The identification of Lynch syndrome in British Columbia

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OBJECTIVE: To determine the prevalence of Lynch syndrome mutations in a Canadian hereditary cancer clinic population, and to determine the effectiveness of the program’s referral criteria and testing algorithm.

METHODS: A retrospective chart review of all patients who were referred for and received genetic counselling at the BC Cancer Agency’s Hereditary Cancer Program for a family history of colon cancer from August 1, 2004, to September 1, 2006, was performed. Charts were reviewed for referral criteria met, cancer history, whether testing was offered and the outcome of testing.

RESULTS: Lynch syndrome was confirmed or highly suspected in 14.3% of index test patients (eight of 56) by the identification of a deleterious mutation or variant likely to be deleterious in either of the hMLH1 or hMSH2 mismatch repair genes. In the program, the two most effective criteria were a personal diagnosis of two or more primary Lynch syndrome-related cancers (one diagnosed at younger than 50 years of age) or two first-degree relatives with a Lynch syndrome-related cancer (both diagnosed at younger than 50 years of age). The respective positive predictive values of these two criteria were calculated to be 66.7% (95% CI 40% to 93%) and 58.3% (95% CI 30.4% to 86.2%).

CONCLUSIONS: The Hereditary Cancer Program developed and successfully implemented an approach that selected individuals at risk for Lynch syndrome with a significant pretest probability of mutation of 14.3%. Improved ascertainment of families with Lynch syndrome will require greater physician awareness of referral criteria, program advances in the testing algorithm and a population-based approach to screening incident colon cancers.

Key Words: Colon cancer, Genetic testing; Hereditary; HNPCC; Lynch syndrome

The Hereditary Cancer Program (HCP) at the BC Cancer Agency (Vancouver, British Columbia [BC]) provides genetic counselling and testing to the population of BC for inherited cancer predisposition. Hereditary colon cancer is the second most frequent reason for referral to the HCP after hereditary breast and ovarian cancer syndrome. Clinical genetic testing for the most common form of hereditary colorectal cancer (CRC) – Lynch syndrome – has been available at the HCP since August 2004. The first aim of the present study was to describe the effectiveness of the referral criteria and testing algorithm in the identification of Lynch syndrome in BC in the two-year period since the inception of this testing. The second aim was to determine the prevalence of Lynch syndrome mutations in a Canadian hereditary cancer clinic population. This will establish a baseline with which to benchmark future improvements in the rate of ascertainment of Lynch syndrome.

Lynch syndrome is the term now used in place of hereditary nonpolyposis colorectal cancer (HNPCC) syndrome to describe families with a germline mutation in a DNA mismatch repair gene. HNPCC was a confusing term applied to heterogeneous families meeting different family history criteria (eg, Amsterdam I, Amsterdam II) (1,2), regardless of genetic etiology. Furthermore, the term excluded single cases and was a misleading descriptor, given the significant extracolonic cancer risks and the presence of polypos, albeit with a much smaller number than in the hereditary polyposis syndromes.

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Lynch syndrome is a dominantly inherited susceptibility to CRC that accounts for up to 5% of all CRCs (3). Lynch syndrome causes up to an 80% lifetime risk of CRC, with an average age at diagnosis of 44 years (4). There is a 40% to 60% lifetime risk of endometrial cancer, with an average age at diagnosis of 46 years (4). Other cancers associated with Lynch syndrome include small bowel, ovarian, renal pelvis, ureter and gastric cancer (5). Given the high lifetime risk of CRC associated with Lynch syndrome, regular colonoscopic surveillance beginning at age 25 is recommended to reduce the morbidity and mortality of cancer in these families (6).

