Current Practices Regarding the Use of *PMS2* Genetic Testing

Master’s Thesis

Presented to

The Faculty of the Graduate School of Art and Sciences  
Brandeis University  
Genetic Counseling Program  
Judith Tsipis, PhD, Advisor  
Karina Brierley, MS, CGC, Carly Pouchet, MS, CGC, Committee

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Sarah Nashed

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ABSTRACT

Current Practices Regarding the Use of PMS2 Genetic Testing

A thesis presented to the Genetic Counseling Program

Graduate School of Arts and Sciences
Brandeis University
Waltham, Massachusetts

By Sarah Nashed

Committee Members: Karina Brierley, M.S., CGC, Carly Pouchet, M.S., CGC, and Judith Tsipis, PhD

Lynch syndrome, a familial cancer syndrome causing colorectal and other cancers, is caused by mutations in the MLH1, MSH2, MSH6, or PMS2 genes. Germline PMS2 testing has only recently become available, and there is no professional consensus regarding the circumstances under which PMS2 testing should be offered to patients. Although PMS2 mutations are not considered to account for a large percentage of Lynch syndrome cases, some authors have suggested that since PMS2 germline testing is not routinely offered to patients with suspected Lynch syndrome, the frequency of PMS2 mutations may be underestimated. The aim of this study was to assess current PMS2 testing practices among genetic counselors who see patients for cancer risk assessment, by exploring factors that influence their decision to offer the test, the sequence in which
Lynch-related tests are most commonly offered, and recommendations for future testing. We recruited genetic counselors who had counseled for Lynch syndrome in the past year through the National Society of Genetic Counselors, the NSGC Cancer Special Interest Group, and the Canadian Association of Genetic Counselors listservs to complete an anonymous, online survey. We received 80 completed surveys, including 60 from genetic counselors who had personally offered \textit{PMS2} germline testing. Among the counselors who reported having offered \textit{PMS2} testing, we found that \textit{PMS2} full sequencing and deletion/duplication testing was offered, on average, in 19\% and 12\%, of their total Lynch syndrome cases, respectively. Most genetic counselors reported offering MSI and IHC for all 4 Lynch-causing genes simultaneously, followed by \textit{MLH1}, \textit{MSH2}, and \textit{MSH6} germline testing. \textit{PMS2} germline testing was considered only in the absence of a positive finding from \textit{MLH1}, \textit{MSH2} and \textit{MSH6} testing. Overall, our findings suggest that genetic counselors do not view \textit{PMS2} germline testing as a first tier test for individuals being evaluated for Lynch syndrome.

\textbf{Keywords:} Lynch syndrome, HNPCC, PMS2, cancer genetic counseling, genetic testing
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INTRODUCTION

Clinical features of Lynch syndrome

Lynch syndrome or Hereditary Non-Polyposis Colorectal Cancer (HNPCC) is an autosomal dominant cancer predisposition syndrome. Approximately two to five percent of all colorectal cancers are due to Lynch syndrome, making it the most common cause of hereditary colorectal cancer. Characteristics of Lynch syndrome include an early age of onset of colorectal cancer compared to the general population: 45 years old versus 63 years old, on average, respectively. Colorectal cancers (CRCs) due to Lynch syndrome are more commonly found on the right side of the colon and their pathology is usually mucinous and poorly differentiated. Endometrial cancer is the second most common cancer characteristic of Lynch syndrome, present in up to 60% of at-risk females. There is also an increased risk for developing other cancers such as ovarian, small bowel, pancreatic, stomach, hepatobiliary, upper uroepithelial, and/or brain. The development of cancer (from tiny polyps to carcinomas) in an individual with Lynch syndrome takes two to three years as opposed to eight to ten years in the general population (Lynch et al, 2009).

Clinical diagnostic criteria for Lynch syndrome

Historically, Lynch syndrome was clinically diagnosed using Amsterdam I criteria, which state that (Vasen et al, 1999):
• There should be at least three relatives with CRC
• One relative should be a first-degree relative of the other two family members
• At least two successive generations should be affected
• At least one CRC should be diagnosed before age 50
• Familial adenomatous polyposis should be excluded in the CRC case
• Tumors should be verified by histopathological exam.

It became apparent that Amsterdam I criteria were too restrictive for diagnosing individuals with Lynch syndrome and that they did not encompass the other cancers that are seen in the spectrum; therefore, Amsterdam II criteria were developed (Vasen et al, 1999). The following are Amsterdam II criteria (Vasen et al, 2007):

• There should be at least three relatives with CRC or with a Lynch syndrome-associated cancer (endometrial, ovarian, small bowel, stomach, pancreatic, brain, hepatobiliary, or uroepithelial)\(^1\)
• One relative should be a first-degree relative of the other two family members
• At least two successive generations should be affected
• At least one CRC should be diagnosed before the age 50
• Familial adenomatous polyposis should be excluded in the CRC case
• Tumors should be verified by histopathological exam.

\(^1\) Difference between Amsterdam I and Amsterdam II criteria is italicized.


**Mismatch repair genes**

Mutations in the DNA mismatch repair (MMR) genes, *MLH1, MSH2, MSH6*, and *PMS2*, predispose an individual to Lynch syndrome. MMR genes code for proteins that repair specific types of errors that may occur during DNA replication, such as base-base mismatches and insertion-deletion loops. The DNA mismatch repair proteins are responsible for repairing these replication mistakes; without repair, the mistakes will be passed on during each cell division (Abdel-Rahman et al, 2006). An individual carrying a germline mutation in one of their MMR genes has normal DNA mismatch repair function. Once the second copy of the same MMR gene acquires a mutation (from environmental factors or cell division error), the cell loses its repairing function and errors remain un-repaired. Consequently, cells completely lacking one of the MMR proteins will accumulate mutations in other genes, some of which can increase the risk for developing cancer (Peltomaki, 2003). The following diagram (Figure A) illustrates the four MMR genes, *MLH1, MSH2, MSH6*, and *PMS2*, repairing a single-base mismatch and an insertion-deletion loop.

![Figure A](Peltomaki, 2001) On the left: the DNA MMR protein complex is repairing a single base mismatch, replacing the T with a C, which appropriately pairs with G. On the right: The DNA MMR protein complex is repairing an insertion-deletion loop by removing the loop and preventing extra bases from being inserted into the sequence, causing microsatellite instability (discussed later).
Figure B demonstrates that MSH2 and MSH6, as well as MLH1 and PMS2, each form a heterodimer to repair DNA damage.

![Mismatch Repair Diagram](image)

**Figure B** (Scherer et al, 2005) The MSH2-MSH6 heterodimer binds to base mismatch replication errors and recruits the MLH1-PMS2 heterodimer to initiate downstream repair events in an ATP-dependent process.

**Lynch syndrome cancer risks**

In families that fulfill Amsterdam I Criteria (strict diagnostic criteria) or Amsterdam II Criteria (revised diagnostic criteria), a germline mutation in one of the four MMR genes is detected 70-80% of the time (Peltomaki, 2003). Although unspecified, the detection rate of MMR gene mutations is significantly lower in families that are Amsterdam criteria negative (Niessen et al, 2009). Of those cases of Lynch syndrome in which mutations could be identified, 85% are due to mutations in the *MLH1* or *MSH2* genes (Peltomaki, 2003). Identifiable *MSH6* mutations are the next most common cause of Lynch syndrome (~10%), followed by identifiable mutations in the *PMS2* gene (~5%)
(Peltomaki, 2003). Deletions and duplications account for 5-10%, 17-50%, and 2-3% of mutations in \textit{MLH1}, \textit{MSH2}, and \textit{MHS6}, respectively, making deletions and duplications rarer than point mutations (Grabowski et al, 2005; van der Kleft et al, 2005).

Mutations in the \textit{MLH1}, \textit{MSH2}, \textit{MSH6}, and \textit{PMS2} genes increase the risk for various cancers. Those who carry a germline mutation in one of these genes have up to an 80% lifetime risk for colorectal cancer compared with a ~5% lifetime risk in the general population (Halvarsson et al, 2006). Female carriers have a 40-60% lifetime risk of developing endometrial cancer compared with a ~3% general population lifetime risk (Lynch et al, 2009). These women also have a 12-15% lifetime risk of developing ovarian cancer compared with a 1-2% general population lifetime risk (Lynch et al, 2009). The lifetime risks for developing the following cancers in those lacking one or more functional MMR proteins are also increased from the general population risks of less than 1%: stomach (13%), small bowel (7%), hepatobiliary tract/pancreatic (7%), uroepithelial, (8%) and brain cancer (5%) (Aarnio et al, 1999).

