectal cancer in this population (Laken *et al.*, 1997).

This finding has raised the issue of genetic predictive testing for the *APC* I1307K polymorphism which could be targeted towards individuals of Jewish Ashkenazi descent to identify those with an increased susceptibility as a prelude to prevention programmes. It is thought, for example, that 360 000 polymorphism carriers live in the United States, but the issue of testing is contentious, as a positive test could carry with it unfavourable psychological effects, insurance difficulties and potential sociological problems linked to selection of a population on the basis of ethnic descent. Moreover, the predictive value of such a marker in isolation is very small.

There is a strong likelihood that different mutational events might result in allelic variants at any of the "major gene" loci which increase the risk of colorectal cancer. The challenge will be to choose candidate genes for detailed sequencing. In future, high-throughput technology will make selection less critical but, at present, it is easy to spend large sums of money and achieve little. For example, a splice site mutation in the *MSH2* gene has been found in normal individuals with no history of hereditary nonpolyposis colorectal cancer. It is therefore possible that some functional effect exists which is associated with increased risk of colorectal cancer but which is not sufficiently high to show up as a positive family history.

One method of targeting is to use single nucleotide polymorphisms (SNPs) to identify haplotypes. Table 2 shows a series of five SNPs at the *MSH2* locus. Four of these are considered neutral, as they involve intronic DNA. The fifth is a coding sequence SNP in exon 6 and may have a phenotypic effect. Of the 32 possible haplotypes in our control population, only nine were identified among 99 chromosomes characterized and three (bold in the table) accounted for 85 of the 99. In other words, common ancient versions of the gene will occur in different populations. It will now be possible to examine these SNP patterns in people with colon cancer to see if the distribution of haplotypes is different from that in the local population. If, for example, there had been a mutation several hundred years ago in an *hMSH2* gene residing on a "ggtat" chromosome, this would be reflected in an overrepresentation of that haplotype in the disease population and would focus sequencing studies on *hMSH2* in affected people with the "ggtat" haplotype.

Interactive genes

In familial adenomatous polyposis, allelic heterogeneity does not appear to account for all of the observed variation in phenotype and other genes are probably involved in the phenotypic expression of this, the most "monogenic" of all cancer susceptibility genes. This is a continuing area of research, but there is evidence for a modifier gene on chromosome 1p35–36 which maps to an equivalent locus in the mouse for a known modifying gene called *Mom-1* (Dobbie *et al.*, 1997). The candidate gene for this is type 2 non-pancreatic *Pla2*, a phospholipase gene, but no mutations in the human homologue have been found to date (Spirio *et al.*, 1996). Identification of modifying genes is a powerful tool in the understanding of a gene's function, and the advent of dense genetic linkage maps has made the dissection of polygenic traits, such as colorectal cancer susceptibility, more practical. A relationship between mucin-producing genes such as *MUC2* and the pathogenesis of colorectal cancer has been suggested (Sternberg *et al.*, 1999). *MUC2* predominates within colorectal goblet cell mucin and is expressed in adenomas and mucinous carcinomas. Down-regulation of *MUC2* is seen in non-mucinous adenocarcinomas arising from adenomas, and cancers that develop *de novo* do not express *MUC2*. When more is known about colorectal cancer mucin, there will be opportunities to study different cell lineages to further explore the pathogenesis and other susceptibility factors in colorectal cancer.

CDX1 and *2* homeotic genes have the characteristics of transcriptional regulatory genes and are down-regulated in about 85% of colorectal cancers. In *CDX2*^{+/-} mice, multiple intestinal polyps are seen in the proximal colon (Chawengsaksophak *et al.*, 1997). However, these polyps are not typical adenomas, and have similar histo-

Haplotype	Number	Haplotype frequency
Table 2. Single nucleotide polymorphisms (SNPs) at the *MSH2* locus		
cgagt	61	0.616
ggagt	14	0.141
ggaat	10	0.101
ggtat	7	0.071
cgagc	3	0.030
ggtgt	0	0.000
ggagc	0	0.000
cgaat	2	0.020
ggaac	2	0.020
Total number of chromosomes typed	99	

