

CHAPTER 7 HIGH-RISK FAMILIAL COLORECTAL CANCER SYNDROMES

Familial Colorectal Cancer includes syndromes in which there is a well-defined inherited genetic basis, as well as those families showing clustering of Colorectal Cancer in which no genetic cause has yet been found. Suspicion of a high-risk syndrome should be raised when two or more close relatives are affected, Colorectal Cancer has been diagnosed at an early age, (the earlier the age, the higher the degree of suspicion), or certain syndrome-specific characteristics are present.

The two best-characterised inherited syndromes are familial adenomatous polyposis (FAP) and hereditary non-polyposis Colorectal Cancer (HNPCC), (also known as Lynch syndrome). Both are inherited as autosomal dominant traits. FAP can usually be diagnosed on the basis of clinical findings alone, but the diagnosis is more difficult in the case of HNPCC. FAP and HNPCC have special significance because of their important contribution to Colorectal Cancer diagnosed before the age of 50 years. The chapter also considers families with a family history of Colorectal Cancer that may indicate a potentially high risk, though not strictly meeting HNPCC criteria. Such families are more numerous than those matching classical diagnostic criteria, and these form a large part of the referrals to family cancer clinics. These families and their management overlap with those considered in Chapter 6, to which the reader is also referred.

7.1 Principles of management

The correct management of individuals with, or at risk of, familial Colorectal Cancer is dependent upon determining which syndrome is present. This is important because risk assessment, genetic counselling, genetic testing, cancer preventive strategies and surgical treatment will differ according to the syndrome.

The diagnosis should be based upon meticulously verified clinical and pathological data concerning representative cancer-affected members of the family pedigree. This diagnosis may ultimately be confirmed by the demonstration of a germline mutation in the causative gene by testing an appropriate family member.

A family-based approach to the problem is facilitated by providing family members with clear and complete information, and by inviting them to participate actively in their own management. Care is focused on the family as well as the individual family member. It aims to reduce cancer morbidity and mortality within an environment that is both supportive and fully appraised of the rapid developments in this complex area.

Cancer mortality is reduced in members of FAP^{1,2,3} and HNPCC^{4,5,6} families who actively participate in regular screening and surveillance programs.

7.2 Multidisciplinary approach

FAP and HNPCC are inherited disorders associated not only with an increased risk of Colorectal Cancer, but also with proliferative disorders in a variety of extracolonic sites. In FAP, the principal life-threatening extracolonic lesions are periampullary adenocarcinoma and intra-abdominal fibromatosis (desmoid tumours).⁷ Additional extracolonic features may include papillary carcinoma of the thyroid, epidermal cysts and mandibular osteomas. Retinal pigmentation (congenital hypertrophy of retinal pigmented epithelium, or CHRPE) is observed commonly and can support the clinical phenotypic diagnosis of FAP.⁸

In HNPCC, extracolonic cancer may affect the endometrium, ovary, stomach, small intestine, renal pelvis and ureter, brain, skin and possibly pancreas.^{9,10} Management of these multi-system disorders does not fall within the traditional boundaries of any clinical discipline, but requires the input of surgeons, gastroenterologists, gynaecologists, oncologists, general practitioners and clinical geneticists. Equally important is expert support from the laboratory-based disciplines, including anatomical pathology,^{1,2,3,4,5,6} and molecular genetics. Registries provide a useful focal point for coordinating the management of these complex disorders. It is difficult for any individual practitioner to offer comprehensive management that is family-based and provides continuity of support to successive generations, encompassing diagnosis, genetic counselling/testing, cancer screening and treatment. Hence the emergence of multidisciplinary familial cancer services.

7.3 Colorectal Cancer family registries

All published data from cancer prevention programs that have reduced cancer incidence in family members with either FAP or HNPCC have used family registries.^{1,2,3,4,5,6} These facilitate the management of familial Colorectal Cancer by providing or supporting the following services:

- ascertainment of families
- construction of extended pedigrees
- verification of clinical and pathological data
- collection of tissue and blood samples
- maintenance of a meticulous, confidential and secure database on behalf of the present and future generations of a family
- liaison with relevant health care professionals
- liaison with other regional registries
- educational support and counselling
- identification of at-risk family members
- coordination of genetic counselling and testing
- coordination of cancer screening
- facilitation of multidisciplinary clinical management
- documentation of extended follow up
- resource for legitimate research translating ultimately into improved patient care.

State-based familial cancer registers have been established in Australia, and are listed in Table 7.4 at the end of this chapter.

7.4 Genetic testing

Genetic testing may provide the ultimate diagnosis of a specific hereditary condition, but it is often not required to achieve a correct working diagnosis. However, a genetic diagnosis (i.e. the identification of a causative mutation) is required if at-risk members of a particular family are to be

offered predictive genetic testing. In FAP, the site of the mutation can also influence the disease phenotype.^{11,12} This may be relevant when considering surgical management.

Genetic testing usually should be undertaken after the family history has been established in detail (just as special investigations *follow* the taking of a history and examination of an individual). Occasionally, blood is taken for DNA extraction and storage on clinical suspicion if the sole surviving affected member is frail or very elderly. Genetic testing should be conducted under the supervision of a clinical geneticist, specialist in cancer genetics, or ethically approved clinical research group, and should be supported by appropriate counselling.^{13,14} Informed consent is mandatory for genetic testing.

An individual believing him/herself to be at risk for an inherited cancer may wish to have a 'genetic test', for obvious reasons. Isolated testing is inappropriate as it is likely to be negative, a proportion of these being false negative results that fail to exclude a genetic risk factor. This can engender a false sense of security with respect to the individual and the individual's family.¹³ Furthermore, a harmless genetic variation (polymorphism) may be incorrectly interpreted as a positive result.