Those who carry a germline mutation in \textit{MLH1}, \textit{MSH2}, or \textit{MSH6} have similar clinical phenotypes. However, there are conflicting views as to whether or not \textit{PMS2} carriers have a less severe phenotype. Hendriks et al (2006) suggested that \textit{PMS2} mutations may have a lower penetrance and cause a less severe phenotype than \textit{MLH1} and \textit{MSH2} mutations. The authors hypothesized that this

“may be explained by the fact that in the absence of PMS2, a functional MLH1 and MLH3 heterodimeric protein can be formed that is able to repair DNA mismatches. In families with \textit{MLH1} or \textit{MSH2} gene defects, no alternative heterodimers can be created, with as a consequence a complete inactivation of the MMR system with massive MSI and a higher risk of cancer” (Hendriks et al, 2006).
However, a study by Niessen et al (2009) found that the average age of onset of colorectal cancer in the PMS2 carriers was 44.8 years, which is not significantly different from the average age of colorectal cancer onset of 45 years for the other MMR genes (Niessen et al, 2009). All of the PMS2 carriers in this study fulfilled the revised Bethesda criteria, had MSI-high\(^2\) tumor(s), lacked PMS2 protein staining by IHC\(^2\), and were Amsterdam Criteria II negative (Niessen et al, 2009). However, since MSI was an inclusion criterion in this study, those PMS2 mutation carriers having a less severe phenotype may have been missed due to the inclusion criteria (Niessen et al, 2009).

\textit{Tumor testing for Lynch syndrome}

\textit{Microsatellite instability}

Mutations in a MMR gene often cause microsatellite instability. Microsatellites are one to six base pair DNA sequences that normally repeat 15 to 30 times in the human genome. When a MMR protein is not properly functioning due to the presence of MMR gene mutations, the microsatellites can shorten or expand during replication, a phenomenon called microsatellite instability (MSI) (Baudhuin et al, 2005). MSI is measured by looking for instability in five markers. If one out of the five markers demonstrate instability, that is considered MSI-L (L=Low). If two or more markers are determined to show instability, the tumor is said to be MSI-H (H=High) (Abdel-Rahman et al 2006). Because microsatellite instability is a characteristic of most Lynch syndrome tumors, a set of guidelines, called the Bethesda guidelines, were created to determine

\[^2\] MSI-high and IHC are discussed in the following sections.
which individuals should have their tumors tested for MSI (Baudhuin et al, 2005). Any one of the following criteria would warrant tumor MSI testing (Vasen et al, 2007):

- Colorectal cancer diagnosed in a patient aged <50 years
- Presence of synchronous, metachronous colorectal or other Lynch syndrome-related tumors, regardless of age
- Colorectal cancer with MSI-H phenotype diagnosed in a patient aged <60 years
- Patient with colorectal cancer and a first-degree relative with a Lynch syndrome-related tumor, with one of the cancers diagnosed at age <50 years
- Patient with colorectal cancer with two or more first-degree or second-degree relatives with a Lynch syndrome-related tumor, regardless of age.

**Immunohistochemical analysis**

Two mutations in any one of the MMR genes are likely to produce a non-functional protein. Immunohistochemical (IHC) analysis performed on a tumor detects the presence or absence of a functional MMR gene protein product and can help identify which of the mismatch repair genes has a mutation, unlike MSI testing. For example, when a tumor has two mutations in *MSH2*, the MSH2 protein is expected to not stain and one can conclude that the *MSH2* gene is likely to have a germline mutation. It is not always that straightforward with the *PMS2* gene, however. There are three situations in which lack of PMS2 protein staining by IHC can be observed, not all of which are due to *PMS2* mutations, as shown in the following table:

---

3 It is possible for IHC analysis to detect a non-functional protein.
<table>
<thead>
<tr>
<th>IHC Result (Protein(s) showing lack of staining)</th>
<th>Mutation found in:</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMS2</td>
<td>PMS2</td>
</tr>
<tr>
<td>PMS2</td>
<td>MLH1</td>
</tr>
<tr>
<td>PMS2 and MLH1</td>
<td>MLH1</td>
</tr>
</tbody>
</table>

(de Jong et al, 2004; Niessen et al, 2009)

As demonstrated in Figure B, MLH1 and PMS2 form a heterodimer, allowing the two protein products to function together and repair DNA damage. A mutation in *MLH1* can break down the MLH1-PMS2 protein heterodimer, thereby causing an absence of both PMS2 and MLH1 proteins, or either protein alone (Niessen et al, 2009). In a study by de Jong et al (2004), 35 tumors which were known to carry a *MLH1* mutation were analyzed; 21 of these tumors lacked both MLH1 and PMS2 staining and eight lacked PMS2 staining only. Twenty-three percent more *MLH1* carriers were identified, leading the authors to conclude that adding IHC for *PMS2* can identify non-*PMS2* carriers as well as those carrying the rarer *PMS2* mutations (de Jong et al, 2004).

A lack of PMS2 protein staining on IHC analysis is therefore complex in its interpretation. The thought was that mutations in *PMS2* do not break down the MLH1 protein; however, there are two cases that prove otherwise. One case is of a *PMS2* carrier with a MSI-high tumor and loss of MLH1 staining (PMS2 IHC was not interpretable), and another case is of a *PMS2* carrier with loss of both PMS2 and MLH1 staining by IHC (Niessen et al, 2009; Southey et al, 2005).

**Efficiency of tumor testing**

Tumor analysis (MSI and IHC testing) provides a more efficient screening method in detecting Lynch syndrome carriers than the use of Amsterdam II criteria alone. IHC results tell on which gene to perform mutational analysis (if it comes back negative
for protein expression) rather than testing all four MMR genes. Even if a lack of PMS2 protein staining leads to a discovery of a \textit{MLH1} mutation, it is still identifying a mutation that can lead to or confirm a diagnosis of Lynch syndrome (Southey et al, 2005).

IHC is generally considered as a front line test by genetic counselors. Among newly diagnosed colorectal cancer patients, the most cost effective genetic testing strategy includes starting with IHC for all four MMR genes alone (Mvundura et al, 2010). IHC analysis for the presence of MLH1, MSH2, MSH6, and PMS2 proteins in the tumor is 89% sensitive (Hendriks et al, 2003).

\textit{Molecular genetic testing}

Because germline mutations in \textit{MLH1, MSH2,} and \textit{MSH6} account for the majority of Amsterdam positive families, germline genetic testing for HNPCC has historically only included these three genes (Hendriks et al, 2006). The association between mutations in \textit{PMS2} and Lynch syndrome has only recently gained attention. \textit{PMS2} is located on chromosome 7p22 and is surrounded by 15 pseudogenes making mutation analysis difficult (Hendriks et al, 2006; Niessen et al, 2009). Recently, however, the use of long-range polymerase chain reaction has made it possible to amplify the \textit{PMS2} gene only, making genetic testing feasible (Senter et al, 2008). Germline testing includes full sequencing of the gene and deletion/duplication analysis. Full sequencing of \textit{PMS2} can now detect point mutations 60% of the time in samples that have abnormal staining by \textit{PMS2} immunohistochemical analysis (Senter et al, 2008). Deletion/duplication analysis detects large deletions, duplications, and rearrangements that cannot be identified by full sequencing. Because the penetrance of \textit{PMS2} mutations remains unknown, it is difficult
to predict how many $PMS2$ carriers are being missed through the current diagnostic criteria and testing methods (Southey et al, 2005).

**Purpose of current study**

The complexities of $PMS2$ testing are evident and there is a lack of literature on the actual use of germline $PMS2$ genetic testing by cancer genetic counselors. It is only within the past year and a half that germline testing for $PMS2$ has become clinically available (Appendix C). Since mutations in $PMS2$ account for only a small proportion of Lynch syndrome families and molecular testing has only recently become available, there is no professional consensus as to if and/or when testing for $PMS2$ should be offered. The aim of this study is to assess the current practices regarding germline testing for $PMS2$ mutations among genetic counselors who see patients for cancer risk assessment. Results of this study will inform genetic counselors about the use of $PMS2$ germline testing and will detail some of the concerns and recommendations practicing genetic counselors have. In addition, results from this study will detail what factors influence ordering $PMS2$ germline testing and the sequence in which tests are most commonly offered.
METHODS

Study design

The study consisted of an anonymous online survey about the use of full sequencing and deletion/duplication analysis for the PMS2 gene in individuals referred for a suspected or clinical diagnosis of Lynch syndrome. The survey (Appendix B) included screening questions to ensure subjects met inclusion/exclusion criteria, questions about choosing germline PMS2 testing and the order in which testing on a new patient might be done, followed by hypothetical scenarios in which respondents were asked whether they would/would not offer PMS2 germline testing. Another group of questions focused on identifying recommendations and concerns for germline PMS2 testing in the future. A final set of questions assessed the participants’ demographics.

There were three main tracks that a participant could follow, labeled as Set A, Set B, and Set C, in the survey. Set A was created for genetic counselors who had previously offered germline PMS2 testing to a patient with a suspected or clinical Lynch syndrome diagnosis. Genetic counselors who had not personally offered germline PMS2 testing before, but a co-worker had, answered Set B questions. Finally, genetic counselors who had not personally offered germline PMS2 testing, nor had a co-worker, answered Set C questions. These tracks were created in order to compare the responses of genetic counselors who had previously offered germline PMS2 testing versus those who had never offered the test.
The survey consisted of open-ended, multiple choice-like (single and multiple answers), and Likert-scale questions. The time estimated to complete the survey was not to exceed twenty-five minutes for the participant. We obtained study approval from the Institutional Review Board of Brandeis University, Waltham, Massachusetts.