Lynch syndrome is caused by a mutation in one of four mismatch repair genes (7-11): hMLH1, hMSH2, hMSH6 and hPMS2. Mutations in the hMLH1 and hMSH2 genes account for approximately 90% of families with Lynch syndrome, while mutations in the hMSH6 gene account for approximately 10% (12). Mutations in the hPMS2 gene (8) are expected to be rare and clinical genetic testing has only recently become available. Genetic testing is costly and complex. The identification of families in whom genetic testing for Lynch syndrome should be considered has traditionally been based on family history criteria, which have evolved over time to improve sensitivity (5,13,14) (Table 1).

An online survey of Canadian genetic counsellors in March 2007, demonstrated significant variability in the eligibility criteria and the genetic testing algorithm for Lynch syndrome.
established using commercially available antibodies (16). The likelihood of Lynch syndrome is exceedingly low if tumour test results are normal, with genetic testing on a blood sample not generally indicated in these cases. Tumours that show MSI, or that are deficient in the MLH1 or MSH2 protein, are triaged for further testing by sequencing of the hMLH1 or hMSH2 genes obtained in a blood sample.

Once a mutation is identified in the index patient, presymptomatic testing (mutation specific) is available to at-risk relatives, affected or unaffected, and is performed by targeted genetic sequencing of a blood sample.

RESULTS
From August 2004 to September 2006, a total of 294 individuals from 207 families underwent genetic counselling for Lynch syndrome assessment. Of these, 47 individuals (16.0%) from 19 families were referred for presymptomatic testing because a Lynch syndrome mutation had already been identified in the family. The majority of referrals (36%) were from the category ‘family members, self or other genetics programs’, 27% were from oncologists, 26% from family doctors and 11% from ‘other’. The large majority of patients were women (71.4%) and the average age at the time of referral was 48.4 years (range 16 to 82 years).

Among the 294 patients, 40% had a personal history of CRC (n=118), with an average age at diagnosis of 56.5 years (range 22 to 82 years). Other cancers in these patients included endometrial (n=15), ovarian (n=12), stomach (n=2), small intestine (n=3), hepatobiliary (n=1), genito-urinary tract tumours (n=8), brain (n=1) and cancers listed as ‘other’ (n=64). Of the 118 patients with CRC, nine had multiple CRCs (7.6%) and 13 (11%) had a second extracolonic primary cancer that is part of the Lynch syndrome spectrum (five cases of endometrial cancer, three cases of genito-urinary tumours and one case each of ovarian, stomach, small intestine, hepatobiliary and brain cancer). Of all patients, 32% met the Amsterdam I criteria, 36% met the Amsterdam II criteria and 29% met the Bethesda criteria.

Figure 1 shows the patient flow in the present study population. Of the 294 patients seen, 46% were eligible for genetic testing. Of the 122 patients who consented to testing, 78 were offered index testing and 44 were offered mutation-specific testing for the mutation previously identified in their family. The uptake of testing in the latter group was 95.7% (95% CI 89.8% to 100%) and among index patients was 88.6% (95% CI 82.0% to 95.3%). Of the 44 presymptomatic tests, 25 patients inherited the specific mutation present in their family (seven MLH1 and 18 MSH2). The remaining 19 individuals did not carry the mutation.

The analysis of index testing is based on the 56 cases with disclosed results. Only five cases did not begin with tumour testing because tissue blocks were unavailable. Twenty-four per cent of tumour test cases (n=12) had abnormal tumour results (either MSI, with or without abnormal IHC) (Figure 1). Germline mutations were identified in five of these cases (42%) and a variant was identified in two additional cases. All cases with a mutation or variant had evidence of MSI and an abnormal IHC result corresponding to the genetic result. Of five other MSI cases, none have been confirmed to have germ-line Lynch syndrome mutations. Some of these cases underwent further investigation (Table 3).

The remaining 39 patients who underwent tumour analysis had normal results (76%) and no further testing was planned (Figure 1). Of these, nine patients met Amsterdam I criteria (23%). The term ‘familial CRC Type X syndrome’ has been proposed for families that are Amsterdam-positive but have no evidence of a germline mismatch repair mutation. In these families, the age of CRC diagnosis is typically later, the penetrance of CRC among relatives is reduced and the excess of extracolonic cancers are not seen, compared with Lynch syndrome families (18).

