THE IMPACT OF SCREENING ON THE CLINICAL COURSE OF LYNCH SYNDROME

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The Impact of Screening on the Clinical Course of

Lynch Syndrome

by

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ABSTRACT

Background & Aims: Lynch syndrome (LS) is an autosomal dominant disorder and is caused by mutations in one of the DNA mismatch repair (MMR) genes, in particular, MLH1, MSH2, MSH6 and PMS2. Lynch syndrome mutation carriers are at a high risk of developing colorectal cancer (CRC) and gynecological cancers, and as such, targeted screening programs have been developed. The primary objective of this thesis was to determine the phenotypic expression of three different MSH2 mutations causing LS in Newfoundland and to examine the impact of screening in this group of MSH2 mutation carriers.

Methods: Age to onset of first CRC, first extracolonic cancers and death were compared for those with an intron 5 splice site mutation, an exon 8 deletion and an exon 4-16 deletion. To determine the impact of colonoscopic screening in male and female MSH2 mutation carriers, CRC incidence and survival in the screened group was compared to that expected, derived from the non-screened group. To correct for survivor bias controls were matched for age at entry into screening and also for gender. Compliance with screening recommendations of colonoscopy every 1-2 years was also addressed. Gynecological cancer incidence and overall survival was compared in females who received gynecological screening and in matched controls. Controls were randomly selected from non-screened mutation carriers who were alive and disease-free at the age the case entered the screening program. One matched control was selected for each case.
**Results:** For all three mutations males had a higher age-related risk of CRC and death compared to females. For the intron 5 splice site mutation carriers, the number of transitional cell cancers of the urinary tract was significantly lower and time to first ovarian cancer was significantly higher than in the carriers of the genomic deletions. Median age to CRC was 58 years in males who received colonoscopic screening whereas expected was 47 years ($P < .0001$), and median survival in screened males was 66 years compared to expected of 62 years ($P = .034$). In females, median age to CRC in the colonoscopic screened group was 79 years, whereas in the non-screened group it was 57 years ($P = .000$), and median survival was 80 years in the screened group compared to expected of 63 years ($P = .001$). Eight of 41 (20%) males and five of 68 (7%) females who had serial screening colonoscopies developed an interval CRC within 2 years of previous colonoscopy. Endometrial or ovarian cancer occurred in 14 of 54 (26%) women in the gynecological screened group. Median age to diagnosis of gynecological cancer was 54 years in the screened group compared to 56 years in matched controls ($p = .50$). Stage I or II cancer was diagnosed in 92% of screened patients compared to 71% in the control group ($P = .17$). Mean survival in the screened group was 79 years compared to 69 years in the matched control group ($P = .11$), likely associated with concomitant colonoscopic screening.

**Conclusions:** The incidence of CRC in $MSH2$ mutation carriers, exposed to the same environment, is not modified by the specific mutation, although there is a suggestion that type of mutation may influence development of some extracolonic cancers. For both
males and females, colonoscopic screening was associated with decreased CRC risk, later age of onset, and better survival than expected if non-screened, however, CRCs continued to occur. CRC development may be further reduced by decreasing the screening interval to one year in \textit{MSH2} mutation carriers and improving compliance and quality of colonoscopic examination. Gynecological screening did not result in earlier gynecologic cancer detection and despite screening two young women died from ovarian cancer suggesting that prophylactic hysterectomy with bilateral salpingo-oophorectomy be considered in female mutation carriers who have completed childbearing.
ACKNOWLEDGEMENTS

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Thank-you to the co-authors of the manuscripts presented in this thesis. As well, I would like to express my gratitude to numerous other colleagues for their advice, encouragement and assistance with this research project.

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<table>
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<th>Description</th>
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<tr>
<td>AFAP</td>
<td>Attenuated familial adenomatous polyposis</td>
</tr>
<tr>
<td>BSO</td>
<td>Bilateral salpingo-oophorectomy</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CRC</td>
<td>Colorectal cancer</td>
</tr>
<tr>
<td>DGGE</td>
<td>Denaturing gradient gel electrophoresis</td>
</tr>
<tr>
<td>FAP</td>
<td>Familial adenomatous polyposis</td>
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<td>FCCTX</td>
<td>Familial colorectal cancer type X</td>
</tr>
<tr>
<td>HNPCC</td>
<td>Hereditary non-polyposis colorectal cancer</td>
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<tr>
<td>HPS</td>
<td>Hyperplastic polyposis syndrome</td>
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<tr>
<td>IHC</td>
<td>Immunohistochemistry</td>
</tr>
<tr>
<td>ICG-HNPCC</td>
<td>The International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer</td>
</tr>
<tr>
<td>LS</td>
<td>Lynch syndrome</td>
</tr>
<tr>
<td>MAP</td>
<td>MUTYH-associated polyposis</td>
</tr>
<tr>
<td>MLPA</td>
<td>Multiplex ligand dependent probe amplification</td>
</tr>
<tr>
<td>MMR</td>
<td>Mismatch repair</td>
</tr>
<tr>
<td>MSI</td>
<td>Microsatellite instability</td>
</tr>
<tr>
<td>MSI-H</td>
<td>Microsatellite instability-high</td>
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<tr>
<td>MSI-L</td>
<td>Microsatellite instability-low</td>
</tr>
<tr>
<td>MSS</td>
<td>Microsatellite stable</td>
</tr>
<tr>
<td>RR</td>
<td>Relative risk</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical package for Social Sciences</td>
</tr>
<tr>
<td>TVU</td>
<td>Transvaginal ultrasound</td>
</tr>
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LIST OF MANUSCRIPTS

The following manuscripts are related to my work in colorectal cancer research:


CO-AUTHORSHIP STATEMENT

This doctoral thesis was comprised of three separate manuscripts, two of which were peer-reviewed and published and another which has been submitted to a journal for review. All three studies were components of a program of research funded by the Canadian Institute for Health Research (CIHR). The aim of The Colorectal Cancer Interdisciplinary Health Research Team (crCIHRt) was to advance knowledge of the determinants, impact and control of CRC. Additional funding was received from the National Cancer Institute of Canada for studies two and three and from Genome Canada and the Atlantic Innovation Foundation for study two. The funding sources had no involvement in the study designs; in the collection, analysis or interpretation of the data; in the writing of the reports; or in the decision to submit the papers for publication.

The first study was an examination of the penetrance of colorectal cancer (CRC) and other extracolonic cancers among Newfoundland families with an inherited mutation causing Lynch syndrome (LS) (Chapter 3). I, along with Dr. Parfrey, was involved in the study concept and design of this project. I collected the data through chart abstraction, entered the information into a database for analysis, performed the statistical analyses, and along with Dr. Parfrey interpreted the findings. I was the principal author and drafted the manuscript. After feedback and review by the other authors, I made the necessary edits and revisions and submitted the manuscript.
In studies two and three I was also the principal author. The second study looked at the impact of colonoscopic screening in LS families identified in chapter 3 (Chapter 4) and the third study looked at the impact of gynecologic screening in this same group of individuals (Chapter 5). For both of these studies, myself and Dr. Parfrey were involved in the study concept and design of the projects. Data for these two studies were collected simultaneously and entered into a large research database which included all demographic, screening, cancer and mortality information available for each family member. I took part in the data collection and entry of this data and also developed my own database of the relevant information required for my projects. I analyzed the data for both studies, and along with Dr. Parfrey interpreted the findings. I drafted the two papers and made the necessary revisions and edits based on feedback from the other authors. After final approval I submitted the manuscripts.
CHAPTER 1

Outline
1.1 OUTLINE

Lynch syndrome (LS) is characterized by autosomal dominant inheritance, early age at onset of colorectal carcinoma, right-sided predominance, and increased incidence of synchronous and metachronous colorectal cancers (CRCs). Additionally, extra-colonic cancers of the endometrium, ovaries, gastrointestinal tract, genitourinary tract, biliary tract, pancreas and brain are frequently observed. Lynch syndrome shows incomplete penetrance (not all mutation carriers will develop a cancer) and variable expressivity (individuals develop different cancers at different ages) leading to phenotypic heterogeneity. Genetic heterogeneity is also a feature of LS as mutations in at least four different mismatch repair (MMR) genes are responsible for LS.

Chapter 2 provides a general overview of the different aspects of LS. History, clinical features, cancer risks, diagnostic strategies, molecular genetics, screening and management of LS are all discussed.

Chapter 3 compares the phenotypic expression of three different founder MSH2 mutations causing LS. The cumulative risk of CRC, extracolonic cancers and death was compared for MSH2 mutation carriers with an intron 5 splice site mutation, an exon 8 deletion and an exon 4-16 deletion. Gender related risks for LS-associated cancers and death, were also measured.
Chapter 4 looks at the impact of colonoscopic screening in \( MSH2 \) mutation carriers with one of three different founder \( MSH2 \) mutations described in Chapter 3. This study evaluated the effectiveness of colonoscopic screening for male and female mutation carriers. Incidence of CRC and survival in the screened group was compared to that expected, derived from the non-screened group, and adjusted for survivor bias. Additionally, compliance with colonoscopic screening recommendations and appropriateness of the screening interval were addressed.

The impact of gynecological screening in LS is discussed in Chapter 5. The effectiveness of gynecological screening was evaluated for females belonging to families with one of the three \( MSH2 \) mutations described in Chapter 3. Incidence of gynecological cancer and overall survival was compared for those who were screened (cases) and for matched controls, who were alive and disease-free at the age the case entered the screening program.

