Review article

Genetic susceptibility to non-polyposis colorectal cancer

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Abstract
Familial colorectal cancer (CRC) is a major public health problem by virtue of its relatively high frequency. Some 15-20% of all CRCs are familial. Among these, familial adenomatous polyposis (FAP), caused by germline mutations in the *APC* gene, accounts for less than 1%. Hereditary non-polyposis colorectal cancer (HNPCC), also called Lynch syndrome, accounts for approximately 5-8% of all CRC patients. Among these, some 3% are mutation positive, that is, caused by germline mutations in the DNA mismatch repair genes that have so far been implicated (*MLH1*, *MSH2*, *MSH6*, *PMS1*, and *PMS2*). Most of the remaining patients belonging to HNPCC or HNPCC-like families are still molecularly unexplained. Among the remaining familial CRCs, a large proportion is probably caused by gene mutations and polymorphisms of low penetrance, of which the I1307K polymorphism in the *APC* gene is a prime example.

Molecular genetic findings have enabled hereditary CRC to be divided into two groups: (1) tumours that show microsatellite instability (MSI), occur more frequently in the right colon, have diploid DNA, harbour characteristic mutations such as transforming growth factor β type II receptor and *BAX*, and behave indolently, of which HNPCC is an example; and (2) tumours with chromosomal instability (CIN), which tend to be left sided, show aneuploid DNA, harbour characteristic mutations such as *K-ras*, *APC*, and *p53*, and behave aggressively, of which FAP is an example.

This review focuses most heavily on the clinical features, pathology, molecular genetics, surveillance, and management including prophylactic surgery in HNPCC. Because of the difficulty in diagnosing HNPCC, a detailed differential diagnosis of the several hereditary CRC variants is provided. The extant genetic and phenotypic heterogeneity in CRC leads to the conclusion that it is no longer appropriate to discuss the genetics of CRC without defining the specific hereditary CRC syndrome of concern. Therefore, it is important to ascertain cancer of all anatomical sites, as well as non-cancer phenotypic stigmata (such as the perioral and mucosal pigmentations in Peutz-Jeghers syndrome), when taking a family cancer history.
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Cancer of the colorectum (CRC) is exceedingly common in most western nations. In the United States, it is expected that 94 700 new cases of cancer of the colon and 34 700 new cases of cancer of the rectum will occur in 1999.[1] During that same period, mortality from colon cancer is projected to be 47 900 and that for rectal cancer 8700.[1] We estimate that at least 10% of this CRC burden (12 940 new cases and 5660 deaths) will be the result of a primary genetic factor.

Hereditary colorectal cancer (CRC) is a major public health problem. More research is needed to aid in its elucidation and the ultimate translation of this knowledge into clinical practice. Identification of the culprit predisposing germline mutations will determine who is or is not a candidate for participation in highly targeted cancer surveillance and management programmes.

Understanding the role of genetics in the aetiology of CRC has increased rapidly during the past decade. This explosion of knowledge has, in a major way, been the result of the prodigious advances in molecular genetics. Indeed, this information has evolved so rapidly that it has outpaced the ability of physicians to keep abreast of these fast breaking events.

How can we help reduce cancer morbidity and mortality among high risk patients? The solution in some instances can be relatively simple through identification of such patients by virtue of their position in their family pedigrees. The identification process begins by systematically recording the family history with emphasis on cancer of all types and corroborated with medical and pathology documents whenever possible. Central to this is a knowledgeable physician who can interpret the pedigree, make a hereditary cancer syndrome

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diagnosis should it be present, and then proceed with highly targeted surveillance and management. Although this is the ideal situation, we nevertheless must deal with the fact that the cancer family history, although potentially the most cost beneficial component of a patient's medical history, is notoriously neglected in the clinical practice setting.[2][3] Ideally, a presumptive hereditary cancer syndrome diagnosis can be confirmed by molecular genetic testing of an affected subject in those disorders where germline mutations have been identified.

Our purpose is to provide an update of hereditary CRC, emphasising its clinical features, pathology, and molecular genetic advances.

Heterogeneity of hereditary CRC

Hereditary CRC can be divided into two groups based upon molecular features. Specifically, tumours ". . .that exhibit microsatellite instability (MIN) tend to occur in the right colon, have diploid DNA, carry characteristic mutations (transforming growth factor β type II receptor, *BAX*), and behave indolently. Hereditary non-polyposis colorectal cancer (HNPCC) epitomises this route of tumour development. Conversely, tumours with chromosomal instability (CIN) tend to be left sided, have aneuploid DNA, carry characteristic mutations (*K-ras*, *APC*, *p53*) and behave aggressively. Familial adenomatous polyposis (FAP) epitomises this type of tumour."[4]

Table 1 provides a list of the several hereditary disorders in which CRC is an integral lesion. However, since space does not allow a discussion of each of these disorders, we will describe briefly the most common hereditary forms, namely (1) FAP and its variants; (2) hereditary non-polyposis colorectal cancer (HNPCC), also known as Lynch syndrome; and (3) familial CRC where low penetrant genes may be important in the aetiology.

Familial adenomatous polyposis (FAP)

FAP is the classical paradigm for hereditary CRC. The disease has been known in medical publications for more than 100 years.[5] In the typical clinical setting, patients have more than 100 (often thousands) colonic adenomas which often begin in the rectosigmoid area of the colon. Prophylactic colectomy is the surgical treatment of choice, since most patients with the FAP trait will manifest colon cancer by 50-60 years of age. Patients are also at increased risk for periampullary carcinoma, papillary thyroid carcinoma, gastric carcinoma, sarcomas, and brain tumours. Desmoid tumours are also sequelae, particularly following intra-abdominal surgery. These tumours may occur in excess in certain FAP families.

When studying FAP families, one must be aware of the syndrome's genotypic and phenotypic heterogeneity. An example of this heterogeneity is the recent discovery of the attenuated familial adenomatous polyposis (AFAP) variant which is characterised by a lesser number of colonic adenomas with proximal colonic predilection and a later age of CRC

onset than occurs in classical FAP.[6][7] Spirio *et al*[8] identified a mutation in the 5' end of the *APC* gene that is aetiological for AFAP. Not all conditions with multiple adenomas are the result of *APC* mutations, as there have been accounts of patients with multiple adenomas who lack mutations in the *APC* gene.[9]

HNPCC (Lynch syndrome)

HISTORY

HNPCC, also termed Lynch syndrome, was originally called cancer family syndrome.[10] Historically, probably the first description of such a family was made by Aldred Warthin, a pathologist, who began studying a family in 1895 which he published in 1913.[11] This family, now known as family G, was restudied by Lynch and Krush in 1971[12] and found to have features of HNPCC.

HNPCC was first delineated as a hereditary cancer syndrome in the mid 1960s,[10] when an autosomal dominant mode of inheritance of CRC was described. The cardinal features of the Lynch syndrome are as follows: (1) autosomal dominant inheritance pattern, as mentioned; (2) gene penetrance for CRC of ≈85-90%; (3) gene carriers develop CRC at an early age (≈45 years); (4) most (≈70%) of the CRCs are proximal to the splenic flexure; (5) multiple CRCs, both synchronous and metachronous, are common; (6) the prognosis is better than that for sporadic CRC; (7) the pathological features of CRC are often distinguishable (but not pathognomic) and include poor differentiation, increased signet cells, medullary features, peritumoural lymphocytic infiltration, Crohn's-like reaction, and tumour infiltrating lymphocytes (TILs) mixed with tumour cells; (8) there is an increased risk for malignancy at several extracolonic sites, particularly the endometrium, ovary, stomach, small bowel, hepatobiliary tract, pancreas, ureter, and renal pelvis.[13] Breast cancer excess may be present in some HNPCC families.[14] In Warthin's family G, gastric cancer was exceedingly common before 1900. However, gastric cancer declined in subsequent generations, paralleling its decline in the general population.[12]

Before the discovery of germline mutations (*MSH2*, *MLH1*, *PMS1*, *PMS2*, known as mismatch repair genes or mutator genes), the diagnosis of HNPCC had to be made exclusively on the presence of clinical findings in concert with a thoroughly documented, and often extended, pedigree. The best estimate of gene carriership and thereby cancer risk was 50% based upon the patient being in the direct line of descent with one or more syndrome cancer affected first degree relatives.

MOLECULAR GENETICS OF HNPCC

We shall not review here the extensive number of publications pertaining to the cloning and characterisation of the MMR genes, nor those on MMR in general. Numerous reviews of these topics are available.[15–22]

From a practical point of view, the molecular diagnosis of HNPCC is usually based on searching the MMR genes for germline

mutations. A good overall picture of the mutational spectrum is provided by Peltomäki[23] and the updated HNPCC Mutational Database that is also accessible through OMIM. As shown in table 2, the database now lists a total of 228 presumptive disease causing mutations and 47 presumptive polymorphisms in the six genes. The list is growing rapidly and will no doubt soon comprise several hundred entries. A major question is the interpretation of missense mutations. These are particularly common in MLH1 and are a frequent source of uncertainty. Short of extensive and cumbersome assays of mismatch repair, there is currently no method that conclusively shows whether a missense mutation causes deficient mismatch repair. Easier in vitro yeast assays have been proposed[24] to fill this gap but apparently need further evaluation.

Founder mutations
There are well known examples of world wide founder mutations, such as the Glu6Val in the β globin gene that accounts for the majority of all sickle cell disease and the ΔF508 mutation of the CFTR gene that accounts for 50-80% of all cystic fibrosis world wide. Each of these mutations arose once in a single chromosome or, as in the case of the sickle cell mutation, a few times, many thousands, or even tens of thousands of years ago, and are believed to have attained world wide distribution and a relatively high allele frequency because they confer or conferred a selective advantage in heterozygotes. Founder mutations occurring in just one or a few human populations are now being described with increasing frequency and in some populations considerable enrichment of some mutations have occurred. Typically, populations displaying founder effects have grown rapidly from a small number of founders and without significant influx of people of different origins. Prime examples are the Finns ("founded" some 2000 years ago[25]), Icelanders (some 1100 years[26]), Ashkenazi Jews (600-800 years[27]), French Canadians, and Amish (250-400 years[28]). There is ample documentation of extremely high allele frequencies for rare disease genes in these populations. The mechanism of gene enrichment in these more recently founded populations is not likely to be any selective advantage but rather genetic drift, that is, the repeated effect of chance at population bottlenecks.

Only very recently has it emerged that mutations contributing to cancer predisposition can be enriched in human populations. A prime example is the genomic deletion of exon 16 of *MLH1* that has been called the "Finland 1" mutation. It occurs in some 40 ostensibly unrelated families in Finland and Sweden. Genealogical studies show that in a geographical cluster in south central Finland many or most of the affected subjects carrying the "Finland 1" mutation and belonging to different families descend from an ancestor who was one of a small number of "founders" of this Finnish subpopulation.[29] This "founding" occurred as late as some 500 years ago. However, other families with the same mutation live several

hundred kilometres farther to the south east and appear to have no genealogical connection. Recently, using a battery of intragenic and flanking polymorphic markers to determine the "age" of these mutations,[30] it could be shown that in the cluster in south central Finland the "age" was perhaps some 16 generations (range), while in the south east it was much older, perhaps greater than 40 generations.[31] These findings are fully consistent with the population history of the Finns and suggest that the "Finland 1" mutation arose or was brought into Finland from the south east and has spread to other parts of the country through repeated intra-Finnish founder effects.

In HNPCC, a number of recurrent mutations have so far been documented. Some of the most common ones are listed in table 3. Of note, when a mutation is seen repeatedly in ostensibly unrelated families, it is not certain to be a founder mutation; instead it could represent a de novo mutation that occurs recurrently. The two can be relatively easily distinguished by haplotype analysis. Founder mutations occur on the same haplotype while recurrent de novo mutations do not.

FREQUENCY OF HNPCC
In the past, to establish the frequency of HNPCC most investigators determined the proportion of all CRC patients that fulfilled the Amsterdam criteria.[32] By this method, estimates varying between approximately 0.5% and 5% were obtained.[16][33-36] Other methods have yielded highly discordant results. For example, Cannon-Albright *et al*[37] suggested that a high proportion of all colorectal tumours resulted from heritable mutations. By large segregation analyses, Houlston *et al*[38] concluded that 13% of CRC cases fit the model of dominant inheritance while Aaltonen *et al*,[39] studying a consecutive cohort of young CRC patients, extrapolated an HNPCC frequency of 0.5-0.9%.