In total, a mutation was detected in the MLH1 gene in two patients and in the MSH2 gene in four patients, for an overall yield of Lynch syndrome in index patients of six of 56 (10.7%). In addition, two patients were found to carry ‘unclassified variants’ in the MLH1 gene (MLH1 p.I32N and MLH1 p.R265S). Both of the tumours in these cases demonstrated MSI and were deficient for the MLH1 protein. Neither of them demonstrated the somatic BRAF (V-ras murine sarcoma viral oncogene homologue B1) gene mutation, which is an effective screening tool to identify noninherited or sporadic MSI-high CRC (19). Both cases had a family history of cancer that met Amsterdam criteria, which further suggests that these variants may represent pathogenic changes. If the unclassified variant cases are included, eight of 56 (14.3%) of the index cases tested were diagnosed with Lynch syndrome.

Performance of HCP criteria
The HCP currently uses four criteria that represent a blend of the Bethesda guidelines and the Amsterdam criteria. The performance of the various criteria is shown in Table 4. The reported values measure the extent to which the eligibility criteria predict whether Lynch syndrome is present in the group.
for whom testing was performed at the HCP. This group is very different from the general population, and the performance measures reported here should not be compared with those in other groups or populations. However, the performance measures can be compared with one another for each of the eligibility criteria in the HCP dataset. Figure 2 shows the sensitivity, positive predictive value (PPV), specificity and negative likelihood ratio with 95% CIs for each criterion.

### TABLE 3
Cases with an abnormal tumour test result (n=12)

<table>
<thead>
<tr>
<th>Case</th>
<th>Personal cancer history, age (years)</th>
<th>Fulfills Amsterdam criteria I or II</th>
<th>Microsatellite instability</th>
<th>IHC MLH1</th>
<th>MLH1</th>
<th>IHC MSH2</th>
<th>MSH2</th>
<th>Germline testing</th>
<th>Other testing requested</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Colorectal, 71, Renal cell cancer, 71</td>
<td>No</td>
<td>High</td>
<td>Intact</td>
<td>Intact</td>
<td>Not requested</td>
<td>No mutation</td>
<td>No mutation</td>
<td>IHC for MS6 and PMS2</td>
</tr>
<tr>
<td>2</td>
<td>Colorectal, 24</td>
<td>No</td>
<td>High</td>
<td>Absent</td>
<td>Intact</td>
<td>No mutation</td>
<td>Not requested</td>
<td>No</td>
<td>BRAF MLPA</td>
</tr>
<tr>
<td>3</td>
<td>Colorectal, 31</td>
<td>No</td>
<td>High</td>
<td>Absent</td>
<td>Intact</td>
<td>No mutation</td>
<td>Not requested</td>
<td>No</td>
<td>BRAF MLPA</td>
</tr>
<tr>
<td>4</td>
<td>Colorectal, 33</td>
<td>No</td>
<td>High</td>
<td>Intact</td>
<td>Intact</td>
<td>Pending</td>
<td>Pending</td>
<td>No</td>
<td>IHC for PMS2 and MS6</td>
</tr>
<tr>
<td>5</td>
<td>Colorectal, 37</td>
<td>No</td>
<td>High</td>
<td>Intact</td>
<td>Intact</td>
<td>No mutation</td>
<td>No mutation</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Colorectal, 40</td>
<td>Yes</td>
<td>High</td>
<td>Intact</td>
<td>Absent</td>
<td>N/A</td>
<td>Mutation</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Colorectal, 32</td>
<td>Yes</td>
<td>High</td>
<td>Intact</td>
<td>Absent</td>
<td>Mutation</td>
<td>N/A</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Colorectal, 33</td>
<td>Yes</td>
<td>High</td>
<td>Intact</td>
<td>Absent</td>
<td>N/A</td>
<td>Mutation</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Colorectal, 44 and synchronous thyroid, 42</td>
<td>Yes</td>
<td>High</td>
<td>Absent</td>
<td>Intact</td>
<td>Mutation</td>
<td>N/A</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Colorectal, 59 (synchronous tumours), Small intestine, 59, Transitional cell carcinoma, renal pelvis, 41</td>
<td>Yes</td>
<td>High</td>
<td>Absent</td>
<td>Intact</td>
<td>N/A</td>
<td>Mutation</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Colorectal, 37</td>
<td>Yes</td>
<td>High</td>
<td>Absent</td>
<td>Intact</td>
<td>Unclassified variant</td>
<td>N/A</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Colorectal, 50, Endometrial, 51</td>
<td>Yes</td>
<td>High</td>
<td>Absent</td>
<td>Intact</td>
<td>Unclassified variant</td>
<td>N/A</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

