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 pigmentation in Peutz-Jeghers syndrome), when taking a family cancer history.

Keywords: colorectal cancer; hereditary non-polyposis colorectal cancer; genetic susceptibility

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. During that same period, mortality from colon cancer is projected to be 47 900 and that for rectal cancer 8700. 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.
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.\(^2\) 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 \( \beta \) 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.\(^\text{84} \)’

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.\(^6\) 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 peripancreatic carcinoma, papillary thyroid carcinoma, gastric carcinoma, sarcomas, and brain tumours. Desmoid tumours are also sequela, 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.\(^7\) Spiro et al 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,\(^13\) 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 \( \approx 85-90\% \); (3) gene carriers develop CRC at an early age (\( \approx 45 \) years); (4) most (\( \approx 70\% \)) 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, uterus, and renal pelvis.\(^15\) Breast cancer excess may be present in some HNPCC families.\(^16\) 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.\(^18-22\)

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 muta-
tional spectrum is provided by Peltomäki37 and
the updated HNPPC 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 cumber-
some 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 proposed38 to fill this gap but appar-
ently 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
cystic fibrosis world wide. Each of these
mutations arose once in a single chromosome
and, 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 dif-
ferent origins. Prime examples are the Finns
(“founded” some 2000 years ago39), Icelanders
(some 1100 years40), Ashkenazi Jews (600-800
years41), French Canadians, and Amish (250-
400 years42). There is ample documentation of
extremely high allele frequencies for rare
disease genes in these populations. The mech-
anism 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 popula-
tion bottlenecks.

Only very recently has it emerged that muta-
tions 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 geographi-
cal cluster in south central Finland many or
most of the affected subjects carrying the “Fin-
land 1” mutation and belonging to different
families descend from an ancestor who was one
of a small number of “founders” of this Finnish
subpopulation.43 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,44 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.45

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 HNPPC, a number of recurrent muta-
tions 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 HNPPC

In the past, to establish the frequency of
HNPPC most investigators determined the propor-
tion of all CRC patients that fulfilled the
Amsterdam criteria.46 By this method, esti-
mates varying between approximately 0.5% and
5% were obtained.47 48 49 Other methods
have yielded highly discordant results. For
example, Cannon-Albright et al47 suggested
that a high proportion of all colorectal tumours
resulted from heritable mutations. By large
segregation analyses, Houlston et al50 con-
cluded that 13% of CRC cases fit the model of
dominant inheritance while Aaltonen et al,51
studying a consecutive cohort of young CRC
patients, extrapolated an HNPPC 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
HNPPC will be fulfilled. This could skew the
results and hamper comparisons between
different series. Moreover, the Amsterdam cri-
teria require the cancer to be colorectal in all
affected family members, thus ignoring fami-
ilies with key members affected by other
HNPPC cancers, in particular of the en-
dometrium. More “relaxed” pedigree criteria
for HNPPC have therefore been proposed.40

A key issue is how HNPPC should be
defined today. Tumours from the great major-
ity of Amsterdam criteria positive families are
MSI positive,41 42 suggesting that mismatch
repair deficiency underlies most HNPPC.
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%-74. In CRC families
that do not fulfil the Amsterdam criteria
Table 1  Hereditary disorders in which CRC is an integral lesion