Chapter 6 provides a general discussion of the thesis. The impact of screening in \( MSH2 \) mutation carriers, the study limitations and the barriers to effective disease management are discussed. The benefits of this study and future directions are also detailed. A summary of the research findings and a list of recommendations are presented in Chapter 7.
CHAPTER 2

Lynch Syndrome
2.1 INTRODUCTION

Colorectal Cancer (CRC) is a major public health problem in the Western world. In Canada, CRC is the third most common cancer, after prostate and lung in men and after breast and lung in women, and is the second and third most common cause of cancer-related death for males and females, respectively. Newfoundland and Labrador has the highest CRC incidence rates among men and women in Canada [Canadian Cancer Society’s Steering Committee 2011] and the highest incidence of inherited CRC in the world [Green 2007].

Colorectal cancer is a common and heterogeneous disease that is influenced by both environmental and genetic factors. The majority of CRC cases are sporadic and account for approximately 70% of the CRC burden. Kindred and twin studies have estimated that the remaining 30% of CRC cases are an inherited form of the disease [Lichtenstein 2000; Zeegers 2008]. Inherited CRC can be divided into familial and hereditary CRC cases, with approximately 15-20% being familial and 5-10% being hereditary [Lynch 2006; Jaspersen 2010]. Familial cases have an increased risk of CRC due to positive family history of CRC but do not meet criteria for known hereditary CRC syndromes. This risk is dependent on the number of affected relatives and their age at diagnosis. The molecular etiology of familial CRC remains poorly understood but is likely caused by alterations in single genes that are less penetrant but more prevalent than genes causing hereditary CRC [Jaspersen 2010]. Hereditary cases have an increased risk due to an inherited susceptibility to CRC and show extensive phenotypic and genotypic heterogeneity. The
The majority of hereditary CRC cases are associated with one of two well-defined inherited syndromes: Lynch syndrome (LS) and familial adenomatous polyposis (FAP).

Lynch syndrome, often called hereditary non-polyposis colorectal cancer (HNPCC), is the most common hereditary CRC syndrome and is responsible for approximately 2 to 5 percent of all CRC cases [de la Chapelle 2005; Hampel 2005a, 2008; Lynch 2009a] and similarly is responsible for about 2 percent of all endometrial cancers [Hampel 2006; Kehoe 2007]. Lynch syndrome is an autosomal dominant condition caused by mutations in DNA mismatch repair (MMR) genes and is characterized by early onset CRC and an increased risk of malignancy for extracolonic cancers. Familial adenomatous polyposis is the second most common inherited syndrome and accounts for approximately 1% of the total CRC burden. Patients with FAP develop hundreds to thousands of colonic adenomas beginning in early adolescence and show close to 100% lifetime risk for CRC without prophylactic management. Attenuated familial adenomatous polyposis (AFAP) is a less severe form of the disease, characterized by fewer (<100) adenomatous polyps of the colon and a reduced lifetime risk for CRC (~70%). Both FAP and AFAP are autosomal dominant disorders caused by inactivating mutations of the *adenomatous polyposis coli* (*APC*) gene [Jass 2008; Jaspersen 2010]. Other rare inherited conditions causing CRC are *MUTYH*-associated polyposis (MAP), hamartomatous polyposis syndromes, namely Peutz-Jeghers syndrome and juvenile polyposis syndrome, and hyperplastic polyposis syndrome (HPS). *MUTYH*-associated polyposis is caused by mutations in the *MUTYH* gene and is inherited as an autosomal recessive condition.
syndromes are caused by germline mutations in \textit{STK11}, \textit{SMAD4} and \textit{BMPRIA} and the etiology of HPS is unknown [Jass 2008]. A precise understanding of the genetics of inherited CRC is important for identifying at-risk individuals so that targeted cancer prevention strategies can be implemented to improve patient outcomes.

2.2 HISTORY OF LYNCH SYNDROME

In 1913, A.S. Warthin published a large pedigree with numerous cases of CRC along with cases of uterine and stomach cancer [Warthin 1913]. This family was designated as ‘cancer Family G’. Family G was updated numerous times over the years, again by Warthin in 1925 [Warthin 1925], and by two of his colleagues, Weller and Hauser, in 1936 [Hauser 1936]. In 1966, Lynch et al. described two additional families, families N and M, whose tumor spectrum was very similar to Family G [Lynch 1966]. In 1971, Lynch revisited family G [Lynch 1971], and along with families N and M, characterized the syndrome, which was later referred to as ‘Lynch syndrome’.

In 1989, the International Collaborative Group on Hereditary Nonpolyposis Colorectal Cancer (ICG-HNPCC) was established to promote international research on the genetic, clinical and pathologic aspects of LS [Lynch 2005]. At this time, LS was largely unknown and the group proposed a new name for the syndrome, hereditary non-polyposis colorectal cancer syndrome (HNPCC), explaining which tumor is mainly involved in the disease [Vasen 1991, 2007a]. It was thought that such a name might promote the recognition of the syndrome. As the syndrome became more well-defined and well-
known, and with the identification of MMR mutations in this syndrome, it was decided that the term ‘hereditary nonpolyposis colorectal cancer’ was no longer ideal and the term ‘Lynch syndrome’ was reintroduced [Vasen 2007a]. Lynch syndrome refers to the autosomal dominant disease caused by germline mutations in one of the DNA MMR genes [Boland 2005]. The name hereditary nonpolyposis colorectal cancer syndrome can still be given to disorders that have similar phenotypes and meet the Amsterdam criteria (a set of clinical criteria developed for standardizing the diagnosis of LS) but who do not have a known DNA MMR defect [e.g., familial colorectal cancer type X (FCCTX)] [Lynch 2009b].

2.3 CLINICAL FEATURES OF LYNCH SYNDROME

Lynch syndrome is an hereditary colon cancer syndrome and is caused by germline mutations in DNA MMR genes, in particular MLH1, MSH2, MSH6, and PMS2 [Wheeler 2000; Lynch 2003; Peltomaki 2004]. Mutations in MSH2 and MLH1 account for approximately 90% of LS cases, mutations in MSH6 account for approximately 10% and mutations in PMS2 are rarely detected [Peltomaki 2004; Jaspers 2010].

Lynch syndrome is an autosomal dominant inherited syndrome with incomplete penetrance and a variable phenotype with respect to tumor site and age at onset. Table 2.1 lists the clinical characteristics of LS. Individuals with LS develop early onset CRC at an average age of 45 years. The tumors are mainly located in the proximal colon and there is an increased incidence of synchronous and metachronous CRCs. Synchronous
tumors are defined as secondary primary tumors detected at the time of diagnosis or occurring within the first six months of diagnosis of the first primary cancer. Metachronous tumors, however, are diagnosed more than six months after the first primary. Mutation carriers are also at an increased risk of extracolonic cancers, especially cancer of the endometrium, and may be at risk for associated malignancies of the brain (glioblastomas) in the Turcot's syndrome variant of LS, and skin (sebaceous gland adenomas, carcinomas, or keratoacanthomas) in the Muir-Torre syndrome variant of LS. Colorectal tumors in LS often show specific pathological characteristics, such as poor differentiation with mucoid features and signet cell excess, Crohn's-like reaction, and an excess of tumor infiltrating lymphocytes [Jass 2004, Lynch 2008b], and the majority exhibit microsatellite instability (MSI), a feature of cancers that arises in the setting of defective MMR genes [Jaspers 2010]. The adenoma to invasive carcinoma sequence is accelerated in LS, however survival, when controlled for age and stage, is improved compared to sporadic CRC in the general population [Watson 1998]. Knowledge of these clinical features is essential for understanding LS and can be used effectively for diagnosis, screening, management and ultimately cancer prevention [Lynch 2008a, 2009a].
Table 2.1 Clinical features of Lynch Syndrome [Lynch 2008a, 2009a]

- **Autosomal Dominant**: Autosomal dominant inheritance pattern
- **Early age at Diagnosis**: Earlier average age at onset of CRC compared to the general population (45 years versus approximately 65 years)
- **Right-sided Predominance**: Proximal (right-sided) CRC involvement (70-85% of CRCs arise proximal to the splenic flexure)
- **Synchronous & Metachronous CRCs**: High risk of synchronous and metachronous CRCs (25-30% of patients develop a second primary CRC within 10 years of their surgical resection for initial CRC if they received a less than subtotal colectomy)
- **Extracolonic Cancers**: Increased risk of extracolonic cancers [namely cancers of the endometrium, ovaries, stomach, small bowel, pancreas, hepatobiliary tract, upper uro-epithelial tract (transitional cell carcinoma of the ureter and renal pelvis), and brain (in the Turcot syndrome variant of Lynch syndrome)]
- **Associated Malignancies**: Other associated malignancies (sebaceous adenomas, sebaceous carcinomas and multiple keratoacanthomas in the Muir-Torre syndrome variant of Lynch syndrome)
- **Tumor Characteristics**: CRC tumors in LS show a pathology that is more often poorly differentiated, with an excess of mucoid and signet-cell features, show a Crohn-like reaction and contain a significant excess of infiltrating lymphocytes within the tumor
- **Better Survival**: Improved survival from CRC in LS as compared to sporadic CRC in the general population
- **Shorter Adenoma to Carcinoma Sequence**: Accelerated carcinogenesis (adenomas can develop into carcinoma within 1-2 years in Lynch syndrome compared with 8-10 years in the general population)
- **Microsatellite Instability**: High frequency of microsatellite instability in CRC tumors (approximately 90% of LS tumors exhibit MSI)
- **MMR Mutation**: Identification of a germline mutation in a MMR gene (*MLH1*, *MSH2*, *MSH6*, and *PMS2*)

2.3.1 **Cancer Risks in Lynch Syndrome**

An accurate estimation of CRC and extracolonic cancer risk for mutation carriers is essential for genetic counseling and the development of appropriate screening programs.