The most appropriate series of steps is to:

- establish a working diagnosis
- consider pre-genetic testing (microsatellite instability and immunohistochemistry) where the pedigree suggests HNPCC but is not clear-cut
- define the causative mutation in an affected individual
- develop a predictive test for the family
- offer predictive testing to at-risk members of the family
- provide appropriate genetic counselling and support for affected and unaffected family members

Although genetic testing is possible and has been achieved for many FAP¹³ and HNPCC^{15,16,17} families, it is a time-consuming and often expensive procedure associated with many pitfalls. It can be recommended only after a family has been thoroughly investigated, and with the involvement of an accredited clinical genetic or cancer genetic service or an ethically approved clinical research group.

7.5 Diagnosis and management of FAP

FAP is an autosomal disorder caused by a germline mutation in the APC gene.^{18,19} Penetrance of an APC mutation, as manifested by the development of Colorectal Cancer, approaches 100% by the age of 50 years in untreated subjects. FAP, however, accounts for less than 1% of all Colorectal Cancer cases. This translates to about 2000 affected and at-risk family members in Australia. In Finland, FAP now accounts for only 0.2% of bowel cancer, which reflects the success of prophylactic colectomy in cancer prevention in that country.²⁰

The diagnosis of a new case is usually made when an individual develops Colorectal Cancer at a relatively early age on a background of colorectal adenomatous polyposis (usually, but not always, considerably more than 100 adenomas). Attenuated FAP is also being increasingly recognised. In this condition there are fewer than 100 adenomas present, often only in the proximal colon. Adenomas and cancer tend to develop at an older age than in classic FAP. Careful review of the family history can identify unaffected family members who are at risk, and genetic testing may clarify risk status for individual family members. All 'at-risk' individuals need to be informed that screening sigmoidoscopy (or colonoscopy in the case of attenuated FAP) is recommended.

At-risk individuals include:

- all first degree relatives, and depending on information available on disease status of intermediate relatives, sometimes more distant relatives of affected individuals, until the family APC mutation has been identified
- once the family APC mutation has been identified, relatives who have been found to carry the family specific mutation, as well as untested close relatives.

The recommended protocol for screening is:

flexible sigmoidoscopy annually or biennially from age 12–15 years to 30–35 years until polyposis develops. Depending on likely compliance, sigmoidoscopy is often done yearly to establish better rapport with the individual and continuity of care. Cancers are exceedingly rare in teenage years, guiding the timing of surgery usually to later teenage years. If no family specific mutation has been identified, and no adenomas have developed, sigmoidoscopy as above until 35 years, and then every three years after the age of 35 years, in view of the diminishing likelihood that the person has inherited the APC mutation. Population-based recommendations can then be introduced from 55 years.²¹

- colonoscopic screening is appropriate for families with attenuated FAP, as recto-sigmoid sparing can occur in this variant of the disease.
- Dye spray scattering (chromo-endoscopy) at flexible sigmoidoscopy or colonoscopy increases detection of polyps and enhances information about the phenotype. This may facilitate decision making about which genes to interrogate in the process of a germline mutation search. Dye spray scattering should be considered in investigations to make the initial diagnosis.

Australian experience indicates that the causative APC gene mutation can be identified in over 85% of FAP families. The APC gene is a large gene, spanning 15 exons on chromosome 5.^{18,22} Mutations in different families are scattered throughout the gene. Most mutations produce a premature stop codon resulting in an abnormally shortened protein product. Such proteins can be readily identified in the laboratory using the protein truncation test.^{23,24} Other mutational analytic strategies may be required to optimise the detection rate (e.g. deletion studies). The exact description of the causative mutation is found by sequencing.

Once a causative APC mutation has been identified for the family, genetic testing may be used to distinguish mutation-positive and mutation-negative family members. Such predictive testing cannot proceed in at-risk members until the specific mutation has been identified in at least one affected family member.²⁵ Individuals shown not to carry the family-specific mutation no longer require intensive screening, with their risk reverting to that of the general population. Since the mutation (and disease) cannot skip generations, there is no need to test the children of such individuals. When a causative gene mutation is identified, at-risk children are generally offered testing when they would otherwise be commencing flexible sigmoidoscopy (e.g. in early to mid teens). Those testing positively will require annual sigmoidoscopy. It is important to check the histological features of a representative sample of any polyps found, because lymphoid polyps are sometimes large and numerous in children and can be mistaken for adenomas. It is usual to plan surgery once there is an endoscopic diagnosis with pathological confirmation.¹⁴

Appropriate surgical options for prophylactic management in FAP include total colectomy and ileorectal anastomosis, or restorative proctocolectomy with pouch formation.^{21,26} The usual age for these procedures is in the later teenage years, or early adulthood at the latest. Lifetime follow up of the rectum every 6 to 12 months after ileorectal anastomosis, or a pouch (after restorative proctocolectomy), is required; development of cancer, severely dysplastic adenomas or

endoscopically uncontrollable numbers of adenomas in the rectum are indications for proctectomy.¹⁴ Pouch surveillance frequency is uncertain; one guideline adopted in Victoria follows Spigelman guidelines (as below, Table 7.1).