**Study sample**

The study included practicing genetic counselors who had some experience with cancer risk assessment for Lynch syndrome cases. To be eligible to participate in the study, the subject must be a genetic counselor who:

- Currently sees patients for cancer risk assessment or who counsels patients for hereditary cancer as part of his/her job
- Saw patients for Lynch syndrome cancer risk assessment between January 2009-January 2010
- Practices in the United States or Canada

**Recruitment**

Three committee members posted the recruitment notice (Appendix A) on three different listservs. Judith Tsipis posted the recruitment notice on the National Society of Genetic Counselors (NSGC) listserv, Karina Brierley posted the recruitment notice on the Cancer Special Interest Group (SIG) listserv and Carly Pouchet posted the recruitment notice on the Canadian Association of Genetic Counsellors (CAGC) listserv. The recruitment notice included a link to the online survey. Each committee member posted the recruitment notice in early February. Ten days after the initial posting, each
committee member posted a reminder, with the exception of the CAGC which did not have a second posting. The survey was open from February 7, 2010 to March 1, 2010 (~3 weeks).

**Data collection**

We collected data through an anonymous online survey hosted by surveymonkey.com. There was no maximum number of respondents at which data collection ceased. At the completion of the data collection period, we downloaded the results into Excel.

**Data analysis**

We analyzed the data obtained on March 1, 2010 using the Predictive Analytics SoftWare (PASW) version 17.0. We used descriptive statistics, correlations, and independent-samples t test to describe and analyze the data acquired. We analyzed open-ended questions by looking for common themes and grouping them accordingly.
RESULTS

One hundred and eighteen individuals responded to the anonymous online survey. Of the 118 respondents, 80 (68%) completed the entire survey and the analysis includes only their responses. Sixty of the 80 respondents had offered germline PMS2 testing to a patient with a suspected or clinical Lynch syndrome diagnosis and they answered Set A questions. Six respondents who had not personally offered germline PMS2 testing before, but one of their co-workers had, answered Set B questions. Finally, 14 respondents had not personally offered germline PMS2 testing nor had any of their co-workers, and they answered Set C questions.

All survey participants were asked how many suspected or clinically diagnosed cases of Lynch syndrome they had seen between January 2009-January 2010. Table 1 shows that the average number (rounded to the nearest whole integer) of such cases seen was 20, while the number of Lynch syndrome cases ranged from 2 to 200 (Table 1).

<table>
<thead>
<tr>
<th>N</th>
<th>Mean # of LS cases</th>
<th>Minimum # of LS cases</th>
<th>Maximum # of LS cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>20</td>
<td>2</td>
<td>200</td>
</tr>
</tbody>
</table>

Table 1: Mean, minimum, maximum number of Lynch syndrome cases seen by surveyed genetic counselors between January 2009-January 2010. Numbers were rounded to the nearest whole integer. (N=number of genetic counselors; LS=Lynch syndrome)

ANALYSIS OF GENETIC COUNSELORS WHO HAD OFFERED PMS2 GERMLINE TESTING (SET A)

Frequency of ordering tests
Genetic counselors who had offered PMS2 genetic testing (Set A) were asked to report the number of times they offered full sequencing for PMS2 to a patient between January 2009-January 2010. Table 2 shows that on average, they offered PMS2 full sequencing three times during that year with a range from one to ten. One respondent was not included in the analysis for this question. He/she reported that they had never offered PMS2 full sequencing, but indicated in a subsequent question that most patients go forward with the testing when offered.

<table>
<thead>
<tr>
<th>Type of test</th>
<th>N</th>
<th>Mean # of times PMS2 test offered</th>
<th>Minimum # of times PMS2 test offered</th>
<th>Maximum # of times PMS2 test offered</th>
</tr>
</thead>
<tbody>
<tr>
<td>FS</td>
<td>59</td>
<td>3</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>DD</td>
<td>60</td>
<td>2</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

*Table 2:* Mean, minimum, and maximum times PMS2 full sequencing and deletion/duplication testing was offered. Numbers were rounded to the nearest whole integer. (N=number of genetic counselors; FS=full sequencing; DD=deletion/duplication testing)

Genetic counselors were also asked to report the number of times they offered deletion/duplication testing for PMS2 to a patient between January 2009-January 2010. Table 2 shows that on average, they offered PMS2 deletion/duplication testing two times during that year with a range from zero to ten.

Further, the order in which participating genetic counselors offered PMS2 full sequencing and deletion/duplication testing showed variability. Approximately 42% reported that they offered PMS2 full sequencing first, and then offered deletion/duplication testing only if full sequencing was negative. Another 40% said they offered both PMS2 full sequencing and deletion/duplication testing together all the time. When the former were asked to explain why they always offered full sequencing first, rather than both tests together, the most common response indicated various lab
restrictions. The second most common reason was there was no need to do further testing as full sequencing had already identified a mutation.

Order of test requisition

When provided with a scenario of a patient who has early onset colon cancer and a family that meets Amsterdam criteria, we asked the genetic counselors to rank the following tests in the order in which they would requisition them. Responders could leave options blank and could number multiple tests with the same number if they would offer them at the same time.

- MSI testing
- IHC for MLH1 and MSH2
- IHC for MLH1, MSH2, and MSH6
- IHC for MLH1, MSH2, MSH6, and PMS2
- Full sequencing for MLH1 and MSH2
- Full sequencing for MLH1, MSH2, and MSH6
- Full sequencing for MLH1, MSH2, MSH6, and PMS2
- Full sequencing for PMS2
- Deletion/Duplication testing for MLH1 and MSH2
- Deletion/Duplication testing for MLH1, MSH2, and MSH6
- Deletion/Duplication testing for MLH1, MSH2, MSH6, and PMS2
- Deletion/Duplication testing for PMS2

Figure 1 illustrates that, for the above scenario, IHC for all four MMR genes or MSI testing would be the most common tests offered first by genetic counselors. Forty-three of 57 genetic counselors said that they would offer MSI first and 39 of 57 genetic counselors said that they would offer IHC for all four MMR genes first. However, the majority (32) of these genetic counselors said that they would offer both MSI and IHC for all four MMR genes at the same time, first. This leaves only 11 genetic counselors who would offer only MSI as a first test and seven genetic counselors who would offer only IHC for all four MMR genes as a first test.
Figure 1: Order of test requisition - Reported first test offered for Lynch syndrome (MSI and/or IHC for MLH1, MSH2, MSH6, PMS2). The red line indicates the 32 genetic counselors who would offer both MSI and IHC (MLH1, MSH2, MSH6, PMS2) simultaneously (FS=Full sequencing, DD=deletion/duplication).

Figure 2 shows that full sequencing for MLH1, MSH2, and MSH6 would be the most common test offered second by genetic counselors. We found that seven out of 19 genetic counselors who would offer full sequencing for MLH1, MSH2, and MSH6 as a second line test would also offer deletion/duplication testing for the same genes at the same time. Another ten genetic counselors said that they would offer full sequencing for MLH1, MSH2, MSH6, and PMS2 as a second line of testing; half of them would simultaneously offer deletion/duplication testing for all four MMR genes. In addition, three out of the four genetic counselors who would offer PMS2 full sequencing as a second test, would do so along with deletion/duplication testing of PMS2.
Figure 2: Order of test requisition - Reported second test offered for Lynch syndrome (FS for MLH1, MSH2, MSH6). The dashed green line indicates the 7 of 19 genetic counselors who would offer FS and DD for MLH1, MSH2, and MSH6 simultaneously. The dashed yellow line illustrates the 5 of 10 genetic counselors who would offer FS and DD for MLH1, MSH2, MSH6 and PMS2 at the same time. The dashed red line shows the 3 out of 4 genetic counselors who would offer FS and DD for PMS2 together. (FS=Full sequencing, DD=deletion/duplication)

The test most commonly offered third in the given scenario would be full sequencing for PMS2 (Figure 3). Six out of the ten genetic counselors who said this would be their next step would also offer deletion/duplication testing of PMS2 simultaneously.
Figure 3: Order of test requisition- Reported third test offered for Lynch syndrome (FS for PMS2). The red line indicates that 6 out of 10 genetic counselors would offer FS and DD for PMS2 together. (FS=Full sequencing, DD=deletion/duplication)

Figure 4 shows that PMS2 deletion/duplication testing would be the most common test offered as a last step. There is no overlap between the three genetic counselors who would offer full sequencing for PMS2 and the eleven who would offer deletion/duplication testing for PMS2 as the last step.
Overall, we found that the order of testing offered would most likely be:

1. MSI and IHC for all four MMR genes simultaneously
2. Full sequencing for MLH1, MSH2, and MSH6
3. Full sequencing for PMS2
4. Deletion/duplication testing for PMS2

**When to offer PMS2 germline testing (All participants)**

Genetic counselors in all sets answered whether or not they thought PMS2 germline testing should always be offered at the same time as full sequencing and/or deletion/duplication testing for other Lynch syndrome genes. Approximately 27% of the genetic counselors who had offered PMS2 germline testing thought that testing for PMS2 germline mutations should always be offered along with germline testing for the other
Lynch syndrome genes (Table 3). Seventy-three percent indicated that they did not think those tests should be offered at the same time. When we pooled Sets B and C, 15% of the genetic counselors who had never personally offered PMS2 germline testing specified that they would like to see PMS2 germline testing offered along with the other Lynch syndrome causing genes. Eighty-five percent of this group disagreed (Table 3).