### TABLE 4
Summary of performance criteria

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% 95% CI</td>
<td>% 95% CI</td>
<td>% 95% CI</td>
<td>% 95% CI</td>
</tr>
<tr>
<td>Amsterdam I</td>
<td>63 29–96</td>
<td>79 67.7–90.7</td>
<td>33 9.5–57.2</td>
<td>93 84.7–100</td>
</tr>
<tr>
<td>Amsterdam II</td>
<td>100 100–100</td>
<td>75 62.8–87.3</td>
<td>40 18.5–61.5</td>
<td>100 100–100</td>
</tr>
<tr>
<td>Bethesda guidelines</td>
<td>100 100–100</td>
<td>21 9.3–32.3</td>
<td>17 6.4–28.3</td>
<td>100 100–100</td>
</tr>
<tr>
<td>HCP1: Personal history of colorectal cancer diagnosed at younger than 40 years of age</td>
<td>50 15.4–84.6</td>
<td>50 35.9–64.1</td>
<td>14 1.3–27.2</td>
<td>86 72.8–94.7</td>
</tr>
<tr>
<td>HCP2: Two primary Lynch cancers (one colorectal cancer), with one diagnosed at younger than 50 years of age</td>
<td>100 100–100</td>
<td>92 84.8–99.5</td>
<td>67 40–93.3</td>
<td>100 100–100</td>
</tr>
<tr>
<td>HCP3: Two first-degree relatives with Lynch cancers (one colorectal cancer), with both diagnosed at younger than 50 years of age</td>
<td>88 64.6–100</td>
<td>90 80.9–98.2</td>
<td>58 30.4–86.2</td>
<td>98 93.3–100</td>
</tr>
</tbody>
</table>

BRAF: V-raf murine sarcoma viral oncogene homologue B1 gene; IHC: Immunohistochemistry; MLPA: Multiplex ligation-dependent probe amplification; MLH(M)H: Mismatch repair proteins/genes; N/A: Not applicable; PMS2: Postmeiotic segregation increased 2 (Saccharomyces cerevisiae) gene

Data are based on a total of 56 index patients. Six mutations and two unclassified variant results are combined in the present analysis. Sensitivity measures the eligibility criteria’s ability to identify carriers who use a genetic testing service. It only applies to carriers and reveals nothing about noncarriers. Sensitivity is between 0 and 1, with a higher value indicating a better service. Specificity measures the eligibility criteria’s ability to exclude noncarriers who use a genetic testing service. It only applies to noncarriers and reveals nothing about carriers. Specificity is between 0 and 1, with a higher value indicating a better service. The positive predictive value (PPV) measures the proportion of carriers among people who are eligible for a genetic testing service. It only applies to eligible people and reveals nothing about ineligible people. PPV is between 0 and 1, with a higher value indicating a better service (The PPV is also called the post-test likelihood of being a carrier given eligibility). The negative predictive value (NPV) measures the proportion of noncarriers among people who are ineligible for a genetic testing service. It only applies to ineligible individuals and reveals nothing about eligible subjects. NPV is between 0 and 1, with a higher value indicating a better service. The NPV is the converse of the post-test probability of being a carrier given ineligibility (ie, 1-NPV). HCP: Hereditary Cancer Program