<table>
<thead>
<tr>
<th>Hereditary form of colorectal cancer (CRC)</th>
<th>Inheritance pattern</th>
<th>Gene</th>
<th>Polyps</th>
<th>Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Familial adenomatous polyposis (FAP)</td>
<td>AD</td>
<td>APC</td>
<td>Adenomatous, often start in distal colon/rectum; usually &gt;100; adenomas may occur in small bowel, gastric polyps common, usually fundic gland polyps</td>
<td>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</td>
</tr>
<tr>
<td>Attenuated familial adenomatous polyposis coli (AFAP)</td>
<td>AD</td>
<td>APC</td>
<td>Ordinary adenomas but also flat adenomas with proximal colonic predominance; may be few (5-10), sometimes &gt;100</td>
<td>CRC with average age onset at 55; occasional periampullary carcinoma</td>
</tr>
<tr>
<td>Ashkenazi Jewish 11307K mutation</td>
<td>AD</td>
<td>11307K mutation in APC</td>
<td>Occasional adenomatous colonic polyps</td>
<td>CRC, “young” but average age of onset not known</td>
</tr>
<tr>
<td>Turcot's syndrome (HNPCC)</td>
<td>AD</td>
<td>(Both FAP (APC) and HNPCC, hPMS2, hMLH1 mutation variants)</td>
<td>Multiple colonic adenomas, but may not be florid</td>
<td>CRC and central nervous system, particularly brain tumours. In APC (FAP families) cerebellar medulloblastomas. In hMLH1 and hMSH2 (HNPCC families) glioblastoma multiforme</td>
</tr>
<tr>
<td>Juvenile polyposis coli</td>
<td>AD</td>
<td>Protein tyrosine phosphate gene (PTEN) SMA/D/1P24</td>
<td>Diffuse hamartomatous polyps (may have adenomatous component) of colon, but may occur in small bowel and stomach</td>
<td>CRC</td>
</tr>
<tr>
<td>Peutz-Jeghers syndrome</td>
<td>AD</td>
<td>Gene encoding serine threonine kinase (STK11) on chromosome 19p13.3 LKB1/STK11</td>
<td>Peutz-Jeghers polyps (may have adenomatous features) in stomach, small bowel, and colon</td>
<td>Stomach, small bowel, colon, sex cord tumours of ovary and testes</td>
</tr>
<tr>
<td>Hereditary mixed polyposis syndrome (IMPS)</td>
<td>AD</td>
<td>Unknown; possible site on chromosome 6q</td>
<td>Atypical colonic juvenile polyps, adenomatous and hyperplastic polyps; usually less than 15 colon polyps</td>
<td>CRC</td>
</tr>
<tr>
<td>Discrete colonic adenomatous polyps and CRC of Burt</td>
<td>AD; may be similar to some familial CRC</td>
<td>APC</td>
<td>Occasional (never florid) adenomatous colonic polyps</td>
<td>CRC, average age in accord with population expectations</td>
</tr>
<tr>
<td>Hereditary non-polyposis colorectal cancer (HNPCC)</td>
<td>AD</td>
<td>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</td>
<td>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</td>
<td>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 45; may show rapid progression from adenoma to CRC</td>
</tr>
<tr>
<td>Familial CRC</td>
<td>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</td>
<td>Unknown</td>
<td>In accord with population expectations</td>
<td>CRC, comparable to general population for age of onset and colonic location</td>
</tr>
<tr>
<td>Inflammatory bowel disease (ulcerative colitis (UC) and Crohn's disease (CD))</td>
<td>Unknown; possible AD in some families; polygenic also likely</td>
<td>Linkage to chromosome 16 (IBD) and 12 (IBD), findings which are tentative</td>
<td>Pseudopolyps (non-adenomatous)</td>
<td>CRC, lymphoma of GI tract</td>
</tr>
</tbody>
</table>

AD=autosomal dominant. CRC=colorectal cancer. IBD=inflammatory bowel disease. UC=familial ulcerative colitis. CD=Crohn’s disease.


(“HNPCC-like” families) the proportion with mutations in MLH1 or MSH2 is lower (8-30%).

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. In at least two HNPCC or HNPCC-like families, MSH6 mutations were implicated and a high proportion of CRC patients with “mild” microsatellite instability was recently briefly described as having germ-line mutations of MSH6. Whether MSH3, further MMR genes, or other genes such as BAX and TGFÎ²RII, will turn out to contribute to inherited predisposition to CRC is not yet clear. It may take time until the entire mutational spectrum of HNPCC is defined. Meanwhile, we propose to define any person or
Table 1 Continued