Obviously, when using the Amsterdam or similar criteria as a sole definition, the smaller the family or the less pedigree information available, the less likely the definition of HNPCC will be fulfilled. This could skew the results and hamper comparisons between different series. Moreover, the Amsterdam criteria require the cancer to be colorectal in all affected family members, thus ignoring families with key members affected by other HNPCC cancers, in particular of the endometrium. More "relaxed" pedigree criteria for HNPCC have therefore been proposed.[40]

A key issue is how HNPCC should be defined today. Tumours from the great majority of Amsterdam criteria positive families are MSI positive,[41][42] suggesting that mismatch repair deficiency underlies most HNPCC. However, when Amsterdam positive families are tested for mutations in MMR genes (usually just in *MLH1* and *MSH2*), only between 45% and 86%[43-46] show a mutation. Even when the *PMS1* and *PMS2* genes were tested in addition, the proportion of mutation positive families was 70%.[47][48] In CRC families that do not fulfil the Amsterdam criteria

Table 1   Hereditary disorders in which CRC is an integral lesion

Hereditary form of colorectal cancer (CRC)	Inheritance pattern	Gene	Polyps	Cancer
Familial adenomatous polyposis (FAP)	AD	*APC* gene at chromosome 5q, mutation distal to 5'	Adenomatous, often start in distal colon/rectum; usually >100; adenomas may occur in small bowel; gastric polyps common, usually fundic gland polyps	CRC, average age onset 39; many cases teens and twenties; cancer of small bowel; stomach (particularly in Japan), papillary thyroid cancer, periampullary carcinoma, sarcoma, brain tumour
Attenuated familial adenomatous polyposis coli (AFAP)	AD	*APC* gene at chromosome 5q, proximal to 5'	Ordinary adenomas but also flat adenomas with proximal colonic predominance; may be few (5–10), sometimes >100	CRC with average age onset at 55; occasional periampullary carcinoma
Ashkenazi Jewish 11307K mutation	AD	11307K mutation in *APC*	Occasional adenomatous colonic polyps	CRC, "young" but average age of onset not known
Turcot's syndrome (HNPCC)	AD	(Both FAP (*APC*) and HNPCC, *hPMS2, hMLH1* mutation variants)	Multiple colonic adenomas, but may not be florid	CRC and central nervous system, particularly brain tumours. In *APC* (FAP families) cerebellar medulloblastomas. In *hMLH1* and *hPMS2* (HNPCC families) glioblastoma multiforme
Juvenile polyposis coli	AD	Protein tyrosine phosphate gene (*PTEN*) SMAD4/DPC4	Diffuse harmatomatous polyps (may have adenomatous component) of colon, but may occur in small bowel and stomach	CRC
Peutz-Jeghers syndrome	AD	Gene encoding serine threonine kinase (*STK11*) on chromosome 19p13.3 *LKB1/STK11*	Peutz-Jeghers polyps (may have adenomatous features) in stomach, small bowel, and colon	Stomach, small bowel, colon, sex cord tumours of ovary and testes
Hereditary mixed polyposis syndrome (IMPS)	AD	Unknown; possible site on chromosome 6q	Atypical colonic juvenile polyps, adenomatous and hyperplastic polyps; usually less than 15 colon polyps	CRC
Discrete colonic adenomatous polyps and CRC of Burt	AD; may be similar to some familial CRC		Occasional (never florid) adenomatous colonic polyps	CRC, average age in accord with population expectations
Hereditary non-polyposis colorectal cancer (HNPCC)	AD	Germline mutations of any of the mismatch repair genes: *hMSH2* at chromosome 2p; *hMLH1* at chromosome 3p; *hPMS1* at chromosome 2q; *hPMS2* at chromosome 7q	Occasional colonic adenomas which are on average larger, more villous, and at younger age than general population. Colonic polyps no more frequent than general population	CRC most common with proximal predominance, an excess of synchronous and metachronous CRC. Others include cancer of the endometrium, ovary, small bowel, stomach, and transitional cell carcinoma of ureter and renal pelvis. Average age of cancer onset is 44; may show rapid progression from adenoma to CRC
Familial CRC	Empirical risk 3 fold increase for CRC in patients with one or more first degree relatives with CRC; likely multifactorial and/or low penetrant genes	Unknown	In accord with population expectations	CRC, comparable to general population for age of onset and colonic location
Inflammatory bowel disease (ulcerative colitis (UC) and Crohn's disease (CD))	Unknown; possible AD in some families; polygenic also likely	Linkage to chromosome 16 (IBD$_1$) and 12 (IBD$_2$), findings which are tentative	Pseudopolyps (non-adenomatous)	CRC, lymphoma of GI tract

AD=autosomal dominant.   CRC=colorectal cancer.   IBD=inflammatory bowel disease.   UC=familial ulcerative colitis.   CD=Crohn's disease.
Adapted with permission from Lynch *et al*. *Eur J Cancer* 1995;**31A**:1039-46.

("HNPCC-like" families) the proportion with mutations in *MLH1* or *MSH2* is lower (8-30%).[43 44 49]

Taking all the evidence together, we tentatively propose that the presence or absence of a germline mutation in an MMR gene should be incorporated into the definition of HNPCC. By this criterion the diagnosis of HNPCC will be missed in some patients because not all MMR genes are studied, and because no mutation detection method is perfect. For example, the role of *MSH6* is not yet fully explored.[50] In at least two HNPCC or HNPCC-like families, *MSH6* mutations were implicated[51 52] and a high proportion of CRC patients with "mild" microsatellite instability was recently briefly described as having germline mutations of *MSH6*.[53] Whether *MSH3*, further MMR genes, or other genes such as *BAX* and *TGFβRII*, will turn out to contribute to inherited predisposition to CRC is not yet clear.[54 55] It may take time until the entire mutational spectrum of HNPCC is defined. Meanwhile, we propose to define any person or

Table 1 Continued

Non-cancer features	Screening	Surgical management/prophylaxis	Presymptomatic DNA testing	Genetic counselling
Gardner's variant-epidermoid cysts of skin, osteomas of mandible, congenital hypertrophy of the retinal pigment epithelium. Desmoid tumours (intrabodiminal) do not metastasise but may kill by direct extension; desmoids may be initiated by surgery (dissected surfaces); adrenal adenomas	Baseline flexible sigmoidoscopy age 10–12, and annually thereafter, for *APC* germline positive. If at risk but not tested for *APC*, same strategy. If eventually found to be *APC* negative, then baseline flexible sigmoidoscopy at age 15–20; if negative, no further screening. Upper endoscopy every 1–3 years starting when colonic polyps first appear. Screen remaining rectal segment (annually) after surgical prophylaxis	Prophylactic subtotal colectomy with low ileorectal anastomosis when phenotype (florid polyposis) identified; may be considered rectal mucosectomy with ileal pouch anal anastomosis if too many rectal polyps to manage or if compliance for rectal segment follow up is poor. Consider sulindac chemoprevention (while reducing polyps, cancer may still occur)	Test for *APC* germline mutation as early as age 10–12	Initiate pre-teens, include parents
Fundic gland polyps in stomach; adenomas in duodenum	Colonoscopy and upper endoscopy, initiate at age 20 and annually for *APC* germline positive or every 2 years if at genetic risk but not tested for *APC*	Prophylactic subtotal colectomy if too many polyps to manage; consider chemo-preventative sulindac	Test for *APC* germline mutation at age 20	Initiate at age 20, include parents
None known	Full colonoscopy, start at age 30–35 in gene carriers	Standard CRC surgery	Ashkenazi *APC* mutation	Start at age 25
Rare examples of multiple café au lait spots and pigmented naevi but not clear if truly integral to the syndrome	Baseline flexible sigmoidoscopy age 10–12 and annual flexible sigmoidoscopy thereafter; consider CT scan or MRI of brain	Prophylactic subtotal colectomy if colonic polyps present, as in FAP. In HNPCC variant, colonoscopy	Two DNA variants: (1) *APC* gene with predominance of cerebellar medulloblastoma, (2) *hMLH1* or *hPMS2* with predominance of glioblastoma multiforme	Initiate age 10–12, include parents
Children may manifest diarrhoea (may be severe)	Initiate colonoscopy age 10–12	Prophylactic subtotal colectomy when phenotype present with too many polyps to manage	Tyrosine phosphate gene (*PTEN*)	Initiate preteens, include parents
Mucocutaneous melanin pigmentation	Baseline colonoscopy and upper endoscopy, initiate age 20; flexible sigmoidoscopy annually thereafter	Consider prophylactic subtotal colectomy if too many polyps to manage and if mixed adenomatous features	Serine threonine kinase (*STK11*) on chromosone 19p13.3	Initiate teens, include parents
None known	Colonoscopy, initiate at age 20, 2–3 years	Polypectomy; consider prophylactic colectomy if polyps too many to manage	None known	Include initiation in teens
None known	Initiate baseline flexible sigmoidoscopy at age 40 and every 3 years thereafter	Standard CRC surgical approach	None known	Initiate at age 25–30
Muir-Torre syndrome variant shows cancer features of HNPCC but includes sebaceous adenomas, sebacous epitheliomas, basal cell epitheliomas with sebaceous differentiation, meibomian gland carcinomas and sebaceous carcinomas; single or multiple keratoacanthomas	Colonoscopy, initiate age 20–25, annually for germline mution carriers; every other year when mutation studies are lacking; endometrial aspiration biopsy at the same time as colonoscopy	Subtotal colectomy for initial CRC; consider option of prophylactic subtotal colectomy for germline carriers; consider prophylactic total abdominal hysterectomy and bilateral salpingo-oophorectomy for patients with initial CRC who have completed their families	Test for germline mutations no earlier than age 18–20	Initiate at age 18, before any consideration for gene testing
None	Baseline flexible sigmoidoscopy at age 35, repeat every 3 years; if two first degree relatives affected or one less than age 50 years, risk is 4–6 fold increased and full colonoscopy every 3–5 years is indicated	Standard surgical procedure for CRC	None known	Initiate at age 30–35
UC: arthritis, pyoderma gangrenosum, annular erythemas, and vascular thromboses, sclerosing cholangitis CD: similar to UC but small bowel involvement prominent, and may involve colon	UC: colonoscopy, annual in patients with chronic pancolitis of 8 or more years duration; check for high grade dysplasia of colonic mucosa. CD: BE may help; *x* ray of small bowel may show rigidity, narrowing submucosal oedema or stenosis, inflammation, "cobblestoned appearance" may see clinical and genetic overlap in UC and CD	Subtotal colectomy for CRC; consider prophylactic subtotal colectomy for patients with persistent high grade dysplasia of colonic mucosa in UC. Proctocolectomy if IBD mandates	None known	Initiate at age 18–20

family as having either mutation positive HNPCC or mutation negative HNPCC. This inevitably means that the entity of mutation negative HNPCC is somewhat loosely defined especially when family size is small. Moreover, new loci will be detected that will eventually show further genes whose mutations contribute to HNPCC or HNPCC-like syndromes.[56]

INCIDENCE OF MUTATION POSITIVE HNPCC
To determine the incidence of HNPCC, unselected consecutive series of colorectal (and other) cancer patients must be studied for mutations in the MMR genes. We know of only one large study of this kind. In a 1.25 million subpopulation of Finland, consecutive CRC tumours were collected prospectively, MSI determined, and *MLH1* and *MSH2* studied for mutation by genomic sequencing in the germline of all patients whose tumours were MSI positive. Among a total of 1050 tumours, 126 were MSI positive (12%) and, among these, 28 had germline mutations in *MLH1* or *MSH2*. Thus, the proportion of patients displaying

Table 2   Number of mutations and polymorphisms in the mismatch repair genes responsible for HNPCC (as of January 1999 at www.nfdht.nl)

Gene	No of disease causing mutations	No of polymorphisms
MLH1	134	15
MSH2	87	22
MSH6	3	5
PMS1	1	—
PMS2	2	5
TGFβRII	1	—
Total	228	47

mutation positive HNPCC was 2.8% in this series. Only part of this study has so far been published.[57]

Obviously, 2.8% is an underestimate. The main reasons are false negative MSI results and false negative mutation analyses. For instance, the lower the proportion of tumour cells in the specimen used for MSI analysis, the greater the risk of a falsely negative MSI result. However, when the pathological procedures are well controlled, as many as 85-95% of all HNPCC tumours are MSI positive.[41 58] Furthermore, mutation analysis by sequencing may simply miss a substitution of a single nucleotide. Perhaps more importantly, large deletions, inversions, and duplications will escape detection and may be more common than previously thought.[59] Of note, most of the other commonly used mutation detection methods, such as SSCP and DGGE, will also miss these types of mutations. Thus, as an MMR gene germline mutation was identified in 2.8% of the CRC patients in the study of Aaltonen *et al*,[57] and as some mutations may have been missed for reasons given above, the frequency of mutation positive HNPCC may be well over 3% in this population. Furthermore, this in turn represents an underestimate of the global HNPCC frequency because only CRC patients were screened. Had patients with endometrial and other HNPCC cancers been screened in the same population at the same time, some further HNPCC cases would undoubtedly have been detected. Hence we conclude that the estimate provided by this study does not differ significantly from previous estimates.

The incidence of MMR gene mutations in the Finnish subpopulation under study can be calculated by extrapolation as follows: if ~3% of all CRC patients have mutation positive HNPCC, and given that the lifetime risk of CRC is 5% in Finland, then the incidence of gene carriership is 3% of 5%, that is, 1 in 660 subjects.

The frequency of HNPCC needs to be studied in different populations and by different methods. It is quite possible that incidences vary. One source of variation might be the presence or absence of founder mutations that may show considerable enrichment in some populations (see above). We assume that the proportion of de novo mutations in HNPCC is low, because no examples have yet been documented. Therefore, in panmixing populations where enriched founder mutations are rare, the expectation is that MMR gene mutation incidences are similar. This assumption should now be tested. If environmental (mainly dietary) factors have an impact on the penetrance and expressivity of HNPCC cancer, then differences in disease incidence and presentation between sporadic and HNPCC cancer may prove highly informative. For instance, the age adjusted incidence of CRC is fivefold higher in the United States than in Mexico.[60] If the penetrance of HNPCC cancer is as high in Mexico as in the United States, the proportion of HNPCC among all CRCs might be much higher in Mexico. Conversely, if penetrance in HNPCC is heavily dependent on environmental factors, then Mexican carriers of MMR gene mutations might show a much lower penetrance. Such populations could prove invaluable in the study of dietary and other factors in CRC and in chemoprevention studies.

Thus, in summary, the overall incidence of HNPCC is the sum of the mutation positive and mutation negative forms. If our best estimate of the former is 3% and if relatively relaxed criteria are used to define the latter, then a figure between 5% and 10% may eventually emerge.