Regular upper gastrointestinal endoscopy (particularly side-viewing duodenoscopy) in the prevention of upper GI malignancy in affected members of FAP families is widely practised from the age of 25 years.²⁷ Upper gastrointestinal endoscopy need not be done in screening of at-risk family members as colorectal adenomas will almost invariably precede duodenal adenomas. Significant duodenal polyposis is uncommon before 40 years of age and is staged using the Spigelman criteria (see Table 7.1).²⁸ The frequency of subsequent surveillance endoscopy is dependent on the stage of polyposis, as per the proposed EuroFAP guidelines (see Table 7.2).^{29,30} Several centres managing FAP are now intervening with endoscopic mucosal resection of duodenal adenomas, at a time when polyps do not extend over more than two duodenal folds. Side-viewing endoscopy is needed to manage the typical polyp in the periampullary region. Consideration should be given to temporary stenting of the pancreatic duct if there is any risk of disturbing it during resection or ablation of polyps. Post-polypectomy haemorrhage is common, so endoscopic skills (such as endoscopic clipping) should be available to manage bleeding polypectomy sites.

It is reasonable to offer gastroduodenoscopy,³⁰ to check the ampulla and the periampullary region, before proceeding to colectomy for treatment of colorectal polyposis, and to then follow the guidelines as above.

Table 7.1 Spigelman staging of duodenal polyposis

Score	1	2	3
Number	1–4	5–20	>20
Max size mm	1–4	5–10	>10
Worst histology	Tubular	tubulovillous	Villous
Dysplasia	Mild	moderate	Severe

Stage 1 = score 1–4; Stage 2 = score 5–6; Stage 3 = score 7–8; Stage 4 = 9–12

Source: Spigelman et al²⁸

Table 7.2 Proposed program for screening, surveillance and treatment for duodenal adenomatosis

Spigelman Stage 0 and I:	Endoscopy at intervals of 5 years
Spigelman Stage II:	Endoscopy at intervals of 3 years
Spigelman Stage III:	Endoscopy at intervals of 1–2 years Consider celecoxib 800 mg daily Consider endoscopic ultrasonography (EUS)
Spigelman Stage IV	Endoscopic ultrasonography (EUS) Consider surgery: Pancreas-sparing or pylorus-sparing duodenectomy

Source: Bulow et al³⁰

One study has shown that screening and surveillance of the upper gastrointestinal tract leads to a moderate gain in life expectancy.³¹ Another, at St Mark's Hospital, London, showed no benefit from surveillance endoscopy, apart from confirming the utility of the above staging system in identifying those for whom prophylactic surgery should be considered.³² A European study provided support for surveillance utilising the Spigelman staging system, demonstrating that most duodenal cancers

developed through a progression of the stages. Their screening and surveillance recommendations are as noted above (EuroFAP Surveillance protocol).³⁰

The role of non-steroidal anti-inflammatory agents (NSAIDs) such as sulindac and celecoxib as cancer chemopreventative agents has been suggested by a number of clinical studies.³³ However, there are reports of subjects developing Colorectal Cancer while on NSAIDs, despite evidence of polyp regression.³⁴ Nevertheless, the specific COX-2 inhibitor celecoxib has been shown to reduce the number of colorectal and duodenal adenomas in a randomised controlled study³⁵ and may therefore have a role as an adjunct to surgical management.³⁶ The large sporadic adenoma trials have reported an increased risk of myocardial infarction and stroke in patients taking polyp suppressive doses of COX-2 inhibitors, and therefore the place of these agents in chemoprevention has come under close clinical and regulatory scrutiny (see Chapter 2). On the other hand, sulindac failed to prevent the onset of adenomas in another randomised controlled trial.³⁷

7.6 MYH-associated polyposis (MAP)

MYH-associated polyposis (MAP) is a recently described condition that results from biallelic germline mutations in the base excision repair gene, mutY homologue (MYH)³⁸. MAP has a phenotype that is very similar to classical or attenuated familial adenomatous polyposis (FAP), but MAP is inherited as a recessive condition, whereas FAP (above) is a dominantly inherited disease caused by germline APC mutation. It is postulated that MYH mutations may act by increasing the frequency of somatic APC mutations. Carriers of biallelic MYH mutations have the multiple polyposis phenotype without a family history in the generations above and below the proband. Biallelic MYH mutation carriers should be treated and followed up as for FAP patients. Relatives of such patients should be counselled as for any other recessive condition, although it is possible that carriers of mono-allelic mutations are at a modestly increased risk of Colorectal Cancer.

7.7 Diagnosis of HNPCC

HNPCC is an autosomal dominant disorder caused by a germline mutation in one of a family of DNA mismatch repair (MMR) genes — principally *hMLH1*, *hMSH2*, *hMSH6* and *hPMS2*.^{39,40,41,42,43,44} The majority of mutation-positive families have mutations in either *hMLH1* or *hMSH2*.⁴⁵ HNPCC probably accounts for about 1–4% of all Colorectal Cancers.^{9,45,46,47,48}

In conjunction with family history, molecular tests have been developed to improve the diagnosis of HNPCC and assist in cancer risk assessment. When the family history suggests HNPCC, the presence of high levels of microsatellite instability (MSI) in the DNA of one or more cancers, or the lack of expression of MMR proteins by tumour testing, especially where that cancer occurs at an early age, may be a good indicator of a germline mutation in one of the MMR genes

7.7.1 Diagnosis based on family history

HNPCC is characterised by an early age of onset of Colorectal Cancer, a predilection for proximal colonic malignancy, and a tendency to develop multiple Colorectal Cancers.^{9,49} It is generally accepted that risk estimates calculated from clinic-based series of HNPCC families place the Colorectal Cancer risk at about 70–90% for men and women by the age of 65 years,⁵⁰ although a lower risk of 30% for women with HNPCC has been described.⁵¹ However, in population-based studies of early onset Colorectal Cancer in Australia, penetrance for Colorectal Cancer by 65 years for males was reported to be only 45% (95% Confidence Intervals 29-62%) and for females 38% (95% CI 19-51%); for any HNPCC related cancers, the risks were 67% (95CI 47-84%), and 72% (CI 48-85%) respectively. (Jenkins MA, personal communication). Another risk estimate for endometrial cancer was 42%,⁵¹ emphasising the need to screen this extracolonic site, especially in *hMSH6*

mutation carriers.⁵² There is also an increased risk of ovarian cancer in female carriers, estimated to be up to 10% over a lifetime.