<table>
<thead>
<tr>
<th>Non-cancer features</th>
<th>Screening</th>
<th>Surgical management/prophylaxis</th>
<th>Presymptomatic DNA testing</th>
<th>Genetic counselling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gardner’s variant-epidermoid cysts of skin, osteomas of mandible, congenital hypertrophy of the retinal pigment epithelium, Desmoid tumours (intra-abdominal) do not metastasise but may kill by direct extension; desmoids may be initiated by surgery (dissected surfaces); adrenal adenomas</td>
<td>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</td>
<td>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)</td>
<td>Test for APC germline mutation as early as age 10–12</td>
<td>Initiate pre-teens, include parents</td>
</tr>
<tr>
<td>Fundic gland polyps in stomach; adenomas in duodenum</td>
<td>Colonoscopy and upper endoscopy, initiate at age 20 and annually for APC germline positive patients or every 2 years if at genetic risk but not tested for APC</td>
<td>Prophylactic subtotal colectomy if too many polyps to manage; consider chemo-preventative sulindac</td>
<td>Test for APC germline mutation at age 20</td>
<td>Initiate at age 20, include parents</td>
</tr>
<tr>
<td>None known</td>
<td>Full colonoscopy, start at age 30–35 in gene carriers</td>
<td>Standard CRC surgery</td>
<td>Ashkenazi APC mutation</td>
<td>Start at age 25</td>
</tr>
<tr>
<td>Rare examples of multiple café au lait spots and pigmented naevi but not clear if truly integral to the syndrome</td>
<td>Baseline flexible sigmoidoscopy age 10–12 and annual flexible sigmoidoscopy thereafter; consider CT scan or MRI of brain</td>
<td>Prophylactic subtotal colectomy if colonic polyposis present, as in FAP. In HNPCC variant, colonoscopy</td>
<td>Two DNA variants: (1) APC gene with predominance of cerebellar medulloblastoma, (2) hMLH1 or hMSH2 with predominance of glialoblastoma multiforme</td>
<td>Initiate age 10–12, include parents</td>
</tr>
<tr>
<td>Children may manifest diarrhoea (may be severe)</td>
<td>Initiate colonoscopy age 10–12</td>
<td>Prophylactic subtotal colectomy when phenotype present with too many polyps to manage</td>
<td>Tyrosine phosphate gene (PTEN)</td>
<td>Initiate pre-teens, include parents</td>
</tr>
<tr>
<td>Muco-cutaneous melanocytic hyperplasia</td>
<td>Baseline colonoscopy and upper endoscopy, initiate at age 20; flexible sigmoidoscopy annually thereafter</td>
<td>Consider prophylactic subtotal colectomy if too many polyps to manage and if mixed adenomatous features</td>
<td>Serine threonine kinase (STK11) on chromosome 19p13.3</td>
<td>Initiate teens, include parents</td>
</tr>
<tr>
<td>None known</td>
<td>Colonoscopy, initiate at age 20, 2–3 years</td>
<td>Polypectomy; consider prophylactic colectomy if polyps too many to manage</td>
<td>None known</td>
<td>Include initiation in teens</td>
</tr>
<tr>
<td>None known</td>
<td>Initiate baseline flexible sigmoidoscopy at age 40 and every 3 years thereafter</td>
<td>Standard CRC surgical approach</td>
<td>None known</td>
<td>Initiate at age 25–30</td>
</tr>
<tr>
<td>Muir-Torre syndrome variant shows cancer features of HNPCC but includes sebaceous adenomas, sebaceous epitheliomas, basal cell epitheliomas with sebaceous differentiation, meibomian gland carcinomas and sebaceous carcinomas; single or multiple keratoacanthomais</td>
<td>Colonoscopy, initiate age 20–25, annually for germline mutation carriers; every other year when mutation studies are lacking; endometrial aspiration biopsy at the same time as colonoscopy</td>
<td>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</td>
<td>Test for germline mutations no earlier than age 18–20</td>
<td>Initiate at age 18, before any consideration for gene testing</td>
</tr>
<tr>
<td>None</td>
<td>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</td>
<td>Standard surgical procedure for CRC</td>
<td>None known</td>
<td>Initiate at age 30–35</td>
</tr>
<tr>
<td>UC: arthritis, pyoderma gangrenosum, annular erythemas, and vascular thromboses, sclerosing cholangitis CD: similar to UC but small bowel involvement prominent, and may involve colon</td>
<td>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</td>
<td>Subtotal colectomy for CRC; consider prophylactic subtotal colectomy for patients with persistent high grade dysplasia of colonic mucosa in UC. Proctocolectomy if IBD mandates</td>
<td>None known</td>
<td>Initiate at age 18–20</td>
</tr>
</tbody>
</table>
Table 2. Number of mutations and polymorphisms in the mismatch repair genes responsible for HNPCC (as of January 1999 at www.med.ku.dk).