CANCER SPECTRUM IN HNPCC

Watson and Lynch[13] described the tumour spectrum in HNPCC. The presence or absence of extracolonic tumours has provided a rationale for subdividing HNPCC into Lynch syndrome I (CRC only) and Lynch syndrome II (CRC and extracolonic tumours). However, the differences in extracolonic involvement are often relative rather than absolute. For example, some families may have many examples of extracolonic tumours while some have few or none, making the distinction between Lynch I and Lynch II problematical (table 1).

The first systematic study of extracolonic cancer in HNPCC[13] compared the observed frequency of cancer at specific sites in more than 1300 high risk members of 23 HNPCC kindreds, with expectations based on general population incidence. Evaluation was made of the hypothesis that there was heterogeneity in cancer frequency among families. Findings showed significantly increased occurrences of cancers of the stomach, small intestine, transitional cell carcinoma of the upper urological tract (renal pelvis and ureter), and ovary. Carcinoma of the colorectum and endometrium, already established as integral to the syndrome, were not included in this assessment. No excess of cancer of the pancreas, lymphatic/haematopoetic system, larynx, breast, brain, or lung/bronchus was detected. In fact, significantly fewer lung/bronchus cases occurred than were expected, even when unverified cases were included.

Table 3   Founder mutations in HNPCC

Gene	Mutation	Population and approximate proportion of all HNPCC in that population		Reference
MLH1	Del exon 16	Finns	50%	45
MLH1	Splice acceptor exon 6 (del exon 6)	Finns	15%	46
MLH1	Ile→Arg, codon 320, exon 4	Finns	10%	46
MLH1	Splice donor exon 14 (breakdown of transcription)	Danes	25%	84
MSH2	943+3 A→T, splice donor exon 5 (del exon 5)	Newfoundlanders	50%	114

There was no evidence of screening examinations for cancer other than colon or endometrium that might have been prompted by family history. For example, a review of the cases of the stomach, small bowel, hepatobiliary system, kidney, ureter, and ovary, showed no cases that were discovered through screening examinations.

"Significant heterogeneity was observed (p<0.01) in the frequencies of endometrial and upper urologic tract cancers. Significant but less noticeable heterogeneity also was observed (p<0.04) in verified ovarian cancer. Cancers of the stomach, hepatobiliary system, and small bowel were distributed homogeneously among families. In the categories that combine organs by systems, significant heterogeneity (p<0.01) was observed in all cancers, all urological cancers, and all female genital cancers. The category of all gastrointestinal system cancers (except colorectal) differed significantly from homogeneous (p<0.05) when only verified cancers were included but not when unverified gastrointestinal tract cancers were included (p<0.08)."[13]

With respect to urological cancer, high risk relatives had three times as many kidney cancers and 22 times as many ureteral cancers as expected. However, there was no evidence of an increased risk of urinary bladder cancer or renal cell cancer. Endometrial cancer was probably the most important of the extracolonic cancers in influencing family ascertainment.

Recently, a study on cancer risk in a cohort of 1763 members of 50 genetically diagnosed families disclosed 360 mutation carriers in whom standard incidence ratios were calculated for the different HNPCC cancers.[61] These were significantly increased for colorectal, endometrial, ovarian, gastric biliary tract, uroepithelial, kidney, and central nervous system cancers. The cumulative cancer incidences by the age of 70 years were: colorectal 82%, endometrial 60%, gastric 13%, and ovarian 12%. For the other tumours associated with increased risk, the cumulative risk incidences were below 4%. Interestingly, as had been suggested before,[13 62] in mutation positive women the incidence of endometrial cancer (60%) exceeded that of colorectal cancer (54%). As soon as additional large studies like this one[61] begin to be published, it will be possible to conclude in a definitive way which cancers belong to the HNPCC spectrum. Clearly, many previous conclusions based on cases in which mutation status was not determined[13 63] are becoming confirmed. For instance, the risks for breast, prostate, and lung cancer do not appear to be raised.

"Bronchogenic carcinoma, common in the general population, occurred very rarely among the high risk family members. In the high risk group, none of the five persons reported to have lung cancer were putative gene carriers (that is, affected with colon or endometrial cancer or having offspring with these cancers). A recent report of cancer in relatives of patients with colon cancer[35] showed that lung cancer occurred only 60% as often in relatives of patients compared with control subjects. Mecklin et al[64] reported lung cancer in only four of 196 cancer affected members of 40 families with HNPCC. We have no evidence that members of the families we studied avoided exposure to carcinogens. In a recent survey of five families with HNPCC, 58% of 497 family members older than 19 years of age who responded indicated that they were current or former cigarette smokers."[13]

Heinimann et al[65] studied 27 Swiss HNPCC families, some meeting the Amsterdam criteria and some not, and some with germline MMR mutations and some not. The findings disclosed an excess of carcinomas of the endometrium, stomach, and brain. Eleven brain tumours were identified in this data set, three of which were glioblastoma multiforme while the remainder were of unknown type. Although an excess of brain tumours was not found in the paper by Watson and Lynch,[13] Vasen et al[66] described an increased risk of brain tumours in HNPCC. Previously Hamilton et al[67] showed that Turcot's syndrome (brain tumours, colonic polyps, and colon cancer) is, in fact, two syndromes, one involving the *APC* gene (CRC, colonic polyps, and medulloblastomas) and one involving HNPCC genes, specifically *MLH1* and *PMS2* (CRC, colonic polyps, and glial malignancies).

Gastric carcinoma was originally found in excess in Warthin's family G described in the early 1900s,[11] but this tumour declined in frequency over time in family G commensurate with its decline in most Western populations.[12] Aarnio et al[68] have elucidated our understanding of gastric cancer arising in HNPCC. Specifically, they found 62 gastric cancers occurring in 51 HNPCC families, encompassing 570 people. They were able to study 24 of the gastric cancers in detail, wherein both *MSH2* and *MLH1* families were represented. However, of keen interest and, indeed, contrary to our expectations about the relative prevalence of extracolonic cancer onset, only one of 22 patients with *MSH2* mutations had gastric cancer while 52 of 489 carriers of *MLH1* mutations manifested gastric cancer. Their average age at diagnosis was 56 years. Nineteen were intestinal cancers while three were defined as diffuse and two were apparently unclassified. Eleven of the 24 tumours showed microsatellite instability. The overall five year survival was 15%, but this improved to 48% with curative surgery which we assume involved lower stage cancers. Gastric carcinoma was the only tumour in 22 subjects and the first tumour in five others.

Carcinoma of the small bowel in HNPCC
Carcinoma of the small bowel is rare and accounts for only about 2% of all gastrointestinal malignancies.[69] However, in HNPCC the lifetime risk of small bowel carcinoma has been calculated to range from 1-4%, which is more than 100 times the risk for the general population.[70 71]

Rodriguez-Bigas et al[72] examined 42 patients from 40 HNPCC families who developed 42 primary and seven metachronous small bowel

cancers. Forty-six were adenocarcinomas while three were carcinoid tumours. The median age at diagnosis of the index small bowel tumour was 49 years. MMR gene mutations were identified in 15 of 42 patients (36%), nine of which were *MLH1* and six were *MSH2* mutations. Small bowel cancer was the first site in 24 patients (57%). Overall five and 10 year survival rates were 44% and 33% respectively. It was concluded that small bowel tumours can be the presenting neoplasms in HNPCC at risk subjects. These lesions occur at an earlier age and appear to have a better prognosis in HNPCC when compared to general population expectations.

Why do we find a pattern and an excess of extracolonic cancers in HNPCC? To date, a comprehensive explanation of these observed findings has not been offered. However, Fearon,[73] in his review of human cancer syndromes, provides an explanation for this phenomenon. Specifically, he suggests that the sites at risk must be exposed to an environmental injury that makes mutation or inactivation of the wild type allele more likely at that location. This would appear to fit the gastric cancer story in HNPCC, given the fact that the earliest reported generations of HNPCC kindreds showed a gastric cancer excess which, interestingly, was even more common in certain families. Of further interest to this genetic/environmental interactive hypothesis, Aarnio *et al*[68] reported that most gastric cancers in HNPCC are intestinal, the very type of gastric cancer that has been most strongly associated with environmental aetiologies.

Muir-Torre syndrome

Diagnosis of the Muir-Torre syndrome (MTS) requires at least a single sebaceous gland neoplasm (adenoma, epithelioma, or carcinoma) or keratoacanthoma(s) or both, in concert with a minimum of one internal malignant neoplasm. The association with sebaceous gland tumours is important in that these cutaneous lesions are exceedingly rare. The first recognition of this association was published in 1966 by Muir *et al*[74] and in 1968 by Torre,[75] leading to the Muir-Torre eponym for this disease. These investigators noted that their patients manifested multiple visceral adenocarcinomas with early age of onset and a clinical course that was relatively benign on occasion. Constant features were the findings of benign and malignant sebaceous neoplasms of the skin and, less frequently, multiple keratoacanthomas. The diagnostic criteria for MTS has been extensively reviewed by Cohen *et al*.[76]

Lynch *et al*[77–79] were the first to describe the cutaneous findings of MTS as part of Lynch syndrome II. More recent publications[21 80–82] have identified *MSH2* and *MLH1* germline mutations as culprit predisposing genes in MTS.

Phenotype-genotype correlations in HNPCC

Vasen *et al*[71] compared the cancer risk in 124 subjects who were carriers of germline *MLH1* mutations with 86 patients with known mutations of *MSH2*. The lifetime risk of CRC was the same for both groups (80%), as was the risk for small bowel carcinoma (at 100 times the general population risk in both groups). Interestingly, endometrial carcinoma appeared to be more common in the subset with *MSH2* mutations (61% *v* 42%), but the difference was not statistically significant. Carriers of the *MSH2* mutations showed an increased risk for transitional cell carcinoma of the renal pelvis, ureter, and adenocarcinoma of the stomach and ovary when compared to *MLH1* mutation carriers.

Lin *et al*[83] described similar findings from the Creighton HNPCC resource. Here, extracolonic cancers were found in 33% of patients with *MSH2* mutations but in only 12% from *MLH1* families (p<0.02).

Gender may also be an important determinant of tumour occurrence in HNPCC. This was evidenced by the work of Dunlop *et al*[62] in their study of 64 HNPCC patients who carried mutations of MMR genes and who were assessed for cancer risk up to the age of 70. Interestingly, the risk was 91% for men and 69% for women. The risk of CRC was strikingly different between the sexes: 74% for men compared with 30% for women (p<0.01). Endometrial carcinoma was actually more common than CRC for female HNPCC patients in this study.

Jäger *et al*[84] postulated that the specific nature of the mutation within the gene may contribute heavily to the phenotype. They described an *MLH1* intron 14 splice donor mutation in five Danish HNPCC families. This mutation resulted in a "silenced" allele in that no abnormal protein was generated. The families with this mutation showed a natural history similar to 16 other HNPCC families with respect to CRC, but only two extracolonic cancers were identified, namely one endometrium and one of the ampulla of Vater, compared with 44 in the other families. The extracolonic cancer to CRC ratio was 2:23 in families with the intron 14 splice donor mutation, compared with 44:91 in the other families. These investigators hypothesised that the silenced allele resulted in a less severe disease, owing to the absence of a dominant negative effect.

Miyaki *et al*[52] identified the *MSH6* germline mutation in a family that did not meet the Amsterdam criteria but which showed a predominance of carcinoma of the endometrium and ovary. Akiyama *et al*[51] also described a germline mutation of *MSH6* in an atypical HNPCC kindred. All three of the proband's colon tumours showed microsatellite instability and mutations of both *MSH6* alleles, indicating that this mutation predisposed to the syndrome in this family.

Finally, Beck *at al*[85] suggested that those cancer families that did not meet the Amsterdam criteria but who have germline mutations often have missense mutations. These investigators speculated that missense mutations result in less severe disease or lower penetrance because of less severe structural change of the encoded protein. To date, no one has provided a comprehensive explanation for the various

patterns of tumour combinations in any of these disorders including, of course, HNPCC.

SURVIVAL OF CRC IN HNPCC

Watson *et al*[86] performed a retrospective cohort study comparing survival characteristics among HNPCC cases (274 cases from 98 HNPCC families) with an unselected hospital series of 820 consecutive CRC cases. Patients were staged according to the TNM system of the American Joint Committee on Cancer and the International Union Against Cancer. When compared with the unselected series, " . . .the HNPCC cases had lower stage disease (p<0.001), and fewer had distant metastases at diagnosis (p<0.001 in an analysis stratified by T classification). In stage stratified survival analysis, the HNPCC cases had a significant overall survival advantage regardless of adjustment for their younger age. A conservative estimate of the hazard ratio (of HNPCC cases to the unselected series) was 0.67 (p<0.0012)." Sankila *et al*[87] reported similar survival data when they studied 175 patients with HNPCC and compared them with 14 086 patients with apparently sporadic CRC, confirming that patients with HNPCC who develop CRC have a better prognosis than patients with sporadic CRC.

The lower stage disease at the time of diagnosis of the HNPCC patients compared to the unselected CRC cases was mainly attributed to rarer distant metastases at the time of diagnosis. Their survival was longer than the unselected CRC patients with same stage tumours. Of keen interest was the fact that the estimated death rate for the HNPCC cases, when adjusted for stage and age differences, was at most two thirds of the rate for the hospital series.