Cancers may occur at other sites as detailed below. Carriers of an *hMSH2* mutation are at a higher risk of cancer than *hMLH1* mutation carriers.⁵³

HNPCC is distinguished clinically from FAP by the paucity of adenomas and by additional pathological features (see below). Adenomas do occur in HNPCC, often at an early age of onset.^{54,55,56}

The original Amsterdam-1 criteria⁵⁷ are clinical criteria formulated to help identify families that should be considered for a diagnosis of HNPCC. A high proportion of families fulfilling the Amsterdam criteria (probably between 60–95%) will have HNPCC.^{58,59} These criteria are:

- at least three cases of Colorectal Cancer in the family (verified)
- one case a first-degree relative to the other two
- at least two successive generations affected
- at least one case diagnosed before the age of 50
- exclusion of FAP.

A memory aid is the 3,2,1 rule: 3 first-degree relatives affected, over 2 generations, with 1 under the age of 50 years.

The Amsterdam II criteria⁶⁰ were introduced in an attempt to increase the sensitivity of diagnosing an HNPCC family, with minimal loss of specificity. These criteria allow ‘extracolonic malignancy’ to be substituted for ‘colorectal malignancy’. The sites of extracolonic malignancies included are: endometrium, small bowel, ureter and renal pelvis. Very rarely, there may be families with these malignancies but no Colorectal Cancer.

However, some families that meet these criteria, or have a very strong family history of cancer suggestive of a dominant susceptibility to bowel cancer, do not have a demonstrable MMR germline mutation. Therefore, they do not have HNPCC as defined by the finding of an MMR germline mutation, based on current knowledge and available technology. Conversely, some families that do not meet the Amsterdam criteria do have HNPCC.^{61,62,63} Families meeting Amsterdam criteria, regardless of molecular information, are usually managed as for HNPCC. However, emerging evidence suggests that in families with a family history that fulfils the Amsterdam –1 criteria, but where tumour testing shows no evidence to suggest MMR deficiency (see 7.7.2), the risk of Colorectal Cancer may be less than for proven HNPCC, and the risk of extracolonic cancers may be absent.⁶⁴ Further evidence may eventually change clinical practice in the management of such families.

7.7.2 Diagnosis based on tumour testing

In conjunction with family history, molecular tests have been developed to improve the diagnosis of HNPCC. When a diagnosis of HNPCC is being considered in an index case, based on a suspicious family or clinical history, testing of tumour tissue using microsatellite instability testing and immunohistochemistry testing may help determine which patients should proceed to formal germline genetic testing.

Microsatellite instability testing (using tumour tissue)

Microsatellites are made up of tandem repeats of short sequences of DNA, 1–5 bases in length. They are found throughout the genome, both in coding and non-coding regions. During DNA replication, errors can occur at these sites, causing a change in size of the microsatellite in the daughter cells — this is termed microsatellite instability (MSI).⁶⁵ Normally these errors are recognised and repaired by the DNA mismatch repair (MMR) system. When the DNA MMR system is defective as in HNPCC, due to a germline mutation in one of the MMR gene alleles coupled with mutation, loss or inactivation of the other normal allele, MSI occurs and can be demonstrated by comparing normal and cancer DNA. Tumours are described as microsatellite stable (MSS), where there is no instability; MSI-high (MSI-H), where two or more of the National Cancer Institute -recommended panel of microsatellites are unstable, or MSI low (MSI-L), where only one of the panel is unstable.⁶⁵ Instability with mononucleotide repeat sequences alone suggests an underlying hMSH6 mutation.

The great majority of Colorectal Cancers in HNPCC are MSI-H and the presence of high levels of microsatellite instability can help corroborate the diagnosis of HNPCC, particularly in small families.^{59,60,62,66,67} Approximately 70% of adenomas in HNPCC will also exhibit MSI-H and show immunohistochemical loss of expression of a DNA mismatch repair protein concordant with the underlying germline mutation.⁵⁹

It is important to note, however, that about 10–15% of the large number of sporadic Colorectal Cancers also exhibit MSI-H.^{68,69} Therefore the finding of MSI-H should be considered in the context of the family history and, importantly, the age of the patient. MSI-H occurs in sporadic Colorectal Cancer as a result of acquired hypermethylation and epigenetic silencing of *hLMHI*⁷⁰

