

## How can we best detect hereditary non-polyposis colorectal cancer?

*New tumour testing methods can improve the accuracy of diagnosis*

Although a strong genetic predisposition to colorectal cancer (CRC) is rare, it is important because of its large contribution to CRC diagnosed before the age of 50 years and because mortality from CRC can be reduced by appropriate management. There are currently limitations in determining whether a case of CRC is “sporadic” or whether it might be related to an inherited predisposition. However, Australian research that examines the utility of new diagnostic tools to help diagnose genetic tendency to CRC may be showing us a way forward.<sup>1,2</sup>

### What is hereditary non-polyposis colorectal cancer?

The two best-characterised high-risk inherited syndromes are familial adenomatous polyposis and hereditary non-polyposis colorectal cancer (HNPCC) — sometimes known as Lynch syndrome. Both are inherited as autosomal dominant traits.

Familial adenomatous polyposis can usually be readily diagnosed on the basis of clinical findings alone, when an individual develops CRC at a relatively young age on a background of colorectal adenomatous polyposis (mostly with more than 100 adenomas). HNPCC cannot usually be readily diagnosed.

HNPCC accounts for about 1%–4% of all CRC, but up to 10% in patients younger than 50 years at diagnosis. It is caused by a germline (heritable) mutation in one of a family of genes known as the DNA mismatch repair genes: *hMLH1*, *hMSH2*, *hMSH6* and *hPMS2*.<sup>3</sup> HNPCC is characterised clinically by an early age of onset of CRC, a predisposition for proximal colonic cancers, and a tendency to develop multiple CRC, along with an increased risk of some extra-colonic malignancies, including cancers of the uterus and ovary, stomach, small bowel, biliary tree, ureter, renal pelvis, pancreas and brain. The family history is often complex.

### What are the difficulties in diagnosing HNPCC?

Traditionally, HNPCC has been suspected in families where the family history of cancer meets the modified Amsterdam criteria.<sup>4</sup> These require a family to have at least three close relatives in two generations diagnosed with cancer of the colon, rectum, endometrium, small bowel, ureter or renal pelvis, with at least one diagnosed before age 50 years.

One problem is that not all “Amsterdam positive” families will be proven to have HNPCC and, conversely, some families with proven HNPCC do not meet these criteria. Family history alone is not enough — a diagnosis of “suspected HNPCC” may be based on verified clinical and pathological information from the family pedigree, but the diagnosis of HNPCC is ultimately confirmed by the demonstration of a family germline mutation in one of the mismatch repair genes. This is done by taking blood from one of the affected family members and searching the mismatch repair genes to determine whether a causative mutation can be found.

However, a mutation cannot be found in every family, as mutations may be missed or mutations may be present in other genes that are not yet identified. This means that if the family

history is strong (Amsterdam positive) and the genetic test (mutation search) fails to identify a mutation in an affected family member, the test result should be considered “inconclusive” and all relatives remain at potentially high risk.

Only when a causative mutation in one of the mismatch repair genes has been identified can other at-risk adult family members be offered “predictive” genetic testing to determine their risk. Importantly, those found not to carry the family mutation should be considered at the average population risk for cancer, and they (and their offspring) can be spared additional cancer screening and concern.

At present, many families with a family history that meets or approaches the Amsterdam criteria are offered this expensive approach to germline genetic testing; few are found to have proven HNPCC.

### How are these difficulties being addressed?

The accurate detection of HNPCC has improved recently for a number of reasons. The first is an increasing awareness of family history as a risk factor for CRC. Clinical practice guidelines assist in estimating CRC risk based on family history.<sup>5,6</sup> For those with a more complex family history, referral to a family cancer clinic may be appropriate. These clinics have been developed to provide cancer risk assessment, surveillance, and prevention strategies, with genetic counselling and testing when appropriate. Finally, we now have improved diagnostic tools, including molecular testing of tumours for microsatellite instability and immunohistochemical staining of tumour tissue for mismatch repair proteins, which can be used to help investigate a history of “suspected HNPCC”. Additional molecular tumour tests are in development.

### Molecular testing for microsatellite instability

Microsatellite instability is one of the hallmarks of HNPCC-related cancers and is due to unrepaired mutations in repetitive sequences of DNA known as microsatellites. CRCs that occur in patients with HNPCC tend to be right-sided, mucinous, poorly differentiated and characterised by the presence of tumour infiltrating lymphocytes. On molecular testing, these tumours show high levels of microsatellite instability.