PATHOLOGY OF HNPCC

Fujiwara *et al*[58] studied 39 HNPCC CRCs (HNPCCa) and 57 sporadic right sided CRCs (SRSCCa). Findings disclosed that "Of HN-PCCa, 95% (37/39) were MSI positive as contrasted with 31% (18/57) of SRSCCa (p=0.000001), but instability tended to be more widespread in SRSCCa (p=0.08). Absence of nuclear *MSH2* mismatch repair gene product by immunohistochemistry was associated with germline *MSH2* mutation (p=0.0007). The prevalence of *K-ras* proto-oncogene mutations was similar in HNPCCa and SRSCCa (30% (11/37) and 30% (16/54)), but no HNPCCa from patients with germline *MSH2* mutations had codon 13 mutations (p=0.02), and two other HNPCCa had multiple *K-ras* mutations attributable to subclones. 18q allelic deletion and *p53* gene product overexpression were inversely related to MSI (p=0.0004 and p=0.0001, respectively). Frameshift mutation of the transforming growth factor β type II receptor gene was frequent in all MSI positive cancers (85%, 46/54), but mutation of the *E2F-4* transcription factor gene was more common in HNPCCa of patients with germline *MSH2* mutation than in those with germline *MLH1* mutation (100% (8/8) versus 40% (2/5),

p=0.04), and mutation of the *Bax* proapoptotic gene was more frequent in HNPCCa than in MSI positive SRSCCa (55% (17/31) versus 13% (2/15), p=0.01). The most common combination of mutations occurred in only 23% (8/35) of evaluable MSI positive cancers."

The authors concluded that their findings depict marked heterogeneity resulting from the accumulation of specific genetic alterations in MSI positive CRCs. Indeed, it is this very genetic heterogeneity that may be responsible for the " . . .heterogeneous clinical and pathological features of MSI positive cancers."

Estimates have shown that the frequency of colonic adenomas in HNPCC is the same as in the general population. However, this important issue remains unresolved. For example, Beck *et al*[9] have suggested that HNPCC patients may develop adenomas earlier and more often than the general population. A St Mark's Hospital study has shown a greater frequency of multiple adenomas in HNPCC when compared to the general population.[88] Jass and Stewart[89] also identified significantly more adenomas in HNPCC patients younger than 50 than in age matched necropsy controls. They found that adenomas in HNPCC were larger, more often villous, and had more high grade dysplasia. These findings are consistent with our hypothesis that adenomas in HNPCC have a greater proclivity for malignant degeneration than sporadic adenomas. Jass[90] advocated the "aggressive adenoma" theory, where adenomas form about as often in HNPCC patients as in the general population; nevertheless, once formed, they progress to carcinoma more quickly or more often or both than their sporadic counterpart. Further evidence in support of this theory has been found in a Finnish study which showed a marked decrease in colon cancer incidence among HNPCC patients who have undergone regular colonoscopic surveillance with removal of adenomas.[91]

Smyrk *et al*[92] and Jass *et al*[93] have made important contributions to the study of the pathology of CRC in HNPCC. CRCs in HNPCC show a tendency towards a solid growth pattern which accounts for the high frequency of poorly differentiated carcinomas in this disorder.[93][94] These tumours resemble the "undifferentiated carcinoma" described by Gibbs[95] and the "medullary carcinoma" described by Jessurun and Manivel.[96] These tumours appear to have a better prognosis than more typical types of CRC. Similar histology features characterise the 15% of sporadic CRCs which express microsatellite instability.[97] This special histology has been referred to as "solid-cribiform" wherein the pattern has a positive predictive value of 53% for MSI+ status.[98]

Smyrk *et al*[92] also described the host lymphoid response, namely the "Crohn's-like reaction," as being more common in HNPCC than in sporadic CRCs. Although this finding is not consistently true in all series,[93] a similar tendency to form lymphoid aggregates around the tumour appears to be a feature of sporadic RER+ colon tumours as well.[97] In the general

population, a Crohn's-like reaction is associated with improved prognosis.[99] It will be important to determine whether this phenomenon accounts for the more favourable prognosis of CRC in HNPCC.[86]

Aberrant crypt foci of the colon

Aberrant crypt foci (ACF) are characterised by lesions in colonic mucosa of mice[100] as large and thick crypts in methyline blue stained specimens that have been treated with a carcinogen (azoxymethane). These crypts, comparable to those in the rodent models, were subsequently reported in colonic mucosa in the human.[101]

Roncucci *et al*[102] studied ACF in colonic mucosa in a cohort of patients with CRC in two Italian provinces. Findings showed that "Density of ACF was higher and crypt multiplicity lower proceeding from proximal to distal large bowel. Microadenomas were observed only in the colon, whereas hyperplastic ACF were more frequent in the rectum." They concluded that the density of ACF correlated with CRC rates in the two Italian provinces where it showed " . . .a positive gradient from proximal to distal large bowel. Histology of ACF suggests that they may be precursors of both hyperplastic and adenomatous polyps. These data provide further evidence of the role of ACF in human colorectal carcinogenesis."

In their review, Roncucci *et al*[102] noted that Augenlicht *et al*[103] described genomic instability at microsatellites which were indicative of DNA MMR deficiency in human ACF. They also noted that Heinen *et al*[104] also found microsatellite instability to be restricted to ACF from right sided colonic mucosa among patients with large bowel cancer. Roncucci *et al*[102] conclude that "Microsatellite instability is also more frequent in right sided colon carcinoma, reinforcing a concept of different pathways for proximal and distal large bowel cancer, and giving support to the hypothesis of ACF involvement in cancer development."

Takayama *et al*[105] have reviewed the subject of ACF foci and, using magnifying endoscopy, studied the prevalence, number, size, and dysplastic features of ACF in concert with their distribution according to age in 171 patients, 131 of whom had a colonic adenoma(s) and 48 had CRC. The authors also prospectively evaluated the prevalence of ACF in 11 subjects, of whom four were normal, six had adenoma, and one had cancer, before and after administration of 100 mg of sulindac three times a day for eight to 12 months. They then compared their results with nine untreated subjects, of whom four were normal and five had adenoma. They all had baseline findings of ACF.

Findings disclosed that of 3155 ACF, 161 were found to be dysplastic. The prevalence and number were found to increase with age. Furthermore "There were significant (p<0.001) correlations between the number of aberrant crypt foci, the presence of dysplastic foci, the size of the foci, and the number of adenomas. After sulindac therapy, the number of foci decreased, disappearing in seven of 11 subjects. In the untreated control group, the number of foci was unchanged in eight subjects and slightly increased in one (p<0.001 for the difference between the groups)." These authors concluded that such ACF, in particular those that are large and harbour dysplastic features, may constitute " . . .precursors of adenoma and cancer."

Further research studies are needed on ACF in HNPCC, since this finding could provide a useful model for elucidating CRC's pathogenesis in HNPCC. In addition, ACF might also provide a pathological "marker" for the effectiveness of chemoprevention studies should they show a reduction in frequency of ACF following exposure to a particular chemopreventive agent.

CLINICAL SURVEILLANCE IN HNPCC

To estimate the effectiveness of surveillance, Vasen *et al*[106] developed a model for estimating life expectancy and health care costs of surveillance for carriers of an HNPCC mutated MMR gene. Colonoscopy was performed every two to three years and was compared to patients who did not receive this CRC surveillance. Estimates were then determined for a lifetime risk of developing CRC in concert with the stage distribution of this cancer among symptomatic subjects who were derived from the Dutch HNPCC Registry. Results indicated that gene carriers under surveillance had an increase in life expectancy of seven years, and also that the cost of surveillance was less than the cost of no surveillance. These investigators concluded that CRC surveillance of HNPCC gene carriers was effective and they recommended that governmental agencies as well as health insurance organisations support such surveillance.

Syngal *et al*[107] examined the life expectancy and quality adjusted life expectancy benefits resulting from endoscopic surveillance and prophylactic colectomy among harbingers of one of the culprit germline mutations for HNPCC. Each of the risk reduction programmes showed large gains in life expectancy for mutation carriers, with benefits "...ranging from 13.5 years for surveillance to 15.6 years for prophylactic proctocolectomy at 25 years of age compared with no intervention. The benefits of prophylactic colectomy compared with surveillance decreased with increasing age and were minimal if colectomy was performed at the time of colorectal cancer diagnosis." These authors concluded that colonoscopic surveillance was effective among HNPCC mutation carriers. However, the choice between prophylactic surgery and surveillance poses a complex decision for the patient.

Because of the early age of CRC onset in HNPCC, coupled with the proximal predilection for CRC, we strongly recommend that colonoscopy be initiated by the age of 20 to 25 in patients at 50% risk for HNPCC based upon their position in the pedigree, or those who are HNPCC germline mutation carriers. Because of the problem of accelerated carcinogenesis, we recommend that colonoscopy be performed every other year in those high risk patients who have not had DNA testing and annually in

those with HNPCC germline mutations. It is important to realise that colonoscopy "miss" rates are as high as 29% for polyps <5 mm in diameter.[108] Järvinen *et al*[91] identified six CRCs among HNPCC germline mutation carriers who were undergoing three yearly colonoscopies, while Vasen *et al*[109] discovered five interval cancers in HNPCC patients within three and a half years following a normal colonoscopy. In reviewing this subject, Church[110] suggests that these interval CRCs develop from normal epithelium within three years or from adenomas that were missed. In order to minimise the "miss" rate, it is mandatory that the preparation be excellent and a meticulous examination of the entire colorectal mucosa be performed. Patients should be advised that colonoscopy is not a perfect screening procedure and hence the option of prophylactic colectomy.[111 112]

Colonoscopy v prophylactic colectomy in HNPCC
Subtotal colectomy as a prophylactic measure among HNPCC patients remains controversial. Patients who harbour one of the culprit germline mutations are offered this option as an alternative to lifetime colonoscopic surveillance. Genetic counselling must be provided so that patients can be in a better position to evaluate the advantages as well as the potential sequelae of these varying management strategies. In the case of prophylactic colectomy, the mortality risk is low, but there is a possible long term morbidity of frequent bowel movements. Patients also need to know that they will require continued endoscopic surveillance of their remaining rectal mucosa since its cancer risk is about 1% per year.[113]

Froggatt *et al*[114] call attention to the risk of CRC in HNPCC being significantly higher (p<0.01) in males as opposed to females at 50 and 60 years of age respectively. In turn, females had a high risk of endometrial cancer (0.5 at 60 years) as well as premenopausal ovarian carcinoma (0.2 at 50 years). These authors appropriately conclude that such intersex differences in colorectal cancer risks in HNPCC " . . .have implications for screening and programmes and for attempts to identify colorectal cancer susceptibility modifiers."[114]

Why is prophylactic subtotal colectomy considered an important option for these high CRC risk HNPCC patients? The answer is predicated upon a number of anecdotal reports of interval CRCs occurring within one to four or five years following surveillance colonoscopy. For example, Lanspa *et al*[115] studied 225 subjects with 313 colon cancers from families on file in the Creighton University Lynch syndrome resource. Six of these patients, from different families, manifested CRCs arising within 4.5 years of a normal colonoscopy. Another 17 patients had metachronous colon cancers within five years of resection (less than subtotal colectomy) of their initial colon cancer. Thus, of 225 CRC patients from Lynch syndrome families, 10.2% had CRC within five years of colonoscopy or colon resection.

Prophylactic surgery in the Lynch syndromes raises the question as to whether the level of risk in the disorder merits preventive colectomy. DeCosse's answer is that "In the added presence of defective HNPCC genes, the answer seems affirmative."[116] In the case of Lynch syndrome II, he states that when surgery is planned for the presence of CRC, prophylactic bilateral oophorectomy and hysterectomy, particularly if the woman is postmenopausal, should also be offered as an option. This is a consideration which we have long recommended.[15 117–119]

The rationale for prophylactic colectomy in HNPCC does not vary significantly from its well accepted orthodoxy for cancer control in FAP. For example, in FAP the average age of CRC onset is about 39 years, whereas in HNPCC this is about 40 to 44 years. Importantly, synchronous and metachronous CRC occurs with approximately the same frequency in germline carriers of the two syndromes. FAP differs with respect to the phenotype of florid polyposis and a higher penetrance of CRC expression than in HNPCC.

Church[112] discusses the advisability of prophylactic colectomy in HNPCC and provides arguments for and against this cancer control practice. Factors favouring prophylactic surgery would be a patient's reduced compliance for colonoscopy and the biology of cancer in HNPCC wherein the adenoma-cancer sequence is accelerated. HNPCC patients with one or more adenomas pose a special consideration for prophylactic colectomy. Special consideration for prophylactic colectomy should be given to a patient with a large colonic polyp that cannot be removed endoscopically, where there is a polyp with intramucosal adenocarcinoma or carcinoma in situ, or the presence of multiple colonic adenomas. When only a small tubular adenoma is present or there are no adenomas, then the decision regarding prophylactic colectomy may be more difficult.

Church[112] points out that patients with ulcerative colitis and FAP, who may otherwise be in reasonably good health but whose colons may show severe dysplasia and multiple adenomas, clearly do not have normal colons. However, in HNPCC, the colon may appear to be normal but it is truly not normal given the fact that "Mismatch repair gene mutations are present in the nucleus of every colonocyte and it is only a matter of time before they are manifest as a tumour." He appropriately asks the question "Why should prophylactic colectomy be routine in one syndrome but not the other? I favour prophylactic colectomy in patients with mutations in the mismatch repair gene associated with HNPCC who are members of a family in which there is a strong clinical pattern of inherited colorectal cancer. . . When cancers appear in young relatives, prophylactic surgery needs to be done early. Patients must not be at an increased risk for complications and must fully understand the rationale behind the recommendation."