MSI-H Colorectal Cancers in HNPCC are likely to be mucinous, poorly differentiated and characterised by the presence of tumour infiltrating lymphocytes.⁷¹ However, there is some evidence that mucinous differentiation and poor glandular differentiation are more typical of sporadic MSI-H Colorectal Cancer, whereas lymphocytic infiltration is more marked in HNPCC-associated MSI-H cancers.⁷² Greater diagnostic precision is achieved by analysis of a combination of clinical, pathological and genetic variables.⁷³ Further molecular approaches may eventually allow a distinction between HNPCC and sporadic MSI-H Colorectal Cancer. For example, a specific BRAF mutation occurs in the majority (but not all) of sporadic MSI-H cancers, but virtually never occurs in HNPCC-associated MSI-H cancers.⁷⁴ Thus, presence of the specific BRAF mutation in a MSI-H tumour makes a sporadic aetiology very likely, but absence of the BRAF mutation is not helpful in this differentiation. Currently, tumour BRAF mutation analysis is not generally available in diagnostic laboratories. Another approach is to assess the degree of methylation (silencing), or more accurately, allele specific methylation of mismatch repair genes shown to lack expression of mismatch repair gene proteins in tumours. Silencing of the promoter of, especially hMLH1, is the dominant mechanism of development of MSI-H Colorectal Cancers. Tumour methylation studies can therefore reveal the pathogenesis of MSI-H cancers, and guide the need for a search for a germline mutation where a MSI-H cancer is identified in a family. Methylation studies of mismatch repair genes may be important in the future, but are currently not generally available in clinical practice.

MSI testing and/or immunohistochemistry (see below) should usually precede the expensive step of formal germline genetic screening of a family (commencing with mutation search in an affected family member). When a family history suggests HNPCC, the presence of MSI-H in the DNA of one or more cancers, especially where that cancer occurs at an early age, is a good indicator of a germline mutation in one of the MMR genes.⁶¹

The Bethesda guidelines were developed to provide selective criteria for testing tumours for MSI *in an individual affected by Colorectal Cancer*.^{75,76} The guidelines were modified after experience was gained in their application (Table 7.3). The Revised Bethesda Guidelines⁷⁷ should be regarded as the

standard for consideration of MSI and/or immunohistochemistry testing. Changes from the original Bethesda Guidelines include no longer recommending testing for patients with endometrial cancer (alone) under 45 years (as the new guidelines refer specifically to testing of colorectal tumours), testing of tumours in families meeting Amsterdam criteria (many centres would move straight to germline mutation testing in an affected member in these families), and adenomas diagnosed in persons under 40 years (as this has proved a poor marker of MSI). The specificity of the new guidelines⁷⁷ has been questioned and tighter guidelines are likely to emerge from this debate⁷⁸

The revised guidelines recommend testing patients with synchronous or metachronous HNPCC-related cancers *regardless of age of onset*, a wider definition of HNPCC-related cancers, increasing the age at diagnosis below which MSI/immunohistochemistry testing should be considered routinely to 50 years, and to 60 years where there are histopathological features suggesting microsatellite instability.

The purpose of the Bethesda criteria is to assist in increasing the yield of HNPCC beyond what may be achieved through the application of the Amsterdam criteria, and help distinguish *individuals* with MSI-H cancers who are likely to have an underlying germline mutation (HNPCC) from the more numerous individuals with sporadic MSI-H cancer, for whom germline screening for a mutation is not warranted. The experience has been that they are sensitive, but not especially specific.

Table 7.3 Revised Bethesda Guidelines for testing colorectal tumours for microsatellite instability

<p>Tumours from individuals should be tested for microsatellite instability (MSI) in the following situations:</p> <ol style="list-style-type: none"> 1. Colorectal Cancer diagnosed in a patient who is less than 50 years of age. 2. Presence of synchronous, metachronous Colorectal Cancer, or other HNPCC-associated tumours^a regardless of age. 3. Colorectal Cancer with the MSI-H^b histology^c diagnosed in a patient who is less than 60 years of age.^d 4. Colorectal Cancer diagnosed in one or more first-degree relatives with an HNPCC-related tumour, with one of the cancers being diagnosed under age 50 years. 5. Colorectal Cancer diagnosed in two or more first- or second-degree relatives with HNPCC-related tumours, regardless of age.
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^a Hereditary nonpolyposis Colorectal Cancer (HNPCC)-related tumours include colorectal, endometrial, stomach, ovarian, pancreas, ureter and renal pelvis, biliary tract, brain (usually glioblastoma as seen in Turcot syndrome) tumours, sebaceous gland adenomas and keratoacanthomas in Muir-Torre syndrome, and carcinoma of the small bowel.

^b MSI-H (microsatellite instability — high) in tumours refers to changes in two or more of the five National Cancer Institute-recommended panels of microsatellite markers.

^c Presence of tumour-infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous/signet-ring differentiation, or medullary growth pattern.

^d There was no consensus among the Workshop participants as to whether to include the age criteria in guideline 3 above; participants voted to keep less than 60 years of age in the guidelines. Sporadic MSI-H cancers are uncommon below the age of 60,⁷² and MSI testing should be considered in subjects up to this age when the morphological features of MSI-H cancers are present.

Source: Umar et al⁷⁷

Clearly, the various guidelines are not exclusive, and overlap. They are relevant for different clinical scenarios — family consultations versus individual presentations. All are presented here for information.

Immunohistochemistry testing using tumour tissue

Immunohistochemistry (IHC) testing uses antibodies to measure expression of the various MMR proteins in a tumour. Loss of expression of a specific protein produced by one of the relevant genes (hMLH1, hMSH2, hMSH6 or hPMS2) may be an indicator of a germline mutation in the gene coding for that protein. Immunostaining is sensitive and highly specific.⁷⁹ The technique pinpoints the gene that is implicated and therefore facilitates the search for the underlying germline mutation.

The Revised Bethesda Guidelines⁷⁷ could also be applied to the selection of cancers for immunohistochemical staining. Indeed, clinical practice world wide is recognizing the value of testing tumours by immunohistochemistry as the first line tumour test, reserving microsatellite instability testing for situations where the family history is highly indicative of HNPCC, but where immunohistochemistry is normal.