<table>
<thead>
<tr>
<th>Should PMS2 germline testing always be offered at the same time as germline tests for the other 3 MMR genes?</th>
<th>N</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Set A (Yes)</td>
<td>60</td>
<td>27% (16/60)</td>
</tr>
<tr>
<td>Set A (No)</td>
<td>60</td>
<td>73% (43/60)</td>
</tr>
<tr>
<td>Set B/C (Yes)</td>
<td>20</td>
<td>15% (3/20)</td>
</tr>
<tr>
<td>Set B/C (No)</td>
<td>20</td>
<td>85% (17/20)</td>
</tr>
</tbody>
</table>

Table 3: Genetic counselors’ opinions on offering PMS2 germline testing at the same time as germline tests for the other 3 MMR genes (N=total sample size)

Some genetic counselors elaborated on their response of a “yes” or “no” to the above question. Below are two comments from those who said “no” to having PMS2 germline genetic testing offered along with testing for the other Lynch syndrome genes:

Set A:

“While it would be helpful to include it and rule it out early on, the likelihood of a PMS2 mutation is low and insurance is not always likely to cover it. I would not want any cost coming back to the patient of PMS2 testing when it was not really indicated, but was just added on to be comprehensive.”

“Although there are some emerging data that we may have underestimated the prevalence of PMS2 mutations in the population, I still suspect it's rare.”

Set C:

“It is a relatively rare cause of this condition--offering it at the same time as the more likely candidates would not be a good use of resources”

The following are comments from two genetic counselors who expressed that they would like to see PMS2 germline testing offered at the same time as testing for other Lynch syndrome genes:

Set A:
“It is part of Lynch and even though it is believed to be attributed to a small amount of Lynch mutations, until we start routinely offering it as germline testing, we will never know how prevalent it really is.”

“Since most companies offering sequencing of the MMR genes (...i.e Myriad or Mayo) only offer MLH1/MSH2/MSH6 it would only make sense to offer the patient the most comprehensive testing which would include PMS2. There may very well be a collection bias of the rarity of PMS2 mutations since most people are not offering PMS2 sequencing on a regular basis. I personally think that all individuals with a suspected diagnosis should first undergo MSI/IHC before proceeding to germline sequencing. However, if there is a strong family history and MSI/IHC is not available or informative I think that if going on to sequencing of the MMR genes PMS2 should always be included.”

**Utility of PMS2 full sequencing and/or deletion/duplication testing**

We asked genetic counselors who had offered germline PMS2 testing how often PMS2 full sequencing and/or deletion/duplication testing confirmed a clinical diagnosis of Lynch syndrome (Figure 5). Out of the 60 genetic counselors queried, seven (11.7%) chose "Not applicable. Testing was declined whenever offered" and were not included in the analysis (Figure 5). Figure 5 illustrates that approximately 43% of the remaining 53 genetic counselors said that germline PMS2 testing “never” confirmed a clinical diagnosis of Lynch syndrome, while eight (15%) stated that germline PMS2 testing very often or always confirmed a clinical Lynch syndrome diagnosis.
Figure 5: Frequency of PMS2 germline testing confirming a clinical Lynch syndrome diagnosis among patients who accepted the offer to test (N=53)

We then asked how often PMS2 full sequencing and/or deletion/duplication testing identified a mutation after IHC showed lack of PMS2 protein staining. Out of 60 genetic counselors, nine (15%) indicated "Not applicable. Testing was declined whenever offered" and were not included in the analysis (Figure 6). The plurality (39.2%) of the remaining genetic counselors (N= 51) stated that PMS2 germline testing "never" identified a mutation after IHC showed lack of PMS2 protein staining, while 16 (31.3%) stated that germline PMS2 testing very often or always identified a mutation after IHC demonstrated lack of PMS2 protein staining.
Figure 6: Frequency of PMS2 germline testing identifying a mutation after IHC showed loss of PMS2 expression among patients who accepted the offer to test (N=51)

ANALYSIS OF GENETIC COUNSELORS WHO HAD NOT OFFERED PMS2 GERMLINE TESTING, BUT A CO-WORKER HAD (Set B; N=6)

Out of the six genetic counselors in this group, three reported that someone other than herself offered full sequencing for PMS2 before; one indicated that a co-worker previously offered PMS2 deletion/duplication testing and two said that a co-worker had previously offered both tests.

When genetic counselors in this group were provided with the same scenario and list of tests as in Set A: Order of test requisition, results were similar, but there was a small sample size (N=6, data not shown).

ANALYSIS OF GENETIC COUNSELORS WHO HAD NOT OFFERED PMS2 GERMLINE TESTING, NOR HAD A CO-WORKER (Set C; N=14)

We asked genetic counselors who had not personally offered germline PMS2 testing nor had any of their co-workers (N=14) to tell us why no one at their institution had offered either type of PMS2 germline testing and provided the following choices:
a. The high cost of tests
b. The sensitivity of the test
c. The fact that \textit{PMS2} mutations are only present in \textasciitilde5\% of HNPCC families
d. The lab that we usually use for HNPCC testing does not offer \textit{PMS2} testing
e. All of our patients seen in the past year have had mutations in \textit{MLH1}, \textit{MSH2}, or \textit{MSH6}
f. Other, please specify

Respondents were able to check all that apply; therefore, the total frequency of responses is greater than 100\%. The most frequent reason given was the fact that \textit{PMS2} mutations are only present in \textasciitilde5\% of Lynch syndrome families (71.4\%) (Table 4).

<table>
<thead>
<tr>
<th>Reason for not offering germline \textit{PMS2} testing</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>The high cost of tests</td>
<td>2/14</td>
</tr>
<tr>
<td>The sensitivity of the test</td>
<td>3/14</td>
</tr>
<tr>
<td>The fact that \textit{PMS2} mutations are only present in \textasciitilde5% of HNPCC families</td>
<td>10/14</td>
</tr>
<tr>
<td>The lab that we usually use for HNPCC testing does not offer \textit{PMS2} testing</td>
<td>7/14</td>
</tr>
<tr>
<td>All of our patients seen in the past year have had mutations in \textit{MLH1}, \textit{MSH2}, or \textit{MSH6}</td>
<td>1/14</td>
</tr>
<tr>
<td>Other (Mostly because 'not indicated')</td>
<td>7/14</td>
</tr>
</tbody>
</table>

\textbf{Table 4}: Frequency of reasons for not offering germline \textit{PMS2} testing (N=14)

**ANALYSIS OF ALL SURVEY PARTICIPANTS**

\textit{Factors potentially influencing the decision to offer \textit{PMS2} germline testing}

We asked all of the genetic counselors to rate a number of factors that might influence the decision to offer germline \textit{PMS2} testing on a 5-point Likert scale ranging from not important=0 to very important=4. The purple bars (or bars to the left) in Figure 7 demonstrate the mean importance rating for each factor in descending order for genetic counselors who previously offered germline \textit{PMS2} testing. We pooled Set B and Set C
respondents as those who have not personally offered germline PMS2 testing previously.

The blue bars (or bars to the right) in Figure 7 represent this group and they illustrate the mean importance rating for each factor, but are not displayed in descending order.

Figure 7. Factors influencing the decision to offer germline PMS2 testing—A comparison between genetic counselors who have offered PMS2 germline testing versus not offered.
For both groups, the most important factor in deciding whether or not to offer germline PMS2 testing was IHC results. Aggressiveness of the patient’s cancer was viewed as the least important factor for both groups. We then asked each group to list any additional factors they thought were important in making the decision to offer germline PMS2 testing. Among those who had offered PMS2 germline testing, the most common response was patient interest in testing for personal and/or family reasons.

"The sense I get of how much the patient wants an 'answer' and how far they are willing/wanting to go. I almost always say it is available, but of course discuss limitations."

Among those who had never offered PMS2 germline testing, nor had any of their co-workers, three respondents specified a lab issue:

"Convenience of testing for PMS2..."

"Reliability of testing lab"

"Does that lab that does the test perform insurance billing?..."

There were no responses to this question among those who had not personally offered PMS2 germline testing, but a co-worker had.

Re-contacting Families

Genetic counselors in all sets were asked if they had re-contacted families suspected of having Lynch syndrome, but who had an unconfirmed genetic diagnosis, to do PMS2 germline testing. Twenty percent of genetic counselors who had offered PMS2 germline testing indicated that they had re-contacted these families and 80% reported that they had not (Table 5). When we pooled Sets B and C, 15% of the genetic counselors who had never personally offered PMS2 germline testing said that they had considered contacting such families, while 85% had not.
Table 5: Re-contacting unconfirmed Lynch syndrome families for PMS2 testing. (N=total sample size)

<table>
<thead>
<tr>
<th>Re-contacting unconfirmed Lynch syndrome families for PMS2 testing</th>
<th>N</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Set A (Yes)</td>
<td>60</td>
<td>20% (12/60)</td>
</tr>
<tr>
<td>Set A (No)</td>
<td>60</td>
<td>80% (48/60)</td>
</tr>
<tr>
<td>Set B/C (Yes)</td>
<td>20</td>
<td>15% (3/20)</td>
</tr>
<tr>
<td>Set B/C (No)</td>
<td>20</td>
<td>85% (17/20)</td>
</tr>
</tbody>
</table>

Among the reasons given by genetic counselors in Set A who said “no” to the above question included not practicing long enough to need to re-contact families, leaving it up to the patient to re-contact them for updates, and expressing a desire to re-contact in the future. One genetic counselor in Set C who responded with a “no” also stated that re-contacting families was “not a bad idea, but haven’t done so yet.”