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The Amsterdam II criteria, Bethesda guidelines and the HCP criterion 2 identified all eight patients with mutations or unclassified variants. The average sensitivity of the eligibility rules (excluding HCP criterion 4) was 0.833 and none of the individual criteria provided significantly different sensitivity than the average ($\chi^2$ tests comparing observed and expected value; all $P>0.05$). The average PPV of the eligibility rules (excluding HCP criterion 4) was 0.383 and none of the individual criteria provided a significantly different PPV from the average ($\chi^2$ tests comparing observed and expected values, all $P>0.05$).

**DISCUSSION**

A two-year period following the start of clinical genetic testing for Lynch syndrome in BC was evaluated. This represents a clinical stream of patients selected initially for referral into the HCP and subsequently selected for tumour analysis and/or genetic testing. The overall yield of Lynch syndrome mutations was 10.7% in individuals who underwent index testing. If the two variants in the $MLH1$ gene were included, the yield increased to 14.3%. Among the 294 individuals assessed for Lynch syndrome (including the 47 patients referred for presymptomatic testing for the known mutation in the family), the overall prevalence of Lynch syndrome mutations was 11.2% (n=33). Excluding the presymptomatic testing referrals, Lynch syndrome was confirmed by a mutation (or strongly suspected with two unclassified variants in $MLH1$) in 3.2% (eight of 247) of all individuals who came for genetic counselling. This yield reflects the real-world scenario of a hereditary cancer clinic and the overall effectiveness of this strategy in detecting Lynch syndrome.

There are several important limitations to the present study. The index test study population includes only eight probands with a Lynch syndrome mutation or unclassified variant. The population that attended an appointment was a self-selected group and was likely different from the population of referred patients who did not follow through with an appointment. There are many reasons why an individual may decide not to follow through on a referral including health, anxiety, not understanding the purpose of the referral, fear of having a genetic risk assessment or the potential for life-insurance discrimination.

Second, the present report highlights obstacles in the ascertainment of new Lynch syndrome families in a real-world clinical scenario; not meeting test criteria, patient refusal of testing, and lack of available tumour tissue blocks. These obstacles are likely to affect mutation-positive and mutation-negative cases equally.

Third, five of 12 cases with abnormal MSI and/or IHC results did not reveal a mutation in $MLH1$ or $MSH2$, some of which are undergoing further testing by multiplex ligation probe amplification to detect large gene rearrangements of $MLH1$ and $MSH2$, $BRAF$ testing to investigate somatic hypermethylation of $MLH1$, and by further evaluation for $MSH6$ mutations via IHC and/or $MSH6$ gene sequencing. While these cases may be due to sporadic methylation of the $MLH1$ gene promoter, as in 15% of all CRCs (20), it is also possible that they were caused by a mutation in $MSH6$ or $PMS2$, or by an undetected mutation in $MLH1$ or $MSH2$, which would...
result in an underestimation of the Lynch syndrome yield. A limitation of the current testing algorithm at the HCP is that IHC for the MSH6 and PMS2 proteins is not currently part of routine tumour analysis.