Chemoprevention in HNPCC
Rüschoff *et al*[120] found that microsatellite instability in CRC cells that are deficient for a

subset of the mismatch repair genes, namely *MLH1*, *MSH2*, and *MSH6*, is markedly reduced following exposure to aspirin or sulindac. These findings were reversible and were independent of proliferation rate and cyclo-oxygenase function. Interestingly, an endometrial cancer cell line did not show any such changes by aspirin/sulindac. The microsatellite instability reduction in these mismatch repair deficient cells was confined to non-apoptotic cells. These authors concluded that their results ". . .suggest that aspirin/sulindac induces a genetic selection for microsatellite instability in a subset of MMR deficient cells and may provide an effective prophylactic therapy for hereditary non-polyposis colorectal cancer kindreds where alteration of the *MSH2* and *MLH1* genes are associated with a majority of cancer susceptibility cases."

ANIMAL MODELS OF HNPCC
Understanding the aetiology, pathogenesis, and control of HNPCC may be accelerated by studying animal models. De Wind *et al*[121] have developed mouse models harbouring a deficiency in the MMR gene *Msh2*. Interestingly, the majority of these *Msh2* deficient mice succumbed to lymphomas at an early age. These malignancies were synergistically enhanced through exposure to ethylnitrosourea. The immunocompromised *Tap1*$^{-/-}$;*Msh2*$^{-/-}$ mice " . . .generally succumbed to HNPCC-like tumours. Together, these data suggest that the HNPCC tumour spectrum is determined by exposure of MMR deficient cells to exogenous mutagens, rather than by tissue specific loss of the wild type MMR allele or by immune surveillance. *Msh2* hemizygous mice had a raised tumour incidence that, surprisingly, was rarely correlated with loss of the *Msh2*$^+$ allele."

These authors developed a model for intestinal tumourigenesis in HNPCC by introducing the Min allele of the *Apc* tumour suppressor gene. Their findings disclosed that there was " . . .loss of the wild type *Msh2* allele in a significant fraction of intestinal tumours in *Apc*$^{+/Min}$;*Msh2*$^{+/-}$ mice. In some of the latter tumours, one area of the tumour displayed loss of the *Msh2*$^+$ allele, but not of the *Apc*$^+$ allele, whereas another displayed the inverse genotype. This apparent biclonality might indicate a requirement for collaboration between independent tumour clones during intestinal tumourigenesis."

MEDICAL LEGAL IMPLICATIONS OF HNPCC
The prodigious advancements in knowledge about genetic risk, natural history, recommended available surveillance and management, DNA testing, and the need for genetic counselling collectively are impacting significantly on the standard of care for HNPCC and FAP, as well as for patients with a variety of other hereditary cancer prone disorders.

Genetic mechanisms
EPIGENETIC FACTORS IN THE CAUSATION OF CRC
The fundamental role of genes in the processes leading to cancer is now well established. Until recently, research into the quantitative and

qualitative expression of genes has focused on changes in gene sequence, commonly referred to as mutations. However, alterations in gene expression can in addition be brought about by mechanisms other than sequence changes; these are referred to as epigenetic changes. Here we briefly review two epigenetic phenomena with cancer relevance: gene silencing by methylation and loss of imprinting. Several recent reviews are available for further reading.[122 123]

Gene silencing by methylation
Cytosine residues can acquire a methyl group in the C-5 position. This occurs on the opposite strands of the palindromic sequence CpG. Many genes have regions rich in CpG doublets, commonly referred to as CpG islands or Hpa tiny fragment or HTF islands. In widely expressed genes, CpG islands are typically, but not exclusively, located in the promoter region. As a rule, CpG islands are unmethylated. When methylation occurs, the binding of transcription factors is inhibited and transcription initiation impeded, leading to silencing of the gene. De novo changes in methylation is one of the mechanisms by which genes are switched on and off during normal development. It is now becoming increasingly clear that, similarly, changes in the methylation pattern is a common phenomenon in cancer.

Recently, findings of interest to hereditary CRC have been unveiled. First, hypermethylation of the promoter region of *APC* was found in CRC[124] but its significance was not assessed, for example, by analysing APC expression or protein. Interestingly, hypermethylation was almost totally confined to tumours in the right sided, as opposed to the left sided, colon. Second, in the mouse model of FAP, the *Apc*^{Min} mouse, it was possible to reduce dramatically the formation of polyps by suppressing genomic methylation.[125] This was accomplished by rendering the mice heterozygous for the DNA methyltransferase gene and by injecting them with the DNA methyltransferase inhibitor 5-aza-2'-deoxycytidine (5-aza-C). While this result did not prove that demethylation of the *Apc* gene specifically accounted for the reduction in polyp number, it raised the provocative possibility that if targeted demethylation could be accomplished, this might suppress tumourigenesis.

Recently, it was shown that in MSI positive sporadic CRCs, hypermethylation of the *MLH1* promoter region is common[126–129] and associated with the absence of immunoreactive MLH1 protein.[127] The hypermethylation could be reversed by treatment with 5-aza-C and this restored MLH1 protein.[127] Somewhat surprisingly, no corresponding findings could be shown for *MSH2*, but some 85% of all MSI positive sporadic (non-HNPCC) tumours could be accounted for by the silencing of *MLH1* in this way. It is well known that 12-15% of all CRCs are MSI positive, but only one quarter or one fifth of them represent HNPCC. Thus, it appears that almost all MSI positive CRCs can be accounted for. A minority, perhaps one fifth, occur in HNPCC patients; most of these have one inherited

mutation in *MLH1* or *MSH2* and a somatic change knocking out the second allele. In a majority, perhaps some four fifths, the mismatch repair deficiency is caused by the biallelic knock out of *MLH1* by somatic hypermethylation of its promoter. Details of this scheme still remain to be worked out. For instance, it has been shown previously that the "second hit" in *MLH1* can be either a mutation[47] or a deletion (loss of heterozygosity; LOH).[130] It now appears that hypermethylation can also produce the "second hit," but it is not clear how often this occurs. Moreover, while in some sporadic cases biallelic hypermethylation of *MLH1* has been documented, it is possible that in others, one allele may be knocked out by methylation, the other by LOH or mutation. This raises the question whether somatic gene specific and allele specific de novo methylation occurs or whether methylation is usually a generalised and biallelic phenomenon.

As most MSI positive sporadic CRCs result from hypermethylation of *MLH1*, this prompts intriguing speculation about the role of epigenetic factors in general. Why do these tumours show a better prognosis[42 86 87 131] and pronounced right sided predominance,[132] and why are they associated with typical age at diagnosis rather than young age?[132 133] What environmental factors contribute to hypermethylation? Is hypermethylation gene specific, organ specific, age specific?[134] Might inhibition or reversal of hypermethylation be a worthwhile preventative or therapeutic strategy? These are important questions that might have an impact on our understanding of the molecular processes in a substantial subset of CRC and, as shown very recently, in gastric cancer[135 136] and endometrial cancer[137] as well.

Imprinting and loss thereof
Imprinting is said to occur when the two alleles in a somatic cell are differentially expressed. Studies on early zygotes and embryos suggest that the modified expression pattern (up or down regulation) is prezygotic, in other words, is introduced by the gamete (egg or sperm). For instance, the insulin-like growth factor 2 (*IGF2*) gene is usually expressed on the paternal, but not the maternal, chromosome. It is believed that the differential imprinting of genes plays an important role in development.[138 139]

Not surprisingly, modifications of imprinting have recently been implicated in cancer. Loss of imprinting (LOI) can be defined either as the activation of a normally silent allele or the silencing of a normally expressed gene.[140 141]

Recently, LOI for *IGF2* was studied in colorectal cancers.[142] Ten of 11 CRCs that were MSI positive showed LOI while only two of 16 that were MSI negative had LOI. Most intriguingly, in those patients whose tumours showed LOI, normal colonic mucosa and, in some instances, blood leucocytes, also showed LOI. In subjects without colon cancer, LOI was rare but did occur in colonic mucosa in two of 16 cases and in blood in two of 15 cases.[142] These findings will require independent confirmation. Moreover, numerous questions need to be answered. Is the *IGF2* gene of importance in CRC or does the LOI phenomenon relate to an entire region that may contain other genes of importance for CRC? If the LOI means activation of the maternal allele (the paternal one is known to be expressed normally), is the main consequence an overall increase in IGF2 activity or some specific effect of the maternal allele? Since, in people whose tumours show LOI, normal mucosa and blood leucocytes also show it, is LOI a body wide phenomenon? As a small number of controls without cancer show LOI, might these people be at increased risk of cancer? What is the basis of the association between microsatellite instability and LOI?

HOMOZYGOSITY FOR MISMATCH REPAIR DEFICIENCY
Recently two reports described examples of people who were homozygous for a mismatch repair gene mutation. In each case, children of consanguineous parents (North African and Turkish, respectively) were homozygous for germline *MLH1* mutations; accordingly, all four parents were heterozygous for an HNPCC mutation. Remarkably, four of five children homozygous for these mutations had neurofibromatosis type 1 that was not known to have occurred in other members of these families. In addition, in one of the families, one homozygous child died at 2 years of age from non-Hodgkin's lymphoma, and one had acute myeloid leukaemia at the age of 6 followed by medulloblastoma at the age of 7.[143] In the other family, one child whose mutational status could not be confirmed died at 2 years of age of acute leukaemia, one child developed non-Hodgkin's lymphoma at 3 years, and the third child developed atypical chronic myeloid leukaemia at 1 year.[144] These reports were brief and gave only scanty clinical and molecular data. Nevertheless, the phenotypes of the four affected children in whom homozygosity for the mutation was proven were fully concordant for both neurofibromatosis and early onset haematological malignancy. Their somatic tissues were, indeed, mismatch repair deficient as shown by the demonstration of MSI in buccal mucosa cells of one subject. One previous example of a subject with constitutional mismatch repair deficiency owing to compound heterozygosity for two different *MLH1* missense mutations has been described.[145] This patient developed breast cancer at the age of 35 and was not reported to have had neurofibromatosis or haematological malignancy.

The paediatric patients homozygous for *MLH1* mutations could not be studied in great detail; for instance, mutation analyses of the *NF1* gene were not done, nor were any molecular studies of the affected haematological cells reported.[143 144] Nevertheless, these data show that normal human development is possible in the presence of deficient mismatch repair in every cell of the zygote. This is consistent with the findings in transgenic mice homozygous for MSH2 or MLH1 deficiency.[121] However, while such mice do develop lymphoma both early and late in life, haematological malignancies have not been reported in

a high proportion of young knock out animals, nor has neurofibromatosis been noted. Clearly, the phenotypic features displayed by these children suggest that mismatch repair deficiency leads to mutations in *NF1*, as well as in so far unidentified genes predisposing to lymphoma and leukaemia. The patient described by Hackman *et al*[145] who was homozygous for two different missense mutations of *MLH1* had a much milder phenotype; this was tentatively explained by assuming that at least one of the mutations allowed residual MMR activity.

LOW PENETRANCE GENES PREDISPOSING TO CRC
I1307K mutation of APC
The report by Laken *et al*[146] is one example of a low penetrant mutation within the *APC* gene. These authors initially studied a 39 year old Ashkenazi Jewish patient who manifested eight adenomatous polyps of the colorectum and who had a family history of CRC. Through an analysis for microsatellite stability in his tumours, hereditary non-polyposis colorectal cancer (HNPCC) was excluded. A truncated APC polypeptide was identified through in vitro synthesised protein (IVSP) assay following in vitro transcription and translation of polymerase chain reaction (PCR) products spanning codons 1099-1693. Surprisingly, sequencing of the relevant region of *APC* showed an absence of the truncating mutations typical of FAP. Instead, a T to A transversion of nucleotide 3920 was identified with the mutation showing a substitution of lysine for isoleucine at codon 1307 (I1307K). The protein truncation was found to be an in vitro phenomenon caused by the A to T transversion resulting in a hypermutable tract. This mutation was not identified in any of the 243 non-Ashkenazim who were examined. In contrast, 6.1% of the 766 Ashkenazi Jewish CRC patients were found to carry the mutation; the difference between these proportions was highly significant.[146]

Next, to determine whether the I1307K mutation was associated with CRC in Ashkenazi Jews, Laken *et al*[146] examined 211 CRC affected Ashkenazim and found that 10.4% of them harboured the I1307K mutation. This was a significantly larger proportion than that seen in Ashkenazim without CRC. In addition, the prevalence of the I1307K mutation was higher in patients under the age of 66 than in those over 66 years old. Laken *et al*[146] also observed the mutation more commonly (28%) in Ashkenazim with CRC and a first or second degree relative with CRC or adenomatous polyps or both than in Ashkenazim with CRC and an unknown or negative family history. They roughly estimated that carrying this mutation resulted in a doubling of CRC risk over the patient's lifetime. They also estimated that the lifetime incidence of CRC in the general Ashkenazi population ranges from 9-15%[147-149]; hence, they suggested that the lifetime risk for CRC in people with I1307K is likely to be in the range of 18-30%. The authors suggested that the risk may be even

higher for those with the mutation and a family history of CRC.