DEMOGRAPHICS

Each participant was asked to voluntarily fill out demographic information including age, gender, year of graduation, number of years practicing cancer genetic counseling, country of practice, and whether he/she work full time or part time. Table 6 summarizes the results.

<table>
<thead>
<tr>
<th>Demographic</th>
<th>N</th>
<th>Mean</th>
<th>Range</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>80</td>
<td>34 years</td>
<td>24-60 years</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>80</td>
<td>-</td>
<td>-</td>
<td>Females: 93.8%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Males: 6.3%</td>
</tr>
<tr>
<td>Country of practice</td>
<td>80</td>
<td>-</td>
<td>-</td>
<td>United States: 90%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Canada: 10%</td>
</tr>
<tr>
<td>Year of graduation</td>
<td>77</td>
<td>-</td>
<td>1979-2009</td>
<td></td>
</tr>
<tr>
<td># of years practicing cancer genetic counseling</td>
<td>80</td>
<td>7 years</td>
<td>1-30 years</td>
<td></td>
</tr>
<tr>
<td>Full-time/part-time work</td>
<td>80</td>
<td>-</td>
<td>-</td>
<td>Full-time: 91.3%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Part-time: 8.8%</td>
</tr>
</tbody>
</table>

Table 6: Demographics- Mean, range, and frequency of age, gender, country of practice, year of graduation, number of years practicing cancer genetic counseling, full/part-time work, as applicable (N=total sample size)
We asked participants to indicate the percent of time at work they spend with cancer, prenatal, pediatrics, or other types of patients. Out of 80 genetic counselors who took the survey, one did not respond to this question and two were not included in the analysis because their percentages did not add up to 100%. Figure 8 shows that 58% of the genetic counselors surveyed exclusively see patients for cancer genetic counseling, while 42% see patients for various other indications (prenatal, pediatrics, etc.) in addition to cancer genetic counseling.

![Figure 8: Demographics- Percent of genetic counselors counseling for cancer genetics only versus cancer plus other types of patients (N=77)](image)

Participants were asked to provide the percent of time they spend on different types of work, such as counseling patients, teaching/education, research/study coordinator, clinical coordinator, management, and/or other types of work. Out of 80 genetic counselors who took the survey, two did not respond to this question and one was not included in the analysis because their percentages did not add up to 100%. Table 8
summarizes the average percent of time spent with the various types of work. All of the participants counsel patients (N=77) and 64 of them do another type of work in addition to counseling.

<table>
<thead>
<tr>
<th>Type of work</th>
<th>Response (out of N=80)</th>
<th>Frequency of GCs who counsel + other work (out of N=64)</th>
<th>Average % of time spent with type of work (includes only those who responded)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Counseling patients</td>
<td>77</td>
<td>-</td>
<td>69.8 (N=77)</td>
</tr>
<tr>
<td>Only counsel patients</td>
<td></td>
<td>13 (out of 64)</td>
<td>100 (N=13)</td>
</tr>
<tr>
<td>Counsels + other work</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Teaching/Education</td>
<td>42 (out of 64)</td>
<td>66%</td>
<td>10.5 (N=42)</td>
</tr>
<tr>
<td>Research/Study Coordinator</td>
<td>27 (out of 64)</td>
<td>42%</td>
<td>20.3 (N=27)</td>
</tr>
<tr>
<td>Clinical Coordinator</td>
<td>20 (out of 64)</td>
<td>31%</td>
<td>13.5 (N=20)</td>
</tr>
<tr>
<td>Management</td>
<td>21 (out of 64)</td>
<td>33%</td>
<td>17 (N=21)</td>
</tr>
<tr>
<td>Other</td>
<td>18 (out of 64)</td>
<td>28%</td>
<td>39.7 (N=18)</td>
</tr>
</tbody>
</table>

Table 7: Frequency of genetic counselors doing more than one type of work and the average amount of time spent with various types of work. (N=sample size)
DISCUSSION

Order of test requisition and factors influencing the decision to offer PMS2 germline testing

There are several tests available to test for Lynch syndrome. MSI testing and IHC analysis require that a tumor be available and are usually used to guide further testing. Full sequencing and deletion/duplication testing determine if there is a mutation present in one of the Lynch associated genes. Overall, we found that the majority of genetic counselors in this study offer testing for Lynch syndrome cases in the following order:

1. MSI and IHC for all four MMR genes simultaneously
2. Full sequencing for *MLH1, MSH2, and MSH6*
3. Full sequencing for *PMS2*
4. Deletion/duplication testing for *PMS2*

It is important to note that no genetic counselor offered *PMS2* germline testing as a first line test. However, three genetic counselors indicated that they would offer germline testing for all four MMR genes at the same time as a first step without IHC and/or MSI results. Less genetic counselors entered a third, fourth, or fifth line of testing which is not surprising as many genetic counselors rely on the results of the IHC to determine the next step.

When we look at what test(s) are offered first by genetic counselors, the majority offer IHC for all four MMR genes and MSI testing together. Of those who would not offer them together, more genetic counselors said they would offer MSI testing first. This
is an unexpected result given the fact that IHC provides more information for germline testing than MSI testing does. It is possible that laboratory differences are affecting this result.

**The use of PMS2 germline testing**

According to this study, *PMS2* germline testing is being widely offered by cancer genetic counselors. Although seeing an average of 20 suspected or clinically diagnosed Lynch syndrome cases in one year is a fairly small amount, the majority of genetic counselors (60/80 or 75%) reported that they had previously offered *PMS2* germline testing to one or more of their patients. Six additional genetic counselors reported that at least one of their co-workers had offered one of the *PMS2* germline tests. However, genetic counselors offered *PMS2* full sequencing and *PMS2* deletion/duplication analysis in only 19% and 12%, respectively, of their total Lynch syndrome cases. While the majority of genetic counselors in this study are offering *PMS2* germline testing, they are offering it in only a small proportion of total Lynch syndrome cases.

The order in which genetic counselors offer each germline test may provide an explanation for why full sequencing is offered more than deletion/duplication testing (difference is not statistically significant). Forty-two percent of genetic counselors reported offering *PMS2* full sequencing first, and then deletion/duplication testing, only if full sequencing was negative. Forty percent reported offering both germline tests simultaneously all the time. If 42% of genetic counselors are offering the tests in a step-wise manner, and a mutation is found through full sequencing, there is no need to go further, hence the smaller proportion of those offering *PMS2* deletion/duplication testing.
This is supported by the comments from genetic counselors stating that lab restrictions and no need for further testing as reasons why they only offered one of the tests.

Although the timing in which the two tests are offered varies, a genetic counselor is more likely to offer PMS2 deletion/duplication testing if PMS2 full sequencing has been offered. There was a significant correlation between the number of times a genetic counselor offered full sequencing for PMS2 and the number of times that same genetic counselor offered deletion/duplication testing for PMS2 ($r=.891$, $p<.001$). Although some genetic counselors only offered PMS2 full sequencing, the strong correlation indicates that both full sequencing and deletion/duplication testing are most often considered together and not as separate tests. This correlation did not take volume of patients into account.

**The future of PMS2 germline testing**

Nearly half (43.4%) of the genetic counselors who had patients accept PMS2 genetic testing when they offered it reported that it never confirmed a clinical diagnosis of Lynch syndrome. Only 15% of the same group stated that PMS2 germline testing very often or always confirmed a clinical Lynch syndrome diagnosis. This is a significant difference that may influence future use of PMS2 germline testing. One genetic counselor expressed her concern for not offering this test regularly:

“It [PMS2] is part of Lynch and even though it is believed to be attributed to a small amount of Lynch mutations, until we start routinely offering it as germline testing, we will never know how prevalent it really is.”

It is important to note, however, that no distinction is made between genetic counselors who offered PMS2 germline testing once and more than once. Therefore, a genetic
counselor may have responded with “always” or “never” confirmed a clinical diagnosis of Lynch syndrome based on one occurrence of offering PMS2 germline testing.

Interestingly, the most frequent reason cited for not offering PMS2 germline testing was the fact that PMS2 mutations are only present in ~5% of Lynch syndrome families. If most genetic counselors are not confirming a clinical diagnosis through this test, many may be less inclined to offer PMS2 germline testing in the future. If this occurs, the level of understanding of the prevalence and effects of PMS2 mutations will likely remain the same.