<table>
<thead>
<tr>
<th>Gene</th>
<th>No of disease causing mutations</th>
<th>No of polymorphisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLH1</td>
<td>134</td>
<td>15</td>
</tr>
<tr>
<td>MSH2</td>
<td>87</td>
<td>22</td>
</tr>
<tr>
<td>MSH6</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>PMS1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>PMS2</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>TGFβRII</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>228</td>
<td>47</td>
</tr>
</tbody>
</table>

Table 3. Founder mutations in HNPCC

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

The frequency 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.

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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.57 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 Lynch13 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 problematic (table 1).

The first systematic study of extracolonic cancer in HNPCC37 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 urinary 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.
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.14 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,11 12 in mutation positive women the incidence of endometrial cancer (60%) exceeded that of colorectal cancer (54%). As soon as additional large studies like this one16 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 determined11 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 cancer17 showed that lung cancer occurred only 60% as often in relatives of patients compared with control subjects. Mecklin et al 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.11 13

Heimann et al 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 described an increased risk of brain tumours in HNPCC. Previously Hamilton et al 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 MSH2 (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 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.17 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.17 19

Rodriguez-Bigas et al examined 42 patients from 40 HNPCC families who developed 42 primary and seven metachronous small bowel
cancers. Forty-six were adenocarcinomas while three were carcinoïd 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,71 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 al86 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, epitheliothenoma, 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 al84 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 have been extensively reviewed by Cohen et al.74 Lynch et al74–76 were the first to describe the cutaneous findings of MTS as part of Lynch syndrome II. More recent publications80–82 have identified MSH2 and MLH1 germline mutations as culprit predisposing genes in MTS.

Phenotype-genotype correlations in HNPCC

Vasen et al83 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 carcinomas appeared to be more common in the subset with MSH2 mutations (61% vs 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 al87 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 al88 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 al64 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 al89 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 al90 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 al91 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 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 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

Fujinawa et al studied 39 HNPCC CRCs (HNPCCa) and 57 sporadic right sided CRCs (SRSCCa). Findings disclosed that “Of HNPCCa, 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 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. Jass and Stewart 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 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.

Smyrk et al and Jass et al 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. These tumours resemble the “undifferentiated carcinoma” described by Gibbs and the “medullary carcinoma” described by Jessurun and Manivel. 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. This special histology has been referred to as “solid-cribriform” wherein the pattern has a positive predictive value of 53% for MSI+ status.

Smyrk et al 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, a similar tendency to form lymphoid aggregates around the tumour appears to be a feature of sporadic RER+ colon tumours as well. In the general
population, a Crohn’s-like reaction is associated with improved prognosis. It will be important to determine whether this phenomenon accounts for the more favourable prognosis of CRC in HNPCC.

Aberrant crypt foci of the colon
Aberrant crypt foci (ACF) are characterised by lesions in colonic mucosa of mice as large and thick crypts in methylene 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.

Roncucci et al. 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. noted that Augenlicht et al. described genomic instability at microsatellites which were indicative of DNA MMR deficiency in human ACF. They also noted that Heinen et al. also found microsatellite instability to be restricted to ACF from right sided colonic mucosa among patients with large bowel cancer. Roncucci et al. conclude that “Microsatellite instability is also more frequent in right sided colon carcinoma, reinforcing the 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. 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. 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. 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 al109 identified six CRCs among HNPCC germline mutation carriers who were undergoing three yearly colonoscopies, while Vasen et al10 identified six CRCs in HNPCC germline mutation carriers 110 who were undergoing five interval cancers in HNPCC patients within three and a half years following a normal colonoscopy. In reviewing this subject, Church110 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

Coloscopy 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 al114 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.”115

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 al115 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.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.

Church112 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.

Church112 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 al120 found that microsatellite instability in CRC cells that are deficient for a
subset of the mismatch repair genes, namely \textit{MLH1}, \textit{MSH2}, and \textit{MSH6}, is markedly reduced following exposure to aspirin or sulindac. These findings were reversible and were independent of proliferation rate and cyclooxygenase 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 \textit{MSH2} and \textit{MLH1} genes are associated with a majority of cancer susceptibility cases.”