7.7.3 Diagnosis by germline genetic testing

Identification of the causative germline mutation in an affected individual allows for other at-risk family members to be offered predictive testing. Genetic testing is not a simple matter, and should not be carried out in isolation. Mutations may occur in any of the four MMR genes. If a causative mutation is found, those carrying the high-risk gene mutation can be targeted for cancer screening or preventive measures. Germline mutational testing of the index case in the family, generally the youngest affected member in a family meeting Amsterdam criteria, or an individual with tumour testing indicative of a mismatch repair mutation, begins the process of characterising the family specific mismatch repair mutation at the molecular level.

7.8 Screening and surgical management of HNPCC

Screening of mutation carriers or individuals affected with HNPCC-related tumours in Amsterdam-positive families should be by full colonoscopy performed annually or at least once every two years, beginning at the age of 25 years or five years earlier than the age of diagnosis of the youngest affected member of the family (whichever is the earliest).⁹ Rapid evolution of the adenoma–carcinoma sequence or other routes of morphogenesis may account for the relatively high frequency of interval cancers.⁸⁰

Screening first-degree relatives of affected members in Amsterdam positive families where the mutation status is unknown is similar, although colonoscopy can be reduced to two-yearly. More distant relatives can be offered 5-yearly colonoscopy. This recommendation is a compromise between a policy to offer all relatives annual screening (to protect them from rapidly growing tumours if they do carry the family specific mutation) and the diminishing risk of carriage as the proximity of the relative to the potential carrier recedes with the degree of relationship. Consideration should be given to screening for extracolonic malignancy in affected and at-risk individuals, particularly gynaecological screening. The efficacy of screening any sites outside the colon and rectum has not been determined, but is expected to be greater in families with an increased burden of extracolonic malignancy. The most common extracolonic site of cancer is the uterus (endometrium).⁸¹ Those to be considered for gynaecological screening would include women with proven MMR mutations and their first-degree relatives who have not been gene tested, women affected by bowel cancer (if uterus or ovaries *in situ*) from Amsterdam–positive families where no mutation has been identified, and untested first- and second-degree relatives of affected members in MSH6 families.⁸²

The Cancer Council of Victoria's VCOG Gynaecological Committee recommends that a screening program should include annual transvaginal ultrasound (TVUS) of the ovaries and uterus in premenopausal women, commencing at age 30–35 or five years before the age at diagnosis of the youngest affected family member. In postmenopausal women, annual CA125 measurement may be

added to transvaginal ultrasound.⁸³ Endometrial sampling and full investigation is required in symptomatic women and those with abnormal endometrial findings as assessed on TVUS during the proliferative phase of the menstrual cycle. Endometrial biopsy is indicated if endometrial thickness is ≥ 9 mm in the proliferative phase of the premenopausal menstrual cycle or if there is endometrial thickness ≥ 4 mm in the postmenopausal woman. Such screening is based on expert evidence but has not been established as reducing either incidence or mortality from endometrial or ovarian cancer.

Screening for other HNPCC-associated cancers in at-risk family members (e.g. gene carriers or untested individuals) should be influenced by the profile of cancers occurring in the family. One guideline is to introduce such screening when there are two or more of a particular tumour type in the family (H Vasen, Netherlands Hereditary Tumour Institute, personal communication). Renal tract screening with urine cytology is particularly non-invasive and cheap. Additional extracolonic sites may be affected more frequently in *hMSH2*⁸¹ and *hMSH6*^{44,84} families. This is particularly pertinent with respect to gastric cancer, which dominated the original (Family G) description of HNPCC by Warthin.⁸⁵

The risk of metachronous Colorectal Cancer is increased in HNPCC. For this reason, extended surgery, for example total colectomy, has been recommended for subjects with proven HNPCC.⁸⁶ However, this is not widely accepted, especially in some parts of Europe, where a controlled surgical trial is being mounted to address the question. In newly suspected cases of HNPCC, MSI testing or immunohistochemical staining of a preoperative biopsy may assist in development of a management plan, including surgery. Age, state of health and the wishes of the patient, together with the site of the cancer, will influence the choice of surgical procedure. Annual endoscopic surveillance of the remaining large bowel mucosa is then required.⁸⁷ Long-term compliance and access to such screening will also influence the surgical procedural choice. Consideration should be given to offering women with proven HNPCC a hysterectomy and possibly an oophorectomy at the time of surgery for Colorectal Cancer, if childbearing is complete.^{9, 14}

Prophylactic colectomy in either at-risk individuals or even those known to be mutation carriers cannot be recommended generally, but is an option in particular instances, especially when screening has led to detection of more than one advanced adenoma.¹⁴ Under such circumstances, consideration should also be given to prophylactic hysterectomy and oophorectomy from the age of 30–35 years or when childbearing is complete. Because the efficacy of gynaecological screening is uncertain, prophylactic gynaecological surgery should be discussed whether or not prophylactic colectomy is being considered as an option.

7.9 Familial clusters of Colorectal Cancer

Since there is evidence that HNPCC can affect some families not meeting the Amsterdam criteria, the possibility that a family cluster of Colorectal Cancer may be due to HNPCC should be reviewed using the Australian Cancer Network⁸⁸ family history guide with review of the clinical and pathological data and testing of tumours for DNA microsatellite instability and/or loss of an MMR protein by immunohistochemistry. These guidelines recommend referral of families for consideration of risk assessment, tumour testing or genetic testing to familial cancer services where:

- three or more first- or second-degree relatives on the same side of the family are diagnosed with bowel cancer, or
- two or more first- or second-degree relatives on the same side of the family are diagnosed with bowel cancer plus any of the following high-risk features:
 - multiple bowel cancers in a family member
 - bowel cancer before age 50 years

- a family member has or has had an HNPCC-related cancer (endometrial, ovarian, stomach, small bowel, renal pelvis or ureter, biliary tract, brain cancer), or
- at least one first- or second-degree relative diagnosed with bowel cancer with a large number of synchronous adenomas (suspected FAP), or
- there is a member of a family in which a gene mutation that confers a high risk of bowel cancer has been identified.