The true prevalence of PMS2 carriers may remain unknown as a minority of genetic counselors have re-contacted families with an unconfirmed, but suspected, genetic diagnosis of Lynch syndrome. Only 15% of those who had never personally offered PMS2 germline testing said that they considered re-contacting such families. There was no significant difference between those who had and had not offered PMS2 germline testing indicating that having offered PMS2 germline testing previously is not related to re-contacting families about PMS2 testing (t(35.6)=.515, p>.05).

The majority of genetic counselors did not think PMS2 germline testing should be offered along with testing for the other MMR genes. An independent-samples t test revealed that there is no difference in opinion between those who had and had not previously offered PMS2 germline testing. This indicates that whether or not a genetic counselor previously offered PMS2 germline testing is not related to wanting or not wanting PMS2 germline testing always be offered with the other three MMR genes in the future (t(39.3)=1.165, p>.05). A genetic counselor in this study is more likely to consider
testing for \textit{PMS2} mutations when mutations in the other three MMR genes have been ruled out.

\textit{Study Limitations}

Since survey participants were required to have seen patients for Lynch syndrome cancer risk assessment, demographics could not be compared to the 2008 NSGC Professional Status Survey (PSS). The PSS includes responses from all genetic counselors in general and is not divided by the type of cases they have counseled for. Therefore, we are unable to determine if the sample in this study is a representative sample.

There is the possibility of ascertainment bias due to the study title. Genetic counselors who had not previously offered \textit{PMS2} germline testing may have interpreted the title as not pertaining to themselves. Additionally, the recruitment notice was posted on three listservs in which some genetic counselors may not belong to and therefore would not know about this study. This may have contributed to the limited sample size.

The online survey design inherently had several flaws that may have affected participant responses. Questions could have been interpreted in different ways leading to inaccurate responses and data. The survey required participants to recall and give estimates of events from 2009 to 2010, which may not have been accurate. Furthermore, hypothetical scenarios did not encompass real or everyday situations. It is difficult to say what one would do in Lynch syndrome cases in general (such as which test to offer and the order of testing requisition) when each case is different from another. Due to the limited utility of the hypothetical scenarios, results were not included. Finally, we did not
ask genetic counselors who had not personally offered PMS2 germline testing before, but one of their co-workers had, why they never offered it themselves.
CONCLUSION

In this study, we examined the current use of PMS2 germline testing among genetic counselors practicing cancer risk assessment for Lynch syndrome cases. This was the first look into genetic counselors’ use of and analysis of factors that influence the decision to offer PMS2 germline testing. Overall, genetic counselors did not consider PMS2 germline testing as a first tier test when counseling patients for Lynch syndrome. Many genetic counselors felt it was more appropriate to wait for other test results (i.e. IHC, MSI, and/or germline testing for the other three MMR genes), which is reflected by the majority of genetic counselors saying that they did not think PMS2 germline testing, should be offered along with testing for MLH1, MSH2, and MSH6 mutations.

It is important to know how prevalent PMS2 mutations are to have a better understanding of the penetrance and phenotype associated with such mutations. More specific cancer screening and/or surgery options can be recommended to patients when more is known about PMS2 mutations. An established set of professional guidelines for when to offer PMS2 germline testing, as well as further research on the phenotype and penetrance of PMS2 mutations, can assist in gaining this valuable information.

Finally, further research is needed to assess the use of PMS2 germline testing when a tumor block is unavailable for tumor analysis. Since a majority of genetic counselors specified that offering PMS2 germline testing would depend on IHC results, it would of interest to explore practices in such situations. Moreover, in the event that
germline testing for *PMS2* mutations becomes more readily available, it would be interesting to see what effect it would have on the use of this testing and the practice of counseling for Lynch syndrome.
REFERENCES


APPENDICES

Appendix A

*Recruitment Notice*

**Are you a genetic counselor who has experience with HNPCC/Lynch syndrome cancer risk assessment?**

I am a second year graduate student in the Genetic Counseling Program at Brandeis University. For my Masters Thesis I am conducting research on the current use of *PMS2* genetic testing for individuals with a known or suspected diagnosis of HNPCC/Lynch syndrome. The aim of this study is to assess the current practices regarding genetic testing for *PMS2* mutations among genetic counselors who see patients for cancer risk assessment.

You are eligible to participate in this study if you are a genetic counselor who:

- Currently see patients for cancer risk assessment or counsel patients for hereditary cancer as part of your job
- Has seen patients for HNPCC/Lynch syndrome cancer risk assessment within the past year (January 2009-January 2010), but do not need to have offered *PMS2* genetic testing
- Practices in the United States or Canada

The study consists of an online survey that will take approximately 20-25 minutes of your time. Participation is completely voluntary and anonymous.

If interested in participating in this study, please access the following website:

[https://www.surveymonkey.com/s/5JFDRV5](https://www.surveymonkey.com/s/5JFDRV5)

If you have any questions or comments please contact me at swnashed@brandeis.edu.

I appreciate your consideration to participate in this study.

Sincerely,

Sarah Nashed
Genetic Counseling Graduate Student
Brandeis University
Waltham, MA
Appendix B

Introduction page to the survey

Thank you for accepting the invitation to participate in this research project. The survey takes approximately 20 minutes to complete. Participation is completely voluntary and anonymous. You may stop at any time.

The purpose of this research project is to assess the current practices regarding genetic testing for PMS2 mutations among genetic counselors who see patients for cancer risk assessment.

Please do not hesitate to contact me with any questions or comments.

Sarah Nashed
Brandeis University Genetic Counseling MS Candidate
swnashed@brandeis.edu

Survey

CURRENT PRACTICES REGARDING THE USE OF PMS2 GENETIC TESTING

Survey Questions

Screening Questions:
1. Do you currently practice in the United States or Canada?
   a. Yes
   b. No → thank you screen

2. Have you seen patients for HNPCC cancer risk assessment within the past year (January 2009 to January 2010)?
   a. Yes
   b. No → thank you screen

3. Do you currently see patients for cancer risk assessment or counsel patients for hereditary cancer as part of your job?
   a. Yes
   b. No → thank you screen

Survey Questions
1. Approximately how many suspected or clinical diagnosis of HNPCC cases have you seen within this past year (between January 2009-January 2010)? (Please provide a specific number) ____

2. Have you ever offered germline PMS2 testing to a patient with a suspected or clinical HNPCC diagnosis?
a. Yes  
b. No

Those who answer “Yes” will follow set A questions.  
Those who answer “No” will move on to question #3

3. Have others at your institution ever offered germline PMS2 testing to patients with a suspected or clinical HNPCC diagnosis?  
a. Yes  
b. No  
c. Do not know

Those who answer “Yes” will move to set B questions.  
Those who answer “Do not know” or “No” will move to set C questions.

Set A (If “Yes”)  
1. Have you ever offered full sequencing for PMS2 to a patient?  
a. Yes  
b. No
1a. If Yes: Approximately how many times have you offered full sequencing for PMS2 to a patient over the past year? _____  
1b. How would you describe patients’ acceptance of going forward with full sequencing for PMS2 after describing its benefits and limitations?  
a. Most accept  
b. About half decline and half accept  
c. Most decline

2. Have you ever offered deletion/duplication analysis for PMS2 to a patient?  
a. Yes  
b. No
2a. If Yes: Approximately how many times have you offered deletion/duplication analysis for PMS2 over the past year? _____  
2b. How would you describe patients’ acceptance of going forward with deletion/duplication analysis for PMS2 after describing its benefits and limitations?  
a. Most accept  
b. About half decline and half accept  
c. Most decline

3. When offering germline testing for PMS2 to a patient, I usually:  
a. Offer full sequencing only  
b. Offer deletion/duplication testing only  
c. Offer full sequencing first, then offer deletion/duplication testing if full sequencing is negative  
d. Offer full sequencing and deletion/duplication testing together always
4. If you have offered only one of the above tests and not the other, please explain why.

5. Please indicate how important each of the following factors are when deciding whether or not to offer germline PMS2 testing.

Not important--Of little importance--Moderately important--Importance--Very important
a. Patient’s insurance coverage
b. Age of cancer diagnosis
c. Aggressiveness of patient’s cancer
d. Cost of the test
e. Type of HNPCC associated cancer patient has
f. Detection rate for PMS2 mutations given abnormal IHC for PMS2
g. Family history of CRC
h. Family history of other HNPCC associated cancers
i. Full sequencing and/or deletion/duplication results for MLH1, MSH2, and MSH6
j. Tumor MSI status
k. Results of IHC
l. Clinical diagnosis of HNPCC

Comments (optional)

5a. Please list any additional factors you think are important in deciding whether or not to offer PMS2 germline testing.
5b. Which one of these is the most important factor in your decision to offer testing?

Scenarios:
Please read the following 4 scenarios. Then rate how likely you would be to recommend full sequencing and/or deletion/duplication analysis for PMS2 in each of the 4 scenarios.