The description of the I1307K change in *APC* and its postulated effect of causing a mild predisposition to cancer aroused widespread interest. This was because if, indeed, a predisposition existed, it would constitute one of the first instances of a definite low penetrance genetic change in human cancer predisposition. Answers are sought to several questions, namely (1) is the I1307K change confined to Ashkenazi Jews; (2) can the observation of its role in predisposition to CRC be confirmed and does it perhaps predispose to other cancers; (3) by what mechanism does the predisposition occur; (4) do I1307K determinations have predictive relevance in clinical practice; (5) might the existence of low or high predisposition genes contribute to differences in cancer incidence between populations; and (6) do other similar polymorphisms exist? Already at least two reviews have addressed some of these questions.[150 151]

(1) It appears that, so far, I1307K has been seen almost exclusively in Ashkenazi Jews. It has been searched for in 392 non-Jewish Norwegians,[152] 148 Finns, 105 African Americans, 54 Hawaii-Japanese, and 38 Italians[153] and not found. In Israel, it occurred in 20/261 (7.7%) of Jews with an Ashkenazi background,[154] confirming the initial approximation of its frequency in this population. In Israeli Jews of non-Ashkenazi extraction, it was (expectedly) found at low frequency, 3/339 (1.3%). Thus, the I1307K polymorphism meets the requirement of a polymorphism that is rare world wide but common in a "founder" population. Whether its enrichment in Ashkenazi Jews is because of a founder effect followed by genetic drift (as assumed for many disease genes in founder populations[155]) or whether it might carry a selective advantage remains to be determined. Firm conclusions are usually extremely hard to reach.

(2) In one study, the I1307K change was deemed not to be associated with CRC in Ashkenazi kindreds that were being studied because they displayed familial aggregation of breast and ovarian cancer.[156] The authors tentatively concluded that no direct predisposition occurred and suggested a role of mismatch repair deficiency instead. In a similar study[157] on ovarian cancer families, a similar conclusion was reached. However, neither the statistical power nor the choice of study populations may have been adequate to detect a predisposing effect of I1307K; in contrast, such an effect was noted by Gryfe *et al*[158] and Rozen *et al*[154] whose data provided confirmation of the interpretations of the original report.[146] Accordingly, carriers of I1307K appear to have an approximately 1.5 to twofold risk of CRC relative to non-carriers.[154 159] Interestingly, in the study by Gryfe *et al*[160] I1307K was also found to contribute to increased numbers of colorectal adenomatous polyps and cancers per patient. Similar results were obtained by Frayling *et al*.[161] Whether or not I1307K predisposes to other cancers, for example, breast cancer, is not

yet clear,[162] but weak association with several cancers has been suggested.

(3) A direct molecular effect of I1307K was shown by Laken *et al*[146] in that among 23 tumours from patients with I1307K and CRC, 11 had a truncating mutation in *APC*. The mutations occurred in the immediate sequence vicinity (29 bases) of codon 1307 and in each case was on the same chromosome as the I1307K change. This provided provocative evidence in favour of a direct effect in cis, and it was reasonable to speculate that the creation of an $(A)_8$ tract instead of $(A)_3 T (A)_4$ might predispose to insertion-deletion mutations through "slippage" at DNA replication. Indirect support for this interpretation was provided by Prior *et al*,[153] who sequenced the codon 1307 region in sporadic CRC tumour DNA from subjects without I1307K and found that the type and location of mutations differed from those reported in I1307K carriers. Moreover, no association with mismatch repair deficiency could be shown in that only one out of 22 tumours in I1307K carriers showed MMR deficiency.[153] Thus, present evidence favours the hypothesis that I1307K predisposes to CRC by making the region around codon 1307 vulnerable to single base insertions and deletions which, in turn, lead to truncation of *APC*, the proposed gatekeeper of colorectal carcinogenesis. Deeper insight into the molecular mechanism, including the effect on chromatin structure, is likely to be forthcoming.

(4) It is probably too early to assess whether the presence or absence of I1307K can be used for counselling purposes. If the lifetime risk of an Ashkenazi Jew to acquire CRC is 12%, and if one postulates that the presence of I1307K increases the risk twofold to 24%, does this have clinical relevance? Since many carriers of I1307K have first or second degree relatives with CRC, the a priori risk of such subjects is already raised (over 12%). How the presence or absence of I1307K would influence the risk of such a person remains to be determined. Moreover, how relatively minor changes in statistical risk have an impact on such things as surveillance behaviour is not clear.

(5) An intriguing question is whether or not differences between populations in the incidence of predisposing polymorphisms (low penetrance genes) might explain differences in disease incidence. For instance, does I1307K contribute to the remarkably high incidence of CRC (9-15% lifetime risk[146]) in Ashkenazi Jews? It is also relevant to ask whether enriched high penetrance founder mutations such as the large genomic deletion of *MLH1* exon 16 in Finns or the 943+3A→T mutation in *MSH2* in Newfoundlanders[114] lead to a higher incidence of CRC in these populations. Assuming that HNPCC accounts for ~3% of all CRC, a modest enrichment (for example, 30%) of an HNPCC mutation in a given population should have a negligible effect on overall CRC incidence (30% of 3% = 0.9% increase). By contrast, if low penetrance gene mutations such as I1307K are common in a population (for example, 7%) and increase the risk of CRC twofold, the overall increase caused by this change alone could be calculated at 7%. Using actual data from a retrospective study of Jewish patients with colorectal carcinomas or adenomas, Gryfe *et al*[160] concluded that I1307K directly contributes to 3-4% of all CRCs in this population. High figures like these are why low penetrance cancer predisposition genes will continue to be actively studied. A recent example is the arginine-72 allele of *p53* that predisposes to HPV associated cancer.[163]

(6) In a retrospective study of germline DNA from 164 patients with multiple colorectal adenomas/carcinoma, Frayling *et al*[161] detected a G to A change that affects codon 1317 predicting a glutamine instead of glutamic acid at this position (E1317Q). This change had been seen earlier in a British family[164] with colon cancer, but did not cosegregate fully with CRC in that family. The E1317Q change has been searched for and not found in 213 British control subjects. Tumour material from four carriers of E1317Q could not be studied so one cannot exclude the possibility that they carry another mutation of *APC* that predisposes to cancer. Limited haplotype analyses were consistent with the E1317Q being a founder mutation so it might be in linkage disequilibrium with another variant or mutation. Thus, while E1317Q may be associated with a raised risk of colorectal tumours, the putative causative mechanism is totally open. It may simply represent a regular missense mutation with low or almost no penetrance.

Summary and conclusions

It is no longer appropriate to discuss the genetics of colorectal cancer without defining the specific hereditary cancer syndrome of concern given the extant phenotypic, genetic, and molecular genetic heterogeneity of hereditary CRC (table 1). Standards of care are emerging where the physician is compelled not only to obtain a sufficiently detailed family history of CRC, but moreover the specific hereditary CRC syndrome must be understood so that appropriate surveillance and management can be offered to the family.

The litigations we discuss in the appendix provide evidence of how the legal system interprets standards of care for hereditary CRC and assigns malpractice awards to plaintiffs when physicians fail to render appropriate care in accord with those surveillance and management needs for the particular hereditary cancer syndrome.

It is clear that we are just beginning to grasp an understanding of hereditary CRC. Molecular genetic advances will undoubtedly help to elucidate this problem further. This research should lead to a better understanding of the magnitude of hereditary CRC, particularly since its familial clustering may involve a variety of low penetrant CRC predisposition genes.

Finally, we fervently believe that the morbidity and mortality of hereditary CRC can be drastically reduced by translating acquired genetic knowledge into the clinical practice setting. Clearly, these extremely important

objectives will be aided once society provides safeguards to protect patients against insurance and employment discrimination and offers affordable screening and management programmes for these hereditary disorders.

1 Landis SH, Murray T, Bolden S, Wingo PA. Cancer statistics, 1999. *CA Cancer J Clin* 1999;**49**:8-31.
2 Lynch HT, Follett KL, Lynch PM, Albano WA, Mailliard JL, Pierson RL. Family history in an oncology clinic. implications for cancer genetics. *JAMA*. 1979;**242**:1268-72.
3 David KL, Steiner-Grossman P. The potential use of tumor registry data in the recognition and prevention of hereditary and familial cancer. *N Y State J Med* 1991;**91**:150-2.
4 Smyrk TC, Lynch HT. Microsatellite instability: impact on cancer progression in proximal and distal colorectal cancers. *Eur J Cancer* 1999;**35**:171-2.
5 Lynch HT, Boman BM, Lanspa SJ, Smyrk T, Lynch JF. Heritage of colonic polyps. In: Herrera L, ed. *Familial adenomatous polyposis*. New York: Alan R Liss, 1990:9-15.
6 Lynch HT, Smyrk T, Lanspa S, Marcus J, Kriegler M, Appelman HD. Flat adenomas in a colon cancer-prone kindred. J *Natl Cancer Inst* 1988;**80**:278-81.
7 Lynch HT, Smyrk T, McGinn T, *et al*. Attenuated familial adenomatous polyposis (AFAP): a phenotypically and genotypically distinctive variant of FAP. *Cancer* 1995;**76**:2427-33.
8 Spirio L, Olschwang S, Groden J, *et al*. Alleles of the APC gene: an attenuated form of familial polyposis. *Cell* 1993;**75**:951-7.
9 Beck NE, Tomlinson IP, Homfray TF, Frayling IM, Hodgson SV, Bodmer WF. Frequency of germline hereditary non-polyposis colorectal cancer gene mutations in patients with multiple or early onset colorectal adenomas. *Gut* 1997;**41**:235-8.
10 Lynch HT, Shaw MW, Magnuson CW, Larsen AL, Krush AJ. Hereditary factors in cancer: study of two large midwestern kindreds. *Arch Intern Med* 1966;**117**:206-12.
11 Warthin AS. Heredity with reference to carcinoma. *Arch Intern Med* 1913;**12**:546-55.
12 Lynch HT, Krush AJ. Cancer family "G" revisited: 1895-1970. *Cancer* 1971;**27**:1505-11.
13 Watson P, Lynch HT. Extracolonic cancer in hereditary nonpolyposis colorectal cancer. *Cancer* 1993;**71**:677-85.
14 Risinger JI, Barrett JC, Watson P, Lynch HT, Boyd J. Molecular genetic evidence of the occurrence of breast cancer as an integral tumor in patients with the hereditary nonpolyposis colorectal cancer syndrome. *Cancer* 1996;**77**:1836-43.
15 Lynch HT, Smyrk T. Hereditary nonpolyposis colorectal cancer (Lynch syndrome): an updated review. *Cancer* 1996;**78**:1149-67.
16 de la Chapelle A, Peltomäki P. Genetics of hereditary colon cancer. *Annu Rev Genet* 1995;**29**:329-48.
17 Peltomäki P, de la Chapelle A. Mutations predisposing to hereditary nonpolyposis colorectal cancer. In: Vande Woude GF, Klein G, eds. *Advances in cancer research*. San Diego, California: Academic Press, 1997:93-119.
18 Kinzler KW, Vogelstein B. Lessons from hereditary colon cancer. *Cell* 1996;**87**:159-70.
19 Kinzler KW, Vogelstein B. Landscaping the cancer terrain. *Science* 1998;**280**:1036-7.
20 Kinzler KW, Vogelstein B. Cancer-susceptibility genes. Gatekeepers and caretakers. *Nature* 1997;**386**:761-3.
21 Kolodner RD, Hall NR, Lipford J, *et al*. Structure of the human MSH2 locus and analysis of two Muir-Torre kindreds for msh2 mutations. *Genomics* 1994;**24**:516-26.
22 Modrich P. Mechanisms and biological effects of mismatch repair. *Annu Rev Genet* 1991;**25**:229-53.
23 Peltomaki P. DNA mismatch repair gene mutations in human cancer. *Environ Health Perspect* 1997;**105**(suppl 4):775-80.
24 Shimodaira H, Filosi N, Shibata H, *et al*. Functional analysis of human MLH1 mutations in Saccaromyces cerevisiae. *Nat Genet* 1998;**19**:384-9.
25 de la Chapelle A, Wright FA. Linkage disequilibrium mapping in isolated populations: the example of Finland revisited. *Proc Natl Acad Sci USA* 1998;**95**:12416-23.
26 Thorlacius S, Struewing JP, Hartge P, *et al*. Population-based study of risk of breast cancer in carriers of BRCA2 mutation. *Lancet* 1999;**352**:1325-6.
27 Goodman RM, Motulsky AG, eds. *Genetic diseases among Ashkenazi Jews*. New York: Raven Press, 1979.
28 McKusick VA, Hostetler JA, Egeland JA. Genetic studies of the Amish: background and potentialities. *Bull Johns Hopkins Hosp* 1964;**115**:203-22.
29 Nyström-Lahti M, Peltomäki P, Aaltonen LA, de la Chapelle A. Genetic and genealogic study of 33 Finnish HNPCC kindreds. *Am J Hum Genet* 1994; **55**(suppl):A356.
30 Risch N, de Leon D, Ozelius L, *et al*. Genetic analysis of idiopathic torsion dystonia in Ashkenazi Jews and their recent descent from a small founder population. *Nat Genet* 1995;**9**:152-9.
31 Moisio AL, Sistonen P, Weissenbach J, de la Chapelle A, Peltomaki P. Age and origin of two common MLH1 mutations predisposing to hereditary colon cancer. *Am J Hum Genet* 1996;**59**:1243-51.
32 Vasen HFA, Mecklin JP, Meera Khan P, Lynch HT. The International Collaborative Group on Hereditary Nonpolyposis Colorectal Cancer (ICG-HNPCC). *Dis Colon Rectum* 1991;**34**:424-5.
33 Moore J, Cowled P. Hereditary non-polyposis colorectal cancer syndrome. *Aust NZ J Med* 1999;**69**:6-13.
34 Mecklin JP, Järvinen HJ, Hakkiluoto A, *et al*. Frequency of hereditary nonpolyposis colorectal cancer: a prospective multicenter study in Finland. *Dis Colon Rectum* 1995;**38**:588-93.
35 Ponz de Leon M, Sassatelli R, Sacchetti C, Zanghieri G, Scalmati A, Roncucci L. Familial aggregation of tumors in the 3-year experience of a population-based colorectal cancer registry. *Cancer Res* 1989;**49**:4344-8.
36 Ponz de Leon M, Sassatelli R, Benatti P, Roncucci L. Identification of hereditary nonpolyposis colorectal cancer in the general population: the 6-year experience of a population-based registry. *Cancer* 1993;**71**:3493-501.
37 Cannon-Albright LA, Skolnick MH, Bishop DT, Lee RG, Burt RW. Common inheritance of susceptibility to colonic adenomatous polyps and associated colorectal cancers. *N Engl J Med* 1988;**319**:533-7.
38 Houlston RS, Collins A, Slack J, Morton NE. Dominant genes for colorectal cancer are not rare. *Ann Hum Genet* 1992;**56**:99-103.
39 Aaltonen LA, Sankila R, Mecklin JP, *et al*. A novel approach to estimate the proportion of hereditary nonpolyposis colorectal cancer of total colorectal cancer burden. *Cancer Detect Prev* 1994;**18**:57-63.
40 Rodriguez-Bigas MA, Boland CR, Hamilton SR, *et al*. A National Cancer Institute workshop on hereditary nonpolyposis colorectal cancer syndrome: meeting highlights and Bethesda Guidelines. *J Natl Cancer Inst* 1997;**89**:1758-62.
41 Aaltonen LA, Peltomaki P, Mecklin JP, *et al*. Replication errors in benign and malignant tumors from hereditary nonpolyposis colorectal cancer patients. *Cancer Res* 1994;**54**:1645-8.
42 Lynch HT, Smyrk TC. Identifying hereditary nonpolyposis colorectal cancer. *N Engl J Med* 1998;**338**:1537-8.
43 Möslein G, Tester DJ, Lindor NM, *et al*. Microsatellite instability and mutation analysis of *hMSH2* and *hMLH1* in patients with sporadic, familial and hereditary colorectal cancer. *Hum Mol Genet* 1996;**5**:1245-52.
44 Wijnen J, Khan PM, Vasen H, *et al*. Hereditary nonpolyposis colorectal cancer families not complying with the Amsterdam criteria show extremely low frequency of mismatch-repair-gene mutations. *Am J Hum Genet* 1997; **61**:329-35.
45 Nystrom-Lahti M, Kristo P, Nicolaides NC, *et al*. Founding mutations and Alu-mediated recombination in hereditary colon cancer. *Nat Med* 1995;**1**:1203-6.
46 Nyström-Lahti M, Wu Y, Moisio AL, *et al*. DNA mismatch repair gene mutations in 55 kindreds with verified or putative hereditary nonpolyposis colorectal cancer. *Hum Mol Genet* 1996;**5**:763-9.
47 Liu B, Nicolaides NC, Markowitz S, *et al*. Mismatch repair gene defects in sporadic colorectal cancers with microsatellite instability. *Nat Genet* 1995;**9**:48-55.
48 Liu B, Parsons R, Papadopoulos N, *et al*. Analysis of mismatch repair genes in hereditary non-polyposis colorectal cancer patients. *Nat Med* 1996;**2**:169-74.
49 Wu Y, Nyström-Lahti M, Osinga J, *et al*. MSH2 and MLH1 mutations in sporadic replication error-positive colorectal carcinoma as assessed by two-dimensional DNA electrophoresis. *Genes Chrom Cancer* 1997;**18**:269-78.
50 Papadopoulos N, Nicolaides NC, Liu B, *et al*. Mutations of GTBP in genetically unstable cells. *Science* 1995;**268**:1915-17.
51 Akiyama Y, Sato H, Yamada T, *et al*. Germ-line mutation of the *hMSH6/GTBP* gene in an atypical hereditary nonpolyposis colorectal cancer kindred. *Cancer Res* 1997;**57**:3920-3.
52 Miyaki M, Konishi M, Tanaka K, *et al*. Germline mutation of *MSH6* as the cause of hereditary nonpolyposis colorectal cancer. *Nat Genet* 1997;**17**:271-2.
53 Hofstra RMW, Wu Y, Berends MJW, *et al*. Frequent involvement of MSH6 germline mutations in HNPCC-suspected families with low microsatellite instability tumors. *Am J Hum Genet* 1998;**63**(suppl):A21.
54 Lu SL, Kawabata M, Imamura T, *et al*. HNPCC associated with germline mutation in the TGF-β type II receptor gene. *Nat Genet* 1998;**19**:17-18.
55 Percesepe A, Kristo P, Aaltonen LA, Ponz de Leon M, de la Chapelle A, Peltomaki P. Mismatch repair genes and mononucleotide tracts as mutation targets in colorectal tumors with different degrees of microsatellite instability. *Oncogene* 1998;**17**:157-63.
56 Tomlinson I, Rahman N, Frayling I, *et al*. Inherited susceptibility to colorectal adenomas and carcinomas: evidence for a new predisposition gene on 15q14-q22. *Gastroenterology* 1999;**116**:789-95.
57 Aaltonen LA, Salovaara R, Kristo P, *et al*. Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. *N Engl J Med* 1998;**338**:1481-7.
58 Fujiwara T, Stolker JM, Watanabe T, *et al*. Accumulated clonal genetic alterations in familial and sporadic colorectal carcinomas with widespread instability in microsatellite sequences. *Am J Pathol* 1998;**153**:1063-78.