When a working diagnosis of HNPCC cannot be justified, screening recommendations for familial clusters of Colorectal Cancer should be followed (see Chapter 6).

Increasing evidence points to a diminished risk for colorectal and especially extracolonic cancers, where there is no evidence of deficient mismatch repair in tumours occurring in the family, despite strong, even Amsterdam positive, family histories of cancer⁶⁴ Screening of at risk relatives in these families may eventually warrant a reduced screening strategy, as the evidence evolves.

7.10 Hyperplastic polyposis and MSI-variable cancers

A particular type of familial clustering of Colorectal Cancer may closely mimic HNPCC and the resemblance may extend to the demonstration of one or more Colorectal Cancers showing evidence of MSI.⁸⁹⁻⁹¹ However, further investigation of such families may show clinical, pathological and molecular features that cannot be explained on the basis of a germline mutation in a DNA MMR gene. These features may include: (i) mixtures of MSI-H, MSI-L and MSS cancers in different family members or even in the same individual; (ii) presence of hyperplastic polyps that may be numerous and/or large (associated adenomas might also be present); (iii) a relatively older age at onset of cancer; (iv) absence of extracolonic HNPCC-associated tumours.^{89,90}

A likely explanation for the finding of MSI-H cancers in such families is silencing of *hMLH1* as a result of somatic DNA methylation. There is now evidence that DNA hypermethylation is involved in the pathogenesis of a subset of hyperplastic polyps, including those found in association with sporadic MSI-H cancers.^{92,93,94,95} Hyperplastic polyps with DNA methylation are likely to be multiple, relatively large (≥ 1 cm) and located in the proximal colon. In these serrated polyps, a specific somatic *BRAF* gene mutation can occur, as seen in sporadic MSI-H Colorectal Cancers which also have promoter methylation of *hMLH1*.⁷⁴ In some instances hyperplastic polyps may occur in sufficient numbers (>20) to warrant a diagnosis of hyperplastic polyposis.⁹⁶

It is possible that the familial clustering of Colorectal Cancer in the context of the previous observations may be explained in part by a familial predisposition to somatic DNA methylation. A polymorphism in the methyltetrahydrofolate reductase gene may be important in this regard.⁹⁷ Screening for a germline mutation in a DNA MMR gene is likely to be unproductive in such families.

Disruption of a DNA MMR gene by inherited mutation is thought to accelerate the development of Colorectal Cancer. It is likely that epigenetic silencing of *hMLH1* or other DNA repair genes such as *MGMT* by DNA methylation may also accelerate cancer progression.^{98,99} When such a ‘methylator’ family is suspected, expert opinion suggests that colonoscopic screening should be the same as for HNPCC in the case of family members affected by either cancer or multiple and/or large hyperplastic polyps (J Jass, personal opinion). Most subjects with hyperplastic polyposis (with or without associated Colorectal Cancer) do not have a family history of Colorectal Cancer. Any genetic basis for this condition is unlikely to be highly penetrant. Therefore, screening of first-degree relatives of affected subjects could be as for subjects in the moderate-risk category (5 yearly colonoscopy -

category 2, see Chapter 6) but may be more intensive if adenomas or multiple small hyperplastic polyps are detected.

7.11 Peutz-Jeghers syndrome and juvenile polyposis

The risk of Colorectal Cancer and some other cancers is also increased in the rarer Peutz-Jeghers syndrome (mucocutaneous pigmentation and multiple hamartomatous polyps)¹⁰⁰ and juvenile polyposis¹⁰¹ (multiple gastrointestinal juvenile polyps). These patients and their families should also be referred to specialist family cancer clinics for advice and coordination of management.

7.12 Summary of recommendations

How should genetic testing be undertaken for high-risk CRC family syndromes?

Guideline — High familial risk syndromes	Level of evidence	Practice recommendation	Refs
After counselling, genetic testing should be undertaken under the supervision of a cancer genetics specialist.	III-2	Recommend	14

What is the role of NSAIDs in the prevention of colorectal neoplasia in high-risk familial syndromes?

Guideline — High familial risk syndromes	Level of evidence	Practice recommendation	Refs
The role of NSAIDs such as sulindac in the prevention of cancer in FAP is unclear. High-dose celecoxib (400 mg twice daily) has been shown to reduce polyp numbers and its use may facilitate the control of polyps, but carries significant cardiovascular morbidity.	II	Equivocal	14, 33, 35, 36

What is the surgical management of FAP?

Guideline — High familial risk syndromes	Level of evidence	Practice recommendation	Refs
The surgical management of FAP is by total colectomy and ileorectal anastomosis or restorative proctocolectomy.	III-2	Recommend	14, 25

When should large bowel screening begin in FAP and what should be offered?

Guideline — High familial risk syndromes	Level of evidence	Practice recommendation	Refs
Screening in FAP is by sigmoidoscopy from 12–15 years of age (the later age is recommended), except in attenuated FAP, where screening is based on colonoscopy, or where there is a family history of very early age of onset of Colorectal Cancer in the family.	III-2	Recommend	1-3, 14

Is duodenal screening recommended in FAP?