1. Patient A:
   - Colon cancer diagnosis at age 38
   - IHC analysis abnormal for MLH1, but normal for MSH2 and MSH6 expression
   - MSI-high tumor
   - Full sequencing and deletion/duplication testing was negative for mutations in the MLH1, MSH2, and MSH6 genes
   - (Please note, no other affected family members or family members’ tumor blocks are available for testing)
How likely would you be to offer testing for *PMS2* mutations if the patient:

Has some family history, Full Seq: Not very likely----------------- Very likely
but does not meet Del/Dup: Not very likely---------------------- Very likely
Amsterdam criteria

Family meets Amsterdam criteria Full Seq: Not very likely--------- Very likely
Del/Dup: Not very likely--------- Very likely

Comments (optional):

2. Patient B:
   - Colon cancer diagnosis at age 38
   - IHC analysis abnormal for MLH1, but normal for MSH2 and MSH6 expression
   - MSI-high tumor
   - Full sequencing and deletion/duplication testing was negative for mutations in the *MLH1*, *MSH2*, and *MSH6* genes.
   - *BRAF* mutation and *MLH1* promoter hypermethylation studies are negative
   - (Please note, no other affected family members or family members’ tumor blocks are available for testing).

How likely would you be to offer testing for *PMS2* mutations if the patient:

Has some family history, Full Seq: Not very likely----------------- Very likely
but does not meet Del/Dup: Not very likely---------------------- Very likely
Amsterdam criteria

Family meets Amsterdam criteria Full Seq: Not very likely--------- Very likely
Del/Dup: Not very likely--------- Very likely

Comments (optional):

3. Patient C:
   - *Endometrial cancer diagnosis at age 38*
   - IHC analysis abnormal for MLH1, but normal for MSH2, and MSH6 expression
   - MSI-high tumor
   - Full sequencing and deletion/duplication testing was negative for mutations in the *MLH1*, *MSH2*, and *MSH6* genes
   - (Please note, no other affected family members or family members’ tumor blocks are available for testing)

How likely would you be to offer testing for *PMS2* mutations if the patient:
Has some family history, Full Seq: Not very likely-------------------Very likely
but does not meet Del/Dup: Not very likely-------------------Very likely
Amsterdam criteria

Family meets Amsterdam criteria Full Seq: Not very likely--------Very likely
Del/Dup: Not very likely--------Very likely

Comments (optional):

4. Patient D:
   • Currently healthy at age 62
   • Colon cancer diagnosis at age 38
   • Tumor block is unavailable
   • Full sequencing and deletion/duplication testing is negative for MLH1, MSH2, and MSH6 mutations
   • (Please note, no other affected family members or family members’ tumor blocks are available for testing).

How likely would you be to offer testing for PMS2 mutations if the patient:

Has some family history, Full Seq: Not very likely-------------------Very likely
but does not meet Del/Dup: Not very likely-------------------Very likely
Amsterdam criteria

Family meets Amsterdam criteria Full Seq: Not very likely--------Very likely
Del/Dup: Not very likely--------Very likely

Comments (optional):

1. The following is a list of tests that can be ordered in possible HNPCC cases. In a patient who has early onset colon cancer and has a family that meets Amsterdam criteria, please number the following tests in the order in which you would requisition them, starting with the number 1 as the test you would offer first. Options that are not used can be left blank. The same number can be used for tests ordered at the same time. Assume there are no financial constraints on the number of tests that can be ordered.

   - MSI testing
   - IHC for MLH1 and MSH2
   - IHC for MLH1, MSH2, and MSH6
   - IHC for MLH1, MSH2, MSH6, and PMS2
   - Full sequencing for MLH1 and MSH2
   - Full sequencing for MLH1, MSH2, and MSH6
   - Full sequencing for MLH1, MSH2, MSH6, and PMS2
   - Full sequencing for PMS2
2. In your experience, how often did PMS2 full sequencing and/or deletion/duplication testing:
   - Confirm a clinical diagnosis of HNPCC
     Never---------Rarely--------Sometimes-------Very often------ Always
   - Identify a mutation after IHC showed loss of PMS2 expression
     Never---------Rarely--------Sometimes-------Very often------ Always
   - Not applicable. Testing was declined whenever offered.

Concluding Questions

1. When offering full sequencing and/or deletion/duplication testing for the HNPCC genes, do you think testing for PMS2 germline mutations at the same time should always be offered as well?
   a. Yes
   b. No
   1a. Please explain

2. Have you re-contacted families suspected of having HNPCC, but who had an unconfirmed genetic diagnosis (i.e. have MSI-high tumors, but tested negative for mutations in MLH1, MSH2, and MSH6) to do PMS2 testing?
   a. Yes
   b. No
   2a. Comments (optional)

3. If you have additional comments about PMS2 germline testing please feel free to share them.

Set B (If “No/Yes”)

1. Please indicate which of the following others at your institution have offered.
   a. Full sequencing for PMS2
   b. Deletion/duplication testing for PMS2
   c. Both full sequencing and deletion/duplication testing for PMS2
   d. I do not know → thank you screen

2. If someone at your institution has only offered one of the above tests, please explain why or check I do not know
   I do not know → thank you screen
3. Please indicate how important each of the following factors would be when deciding whether or not to offer germline *PMS2* testing.

Not important--Of little importance--Moderately important--Importance--Very important

a. Patient’s insurance coverage
b. Age of cancer diagnosis
c. Aggressiveness of patient’s cancer
d. Cost of the test
e. Type of HNPCC associated cancer patient has
f. Detection rate for *PMS2* mutations given abnormal IHC for PMS2
g. Family history of CRC
h. Family history of other HNPCC associated cancers
i. Full sequencing and/or deletion/duplication results for *MLH1*, *MSH2*, and *MSH6*
j. Tumor MSI status
k. Results of IHC
l. Clinical diagnosis of HNPCC

Comments (optional)

3a. Please list any additional factors you think would be important in deciding whether or not to offer *PMS2* germline testing.

3b. Which one of these would be the most important factor in your decision to offer testing?

**Scenarios:**

Please read the following 4 scenarios. Then rate how likely you would be to recommend full sequencing and/or Deletion/Duplication analysis for *PMS2* in each of the 4 scenarios.

1. **Patient A:**
   - Colon cancer diagnosis at age 38
   - IHC analysis abnormal for MLH1, but normal for MSH2 and MSH6 expression
   - MSI-high tumor
   - Full sequencing and deletion/duplication testing was negative for mutations in the *MLH1*, *MSH2*, and *MSH6* genes
   - (Please note, no other affected family members or family members’ tumor blocks are available for testing)

How likely would you be to offer testing for *PMS2* mutations if the patient:

Has some family history, Full Seq: Not very likely-----------------------Very likely
but does not meet Del/Dup: Not very likely-----------------------Very likely
Amsterdam criteria

Patient B:

- Colon cancer diagnosis at age 38
- IHC analysis abnormal for MLH1, but normal for MSH2 and MSH6 expression
- MSI-high tumor
- Full sequencing and deletion/duplication testing was negative for mutations in the MLH1, MSH2, and MSH6 genes.
- BRAF mutation and MLH1 promoter hypermethylation studies are negative
- (Please note, no other affected family members or family members’ tumor blocks are available for testing).

How likely would you be to offer testing for PMS2 mutations if the patient:

| Has some family history, but does not meet Amsterdam criteria | Full Seq: Not very likely----------Very likely | Del/Dup: Not very likely----------Very likely |

Patient C:

- Endometrial cancer diagnosis at age 38
- IHC analysis abnormal for MLH1, but normal for MSH2, and MSH6 expression
- MSI-high tumor
- Full sequencing and deletion/duplication testing was negative for mutations in the MLH1, MSH2, and MSH6 genes
- (Please note, no other affected family members or family members’ tumor blocks are available for testing).

How likely would you be to offer testing for PMS2 mutations if the patient:

| Has some family history, but does not meet Amsterdam criteria | Full Seq: Not very likely----------Very likely | Del/Dup: Not very likely----------Very likely |

Comments (optional):
Comments (optional):

4. Patient D:
   - Currently healthy at age 62
   - Colon cancer diagnosis at age 38
   - Tumor block is unavailable
   - Full sequencing and deletion/duplication testing is negative for MLH1, MSH2, and MSH6 mutations
   - (Please note, no other affected family members or family members’ tumor blocks are available for testing).

How likely would you be to offer testing for PMS2 mutations if the patient:

- Has some family history, Full Seq: Not very likely------------------------Very likely
- but does not meet Del/Dup: Not very likely------------------------Very likely

Amsterdam criteria

- Family meets Amsterdam criteria Full Seq: Not very likely--------Very likely
- Del/Dup: Not very likely--------Very likely

Comments (optional):

1. The following is a list of tests that can be ordered in possible HNPCC cases. In a patient who has early onset colon cancer and has a family that meets Amsterdam criteria, please number the following tests in the order in which you would requisition them, starting with the number 1 as the test you would offer first. Options that are not used can be left blank. The same number can be used for tests ordered at the same time. Assume there are no financial constraints on the number of tests that can be ordered.