The most efficient way to calculate these risks would be to use a cohort of proven mutation carriers, however, many risk estimates provided in the literature are based on *proven and presumed mutation carriers*. Also, risk estimates may be biased due to the way families were ascertained. The majority of early studies included families who met the Amsterdam criteria or included high risk families referred to clinical genetics departments. Therefore, families without an apparent clustering of CRC due to small sibships, few cancer cases, unrelated deaths, non-paternity, adoption or insufficient pedigree information, were less frequently included. Analysis of this high risk group results in an overestimation of the cumulative cancer risks [Barrow 2009]. A few studies have attempted to correct for ascertainment bias using different evaluation models and have found lower risk estimates than previously reported [Carayol 2002; Quehenberger 2005].

Lifetime risk of LS cancers varies considerably due to the numerous ways in which risk estimates are calculated. Some studies report risks associated with particular genes while others present combined risk estimates. The lifetime risk of CRC in mutation carriers ranges from 15-100% (Table 2.2) and is dependent on gender, method of ascertainment and the MMR genes involved. Male carriers in all four MMR mutations are at an increased risk of developing CRC compared to female mutation carriers. The lifetime risk for endometrial cancer is also very high and ranges from 15-71%. For other LS-related cancers, the lifetime risk ranges anywhere from 1-32% and is highest for urinary tract, ovarian and gastric cancers (Table 2.2).
Table 2.2 Lifetime risk of cancer in Lynch Syndrome

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Affected Mismatch Repair Gene</th>
<th>MLH1/MSH2*</th>
<th>MLH1</th>
<th>MSH2</th>
<th>MSH6</th>
<th>PMS2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorectal cancer</td>
<td>Male</td>
<td>27-100</td>
<td>58-94</td>
<td>53-96</td>
<td>36-69</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>22-83</td>
<td>50-63</td>
<td>39-68</td>
<td>18-30</td>
<td>15</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td></td>
<td>28-60</td>
<td>19-42</td>
<td>24-61</td>
<td>49-71</td>
<td>15</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td></td>
<td>6-14</td>
<td>3-6</td>
<td>8-21</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td></td>
<td>9-13</td>
<td>2-11</td>
<td>4-8</td>
<td>10</td>
<td>NA</td>
</tr>
<tr>
<td>Small bowel cancer</td>
<td></td>
<td>3</td>
<td>5-7</td>
<td>1-5</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Urinary tract cancer</td>
<td></td>
<td>3-4</td>
<td>1-3</td>
<td>4-32</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Biliary tract cancer</td>
<td></td>
<td>1-2</td>
<td>3</td>
<td>0.4</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Brain</td>
<td></td>
<td>3-4</td>
<td>1</td>
<td>1-6</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Lifetime risk for MLH1 and MSH2 mutation carriers combined


Several studies have reported differences in the clinical cancer phenotype between MMR mutations. Carriers of MSH2 mutations may be at an increased risk of developing extracolonic cancers compared to individuals with MLH1 mutations [Lin 1998; Vasen 2001] and may also have an increased incidence of Muir-Torre syndrome. MLH1 carriers may have an increased lifetime risk of developing CRC when compared with carriers of MSH2, MSH6 and PMS2 mutations [Lynch 2006, 2008a]. The mean age at onset of CRC in MLH1 and MSH2 mutation carriers is approximately 45 years. For carriers of MSH6 and PMS2 mutations, the mean age at diagnosis of CRC is delayed by 5-10 years and 15 years, respectively [Plaschke 2004; Senter 2008; Ramsoekh 2009]. MSH6 mutation carriers show a lower expression of CRC when compared to MLH1 and MSH2 mutation carriers but have an increased lifetime risk of developing endometrial cancer [Hendricks
2004; Barrow 2009; Ramsoekh 2009]. Carriers of PMS2 mutations have a substantially reduced risk of developing CRC or endometrial cancer compared to other MMR mutation carriers [Senter 2008].

2.3.2 CRC Survival in Lynch Syndrome

Several studies have looked at the survival of patients with LS-associated CRC compared to sporadic CRC. A Finnish study compared the survival of 175 patients with suspected LS to a population based cohort of over 14,000 patients with apparently sporadic CRC diagnosed at less than 65 years [Sankila 1996]. The overall 5-year cumulative survival rate was found to be 65% for patients with LS compared to 44% for patients with sporadic colorectal cancer. A further analysis of those with a confirmed MLH1 germline mutation (the rest were diagnosed by the Amsterdam criteria) revealed an even larger survival advantage over those with sporadic CRC. This survival advantage for LS patients compared to sporadic CRC patients was found in every strata studied and was not due to a screening advantage for LS patients. After adjustment for stage at diagnosis, the survival rates were still better among patients with localized (Dukes’ A & B) (85% for LS versus 68% for sporadic) and non-localized (Dukes’ C & “D”) (40% for LS versus 18% for sporadic) tumors, disproving the view that the good prognosis observed in patients with LS-associated CRC is due to a more favorable stage at diagnosis.

In 1997, a Danish study evaluated CRC tumor parameters and survival in LS [Myrhøj 1997]. They compared 108 individuals fulfilling the Amsterdam criteria to 870
individuals with sporadic CRC diagnosed at less than 40 years. LS-associated CRC was found to behave differently than sporadic CRC with more right sided carcinomas (68% versus 49%), more synchronous (7% versus 1%) and metachronous (29% versus 5%) lesions, more localized carcinomas (Dukes’ A and B) (62% versus 39%) and a significantly higher crude 5-year survival rate (56% versus 30%). The metastatic tendency was less in LS-associated CRC than sporadic CRC and survival was substantially better in LS patients compared to young sporadic patients. However, after stratification into localized carcinomas (Dukes’ A & B) and those with regional lymph node metastases (Dukes’ C), they found the crude 5-year survival rate only differed significantly between those with Dukes’ C carcinomas. The survival rates for localized tumors were 69% and 66%, respectively, for LS patients versus sporadic patients, and for Dukes’ C carcinomas it was 61% versus 21% (p<0.01).

Watson et al. [Watson 1998] compared stage and survival in a retrospective cohort of LS family members with CRC to an unselected hospital series of patients with sporadic CRC. Previous work had shown LS-associated CRC to have a better prognosis than sporadic CRC but it had been unclear whether this could be due to differences in stage at diagnosis. Two hundred and seventy four individuals, who met either the Amsterdam criteria or were known to carry an MMR mutation, were compared to 820 consecutive individuals with sporadic CRC. LS CRC cases were found to have significantly lower stage disease at diagnosis than sporadic CRC cases, due mainly to the fact that distant metastases at diagnosis were rare in LS. In stage-stratified survival analysis, LS cases
were found to have a significant overall survival advantage over sporadic cases regardless of adjustment for their younger age (HR=0.63; p<0.002).

2.3.3 Gynecological Cancer Survival in Lynch Syndrome

Various studies have shown that LS-associated CRC has better survival estimates than corresponding sporadic CRC [Sankila 1996; Myrhoj 1997; Watson 1998]. Similarly, work has been done to determine whether the survival rate of patients with gynecological cancer due to LS differs from sporadics.

Boks et al. [Boks 2002] compared the survival rates of LS-associated endometrial cancer with sporadic endometrial cancer. The study group involved 50 women with a germline mutation or who met the Amsterdam Criteria II. These women were matched for age at diagnosis and FIGO stage to 100 controls with sporadic cancer. The majority of study patients (78%) presented with stage I cancer. The overall 5-year cumulative survival rates (88% vs. 82%) and the tumor histologic subtypes were found to be similar for women with LS and for matched controls suggesting a likeness in the biological behavior of LS-associated and sporadic endometrial cancer.

Conflicting results have been published regarding LS-associated ovarian cancer survival compared to sporadic ovarian cancer. Crijnen et al. [Crijnen 2005] compared the survival of patients with ovarian cancer due to LS with a control group matched for age (age +/- 5 years), stage at diagnosis (FIGO stage) and year of diagnosis (diagnosis year +/- 5 years).
For cases diagnosed before 1970, the difference in year of diagnosis was somewhat larger. Twenty six women that met either the Amsterdam II criteria or who had an MMR gene mutation identified were compared to 52 controls from a population-based registry. The mean age at diagnosis was significantly lower for LS-associated ovarian cancer compared to all cases of ovarian cancer in the registry (50 vs. 61). Also, when compared to all cases in the registry, significantly more LS-associated ovarian cancers were diagnosed at an early stage (I or II) (77% vs. 39%). None of the stage I cases were detected by screening, hence early detection cannot explain this finding. This study found that the overall cumulative 5-year survival rate was similar for both LS-associated ovarian cancer and sporadic ovarian cancer (64.2% vs. 58.1%), suggesting a likeness in the biological behavior of ovarian cancer due to LS and sporadic ovarian cancer.