In the present study, HCP criteria 2 (individual with two or more primary Lynch syndrome cancers, with at least one being colorectal and one diagnosed at younger than 50 years of age) and 3 (Lynch syndrome cancer younger than 50 years of age in two first-degree relatives, with at least one being CRC) outperformed both Amsterdam I and II criteria as well as the Bethesda guidelines, if both high sensitivity and PPV were sought (Figure 2). HCP criterion 2 identified all cases and HCP criterion 3 identified seven of eight. In a meta-analysis conducted by Kievit et al (21), the sensitivity of the Amsterdam I and Amsterdam II criteria were 54% to 91% and 78%, respectively. The PPVs were 61% and 46%, respectively. The high sensitivity and low PPV of the Bethesda criteria in our population are similar to previous reports (22). The 33% PPV of the Amsterdam criteria in our study is lower than the 50% to 92% reported in other studies (23-25). This may be due to the fact that our program does not require confirmation of diagnoses by pathology reports before offering testing. A more detailed review of records may have shown that some of these patients did not meet the Amsterdam criteria.

Endometrial cancer is the sentinel Lynch syndrome cancer in some families. A limitation to the testing algorithm in BC described in the present study is that it required colorectal tumour tissue for preliminary MSI and IHC testing, thereby precluding testing in endometrial-only Lynch syndrome families. Seventy-five per cent of endometrial tumours in Lynch syndrome demonstrate MSI compared with 25% to 45% of sporadic endometrial cancer, making MSI an effective prescreening tool for Lynch syndrome (26). Lu et al (27) found a 32% positive predictive value for Lynch syndrome in MSI-high endometrial cancers diagnosed at younger than 50 years of age. The clinical utility of MSI in other Lynch syndrome tumours, such as sebaceous adenomas, has also been proposed and is under current evaluation.

**FUTURE DIRECTIONS FOR THE IDENTIFICATION OF LYNCH SYNDROME IN BC**

The population of BC is currently estimated to be 4.380 million (Statistics Canada, 2007). Using a background Lynch syndrome mutation prevalence of one in 531 (28), this would yield an estimated 8249 Lynch syndrome patients. The provincial clinical database includes slightly more than 100 patients with confirmed Lynch syndrome mutations (42 MLH1 mutation carriers, 52 MSH2 carriers and three MSH6 carriers).

In 2008, an important step was taken toward population-based screening for Lynch syndrome in the province. All newly diagnosed colorectal cancers for individuals younger than 50 years of age can now be referred for MSI analysis by any physician, regardless of family history and with referral to the HCP. If the next step was for MSI to become part of routine reporting on all newly diagnosed CRCs in individuals younger than 50 years of age, this testing would be expected to have close to a 100% sensitivity in identifying Lynch syndrome, based on the high prevalence of MSI in Lynch tumours. Alternatively, IHC for the MSH2, MLH1, PMS2 and MSH6 proteins could be offered. This would provide similar sensitivity and also provide data for direct genetic testing. However, because loss of MSH2 expression in a CRC almost invariably indicates a germline mutation, there is a greater need for informed consent for IHC as opposed to MSI testing.

There are approximately 200 cases of CRC diagnosed in individuals younger than 50 years of age in BC each year. Using a prevalence of mutations of 8.7% in unselected CRC diagnosed at younger than 50 years of age (29), if MSI testing was performed on all cases, up to 16 new patients with Lynch syndrome may be identified per year. We used a simulation model (30) to determine the effect of additional Lynch syndrome genetic testing for all CRC cases diagnosed in individuals younger than 50 years of age in BC. Simulations indicated that the additional testing strategy would increase sensitivity by approximately 4.0%, increase PPV by approximately 0.3% and decrease specificity by approximately 0.1%.

Currently, approximately 15% of all referrals to the HCP are for hereditary colon cancer as opposed to 77% for hereditary breast and ovarian cancer, a condition for which our program has offered testing since 1999. This study provides a baseline analysis of the yield of Lynch syndrome testing in BC by which future improvements will be benchmarked. Improving the effectiveness of identifying Lynch syndrome in BC can be achieved with a two-pronged approach: greater physician education of Lynch syndrome awareness of the HCP’s service and a population-based approach to screening incident colorectal cancers in patients younger than 50 years of age.

**REFERENCES**

Identifying Lynch syndrome families in British Columbia