   _MSI testing
   _IHC for MLH1 and MSH2
   _IHC for MLH1, MSH2, and MSH6
   _IHC for MLH1, MSH2, MSH6, and PMS2
   _Full sequencing for MLH1 and MSH2
   _Full sequencing for MLH1, MSH2, and MSH6
   _Full sequencing for MLH1, MSH2, MSH6, and PMS2
   _Full sequencing for PMS2
   _Deletion/Duplication testing for MLH1 and MSH2
   _Deletion/Duplication testing for MLH1, MSH2, and MSH6
   _Deletion/Duplication testing for MLH1, MSH2, MSH6, and PMS2
   _Deletion/Duplication testing for PMS2
Concluding Questions:

1. When offering full sequencing and/or deletion/duplication testing for the HNPCC genes, do you think testing for \textit{PMS2} germline mutations at the same time should \textbf{always} be offered as well?
   
a. Yes
   
b. No

1a. Please explain

2. Have you considered re-contacting families suspected of having HNPCC, but who had an unconfirmed genetic diagnosis (i.e. have MSI-high tumors, but tested negative for mutations in \textit{MLH1}, \textit{MSH2}, and \textit{MSH6}) to do \textit{PMS2} testing?
   
a. Yes
   
b. No

2a. Comments (optional)

3. If you have additional comments about \textit{PMS2} germline testing please feel free to share them.

Set C (If “No/No”)

1. Which of the following help explain why no one at your institution has offered full sequencing and/or deletion/duplication testing for \textit{PMS2} mutations? (Check all that apply)
   
a. The high cost of tests
   
b. The sensitivity of the test
   
c. The fact that \textit{PMS2} mutations are only present in \textasciitilde5\% of HNPCC families
   
d. The lab that we usually use for HNPCC testing does not offer \textit{PMS2} testing
   
e. All of our patients seen in the past year have had mutations in \textit{MLH1}, \textit{MSH2}, or \textit{MSH6}
   
f. Other, please specify

2. Please indicate how important each of the following factors would be when deciding whether or not to offer germline \textit{PMS2} testing.

Not important--Of little importance--Moderately important--Importance--Very important
   
a. Patient’s insurance coverage
   
b. Age of cancer diagnosis
   
c. Aggressiveness of patient’s cancer
   
d. Cost of the test
   
e. Type of HNPCC associated cancer patient has
   
f. Detection rate for \textit{PMS2} mutations given abnormal IHC for \textit{PMS2}
   
g. Family history of CRC
   
i. Family history of other HNPCC associated cancers
j. Full sequencing and/or deletion/duplication results for \textit{MLH1, MSH2}, and \textit{MSH6} 
k. Tumor MSI status 
l. Results of IHC 
k. Clinical diagnosis of HNPCC 

Comments (optional)

2a. Please list any additional factors you would think are important in deciding whether or not to offer \textit{PMS2} germline testing.
2b. Which one of these would be the most important factor in your decision to offer testing?

**Scenarios:**

Please read the following 4 scenarios. Then rate how likely you would be to recommend full sequencing and/or Deletion/Duplication analysis for \textit{PMS2} in each of the 4 scenarios.

1. **Patient A:**
   - Colon cancer diagnosis at age 38
   - IHC analysis abnormal for MLH1, but normal for MSH2 and MSH6 expression
   - MSI-high tumor
   - Full sequencing and deletion/duplication testing was negative for mutations in the \textit{MLH1, MSH2}, and \textit{MSH6} genes
   - (Please note, no other affected family members or family members’ tumor blocks are available for testing)

   How likely would you be to offer testing for \textit{PMS2} mutations if the patient:
   
   Has some family history, Full Seq: Not very likely----------------------Very likely 
   but does not meet Del/Dup: Not very likely----------------------Very likely 
   Amsterdam criteria

   Family meets Amsterdam criteria Full Seq: Not very likely-------Very likely 
   Del/Dup: Not very likely---------Very likely

   Comments (optional):

2. **Patient B:**
   - Colon cancer diagnosis at age 38
   - IHC analysis abnormal for MLH1, but normal for MSH2 and MSH6 expression
   - MSI-high tumor
   - Full sequencing and deletion/duplication testing was negative for mutations in the \textit{MLH1, MSH2}, and \textit{MSH6} genes.
- BRAF mutation and MLH1 promoter hypermethylation studies are negative
- (Please note, no other affected family members or family members’ tumor blocks are available for testing).

How likely would you be to offer testing for PMS2 mutations if the patient:

Has some family history, Full Seq: Not very likely---------------------Very likely
but does not meet Del/Dup: Not very likely---------------------Very likely

Amsterdam criteria

Family meets Amsterdam criteria Full Seq: Not very likely-------Very likely
Del/Dup: Not very likely--------Very likely

Comments (optional):

3. Patient C:
- Endometrial cancer diagnosis at age 38
- IHC analysis abnormal for MLH1, but normal for MSH2, and MSH6 expression
- MSI-high tumor
- Full sequencing and deletion/duplication testing was negative for mutations in the MLH1, MSH2, and MSH6 genes
- (Please note, no other affected family members or family members’ tumor blocks are available for testing)

How likely would you be to offer testing for PMS2 mutations if the patient:

Has some family history, Full Seq: Not very likely---------------------Very likely
but does not meet Del/Dup: Not very likely---------------------Very likely

Amsterdam criteria

Family meets Amsterdam criteria Full Seq: Not very likely-------Very likely
Del/Dup: Not very likely--------Very likely

Comments (optional):

4. Patient D:
- Currently healthy at age 62
- Colon cancer diagnosis at age 38
- Tumor block is unavailable
- Full sequencing and deletion/duplication testing is negative for MLH1, MSH2, and MSH6 mutations
- (Please note, no other affected family members or family members’ tumor blocks are available for testing).

How likely would you be to offer testing for PMS2 mutations if the patient:
Has some family history,  Full Seq: Not very likely-------------------Very likely but does not meet Del/Dup: Not very likely-------------------Very likely Amsterdam criteria

Family meets Amsterdam criteria  Full Seq: Not very likely------Very likely Del/Dup: Not very likely------Very likely

Comments (optional):

Concluding Questions:
1. When offering full sequencing and/or deletion/duplication testing for the HNPCC genes, do you think testing for PMS2 germline mutations at the same time should always be offered as well?
   a. Yes
   b. No
1a. Please explain

2. Have you considered re-contacting families suspected of having HNPCC, but who had an unconfirmed genetic diagnosis (i.e. have MSI-high tumors, but tested negative for mutations in MLH1, MSH2, and MSH6) to do PMS2 testing?
   a. Yes
   b. No
2a. Comments (optional)

3. If you have additional comments about PMS2 germline testing please feel free to share them.

Demographic Questions:
Please answer the following demographic questions.

1. Please indicate your age: ____________ years

2. Please indicate your gender:
   a. Female
   b. Male

3. Do you currently work full time or part time?
   a. Full time
   b. Part time

4. How many years have you been practicing cancer genetic counseling?
   ________ years
5. In an average week, what percent of your time at work do you spend with (should add to 100%):
   - Cancer patients ______%  
   - Prenatal patients ______%  
   - Pediatrics patients ______%  
   - Other, please specify: _____, ______%  
   - Other, please specify: _____, ______%  

6. In an average week, what percent of your time at work do you spend (should add to 100%):
   - Counseling patients ______%  
   - Teaching/Education ______%  
   - Research/Study Coordinator ______%  
   - Clinical Coordinator ______%  
   - Management ______%  
   - Other, please specify _____, _____%  

7. Do you practice in the United States or Canada?
   a. If U.S., in what region?
      - Region I: CT, MA, ME, NH, RI, VT
      - Region II: DC, DE, MD, NJ, NY, PA, VA, WV, Puerto Rico, Virgin Islands
      - Region III: AL, FL, GA, KY, LA, MS, NC, SC, TN
      - Region IV: AR, IA, IL, IN, KS, MI, MN, MO, ND, NE, OH, OK, SD, WI
      - Region V: AZ, CO, MT, NM, TX, UT, WY
      - Region VI: AK, CA, HI, ID, NV, OR, WA
   b. If Canada, in what region?
      - Region I: Canadian Maritime Provinces
      - Region II: Quebec
      - Region III: Ontario
      - Region IV: Alberta, Manitoba, Saskatchewan
      - Region: V: British Colombia

8. What is your work setting? Check all that apply
   a. Cancer institute
   b. University medical center
   c. Private hospital
   d. Public hospital
   e. Physician’s private practice
   f. Private practice
   g. Diagnostic laboratory
   h. Other, please specify
9. In what year did you graduate from a genetic counseling masters program?

10. Did you have a formal cancer genetics class as part of your genetic counseling program?
   a. Yes
   b. No

Conclusion page of Survey

Thank you for taking the time to participate in this survey!
### Appendix C

*Labs offering PMS2 germline testing (United States and Canada)*

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Full Sequencing of PMS2</th>
<th>Deletion/Duplication analysis of PMS2</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARUP- Utah, USA</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Baylor College of Medicine- Texas, USA</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>City of Hope- California, USA</td>
<td>YES</td>
<td>NO</td>
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<tr>
<td>Emory University- Georgia, USA</td>
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<td>Huntington Medical Research Institutes- Californi, USA</td>
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<td>YES</td>
</tr>
<tr>
<td>London Health Sciences Centre- Ontario, Canada</td>
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<td>YES</td>
</tr>
</tbody>
</table>

(Genetests.org 8/9/2010)