More recently, a study by Grindedal et al. [Grindedal 2010] described ovarian cancer survival in carriers of pathogenic mutations in one of the MMR genes. One hundred and forty-four mutation carriers were compared to both BRCA-associated and sporadic ovarian cancer cases. Ten year survival specific for deaths due to ovarian cancer was found to be 81% for MMR mutation carriers compared to only 36%-47% for BRCA mutation carriers and the general population. However, 80% of ovarian cancers diagnosed in MMR mutation carriers were found to be stage I or II contrasting with the more than two thirds of ovarian cancer cases diagnosed as stage III or IV in BRCA-associated and sporadic ovarian cancer. Again this low stage at diagnosis for LS-associated ovarian cancer could not be explained by screening as most of the women had
not attended screening. When comparing stage at cancer diagnosis, ten year ovarian
cancer free survival for stage I and II cancers was found to be similar for both MMR
mutation carriers and *BRCA* mutation carriers (87% for both), however, ovarian cancer
free survival for advanced stage ovarian cancers (stage III and IV) was found to be 53%
for MMR mutation carriers and only 19% for *BRCA* carriers. When compared to the
general population, 5 year ovarian cancer survival for advanced stage ovarian cancer was
found to be 59% for MMR mutation carriers compared to 28% in the general population.
They suggest that survival for advanced stage ovarian cancer may be better for MMR
mutation carriers compared to *BRCA* carriers and the general population and that the
MMR genes may predispose to a biologically different type of ovarian cancer that is
characterized by early stage and more favorable prognosis.

### 2.4 MOLECULAR BASIS OF Lynch SYNDROME

Lynch syndrome is due to a germline mutation in one of several MMR genes. However,
because searching for one of these MMR mutations is difficult and expensive, molecular
prescreening can be performed. Microsatellite instability of the tumor, and loss of one or
two of the MMR proteins in the tumor compared to normal tissue, are two tumor
characteristics that can be used to identify CRC patients most likely to have LS.

#### 2.4.1 Microsatellite Instability

Microsatellite instability is a hallmark of LS and is detected in more than 90% of tumors
in LS [Aaltonen 1993, 1994]. Microsatellites are short repetitive DNA sequences that
occur throughout the genome and MSI is caused by failure of the DNA MMR system to correct for errors in microsatellite repeat sequences that occur during DNA replication. International guidelines for the evaluation of MSI recommend using a set of five microsatellite markers, namely, D2S123, D5S346, D17S250, BAT25 and BAT26 [Boland 1998] and additionally BAT40 may be tested to increase the sensitivity of the test [Hendricks 2003]. Comparison of marker size in tumor tissue and unaffected tissue from the same individual is scored as MSI-high (MSI-H) if 30% or more of the tumor markers show instability, MSI-low (MSI-L) if less than 30% show instability, or microsatellite stable (MSS) if none of the markers show instability. Because the majority of CRCs from patients with LS exhibit MSI, it can be helpful in diagnosing LS. However, MSI status alone cannot be used as a test for LS cancers because MSI is not specific to LS as it also occurs in up to 15% of sporadic CRCs [Aaltonen 1994; Moslein 1996; Herman 1998; Lindor 2002]. Sporadic MSI-H CRCs are due to hypermethylation of the MLH1 promoter region, whereas, MSI-H tumors in LS are the result of a germline gene mutation [Jasperson 2010].

2.4.1.1 Prediction of MSI in Colorectal Cancers

As was stated previously, high frequency microsatellite instability is a feature of CRCs that arise in LS. However, testing of all tumors for microsatellite status in a population-based setting would be a very non-specific and expensive way to identify LS cases. In 2007, Jenkins et al. [Jenkins 2007] identified pathology features (based on the tumor histology criteria included in the Revised Bethesda Guidelines, namely, the presence of
tumor infiltrating lymphocytes, a Crohn’s-like lymphocytic reaction, mucinous or signet ring differentiation and a medullary or undifferentiated and solid growth pattern) and other clinical features (age at diagnosis and anatomic site of colon in the tumor) that independently predict MSI-H status. Identification of these features led to the development of the MsPath (Microsatellite instability by Pathology) model which uses easily assessable clinicopathologic characteristics to calculate a MsPath score. This simple scoring system can then be used to determine which tumors are to be selected for MSI or immunohistochemistry (IHC) testing and improves upon the sensitivity and specificity of the Revised Bethesda Criteria.

A more recent study by Hyde et al. [Hyde 2010], improved upon the existing MsPath model by analyzing and scoring additional histologic features (such as peritumoral lymphocytic reaction and increased proportion of plasma cells in the tumor stroma). From this analysis they developed the Pathological Role in the Determination of Instability in Colorectal Tumors (PREDICT) model. This model was superior in both sensitivity and specificity compared to the MsPath model. The authors conclude that histologic evaluation is superior to family history for identifying MSI-H CRCs and is an efficient and cost-effective method compared with collection of a detailed family history and confirmation of cancer diagnoses. Pathological evaluation is also more useful in situations where family history data is limited and uninformative for identifying possible LS families. The PREDICT model can direct MSI testing to only those tumors likely to
be MSI-H, reducing the number of tumors to be tested. Prediction of MSI-H tumors is an important first step in identifying CRC patients most likely to have LS.

2.4.2 Immunohistochemistry

Immunohistochemistry analysis uses specific antibodies to identify the presence or absence of MMR proteins (MLH1, MSH2, MSH6 and PMS2) in tumor tissue. Immunohistochemistry is considered abnormal when one or more of the proteins is absent in the tumor tissue [Thibodeau 1996; Muller 2001; de Jong 2004]. Since the MMR proteins form heterodimer complexes, distinct IHC patterns can be expected (Table 2.3).

The MSH2 protein can form a heterodimer with either MSH6 or MSH3, whereas MSH6 can only pair with MSH2. Therefore, the specific IHC pattern observed in tumors of MSH2 carriers consists of absent staining of MSH2 and MSH6 and normal staining of MLH1 and PMS2, whereas, if there is a germline mutation in MSH6, MSH2 is normally stable because it can pair with MSH3 and the tumor will generally exhibit the absence of MSH6 only. Similarly, MLH1 can pair with PMS1, PMS2 or MLH3, but PMS2 can only pair with MLH1. Therefore, the IHC pattern for colorectal tumors from carriers of an MLH1 mutation consists of absent staining for MLH1 and PMS2 and normal staining for MSH2 and MSH6, whereas, the IHC pattern for tumors from PMS2 mutation carriers generally consists of only absent staining of the PMS2 protein [Vasen 2007a; Hampel 2009]. These IHC patterns are a general rule of thumb for determining which MMR gene is causing LS but inconsistent findings are not uncommon [Woods 2010].
Immunohistochemistry is especially indicative of MMR mutations that result in truncation of the protein (such as nonsense, frameshift, splice site mutations and large genomic rearrangements) but is not always diagnostic in the case of missense mutations as the protein can be functionally abnormal but still be detected by IHC [Ramsoekh 2007; Hendricks 2006].

**Table 2.3 IHC patterns associated with MMR mutations** [Ramsoekh 2007]

<table>
<thead>
<tr>
<th>IHC Staining</th>
<th>MLH1</th>
<th>MSH2</th>
<th>MSH6</th>
<th>PMS2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLH1</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MSH2</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MSH6</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>PMS2</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

MMR, mismatch repair; IHC, immunohistochemistry

### 2.4.3 Performance of Molecular Prescreening

Several studies have evaluated the results of MSI or IHC analysis in CRC tissue for the identification of MMR gene mutations. Palomaki et al. [Palomaki 2009] performed an evidence-based review of MSI and IHC as prescreening tests for the detection of MMR mutations and provided sensitivities and specificities for each test. They found that the sensitivity of MSI was 89% for patients with MLH1 and MSH2 mutations, and 77% for patients with MSH6 mutations. Sensitivities were lower if less than three mononucleotide repeats were included in the panel of microsatellites tested. Specificity of MSI testing was found to be 90%. The sensitivity of IHC was 83% regardless of the underlying MMR
gene involved, and specificity was 89%. This review, however, did not assess the sensitivity of MSI and IHC on the same tumor to screen for LS.

Several prospective studies have evaluated the effectiveness of using both MSI and IHC on the same tumor for the detection of MMR mutations. One study to perform such an analysis was conducted by Pinol et al. [Pinol 2005] and evaluated over 1200 tumors. This population based study found that the sensitivity and specificity of MSI-H status was 91% and 94%, respectively, for patients with MLH1 and MSH2 mutations. The sensitivity of IHC was lower at 82% but specificity was the same at 94%. In this study, MSI missed one MSH2 mutation (the tumor was MSS) and IHC missed one MLH1 mutation and one MSH2 mutation. Another study performed MSI and IHC for the four MMR proteins on 500 tumors from unselected CRC patients [Hampel 2008]. They found sensitivities of 100% and 94% and specificities of 90% and 88%, respectively, for MSI and IHC screening. A third study evaluated a population-based sample of unselected CRC patients diagnosed at age less than 45 years [Southey 2005]. In this higher risk group, MSI-H status was found to have a sensitivity of 72% and a specificity of 94% for patients with MLH1, MSH2, MSH6 and PMS2 mutations. IHC analysis was found to have a sensitivity of 100% and a specificity of 94%. Of the five mutations missed by MSI analysis, four were MSH6 mutations with MSI-L status and one was a PMS2 mutation which was MSS.

A combination of MSI and IHC provides the most optimal selection for mutation analysis, however there is no consensus on whether MSI or IHC should be used as the
first step. MSI can indicate the presence of undiscovered pathogenic genes, whereas IHC can only detect loss of protein expression for already identified genes. Therefore, it has been suggested that IHC be used as the first step in families with a high probability of carrying a mutation (e.g., families that fulfill the Amsterdam criteria) because the result can indicate which gene is mutated and direct mutational testing [Vasen 2007b]. MSI analysis can be performed first on families that fulfill the Bethesda criteria. In these cases, MSI can provide information on loss of MMR function for both the known MMR genes and for those yet to be identified. In those found to have MSI-H and MSI-L tumors, IHC can then be performed as a second step [Hendricks 2006; Vasen 2007a, 2007b]. Other factors to be considered are time and cost. IHC is a faster, less expensive test than MSI and can direct mutational testing to the affected gene. Therefore, due to the fact that IHC has been shown to be almost equally effective as MSI [Pinol 2005; Hampel 2008] some studies advocate IHC as the first step.