(unpublished data)

logical characteristics to those seen in the *Min* mouse. These polyps occasionally contain true metaplasia and occasional large pedunculated tubulovillous colonic adenomas are seen. The fact that polyps are seen mostly in the proximal colon suggests that lowering levels of *CDX2* would induce exaggerated cell growth leading to tumour formation, and expression would stimulate cell differentiation and growth arrest (Yagi *et al.*, 1999). However, because the tumours display an unusual histological pattern and human mutations have not been identified, the role of this gene in human carcinogenesis remains unknown.

Environmentally sensitive genetic polymorphisms
A functional polymorphism in the methylene tetrahydrofolate reductase gene (*MTHFR*) makes the enzyme more thermolabile and appears to confer a 50% reduction in colorectal cancer risk in the US population. This polymorphism ([667]C-T, *alaval*), found in 10–15% of the study population, only provided protection when adequate folate was present in the diet (Ma *et al.*, 1997). Figure 5 shows the competing pathways in folate metabolism. Before the report that this polymorphism was protective against colorectal cancer in homozygotes, it might have been expected that the reverse

would be true. The observation could indicate that malignancies are more susceptible to disturbance of the folate pathway and that the homozygotes for the thermolabile variant are relatively protected by the less efficient folate pathway. It is of potential importance that protection against colorectal cancer in those homozygous for the thermolabile variant was observed only in study subjects reporting zero to modest alcohol intake. With high alcohol intake, no protection was seen, indicating an important diet–gene interaction (Ma *et al.*, 1997).

The importance of gene–environment interactions in assessing cancer susceptibility was illustrated by the β-carotene trials. Intervention studies designed to test the hypothesis that supplementation with β-carotene would reduce cancer risk showed no protection. However, the trials showed increased risk of lung cancer in smokers given β-carotene (Mathers & Burn, 1999). This effect is thought to be due to increased cell proliferation and squamous metaplasia in the lung, effects that were enhanced by tobacco smoke, and is associated with suppression of retinoic acid receptor β gene expression and over-expression of *c-Jun* and *c-Fos* genes (Wang *et al.*, 1999).
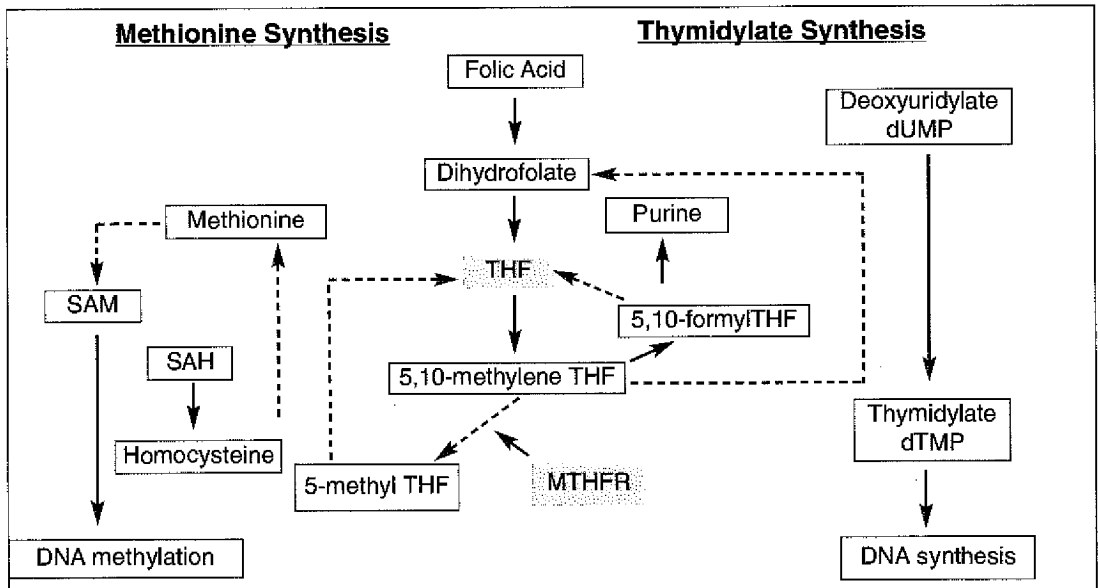
Figure 5. Competing pathways in folate metabolism
THF, tetrahydrofolate; MTHFR, methylene tetrahydrofolate reductase