\textbf{ANIMAL MODELS OF HNPCC}

Understanding the aetiologia, pathogenesis, and control of HNPCC may be accelerated by studying animal models. De Wind \textit{et al.} have developed mouse models harbouring a deficiency in the MMR gene \textit{Msh2}. Interestingly, the majority of these \textit{Msh2} deficient mice succumbed to lymphomas at an early age. These malignancies were synergistically enhanced by aspirin/sulindac. The microsatellite trial cancer cell line did not show any such changes independent of proliferation rate and cyclo-
dac. These findings were reversible and were reduced following exposure to aspirin or sulindac.

\textbf{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.

\textbf{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 HpaI 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 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 \textit{APC} was found in CRC\textsuperscript{124} but its significance was not assessed, for example, by analysing \textit{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 \textit{Ap}c\textsuperscript{Min} mouse, it was possible to reduce dramatically the formation of polyps by suppressing genominc methylation. \textsuperscript{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 \textit{Ap}c gene specifically accounted for the reduction in polyph 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 \textit{MLH1} promoter region is common\textsuperscript{126-129} and associated with the absence of immunoreactive MLH1 protein.\textsuperscript{130} The hypermethylation could be reversed by treatment with 5-aza-C and this restored MLH1 protein.\textsuperscript{131} Somewhat surprisingly, no corresponding findings could be shown for \textit{MSH2}, but some 85% of all MSI positive sporadic (non-HNPCC) tumours could be accounted for by the silencing of \textit{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 or a deletion (loss of heterozygosity; LOH). 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 and pronounced right sided predominance, and why are they associated with typical age at diagnosis rather than young age? What environmental factors contribute to hypermethylation? Is hypermethylation gene specific, organ specific, age specific? 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 and endometrial cancer 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.

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.

Recently, LOI for IGF2 was studied in colorectal cancers. Ten of 11 CRCs that were MSI positive showed LOI while both of 2 LOI, 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. 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. 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. 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. 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. 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. However, while such mice do develop lymphoma both early and late in life, haematological malignancies have not been reported in

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*Note: The text continues with further details and references.*
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 NTF1, as well as in so far unidentified genes predisposing to lymphoma and leukaemia. The patient described by Hackman et al\(^1\) 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\(^2\) 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.\(^3\)

Next, to determine whether the I1307K mutation was associated with CRC in Ashkenazi Jews, Laken et al\(^4\) 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\(^5\) also observed the mutation more commonly (28%) in Ashkenazim with CRC and a first or second degree relative with CRC or adenomatous polyposis 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\%\(^6\); 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 putative 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.\(^7\)-\(^11\)

(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,\(^12\) 148 Finns, 105 African Americans, 54 Hawaii-Japanese, and 38 Italians\(^13\) and not found. In Israel, it occurred in 20/261 (7.7%) of Jews with an Ashkenazi background,\(^14\) 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 worldwide 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\(^15\)) 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.\(^16\) The authors tentatively concluded that no direct predisposition occurred and suggested a role of mismatch repair deficiency instead. In a similar study\(^17\) 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\(^18\) and Rozen et al\(^19\) whose data provided confirmation of the interpretations of the original report.\(^20\) Accordingly, carriers of I1307K appear to have an approximately 1.5 to twofold risk of CRC relative to non-carriers.\(^21\)-\(^25\) Interestingly, in the study by Gryfe et al\(^26\) I1307K was also found to contribute to increased numbers of colorectal adenomatous polyps with cancers per patient. Similar results were obtained by Frayling et al.\(^27\) Whether or not I1307K predisposes to other cancers, for example, breast cancer, is not
yet clear, but weak association with several cancers has been suggested.

(3) A direct molecular effect of I1307K was shown by Laken et al. 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), tract instead of (A), T (A), might predispose to insertion-deletion mutations through “slippage” at DNA replication. Indirect support for this interpretation was provided by Prior et al. 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. 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 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.

(6) In a retrospective study of germline DNA from 164 patients with multiple colorectal adenomas/carcinoma, Frayling et al. 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 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 accordance 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 morbid 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.

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Genetic susceptibility to non-polyposis colorectal cancer

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