Although the majority of tumors from patients with LS show high levels of MSI, restricting mutation analysis to MSI-H tumors only can miss a proportion of *MSH6* and *PMS2* mutation carriers. Several studies have shown that LS patients with an *MSH6* mutation had tumors that were MSI-L [Southey 2005; Barnetson 2006; Niessen 2006] and MSS [Barnetson 2006]. Similarly, patients with a *PMS2* mutation may have a lower rate of MSI-H tumors than patients with *MLH1* and *MSH2* mutations [Southey 2005]. Also, in cases with a strong family history suggestive of LS but a MSS tumor, MSI analysis on a second tumor from the family should be performed to exclude the
possibility of a phenocopy [Vasen 2007a]. A phenocopy occurs when an individual shows the features characteristic of a particular genotype but is produced environmentally rather than genetically.

Microsatellite instability and IHC is preferably performed on CRC tumor tissue, however, if this is not possible other tumor tissues can be analyzed (e.g. endometrial cancer). Unfortunately, the value of MSI/IHC in other LS-associated tumors is largely unknown. An American study looked at the feasibility of molecular screening among unselected endometrial cancer patients and found that MSI status in these patients was less sensitive compared with MSI performed on CRC tumors [Hampel 2006]. Of the 10 deleterious germline mutations identified (1 MLH1, 3 MSH2 and 6 MSH6), three MSH6 mutations were missed by MSI analysis (2 were MSI-L and 1 was MSS). Similar results were found in other studies and may be directly owing to the fact that a large proportion of endometrial carcinomas in MSH6 mutation carriers exhibit a MSI-L or MSS phenotype [de Leeuw 2000].

2.4.4 MLH1 Promoter Methylation and BRAF Testing

Sporadic MSI-H tumors are typically due to somatic hypermethylation of the MLH1 promoter region which leads to epigenetic silencing of the gene. Large population based studies have shown that a high proportion of MSI/hMLH1 loss cases to be sporadic [Pinol 2005; Hampel 2008]. Therefore, in tumors with an MSI-H phenotype and absent staining of MLH1, direct measurement of the methylation status of MLH1 in the tumor
should be performed as well as genetic analysis of the \textit{BRAF} gene. Mutations in the \textit{BRAF} gene can cause disease in two ways. First, mutations can be inherited and cause birth defects. Second, mutations can appear later in life and cause cancers, such as CRC. \textit{BRAF} mutations have been identified in approximately 70\% of CRC tumors from individuals with \textit{MLH1} promoter methylation but have yet to be identified in patients with a \textit{MLH1} germline mutation [Palomaki 2009; Woods 2010]. Thus, performing additional \textit{BRAF} testing and \textit{MLH1} promoter methylation analysis can differentiate between sporadic and LS-associated MSI-H tumors.

\textbf{2.5 \hspace{1em} DIAGNOSIS OF LYNCH SYNDROME}

Lynch syndrome is defined in terms of having a germline mutation in one of the DNA MMR genes. However, it is not feasible to test every CRC patient for one of these mutations [Lynch 2009a]. Diagnosis of LS is made even more difficult due to the fact that LS has a variable phenotype with respect to tumor site, age of onset and penetrance of disease. Identification of family members with an MMR gene mutation is very important for screening and management purposes as screening can be restricted to these individuals, whereas those without a mutation may be spared intensive surveillance [Ramsoekh 2007; Vasen 2007b]. There is no "gold standard" test for diagnosing LS but several strategies for identification of individuals with LS have been proposed. Family history based clinical criteria have been used to identify high risk families for further analysis, universal screening of all colorectal tumors has been proposed as a possible strategy for LS identification, algorithms have been designed for evaluation of patients
with suspected LS and models have been developed to predict the likelihood of carrying a germline mutation.

2.5.1 Clinical Diagnostic Criteria

Identification of families at risk for LS should be based on clinical and family history criteria. Historically, the Amsterdam criteria were used in clinical practice to identify high risk families for further analysis. More than 50% of families with LS, however, fail to meet these criteria. As a result, the Amsterdam II and Bethesda guidelines were developed to try to identify a larger proportion of individuals at risk for LS.

2.5.1.1 Amsterdam I and II

In 1990, the ICG-HNPCC established research criteria for the diagnosis of LS [Vasen 1991]. These criteria, known as the Amsterdam criteria, included the following: 1) at least three relatives with CRC, one of them a first degree relative of the other two; 2) at least two successive generations affected; and 3) at least one CRC should be diagnosed before the age of 50. Also, all tumors should be verified by pathological examination and FAP should be excluded. These criteria were developed to standardize the diagnostic criteria for LS and to provide a basis for uniformity in collaborative studies.

The initial Amsterdam criteria (Amsterdam criteria I) did not account for the extracolonic cancers found in LS and missed a number of at-risk individuals. For these reasons the Amsterdam criteria were revised in 1999 and included several extra colonic tumors.
These criteria, known as the Amsterdam criteria II, differed from the original criteria by including families with three relatives with an HNPCC-associated cancer (CRC, endometrial, small bowel, ureter or renal pelvis) as opposed to three relatives with CRC [Vasen 1999]. The Amsterdam criteria II were used in clinical practice to select individuals for mutation analysis of the MMR genes, however, these criteria were too stringent and many LS families were missed. In a large population-based review that performed MSI and DNA MMR gene testing on consecutive CRCs, only 42% of mutation carriers met the Amsterdam criteria II [Barnetson 2006]. Therefore, a family which does not fulfill these criteria may still have a mutation in one of the MMR genes and should not be falsely reassured and excluded from genetic counseling, DNA testing or surveillance [Ramsoekh 2007].

2.5.1.2 Bethesda Guidelines

In 1996, the National Cancer Institute hosted an international workshop on HNPCC and proposed a set of guidelines that were later updated in 2004 [Rodriguez-Bigas 1997; Umar 2004]. The Bethesda guidelines were developed to select patients whose colorectal tumors should be tested for MSI. Tumors found to have microsatellite instability were subsequently tested for an MMR gene mutation. The revised Bethesda guidelines are listed in Table 2.4.
Table 2.4 The revised Bethesda guidelines for testing colorectal tumors for microsatellite instability (MSI)  

| Tumors from individuals should be tested for MSI in the following situations: |
| 1. Colorectal cancer diagnosed in a patient who is less than 50 years of age. |
| 2. Presence of synchronous or metachronous colorectal or other HNPCC-associated tumors, regardless of age. |
| 3. Colorectal cancer with MSI-H\textsuperscript{b} histology\textsuperscript{c} diagnosed in a patient who is less than 60 years of age. |
| 4. Colorectal cancer diagnosed in a patient with one or more first degree relatives with an HNPCC-related tumor, with one of the cancers being diagnosed under age 50 years. |
| 5. Colorectal cancer diagnosed in a patient with two or more first- or second-degree relatives with HNPCC-related tumors, regardless of age. |

\textsuperscript{a} Hereditary nonpolyposis colorectal cancer (HNPCC)-associated tumors include colorectal, endometrial, stomach, ovarian, pancreas, ureter or renal pelvis, biliary tract, and brain (usually glioblastoma as seen in Turcot syndrome) tumors, sebaceous gland adenomas and keratoacanthomas in Muir–Torre syndrome, and carcinoma of the small bowel.

\textsuperscript{b} MSI-H = microsatellite instability-high in tumors refers to changes in two or more of the five National Cancer Institute-recommended panels of microsatellite markers.

\textsuperscript{c} Presence of tumor infiltrating lymphocytes, Crohn’s-like lymphocytic reaction, mucinous/signet-ring differentiation, or medullary growth pattern.

\textsuperscript{d} There was no consensus among the Workshop participants on whether to include the age criteria in guideline 3 above; participants voted to keep less than 60 years of age in the guidelines.

Several studies have looked at whether the Amsterdam criteria II and revised Bethesda guidelines are adequate for identifying LS patients. To determine if these criteria are appropriate, studies have looked at the proportion of cases with an MMR gene mutation (mainly \textit{MLH1} and \textit{MSH2} mutations) that would be missed in a series of unselected patients with CRC. Vasen et al. [Vasen 2007b] evaluated six studies [Aaltonen 1998; Debnik 2000; Salovaara 2000; Cunningham 2001; Hampel 2005a; Pinol 2005] and found that the sensitivity of the Amsterdam criteria II for detection of LS mutation...
carriers was around 40%, while the sensitivity of the revised Bethesda guidelines was approximately 90%. The Bethesda guidelines have a high sensitivity, however, due to their less restrictive nature they have a low specificity; 80% or more of patients who meet the Bethesda guidelines will not have LS [Vasen 2007b].

2.5.2 Universal Testing Strategies

Several factors, including small families, unrelated deaths, variable phenotype, and erroneous reporting have all limited the utility of the Amsterdam criteria and Bethesda guidelines in identifying LS families. Due to the fact that a substantial proportion of individuals with LS would be missed using these clinical diagnostic criteria, studies have looked at performing MSI and/or IHC on all CRC patients to screen for MMR deficiency [Cunningham 2001; Hampel 2005a, 2008; Julie 2008]. Similar work has also been done among endometrial cancer patients [Hampel 2006].