Exposure to environmental carcinogens such as aromatic amines found in well cooked or preserved meat and cigarette smoke are associated with increased risk of colorectal cancer. Their metabolism is complex but central to most is activation or detoxification of amylines and heterocyclic amines. Acetylation of heterocyclic amines by N-acetyltransferases (NAT) is likely to be of major importance. Acetylation of hetero-cyclic amines by *NAT1* and *NAT2* gene products can lead to the formation of reactive carcinogenic intermediates or to detoxification. This means that the association of cancer risk and enzyme activity could go either way. Acetylation activity varies as a result of the sequence polymorphism in the *NAT2* gene, and if two non-functional alleles are inherited (slow acetylator alleles), there is no NAT2 activity. Several studies show that NAT2 rapid acetylation phenotypes are associated with an increased risk of colorectal cancer. An increased colorectal cancer risk of 1.9 results from a variation in NAT1, which is again due to a rapid acetylation genotype (Hein *et al.*, 2000). There is an association between fast acetylator status and cancer in those with high intakes of cooked meat (a source of heterocyclic

amines). Conversely, slow acetylators who smoke and drink are at increased risk. These genetic polymorphisms provide a good example of how complex the interaction between genotype and environmental factors can be. It must also be remembered that "multiple slices" of a data-set might point to apparent interactions as a random event, resulting in claims and counter-claims on their predictive significance.

Prevention and colorectal cancer genotype
Familial adenomatous polyposis has been identifi-able in families for over a hundred years because of the presence of multiple polyps, and more recently, by genetic testing for mutations or specific patterns in and around the *APC* gene. In the Northern Region of England, using a registry approach, and by applying clinical and genetic criteria, almost all individuals at high risk have been recruited to screening programmes (Burn *et al.*, 1991). One of the biggest advantages of a proactive approach towards offering genetic testing is the relief of uncertainty and the reduction of unnecessary colonoscopic surveil-lance in those not at high genetic risk. At the level of population health care, there are benefits in

being able to target screening effectively to those at greatest risk.

There are many differences between making a genetic diagnosis of hereditary colorectal cancer risk and a clinical diagnosis of colorectal cancer. Firstly, hereditary cancer involves probability statements describing inheritance and penetrance. Little is known about the role of environmental influences in these families, and this contributes to the high levels of uncertainty accompanying predictive testing for late-onset disorders. Secondly, there are psychosocial implications for the individual and for the family. How people respond to the information will depend on many variables such as their personality, defence mechanisms and understanding of the disease and its consequences. The effect of a genetic diagnosis can pass through a family like ripples on a pond and extended families will become more aware of, and discuss, cancer diagnoses. Family beliefs about inheritance may be very different from accepted patterns of Mendelian inheritance. For example, some believe that only males are affected by hereditary nonpolyposis colorectal cancer.

It is easy to assume that those found not to carry a predisposing gene will be unconditionally pleased. However, some individuals react in a negative way to such information. This may be due to having to alter life plans built around an assumption that they will become ill, or to guilt about escaping the family disease. It would also be logical to assume that those found to be gene carriers would present themselves for bowel examination. This does not always happen and is a source of frustration to surgical and endoscopy staff when appointments are missed repeatedly. The reasons for this reaction by patients are complex, but may include fear of surgery, fear of the examination, or a hedonistic personality which does not easily accept hospitalization and potential illness. These reasons can differ greatly from a non-familial case where the patient is attending with symptoms (Rossi & Srivastava, 1996).