The Bethesda guidelines<sup>7</sup> were developed to provide criteria for testing tumours for microsatellite instability in an individual affected family member to assist in the diagnosis of HNPCC. These guidelines suggest that all people with CRC diagnosed before the age of 50 years should have microsatellite instability testing to look for possible HNPCC. However, another limitation presents itself. Although high levels of microsatellite instability are seen in most HNPCC-related tumours, microsatellite instability is not exclusive to HNPCC. Some people develop sporadic cancers with high levels of microsatellite instability, unrelated to an inherited defect in mismatch repair; around 10%–15% of sporadic colorectal cancers will exhibit high levels of microsatellite instability. This tends to occur in older women with right-sided cancers, often mucinous,

with poor glandular differentiation (as per HNPCC), but with no associated HNPCC family history.

In patients with sporadic CRC with high levels of microsatellite instability, the defect in mismatch repair is not heritable and occurs only in the tumour as a result of epigenetic silencing of the promoter region of *hMLH1*. It does not alter the risk of CRC in offspring, so it is important to differentiate this from HNPCC. Tumour *BRAF* gene mutations occur in most of these sporadic cancers, but virtually never in HNPCC-associated cancers with high levels of microsatellite instability. The presence of a *BRAF* gene mutation in a tumour with high levels of microsatellite instability makes it more likely to be a sporadic CRC; however, the use of tumour testing for *BRAF* mutation is speculative at this stage and it is not yet readily available.

### Immunohistochemical staining

Another approach used to improve the accuracy of diagnosis of HNPCC is the use of immunohistochemical staining in suspected cases. Immunohistochemical staining uses antibodies to the proteins encoded by the four relevant mismatch repair genes (*hMLH1*, *hMSH2*, *hMSH6* and *hPMS2*) to test for the expression of these proteins in tumour tissue. Absence of mismatch repair proteins is often seen in HNPCC and may be specifically related to the gene in which a germline mutation may subsequently be found; that is, the absence of *hMSH2* protein in cancer cells usually indicates a germline mutation in *hMSH2*. However, once again, this absence of staining is not specific to HNPCC — loss of expression of *hMLH1* due to somatic inactivation of *hMLH1* does occur in sporadic cancers.

### How is Australian research contributing in this field?

Southey et al have recently conducted an analysis of the use of tumour testing to prioritise mismatch repair germline testing in an Australian population-based study of early onset (< 45 years old) CRC.<sup>1</sup> In 131 patients, unselected for family history, they found 18 with germline mutations in one of the mismatch repair genes (indicating HNPCC). Based on family history alone (the Amsterdam criteria), only half of these would have been identified, indicating that tumour testing (specifically immunohistochemical staining) is a very useful adjunct to diagnosis, and more sensitive than family history. Ward et al, also Australian, have since published similar findings.<sup>2</sup>

Immunohistochemical staining has an advantage over microsatellite instability testing in that it is relatively inexpensive, can be conducted in a routine pathology laboratory (rather than a molecular laboratory), and the immunohistochemical staining test identifies the specific mismatch repair gene in which to search for a germline mutation, thereby saving money by directing the mutation search. However, although immunohistochemical staining is promising as a tool to increase diagnostic accuracy for HNPCC, it may be too early to apply this test routinely at diagnosis to all patients with CRC.

There are still some problems with tumour testing. Some patients have loss of staining on immunohistochemical staining without a demonstrable mismatch repair germline mutation, indicating a lack of specificity, although this may change as germline testing improves. Immunohistochemical staining utilises antibodies that are not commonly used in pathology laboratories, and staining may be patchy and difficult to interpret for those who

do not use these antibodies routinely. Moreover, there is generally a lack of understanding concerning the interpretation of loss of stain — loss of *hMLH1* in a patient with an older onset CRC, without family history, usually indicates a sporadic cancer rather than HNPCC, but some of these families are now inappropriately being referred to family cancer clinics as “possible HNPCC”. Finally, some argue that tumour testing is a surrogate genetic test and so requires genetic counselling and fully informed consent. However, tumour testing should be simply viewed as a useful adjunct to HNPCC diagnosis, in that an abnormal finding on immunohistochemical staining raises the possibility, rather than the certainty, of a familial predisposition to CRC.

### Where to from here?

Tumour testing could now be used as a “triage” measure to assist referral to a family cancer clinic for further assessment and germline testing, if appropriate. It is especially applicable when the family history approaches, but does not quite meet, the Amsterdam criteria for suspecting HNPCC or when CRC is diagnosed at an early age (younger than 50 years). However, before this can become routine, immunohistochemical staining testing for HNPCC may need to be standardised, and laboratories validated through the Royal College of Pathologists of Australia to ensure reliable results.

Even so, for now, the best diagnostic precision is still achieved by a combined analysis of all the variables: family history, and clinical and pathological findings, as well as results of genetic tests.

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