One of the first studies to examine the frequency of inherited CRC due to MMR deficiency found that 51 of 257 (20%) unselected patients referred for CRC resection had evidence of defective MMR (MSI-H) [Cunningham 2001]. Of the 51 patients with defective MMR, seven were found to have a germline mutation, and of these seven individuals, only three had family histories that met the Amsterdam criteria. In one of the largest studies to evaluate a universal screening approach, 208 of 1066 (20%) patients were found to have MSI (135 MSI-H, 73 MSI-L), and 23 of these patients had a germline mutation causing LS [Hampel 2005a]. Among the 23 individuals found to have LS, only
three met the Amsterdam criteria, 18 met the Bethesda criteria and five did not meet either of these criteria. Similarly, a study evaluating 214 consecutive, newly diagnosed, CRC patients found that of the eight patients identified with a germline mutation, only two of eight and six of eight met the Amsterdam II and revised Bethesda criteria, respectively [Julie 2008]. These studies show that 2 to 4% of unselected CRC cases are due to LS and that a substantial proportion of cases (more than 20%) would be missed using only the Amsterdam or Bethesda criteria. However, to find these extra cases would require a complex and expensive effort and more research is needed to determine the feasibility and cost effectiveness of these strategies.

A recent study evaluating a comprehensive screening approach also compared the suitability of MSI or IHC as the primary screening method in detecting LS-associated CRC [Hampel 2008]. Among the 500 CRC patients studied, 18 were found to have a germline mutation. All 18 patients with LS had MSI-H tumors and 17 of 18 patients with LS were correctly predicted by IHC analysis. This study showed that MSI and IHC were quite similar in having high sensitivity to detect LS in population-based studies. In large-scale screening studies, performing IHC instead of MSI as the primary screening method, will lead to considerable savings in time, effort and cost.

Due to the high risk of endometrial cancer among LS patients, work has also been done to determine the frequency of LS among endometrial cancer patients. One such study evaluated the feasibility of molecular screening among 543 unselected endometrial
cancer patients [Hampel 2006]. All tumors underwent MSI testing and 118 patients were found to have MSI-positive tumors (98 MSI-H, 20 MSI-L). Of those with a MSI-positive tumor, nine were found to have a deleterious germline mutation. One additional patient with a MSI-negative tumor was also found to have a mutation. Of the 10 LS patients identified, three met the Amsterdam II criteria, two met the revised Bethesda criteria, and seven did not meet either of these criteria. This study shows that approximately 2% of unselected endometrial cancer cases are due to LS and that 70% of cases would be missed using only clinical diagnostic criteria.

2.5.3 Mutation Prediction Models

Due to the limitations of the Amsterdam and Bethesda criteria in predicting patients with LS, strategies involving universal testing of all CRC tumors for MSI and/or IHC were undertaken. These studies demonstrated that a substantial proportion of LS patients would be missed if only clinical diagnostic criteria were used but that it would require a complex and expensive effort to find additional patients using a comprehensive molecular screening approach. Even if universal MSI/IHC screening of all CRC tumors was feasible, it may still fail to identify cases in which MMR mutations disrupt MMR function but do not result in MSI or when IHC results are found to be normal despite a nonfunctional MMR protein.

In recent years predictive models have been developed in an attempt to improve upon the Amsterdam and Bethesda criteria and to reduce the burden of population-based molecular
screening in detecting patients with LS. These models utilize personal and family history to predict the likelihood that an individual carries an MMR gene mutation. A major advantage of prediction models is that these models provide a quantitative estimation of the likelihood of an individual carrying a mutation instead of a bivariate (yes/no) assessment as given by clinical diagnostic criteria. Models vary widely with respect to the MMR genes they predict, the patient populations used to develop and validate the model, the clinical variables used to predict risk and in the statistical methodology used.

Wijnen et al. [Wijnen 1998] were the first to develop a multivariable model for prediction of $MLH1$ and $MSH2$ mutations. They identified mean age at diagnosis of CRC within a family, fulfillment of the Amsterdam criteria, and presence or absence of endometrial cancer in the family, as significant predictors of the presence of $MLH1$ and $MSH2$ mutations. More recently, the MMRpredict [Barnetson 2006], PREMM$_{1,2}$ [Balmana 2006] and MMRpro [Chen 2006] models have been developed and validated. The MMRpredict and MMRpro models estimate the probability of carrying a deleterious mutation in MMR genes $MLH1$, $MSH2$, and $MSH6$, whereas the PREMM$_{1,2}$ model was originally developed to predict mutations in $MLH1$ and $MSH2$ genes. The PREMM$_{1,2}$ has recently been expanded to include $MSH6$ mutations (PREMM$_{1,2,6}$) [Kastrinos 2011].

2.5.3.1 Validation of Models

The performance of the above mentioned prediction models in clinical practice and their ability to predict mutation carrier status in cohorts with varying risks of CRC have
recently been published [Balmana 2008; Green 2009; Pouchet 2009; Monzon 2010]. Two studies have looked at mutation prediction models in low risk settings and found varying results. Balmana et al. [Balmana 2008] evaluated the PREMM\(_{1,2}\) and MMRpredict models in a large, low-risk, population-based cohort of over 1200 newly diagnosed CRC cases and were unable to demonstrate that either of these models were superior to each other or the revised Bethesda guidelines at predicting an MMR mutation. This study, however, included only eight mutation carriers and no statistical comparisons were made. Another study compared the diagnostic utility of four models (Leiden, MMRpredict, PREMM\(_{1,2}\) and MMRpro) in a population based cohort of over 700 consecutive patients with CRC [Green 2009]. After correcting for family size, Green et al. found that the MMRpredict model was better at predicting patients who were at high risk of carrying an MMR mutation.

Pouchet et al. [Pouchet 2009] and Monzon et al.[Monzon 2010] looked at the performance of these models in predicting a LS-causing mutation in individuals with a family history of CRC. Pouchet et al. evaluated three models (MMRpredict, PREMM\(_{1,2}\) and MMRpro) and found that all three carried out well in a cancer genetics setting, with PREMM\(_{1,2}\) having slightly better predictive abilities. Monzon et al. evaluated these three models along with the Wijnen and Myriad Genetics models [Myriad Genetics Laboratory] and found similar results, with the PREMM\(_{1,2}\) model having the best predictive performance. More studies should be performed to corroborate these findings in both low-risk and moderate- to- high risk CRC populations.
2.5.4 Mutation Analysis

Due to the heterogeneity of the mutation spectrum in MMR genes, germline mutation testing in LS is very expensive and time consuming and is normally only recommended when high risk individuals have been identified. Therefore, mutation analysis is normally performed after MSI and/or IHC have been shown to be indicative of a germline mutation or if there is a very high suspicion of a mutation due to family history [Ramsoekh 2007]. When MSI and IHC analysis do not indicate abnormalities, germline mutation testing is not useful.

The presence of a deleterious germline mutation in one of the MMR genes confirms the suspected clinical diagnosis of LS. Germline mutations resulting in LS have been found in 4 MMR genes: MSH2 [Fishel 1993], MLH1 [Bronner 1994], PMS2 [Nicolaides 1994] and MSH6 [Akiyama 1997; Miyaki 1997]. Both point mutations and large genomic deletions have been identified and, as such, gene testing should include both full sequencing of the gene and large rearrangement testing. Large deletions account for approximately 26% of MSH2 mutations, 22% of MLH1 and PMS2 mutations and 7% of MSH6 mutations [Hampel 2009].

Mutation analysis is performed in DNA from blood derived lymphocytes and should be performed on the youngest family member with CRC. Gene testing can be carried out using a variety of techniques: denaturating gradient gel electrophoresis (DGGE), multiplex ligand dependent probe amplification (MLPA) for the detection of large
genomic deletions, and direct sequencing. Once a germline mutation is identified in an affected individual, presymptomatic diagnostic testing can be offered to healthy family members. This type of testing has lower cost and higher accuracy than the initial gene testing needed to identify the specific mutation [Lynch 2009b]. A mutation in any of the four MMR genes is diagnostic for LS, however, sometimes the result of genetic testing is less clear. Genetic variants of uncertain significance are detected fairly frequently (~7%) in the MMR genes and can make interpretation of the genetic test results difficult [Hampel 2009]. To determine whether the mutation is likely to be deleterious or if the variant is tracking with cancer in the family, genetic testing for these variants can be carried out in other affected family members, but even these results may be inconclusive. Until the genetic variant has been determined to cause LS, predictive testing should not be offered to at-risk relatives.

2.5.5 Familial Colorectal Cancer Type X (FCCTX)

Almost half the families meeting the Amsterdam-I criteria do not have LS by the current definition; they do not have MSI or show abnormal staining for the MMR proteins. Clustering of CRC by chance or yet-to-be defined genetic defects may be responsible for the disease pattern seen in these families. The term ‘familial colorectal cancer type X (FCCTX)’ has been used to describe families that fulfill the Amsterdam-I criteria but who have no evidence of deficient MMR [Lindor 2009]. Lindor et al. [Lindor 2005] compared cancer risks in Amsterdam I families with an MMR mutation to Amsterdam I families without a MMR mutation. Families in the latter group did not share the same
cancer incidence as LS families (i.e., MMR deficiency): CRC risks were lower and risks for other LS-associated cancers did not appear to be increased. Other studies have shown a later age of onset for CRC and a greater proportion of left-sided tumors versus right-sided tumors for FCCTX families compared to LS families [Llor 2005; Mueller-Koch 2005; Valle 2007; Woods 2010]. Slower progression from adenoma to carcinoma and fewer synchronous, metachronous and extracolonic cancers have also been reported. These differences in the clinical features and the differences in the molecular features of the FCCTX tumors [Abdel-Rahman 2005] distinguish this group from those with LS and from those with sporadic CRC.