Guideline — High familial risk syndromes	Level of evidence	Practice recommendation	Refs
Duodenal screening in FAP is recommended perioperatively at the time of colectomy, from 25 years of age, or earlier should there be a family history of duodenal cancer at an early age.	III-2	Recommend	14, 26-32

How should FAP family members not carrying their family mutation be advised?

Guideline — High familial risk syndromes	Level of evidence	Practice recommendation	Refs
Members of proven FAP families who are documented to NOT carry the family specific APC mutation are no longer at high risk.	III-2	Recommend	25

When should large bowel screening of at-risk members in proven HNPCC families be offered?

Guideline — High familial risk syndromes	Level of evidence	Practice recommendation	Refs
Screening of at-risk members in proven HNPCC families should be by annual or 2-yearly colonoscopy, commencing around the age of 25 years or five years before the earliest age of cancer diagnosis in the family, whichever comes first. Annual screening should be offered to individuals carrying a germline mutation and for clinically affected individuals in Amsterdam families where mutation status is unknown.	III-2	Recommend	4-6, 9, 14

What screening is recommended for extracolonic cancers in HNPCC?

Guideline — High familial risk syndromes	Level of evidence	Practice recommendation	Refs
Consideration should be given to screening extracolonic sites in HNPCC, especially in families with clusters of extracolonic cancers. Routine screening of the uterus and ovaries (see text) should begin at around 30–35 years, or five years earlier than the youngest relative affected with uterine or ovarian cancer, whichever comes first. Referral to a gynaecological oncologist is advised for women in these families. Gastroscopy should be added to colonoscopy (on the same day, where possible, for patient convenience) if there is any family history of gastric cancer. Annual urine cytology and renal ultrasound is recommended in families with tumours of the renal collecting system.	III-3	Recommend	9

How should tumour testing (MSI and IHC) be used in affected individuals from families suspected to have HNPCC?

Guideline — High familial risk syndromes	Level of evidence	Practice recommendation	Refs
The Revised Bethesda Guidelines could be applied to the selection of cancers for microsatellite instability (MSI) testing and immunohistochemical staining.	III-2	Recommend	77, 78

How should HNPCC family members not carrying their family mutation be advised?

Guideline — High familial risk syndromes	Level of evidence	Practice recommendation	Refs
Members of proven HNPCC families who test negatively for the family mismatch gene mutation do not have an additional risk associated with this mutation.	III-2	Recommend	14

What surveillance is recommended in hyperplastic polyposis and for MSI-variable cancers?

Guideline — High familial risk syndromes	Level of evidence	Practice recommendation	Refs
Affected subjects in familial clusters characterised by mixtures of MSI-H, MSI-L and MSS cancers and/or the finding of multiple/large hyperplastic polyps should be screened by colonoscopy according to HNPCC recommendations, though first-degree relatives unaffected by cancer may be screened according to intermediate risk-guidelines.	IV	Equivocal	92, 93

Table 7.4 Hereditary Bowel Cancer Registers in Australia and New Zealand

FamilialCancer@yahoo.com		
NSW and ACT Hereditary Cancer Registers (FAP, polyposis syndromes and HNPCC)	Cancer Council NSW 153 Dowling Street Woolloomooloo, NSW 2011 PO Box 572 Kings Cross NSW 1340 Australia	Tel: 02 9334 1807 Fax: 02 9334 1867 Email: hcr@nswcc.org.au Website: < www.nswcc.org.au > Cancer Helpline: 13 11 20 or 1800 422 760
Queensland Familial Bowel Cancer Registry inc Queensland Familial Adenomatous Polyposis Register (FAP and polyposis syndromes) and HNPCC	C/- Queensland Familial Bowel Cancer Registry QLD Clinical Genetic Service Block 65 via Back Road, Royal Brisbane Hospital HERSTON QLD 4029	Tel: 07 3636 5117 Fax: 07 3636 9164 Website: www.cancersa.org.au FAP: Ngaire_knight@health.qld.gov.au IHNPCC: Vivianne_geldard@health.qld.gov.au
Familial Cancer Unit (FAP, polyposis syndromes and HNPCC and other familial cancers)	Familial Cancer Unit SW7 Women's and Children's Hospital 72 King William Road NORTH ADELAIDE SA 5006	Tel: 08 8161 6995 Fax: 08 8161 7984 Email: famcancer@mail.wch.sa.gov.au
Victorian Family Cancer Register/ FAP Registry	The Cancer Council Victoria I Rathdowne Street CARLTON VIC 3053	Tel: 03 9635 5374 03 9635 5414 03 9635 5176 Fax: 03 9635 5270 Email: enquiries@cancervic.org.au Website: < www.cancervic.org.au >
Familial Cancer Registry of Western Australia FAP, polyposis syndromes HNPCC	Familial Cancer Program Genetic Services of WA 374 Bagot Road SUBIACO WA 6008	Tel: 08 9340 1603 or 9340 1713 Fax: 08 9340 1725 Website: < www.cancerwa.asn.au >
Tasmanian Bowel Cancer Register	W.D. Booth Centre PO Box 1963 LAUNCESTON TAS 7250	Tel: 03 6348 7006 Fax: 03 6348 7905 Email: enquiries@cliffordcraig.org.au Website: < www.cliffordcraig.org.au >
New Zealand Familial Bowel Cancer Registry (FAP, polyposis syndromes and HNPCC)	Northern Regional Genetic Services Lower Ground Floor Building 18 Private Bag 92024 GRAFTON, AUCKLAND NZ	Tel: + 649 307 4949, ext 5436 Fax: + 649 307 4978

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