59 Wijnen J, van der Klift H, Vasen H, *et al*. *MSH2* genomic deletions are a frequent cause of HNPCC. *Nat Genet* 1998; **20**:326-8.

60 Landis SH, Murray T, Bolden S, Wingo PA. Cancer statistics, 1998. *CA Cancer J Clin* 1998;**48**:6-29.

61 Aarnio M, Sankila R, Pukkala E, *et al*. Cancer risk in mutation carriers of DNA-mismatch-repair genes. *Int J Cancer* 1999;**81**:214-18.

62 Dunlop MG, Farrington SM, Carothers AD, *et al*. Cancer risk associated with germline DNA mismatch repair gene mutations. *Hum Mol Genet* 1997;**6**:105-10.

63 Mecklin JP, Jarvinen HJ. Tumor spectrum in cancer family syndrome (hereditary nonpolyposis colorectal cancer). *Cancer* 1991;**68**:1109-12.

64 Mecklin JP, Jarvinen HJ, Peltokallio P. Cancer family syndrome: genetic analysis of 22 Finnish kindreds. *Gastroenterology* 1986;**30**:328-33.

65 Heinimann K, Müller H, Weber W, Scott RJ. Disease expression in Swiss hereditary non-polyposis colorectal cancer (HNPCC) kindreds. *Int J Cancer (Pred Oncol)* 1997;**74**:281-5.

66 Vasen HFA, Sanders EACM, Taal BG, *et al*. The risk of brain tumours in hereditary non-polyposis colorectal cancer (HNPCC). *Int J Cancer* 1997;**74**:551-5.

67 Hamilton SR, Liu B, Parsons RE, *et al*. The molecular basis of Turcot's syndrome. *N Engl J Med* 1995;**332**:839-47.

68 Aarnio M, Salovaara R, Aaltonen LA, Mecklin J-P, Järvinen HJ. Features of gastric cancer in hereditary non-polyposis colorectal cancer syndrome. *Int J Cancer (Pred Oncol)* 1997;**74**:551-5.

69 Ashley SW, Wells SA. Tumors of the small intestine. *Semin Oncol* 1988;**15**:116-28.

70 Aarnio M, Mecklin JP, Aaltonen LA, Nystrom-Lahti M, Jarvinen HJ. Life-time risk of different cancers in hereditary non-polyposis colorectal cancer (HNPCC) syndrome. *Int J Cancer* 1995;**64**:430-3.

71 Vasen HFA, Wijnen JT, Menko FH, *et al*. Cancer risk in families with hereditary nonpolyposis colorectal cancer diagnosed by mutation analysis. *Gastroenterology* 1996;**110**:1020-7.

72 Rodriguez-Bigas MA, Vasen HFA, Lynch HT, *et al*. Characteristics of small bowel carcinoma in hereditary nonpolyposis colorectal carcinoma. *Cancer* 1998;**83**:240-4.

73 Fearon ER. Human cancer syndromes: clues to the origin and nature of cancer. *Science* 1997;**278**:1043-50.

74 Muir EG, Bell AJ, Barlow KA. Multiple primary carcinomata of the colon, duodenum, and larynx associated with keratoacanthomata of the face. *Br J Surg* 1966;**54**:191-5.

75 Torre D. Multiple sebaceous tumors. *Arch Dermatol* 1968;**98**:549-51.

76 Cohen PR, Kohn SR, Kurzrock R. Association of sebaceous gland tumors and internal malignancy: the Muir-Torre syndrome. *Am J Med* 1991;**90**:606-13.

77 Lynch HT, Lynch PM, Pester J, Fusaro RM. The cancer family syndrome: rare cutaneous phenotypic linkage of Torre's syndrome. *Arch Intern Med* 1981;**141**:607-11.

78 Lynch HT, Fusaro RM, Roberts L, Voorhees GJ, Lynch JF. Muir-Torre syndrome in several members of a family with a variant of the cancer family syndrome. *Br J Dermatol* 1985;**113**:295-301.

79 Lynch HT, Fusaro RM. Muir-Torre syndrome: heterogeneity, natural history, diagnosis, and management. *Prob Gen Surg* 1993;**10**:1-14.

80 Bapat B, Xia L, Madlensky L, *et al*. The genetic basis of Muir-Torre syndrome includes the *hMLH1* locus. *Am J Hum Genet* 1996;**59**:736-9.

81 Kruse R, Lamberti C, Wang Y, *et al*. Is the mismatch repair deficient type of Muir-Torre syndrome confined to mutations in the hMSH2 gene? *Hum Genet* 1996;**98**:747-50.

82 Kruse R, Rütten A, Lamberti C, *et al*. Muir-Torre phenotype has a frequency of DNA mismatch-repair-gene mutations similar to that in hereditary nonpolyposis colorectal cancer families defined by the Amsterdam criteria. *Am J Hum Genet* 1998;**63**:63-70.

83 Lin KM, Shashidharan M, Ternent CA, *et al*. Colorectal and extracolonic cancer variations in MLH1/MSH2 hereditary nonpolyposis colorectal cancer kindreds and the general population. *Dis Colon Rectum* 1998;**41**:428-33.

84 Jäger AC, Bisgaard ML, Myrhoj T, Bernstein I, Rehfeld JF, Nielsen FC. Reduced frequency of extracolonic cancers in hereditary nonpolyposis colorectal cancer families with monoallelic *hMLH1* expression. *Am J Hum Genet* 1997;**61**:129-38.

85 Beck NE, Tomlinson IPM, Homfray T, Hodgson SV, Harocopos CJ, Bodmer WF. Genetic testing is important in families with a history suggestive of hereditary non-polyposis colorectal cancer even if the Amsterdam criteria are not fulfilled. *Br J Surg* 1997;**84**:233-7.

86 Watson P, Lin K, Rodriguez-Bigas MA, *et al*. Colorectal carcinoma survival among hereditary nonpolyposis colorectal cancer family members. *Cancer* 1998;**83**:259-66.

87 Sankila R, Aaltonen LA, Jarvinen HJ, Mecklin JP. Better survival rates in patients with MLH1-associated hereditary colorectal cancer. *Gastroenterology* 1996;**110**:682-7.

88 Hodgson SV, Harocopos C, Gaglia P. Screening results in a family cancer clinic: five years experience. *Anticancer Res* 1993;**13**:2581-6.

89 Jass JR, Stewart SM. Evolution of hereditary non-polyposis colorectal cancer. *Gut* 1992;**33**:783-6.

90 Jass JR. Colorectal adenoma progression and genetic change: is there a link? *Ann Med* 1995;**27**:301-6.

91 Järvinen HJ, Mecklin JP, Sistonen P. Screening reduces colorectal cancer rate in families with hereditary nonpolyposis colorectal cancer. *Gastroenterology* 1995;**108**:1405-11.

92 Smyrk TC, Lynch HT, Watson PA, Appelman HD. Histologic features of hereditary nonpolyposis colorectal carcinoma. In: Utsunomiya J, Lynch HT, eds. *Hereditary colorectal cancer.* Tokyo: Springer-Verlag, 1990:357-62.

93 Jass JR, Smyrk TC, Stewart SM, Lane MR, Lanspa SJ, Lynch HT. Pathology of hereditary non-polyposis colorectal cancer. *Anticancer Res* 1994;**14**:1631-4.

94 Mecklin JP, Jarvinen HJ. Clinical features of colorectal carcinoma in cancer family syndrome. *Dis Colon Rectum* 1986; **29**:160-4.

95 Gibbs NM. Undifferentiated carcinoma of the large intestine. *Histopathology* 1977;**1**:77-84.

96 Jessurun MR, Manivel JC. Cecal, poorly differentiated adenocarcinomas, medullary-type. *Mod Pathol* 1992;**5**: 43A.

97 Kim H, Jen J, Vogelstein B, Hamilton SR. Clinical and pathological characteristics of sporadic colorectal carcinomas with DNA replication errors in microsatellite sequences. *Am J Pathol* 1994;**145**:148-56.

98 Thibodeau SN, French AJ, Roche PC, *et al*. Altered expression of hMSH2 and hMLH1 in tumors with microsatellite instability and genetic alterations in mismatch repair genes. *Cancer Res* 1996;**56**:4836-40.