2.6 GENETIC TESTING & SCREENING IN LYNCH SYNDROME

The key to managing and preventing cancer development in LS is early diagnosis through a comprehensive family history, followed by germline mutation testing if appropriate, and targeted screening and management for patients with mutations [Lynch 2009b].

2.6.1 Genetic Testing and Counseling

There are multiple benefits to genetic testing in LS, including a more accurate diagnosis, determination of risk for other family members and targeted screening and surveillance options for positive gene carriers. However, genetic testing often provides a diagnosis long before any symptoms are experienced and this can have considerable psychosocial and management consequences. It is therefore recommended that individuals receive appropriate counseling and provide informed consent before genetic testing. The
informed consent document should contain a general description of the test, including the benefits, risks and limitations of testing, and the meaning of positive, negative and uninformative results.

The protocol for genetic testing of the American Society of Clinical Oncology recommends that three sessions be performed [ASCO 2003]. During the first session, issues relating to the reasons for testing, the clinical features of the hereditary CRC syndrome, the mode of inheritance, the consequences of the test results, the options for treatment in case of a positive test result, and the DNA testing procedure itself are discussed. In the second session blood samples are taken and in the third session the test results are disclosed. The third session also involves reviewing preventative surveillance and surgical recommendations, exploring the psychosocial impact of the result, and discussing the importance of disclosure to family members and the impact the results may have on them [Aronson 2009].

As was stated earlier, once a mutation has been detected in an affected individual, healthy family members can undergo mutation-specific testing. One of the strongest motivators for presymptomatic testing is the relief from uncertainty. Other motivators include the desire to define the risk to their children and the need to determine appropriate surveillance and management options for themselves. Deterrents to genetic testing include concerns about loss of health insurance, impact on the family and the psychologic impact of a positive test result [Aronson 2009]. A positive test result may lead to
emotional distress regarding personal cancer risk and the need for frequent surveillance or prophylactic surgery. A negative test may result in emotional relief and avoidance of unnecessary surveillance but may also lead to feelings of guilt towards affected relatives [Ramsoekh 2007]. Genetic testing has both benefits and disadvantages, but it is necessary for providing targeted screening and management programs for at-risk family members.

2.6.2 Screening in Lynch Syndrome

Screening and management programs for LS are based on knowledge of the natural history of disease and the cardinal features of LS. Cancer-related morbidity and mortality may be significantly reduced through highly targeted screening programs that take into account the types of cancers involved, the age at onset of these cancers and the risk of developing these cancers for affected individuals. Presently, screening for CRC in LS requires complete colonoscopy to the cecum every 1 to 2 years starting at ages 20 to 25 years, or 10 years younger than the youngest age of the person diagnosed in the family. However, due to the attenuated phenotype (later age at CRC onset and lower penetrance) seen in families with an MSH6 or PMS2 mutation, the recommendation is to start at age 30 years [Lindor 2006; Lynch 2008a; Senter 2008]. Gynecologic screening requires endometrial biopsy, transvaginal ultrasound, and CA 125 testing, every 1 to 2 years starting at ages 30-35 years [Lindor 2006; Lynch 2008a; Schmeler 2008; Meyer 2009]. Other screening procedures include endoscopy for families with a history of gastric cancer and urine cytology and ultrasound for evaluation of the ureter and renal pelvis.
Furthermore, education and genetic counselling regarding LS should be initiated at age 21 years [Lindor 2006; Lynch 2008a].

2.6.2.1 Colon Screening

Although LS accounts for only a small percentage of the total CRC burden, the high risk of cancer among affected family members makes screening an important clinical endeavor [Johnson 2006]. Detection and treatment of CRC at an early stage can save lives, however, prevention of CRC by colonoscopic screening may have a far greater impact on morbidity, mortality and the economic burden to the healthcare system [Helm 2003; Green 2009; Kopciuk 2009].

Determining the impact of screening in LS is difficult because ethically it is not possible to randomly allocate mutation carriers to “no screening”. As such, there are no randomized, controlled, clinical trials looking at the effectiveness of screening in LS [Johnson 2006; Stupart 2009]. Several observational studies on the efficacy of regular colonoscopic screening have been reported [de Vos tot Nederveen Cappel 2002; Dove-Edwin 2005, 2006; de Jong 2006b; Mecklin 2007; Engel 2010; Vasen 2010].

Currently, two cohort studies provide the best available evidence in support of colonoscopic screening in LS [Jarvinen 2000; Stupart 2009]. A prospective cohort study by Jarvinen et al. [Jarvinen 2000] evaluated the efficacy of screening in a prospective cohort study over 15 years. This study involved a heterogeneous group of patients and
included patients with mutations of several mismatch repair genes and also patients with no known mutation but who met the clinical criteria for LS. This study demonstrated a significant difference in the rate of CRC and death between those who were screened every 3 years and those who did not receive screening. Colonoscopic screening was found to decrease the CRC rate by 62%, prevent CRC deaths, and to decrease overall mortality by about 65% in LS families. A more recent prospective cohort study by Stupart et al. [Stupart 2009] investigated whether screening colonoscopy improves survival in subjects who carry a single MLH1 germline mutation. This study showed that screening colonoscopy was associated with improved overall and CRC-related survival and that the median age at onset of CRC was delayed by more than 20 years in the screened group.

The previous two studies both provide evidence of the benefit of colonoscopic screening in LS but neither adjusted for survivor bias nor evaluated screening based on gender. Survivor bias is inherent in screening studies as only those who are alive and disease-free can enter primary screening programs leading to an overestimation of the impact of screening. Due to the phenotypic difference in age at onset and lifetime risk of CRC between male and female mutation carriers [Green 2002; Stuckless 2007; Kopciuk 2009], and the potential differences in enrollment and compliance between males and females, screening effectiveness may differ by gender.
Screening may never completely prevent CRC from developing, but it may be possible to further improve the reduction in the number of CRCs diagnosed by reducing the screening interval to one year. Previously, Lynch et al. [Lynch 2008a], argued for a shorter screening interval due to the relatively high incidence of CRC in screened subjects with LS. Given the high incidence of CRC and the accelerated adenoma-carcinoma sequence in LS [de Jong 2004b], annual screening colonoscopies may be appropriate for this group. In 2006, de Jong et al. [de Jong 2006] studied colonoscopic screening in 215 mutation carriers aged 40-60 years. Of the 34 screen-detected CRCs identified, 13 were diagnosed within two years of a previously normal screening colonoscopy. More recently, a study by Vasen et al. [Vasen 2010] evaluated the effectiveness of a one-to-two year screening interval in reducing the risk of CRC in LS. Thirty-three of 745 (4.4%) mutation carriers developed CRC under surveillance. Of these 33, 14 developed their cancer within the 1-2 year recommended screening interval and an additional two developed it within one year. Another recent prospective cohort study by Engel et al. [Engel 2010] evaluated the efficacy of annual colonoscopies in detecting adenomas and CRCs. This study found that 19 of 43 interval cancers detected by screening were preceded by a normal colonoscopic exam within the recommended interval of 12 months. In the absence of a prospective controlled trial to determine the optimal screening interval, these studies provide evidence for reducing the screening interval.
Improvement in the technique of colonoscopy examinations may also reduce CRC development. As was stated earlier, Engel et al. [Engel 2010] found that 19 individuals developed CRC within 1 year of their previous colonoscopy. These tumors were likely to have been missed lesions resulting from poor quality colonoscopic examinations. Studies have shown that more than 50% of adenomas in LS are missed by conventional colonoscopy and that many of these missed lesions are small, flat adenomas [Hurlestone 2005; Lecomte 2005; East 2008; Stoffel 2008]. Given the accelerated adenoma to carcinoma sequence in LS, improved detection of these small lesions may be very important in preventing CRC tumors from developing.

Hurlstone et al. [Hurlestone 2005] and Lecomte et al. [Lecomte 2005] compared back-to-back examinations in which standard colonoscopy was followed by chromoendoscopy, performed by spraying dye (indigo carmine solution) on the colorectal mucosa during colonoscopy, and found that chromoendoscopy more than doubled the number of adenomas detected in individuals with LS. East et al. [East 2008] found that using narrow band imaging (a novel endoscopic technology that highlights superficial capillaries in the mucosa and improves contrast for adenomas) as the second exam nearly doubled the number of adenomas detected in LS patients. Stoffel et al. [Stoffel 2008] compared chromoendoscopy versus standard colonoscopy with intensive inspection (lasting > 20 minutes) for detecting polyps missed by conventional colonoscopy. They found that the second examinations more than doubled the number of adenomas detected but that there was no significant difference between the use of chromoendoscopy and intensive
inspection in detection of additional adenomas. More recently, a study by Ramsoekh et al. [Ramsoekh 2010] found that the use of autofluorescence endoscopy, a technique specifically designed to probe large areas of mucosa using short wavelength light (typically blue light) to detect neoplasias, resulted in a 34% increase in the adenoma detection rate. Prevention of CRC in this high risk group is important and further evaluation is needed to determine the most effective colonoscopic imaging technique for detecting adenomas.

2.6.2.2 Gynecological Screening

Endometrial cancer is the most common extracolonic tumor associated with LS and in some mutation carriers the risk of developing endometrial cancer exceeds the risk of CRC development [Dunlop 1997; Aarnio 1999; Hendricks 2004; Hampel 2005; Stuckless 2007]. Female mutation carriers are also at an increased risk of developing ovarian cancer. Various studies have shown colonoscopic screening in LS mutation carriers to reduce the risk of CRC development and improve survival [Jarvinen 2000; de Jong 2006b; Stupart 2009]. However, unlike CRC, the efficacy of screening for gynecological cancers in LS remains controversial.