There are many ethical issues surrounding hereditary colon cancer, whether it is a clinical or a genetic diagnosis. Firstly, equitable access to services is a guiding principle for health services in the United Kingdom, but this is difficult to apply when only some regions have active registers and recall systems with individual counselling services.

Bearing in mind the European history of eugenics during the early part of the 20th century, there must be caution in pursuing equity by using forms of coercion such as laws and social pressures. Where efforts are made to make contact with family members who are at risk in an attempt to offer equitable services, there is a risk of straying over the invisible line between voluntary and enforced testing. For example, even if DNA testing is undertaken, each person should be able to withdraw at any time or choose not to take any action if the result is positive. Other issues, such as those concerning confidentiality and privacy, must be discussed with family members, so together with the genetic and clinical information and exploration of psychosocial issues, much careful counselling is required. The common thread to all of this discussion is the protection of individual rights, and a multi-professional approach with specialist genetic counselling available for all is the best way to offer such a service.

Research into other risk factors and possible interventions described earlier can be undertaken with subjects at high risk as opposed to general population cohorts. This means that smaller numbers are required, as interim or end-point results such as adenomas are likely to be more frequent. Two such trials are under way using patients with familial adenomatous polyposis and mismatch repair gene mutations, in which aspirin and resistant starch are being tested in randomized controlled trials (Burn *et al.*, 1995, 1998). How much relevance the results of these trials will have to the rest of the population remains to be established, but both interventions were selected because of their favourable effect in observational studies of the general population.

Conclusions

All colorectal cancer involves multiple somatic genetic changes. Based on family history data, it is likely that in at least a half, and probably three quarters, of colorectal cancer in developed countries, these changes occur through random acquisition of mutations due to environmental influences. If and when diet improves and people take more exercise, the importance of germline defects will grow. Even now, there are very large numbers of people at significantly elevated risk of cancer who will be identifiable using molecular

genetic tests for pathological mutations in *APC* and the mismatch repair genes. It is likely that there will be value in searching for less penetrant defective alleles in these genes, as this might influence treatments and screening strategies. Perhaps more importantly, knowledge of a specific personal risk factor is liable to stimulate a greater interest in chemoprevention and lifestyle factors which could lead to reduced risk.

On the negative side, this possibility also raises psychosocial issues, including the risk of making healthy people "sick" by describing them as susceptible to cancer and by identifying high-risk ethnic groups such as Ashkenazi Jews. Ethical issues relating to providing equitable screening opportunities across populations, and financial concerns when insurance risks can be stratified by susceptibility factors or medical liability of the clinician become more complex. In the United States, physicians are being pressurized into assuming the additional responsibility of establishing a family history of cancer across several generations. This in turn creates a duty for a health care worker to provide counselling to extended families, and several lawsuits have been instituted claiming negligence when a family history has not been given adequate consideration or has not been communicated to family members at risk (Nelson, 1996; Severin, 1999). In addition to legal pressures to include genealogy in colorectal cancer care, there are economic factors associated with identification and monitoring of susceptible individuals (Bolin, 1996; Brown & Kessler, 1996; Smith & DuBois, 1997). Where there are advantages to a community or society in providing a health care intervention, there will always be a risk of eugenic policies creeping into practice.

Establishing genetic susceptibility for colorectal cancer will soon become a reality, and the advantages to a member of a hereditary nonpolyposis colorectal cancer or familial adenomatous polyposis family member in finding out they are not a gene carrier can be enormous. However, the situation is more complex where the predictability of the genotype is less certain, and this is likely to be the situation for most genetic susceptibility testing (Lynch *et al.*, 1999). The needs and views of the individual must always take precedence over societal needs if maximum uptake of screening alongside freedom of choice is to be assured. Even more important is the recognition that few risk factors will be sufficiently predictive to justify extension from the realm of primary research to the clinic. In many cases, groups of individuals chosen for their susceptible genotype will be used to test which environmental changes might benefit the whole community.

Acknowledgements

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