99 Graham DM, Appelman HD. Crohn's-like lymphoid reaction and colorectal carcinoma: a potential histologic prognosticator. *Mod Pathol* 1990;**3**:332-5.

100 Bird RP. Observation and quantification of aberrant crypts in the murine colon treated with a colon carcinogen: preliminary findings. *Cancer Lett* 1987;**37**:147-51.

101 Roncucci L, Stamp D, Medline A, Cullen JB, Bruce WR. Identification and quantification of aberrant crypt foci and microadenomas in the human colon. *Hum Pathol* 1991;**22**: 287-94.

102 Roncucci L, Modica S, Pedroni M, *et al*. Aberrant crypt foci in patients with colorectal cancer. *Br J Cancer* 1998;**77**: 2343-8.

103 Augenlicht LH, Richards C, Corner G, Pretlow TG. Evidence for genomic instability in human colonic aberrant crypt foci. *Oncogene* 1996;**12**:1762-7.

104 Heinen CD, Shivapurkar N, Tang Z, Groden J, Alabaster O. Microsatellite instability in aberrant crypt foci from human colons. *Cancer Res* 1996;**56**:5339-41.

105 Takayama T, Katsuki S, Takahashi Y, *et al*. Abberant crypt foci of the colon as precursors of adenoma and cancer. *N Engl J Med* 1998;**339**:1277-84.

106 Vasen HFA, van Ballegooijen M, Buskens E, *et al*. A cost-effectiveness analysis of colorectal screening of hereditary nonpolyposis colorectal carcinoma gene carriers. *Cancer* 1998;**82**:1632-7.

107 Syngal S, Weeks JC, Schrag D, Garber JE, Kuntz KM. Benefits of colonoscopic surveillance and prophylactic colectomy in patients with hereditary nonpolyposis colorectal cancer mutations. *Ann Intern Med* 1998;**129**:787-96.

108 Rex DK, Cutler CS, Lemmel GT, *et al*. Colonoscopic miss rates of adenomas determined by back-to-back colonoscopies. *Gastroenterology* 1997;**112**:24-8.

109 Vasen HFA, Nagengast FM, Khan PM. Interval cancers in hereditary non-polyposis colorectal cancer (Lynch syndrome). *Lancet* 1995;**345**:1183-4.

110 Church J. Hereditary colon cancers can be tiny: a cautionary case report of the results of colonoscopic surveillance. *Am J Gastroenterol* 1998;**93**:2289-90.

111 Lynch HT. Is there a role for prophylactic subtotal colectomy among hereditary nonpolyposis colorectal cancer germline mutation carriers? *Dis Colon Rectum* 1996;**39**: 109-10.

112 Church JM. Prophylactic colectomy in patients with hereditary nonpolyposis colorectal cancer. *Ann Med* 1996; **28**:479-82.

113 Rodriguez-Bigas MA, Vasen HFA, Pekka-Mecklin J, *et al*. Rectal cancer risk in hereditary nonpolyposis colorectal cancer after abdominal colectomy. *Ann Surg* 1997;**225**:202-7.

114 Froggatt NJ, Green J, Brassett C, *et al*. A common MSH2 mutation in English and North American HNPCC families: origin, phenotypic expression, and sex specific differences in colorectal cancer. *J Med Genet* 1999;**36**:97-102.

115 Lanspa SJ, Jenkins JX, Cavalieri RJ, Smyrk TC, Watson P, Lynch J. Surveillance in Lynch syndrome: how aggressive? *Am J Gastroenterol* 1994;**89**:1978-80.

116 DeCosse JJ. Surgical prophylaxis of familial colon cancer: prevention of death from familial colorectal cancer. *J Natl Cancer Inst (Monogr)* 1995;**17**:31-2.

117 Lynch HT, Smyrk TC, Watson P, *et al*. Genetics, natural history, tumor spectrum, and pathology of hereditary nonpolyposis colorectal cancer: an updated review. *Gastroenterology* 1993;**104**:1535-49.

118 Lynch HT, Smyrk T, Lynch J. An update of HNPCC (Lynch syndrome). *Cancer Genet Cytogenet* 1997;**93**:84-99.

119 Lynch HT, Smyrk T. An update of Lynch syndrome. *Curr Opin Oncol* 1998;**10**:349-56.

120 Rüschoff J, Wallinger S, Dietmaier W, *et al*. Aspirin suppresses the mutator phenotype associated with hereditary nonpolyposis colorectal cancer by genetic selection. *Proc Natl Acad Sci USA* 1998;**95**:11301-6.

121 de Wind N, Dekker M, van Rossum A, van der Valk M, te Riele H. Mouse models for hereditary nonpolyposis colorectal cancer. *Cancer Res* 1998;**58**:248-55.

122 Baylin SB, Herman JG, Graff JR, Vertino PM, Issa JP. Alterations in DNA methylation: a fundamental aspect of neoplasia. *Adv Cancer Res* 1998;**72**:141-96.

123 Jones PA, Laird PW. Cancer epigenetics comes of age. *Nat Genet* 1999;**21**:163-7.
124 Hiltunen MO, Alhonen L, Koistinaho J, *et al*. Hypermethylation of the *APC* (adenomatous polyposis coli) gene promotor region in human colorectal carcinoma. *Int J Cancer* 1997;**70**:644-8.
125 Laird PW, Jackson-Grusby L, Fazeli A, *et al*. Suppression of intestinal neoplasia by DNA hypomethylation. *Cell* 1995;**81**:197-205.
126 Kane MF, Loda M, Gaida GM, *et al*. Methylation of the *hMLH1* promoter correlates with lack of expression of hMLH1 in sporadic colon tumors and mismatch repair-defective human tumor cell lines. *Cancer Res* 1997;**57**:808-11.
127 Herman JG, Umar A, Polyak K, *et al*. Incidence and functional consequences of *hMLH1* promoter hypermethylation in colorectal carcinoma. *Proc Natl Acad Sci USA* 1998;**95**:6870-5.
128 Cunningham JM, Christensen ER, Tester DJ, *et al*. Hypermethylation of the hMLH1 promoter in colon cancer with microsatellite instability. *Cancer Res* 1998;**58**:3455-60.
129 Veigl ML, Kasturi L, Olechnowicz J, *et al*. Biallelic inactivation of hMLH1 by epigenetic gene silencing, a novel mechanism causing human MSI cancers. *Proc Natl Acad Sci USA* 1998;**95**:8698-702.
130 Hemminki A, Peltomäki P, Mecklin JP, *et al*. Loss of the wild type *MLH1* gene is a feature of hereditary nonpolyposis colorectal cancer. *Nat Genet* 1994;**8**:405-10.
131 Bubb VJ, Curtis LJ, Cunningham C, *et al*. Microsatellite instability and the role of hMSH2 in sporadic colorectal cancer. *Oncogene* 1996;**12**:2641-9.
132 Lothe RA, Peltomaki P, Meling GI, *et al*. Genomic instability in colorectal cancer: relationship to clinicopathological variables and family history. *Cancer Res* 1993;**53**:5849-52.
133 Senba S, Konishi F, Okamoto T, *et al*. Clinicopathologic and genetic features of nonfamilial colorectal carcinomas with DNA replication errors. *Cancer* 1998;**82**:279-85.
134 Ahuja N, Li Q, Mohan AL, Baylin SB, Issa JPJ. Aging and DNA methylation in colorectal mucosa and cancer. *Cancer Res* 1998;**58**:5489-94.
135 Leung SY, Yuen ST, Chung LP, Chu KM, Chan ASY, Ho JCI. hMLH1 promoter methylation and lack of hMLH1 expression in sporadic gastric carcinomas with high-frequency microsatellite instability. *Cancer Res* 1999;**59**:159-64.
136 Fleisher AS, Esteller M, Wang S, *et al*. Hypermethylation of the *hMLH1* gene promoter in human gastric cancers with microsatellite instability. *Cancer Res* 1999;**59**:1090-5.
137 Esteller M, Levine R, Baylin SB, Ellenson LH, Herman JG. *MLH1* promoter hypermethylation is associated with the microsatellite instability phenotype in sporadic endometrial carcinomas. *Oncogene* 1998;**16**:2413-17.
138 Cattanach BM, Beechey CV. Autosomal and X-chromosome imprinting. *Development* 1990;**suppl**:63-72.
139 Sapienza C, Paquete J, Tran TH, Peterson A. Epigenetic and genetic factors affect transgene methylation imprinting. *Development* 1989;**107**:165-8.
140 Rainier S, Johnson LA, Dobry CJ, Ping AJ, Grundy PE, Feinberg AP. Relaxation of imprinted genes in human cancer. *Nature* 1993;**362**:747-9.
141 Feinberg AP. Genomic imprinting and cancer. In: Vogelstein B, Kinzler KW, eds. *The genetic basis of human cancer*. New York: McGraw Hill, 1998:95-107.
142 Cui H, Horon IL, Ohlsson R, Hamilton SR, Feinberg AP. Loss of imprinting in normal tissue of colorectal cancer patients with microsatellite instability. *Nat Med* 1998;**4**:1276-80.
143 Wang Q, Lasset C, Desseigne F, *et al*. Neurofibromatosis and early onset of cancers in *hMLH1*-deficient children. *Cancer Res* 1999;**59**:294-7.
144 Ricciardone MD, Özçelik T, Cevher B, *et al*. Human *MLH1* deficiency predisposes to hematological malignancy and neurofibromatosis type I. *Cancer Res* 1999;**59**:290-3.
145 Hackman P, Tannergard P, Osei-Mensa S, *et al*. A human compound heterozygote for two *MLH1* missense mutations. *Nat Genet* 1997;**17**:135-6.
146 Laken SJ, Petersen GM, Gruber SB, *et al*. Familial colorectal cancer in Ashkenazim due to a hypermutable tract in *APC*. *Nat Genet* 1997;**17**:79-83.
147 Greenwald P, Korns RF, Nasca PC, Wolfgang PE. Cancer in United States Jews. *Cancer Res* 1975;**35**:3507-12.
148 Bat L, Pines A, Ron E, Rosenblum Y, Niv Y, Shemesh E. Colorectal adenomatous polyps and carcinoma in Ashkenazi and non-Ashkenazi Jews in Israel. *Cancer* 1986;**58**:1167-71.
149 Kune S, Kune GA, Watson L. The Melbourne Colorectal Cancer Study: incidence findings by age, sex, site, migrants and religion. *Int J Epidemiol* 1986;**15**:483-93.
150 White RL. Excess risk of colon cancer associated with a polymorphism of the *APC* gene? *Cancer Res* 1998;**58**:4038-9.
151 Gruber SB, Petersen GM, Kinzler KW, Vogelstein B. Cancer, crash sites, and the new genetics of neoplasia. *Gastroenterology* 1999;**116**:210-12.
152 Lothe RA, Hektoen M, Johnsen H, *et al*. The *APC* gene I1307K variant is rare in Norwegian patients with familial and sporadic colorectal or breast cancer. *Cancer Res* 1998;**58**:2923-4.
153 Prior TW, Chadwick RB, Papp AC, *et al*. The I1307K polymorphism of the *APC* gene in colorectal cancer. *Gastroenterology* 1999;**116**:58-63.
154 Rozen P, Shomrat R, Strul H, Naiman T, Karminsky N. Prevalence of the I1307K *APC* gene variant in Israeli Jews of differing ethnic origin and risk for colorectal cancer. *Gastroenterology* 1999;**116**:54-7.
155 de la Chapelle A. Disease gene mapping in isolated human populations: the example of Finland. *J Med Genet* 1993;**30**:857-65.
156 Petrukhin L, Dangel J, Vanderveer L, *et al*. The I1307K *APC* mutation does not predispose to colorectal cancer in Jewish Ashkenazi breast and breast-ovarian cancer kindreds. *Cancer Res* 1997;**57**:5480-4.
157 Abrahamson J, Moslehi R, Vesprini D, *et al*. No association of the *I1307K APC* allele with ovarian cancer risk in Ashkenazi Jews. *Cancer Res* 1998;**58**:2919-22.
158 Gryfe R, Di Nicola N, Lal G, Gallinger S, Redston M. Inherited colorectal polyposis and cancer risk of the *APC I1307K* polymorphism. *Am J Hum Genet* 1999;**64**:378-84.
159 Woodage T, King SM, Wacholder S, *et al*. The *APC* I1307K allele and cancer risk in a community-based study of Ashkenazi Jews. *Nat Genet* 1998;**20**:62-5.
160 Gryfe R, Di Nicola N, Gallinger S, Redston M. Somatic instability of the *APC I1307K* allele in colorectal neoplasia. *Cancer Res* 1998;**58**:4040-3.
161 Frayling IM, Beck NE, Ilyas M, *et al*. The *APC* variants I1307K and E1317Q are associated with colorectal tumors, but not always with a family history. *Proc Natl Acad Sci USA* 1998;**95**:10722-7.
162 Redston M, Nathanson KL, Yuan ZQ, *et al*. The *APC* I1307K allele and breast cancer risk. *Nat Genet* 1998;**20**:13-14.
163 Storey A, Thomas M, Kalita A, *et al*. Role of a p53 polymorphism in the development of human papillomavirus-associated cancer. *Nature* 1998;**21**:229-34.
164 White S, Bubb VJ, Wyllie AH. Germline APC mutation in (Gln^{1317}) in a cancer-prone family that does not result in familial adenomatous polyposis. *Genes Chrom Cancer* 1996;**15**:122-8.