The survival rate of LS-associated endometrial cancer has previously been proven to be favorable with an overall 5-year cumulative survival rate of 88% [Boks 2002]. Given the early stage at presentation and the good prognosis of endometrial cancer, it is unknown
whether screening for this cancer is necessary at all or if it would improve morbidity or mortality in female LS carriers.

Annual or biennial screening for endometrial cancer using TVU (or in some cases transabdominal ultrasound) was evaluated in 269 women from families suspected of having LS [Dove-Edwin 2002]. During a 13 year observation period, the study detected no premalignant lesions or endometrial cancers, but two interval cancers were detected approximately 5 months and almost 2 years after a normal ultrasound. Both tumors were diagnosed at an early stage (FIGO stage I). FIGO staging is a tumor staging system established and revised by the International Federation of Gynecology and Obstetrics (FIGO) that takes into account the postoperative surgical pathology of the specimen. A study of 41 women from LS families, who underwent screening by transvaginal ultrasound followed by aspiration biopsy in suspected cases, found three malignant lesions (with complex atypical hyperplasia) after a median follow-up of 5 years [Rijcken 2003]. No endometrial cancers were detected by screening but one interval cancer was diagnosed as a result of clinical symptoms 8 months after a normal transvaginal ultrasound. This tumor was diagnosed at an early stage (FIGO stage IB). Another study reported the results of screening by TVU combined with aspiration biopsy for the detection of endometrial cancer [Renkonen-Sinisalo 2006]. This study evaluated 175 LS mutation carriers and found 11 screen detected cancers and 14 premalignant lesions which may become cancerous. Two interval cancers were also diagnosed 3 and 31 months after a previous screening visit. One additional endometrial cancer was found
during prophylactic hysterectomy that was performed in addition to a colectomy for cancer. Out of the 11 screen-detected cancers, six would have been missed without routine endometrial sampling. Women in the screened group were also compared to 83 mutation carriers with endometrial cancer who did not undergo screening. The stage distribution and mortality tended to be more favorable in the 14 endometrial cancer cases in the screened group (no deaths) compared to the 83 symptomatic mutation carriers (7 deaths). This difference in survival curves (100% vs. 92% at 10 years), however, was not statistically significant.

More recently, a study of 100 women belonging to suspected Lynch syndrome families found three atypical hyperplasias and one endometrial cancer in 64 visits where routine endometrial samplings were performed [Gerritzen 2009]. One case of atypical hyperplasia and two endometrial cancers were also detected in 28 samples performed because of abnormal screening results in 221 visits. These studies suggest that endometrial cancer screening with routine endometrial biopsy is more efficient in diagnosing endometrial (pre)malignancies than TVU alone. They provide guidance on how to perform screening but they do not fulfill the criteria for making evidence-based decisions. Although screening for endometrial cancer is controversial due to early stage at diagnosis and good prognosis, there may be benefit if the cancers can be detected at an even earlier stage when hysterectomy alone is effective. Broaddus et al. [Broaddus 2006] found that 22% of their L.S-associated endometrial cancers were stage II or III and would require additional adjuvant radiation therapy or chemotherapy. Detection of very early
stage cancers can therefore provide the opportunity to avoid extensive adjuvant therapy/treatment.

A recent population-based randomized controlled trial found that annual screening of women with CA125 testing and TVU compared to usual care did not reduce mortality from ovarian cancer (mortality RR=1.18; 95% CI 0.82-1.20 [Buys 2011]. Annual screening of women at increased familial risk of ovarian cancer, using ultrasound and CA-125 measurements, has not led to early stage detection or improved survival [Evans 2009]. Few studies have looked at the usefulness of these tests for ovarian cancer screening in LS families only. Rijcken et al. [Rijcken 2003] found no abnormalities on TVU nor did they find any elevated CA-125 values. No ovarian cancers were diagnosed through screening nor were any interval cancers detected. Another study by Renkonen-Sinisalo et al. [Renkonen-Sinisalo 2006] found four endometroid-type ovarian cancers, however, none were detected by screening. Two cancers were diagnosed by symptoms 2 and 5 months after normal surveillance visits (stages III and I), and two were detected incidentally during an operation performed for endometrial cancer or complex hyperplasia (both stage I). A third study detected one ovarian cancer through screening, however, this cancer was a FIGO stage IIIC cancer [Gerritzen 2009]. Of the five ovarian cancers diagnosed in these three studies, only one was detected through screening and it was found at an advanced stage suggesting no benefit of ovarian cancer screening in this population.
2.6.2.3 Screening for Extracolonic Cancers

Other cancers associated with LS include cancer of the stomach, ureter, renal pelvis, small bowel, bile ducts and tumors of the brain. However, the incidence of many of these cancers is too small to warrant routine screening [Watson 2008]. Experts have suggested that if a family shows a clustering of gastric cancer, surveillance by means of endoscopy examination can be considered, or if a clustering of urothelial cancers is seen, screening for these cancers starting at age 30 to 35 years using annual urine analysis with cytology and renal ultrasounds can be performed. However, screening by these methods is unproven and no data exist on the effectiveness of these approaches [Vasen 2007a; Grover 2010].

2.6.3 Screening in Families Without an Identified Mutation

2.6.3.1 FCCTX

As was stated earlier, FCCTX families are characterized by a more advanced age of onset of CRC and by absence of endometrial cancer or other extracolonic tumors when compared to LS families. Lindor et al. [Lindor 2005] reported that the risk of developing CRC in these families was only increased by a factor of 2.3 and no increased risk was seen for other cancers. They suggest that these FCCTX families are comprised of (1) some cancer aggregation occurring by chance alone, (2) some aggregation related to shared lifestyle factors, and (3) some yet to be defined genetic syndromes. They propose that these families should be managed based on a customized assessment of the pedigree and not automatically screened based on the screening algorithms for LS families.
Another study compared the results of colonoscopic surveillance in families with clustering of CRC with and without MSI [Dove-Edwin 2006]. They found that both groups were at an equal risk of developing high-risk adenomas but that CRC was only identified in families with MSI tumors. They propose that FCCTX families require colonoscopic surveillance but that the interval could be lengthened because the risk of (interval) cancer is low. Based on these studies, Vasen et al. [Vasen 2007b] have suggested the following cancer screening recommendations for FCCTX families: (1) colonoscopy at 3-5 year intervals, starting 5-10 years before the first diagnosis of CRC or at > 45 years, and (2) screening for cancer of the endometrium is not indicated. In families that meet Amsterdam criteria I, but in whom tumor MSI testing or genetic testing is not feasible, screening recommendations should be the same as those proposed for LS families.

2.6.3.2 Novel Genes

Given that a large proportion of high-risk families have no detectable mutations in the most commonly mutated MMR genes, it is possible that other colorectal cancer predisposition genes could be responsible for disease in these families [Woods 2005; Woods 2010]. It is also possible that current mutation detection methods are inadequate to detect large deletions or rearrangements. Families which are highly suspicious for LS but in whom no known mutation was found should follow the same screening recommendations as those proposed for families with a known MMR mutation.
2.7 SURGICAL MANAGEMENT OF LYNCH SYNDROME

2.7.1 Treatment for CRC

Lynch syndrome patients have an increased risk of developing multiple (synchronous and metachronous) CRCs and this has an impact on surveillance and management options for these individuals. The options for surgical management of a colon cancer in LS patients include either a standard segmental colectomy or a subtotal (total) colectomy with ileorectal anastomosis.

Several studies have looked at whether a subtotal colectomy instead of a segmental resection might be the preferred treatment in LS patients. A Finnish study reported that over a 7 year period, metachronous CRCs developed in 41% (15/37) of HNPCC patients who underwent a segmental resection compared to 24% (4/17) of patients who underwent a subtotal colectomy [Mecklin 1993]. In a Dutch study of proven mutation carriers, the 10-year cumulative risk of developing CRC was found to be 16% after partial colectomy and 3.4% after subtotal colectomy [de Vos tot Nederveen Cappel 2002]. More recently, a retrospective study examined patients who had undergone segmental colectomy compared to patients who had a total or subtotal colectomy with ileorectal or ileosigmoid anastomosis [Kalady 2010]. After a median follow-up of almost 7 years, high risk adenomas were detected in 22% of patients who underwent segmental colectomy compared to 11% in the total/subtotal colectomy group. Similarly, 25% of patients in the segmental colectomy group developed a metachronous CRC versus 8% in the total/subtotal colectomy group.
In a study by de Vos tot Nederveen Cappel et al. [de Vos tot Nederveen Cappel 2003], a decision analysis (Markov model) was performed to compare the life expectancy of patients undergoing a subtotal colectomy or a segmental colon resection. The results indicated that a subtotal colectomy performed at a young age (< 47 years) would lead to an increased life expectancy of up to 2.3 years. However, a subtotal colectomy in a 67 year old would lead to an increased life expectancy of up to 0.3 years. A study by Natarajan et al. [Natarajan 2010] found that time to second CRC was shorter for those who underwent limited resection (controls) at initial CRC diagnosis compared to those who underwent a subtotal colectomy(cases). Time to second CRC ranged from 16 to 175 months for the cases and 6 to 160 months for the controls. In view of these findings and the substantial risk of developing a metachronous tumor, it has been suggested that subtotal colectomy with ileorectal anastomosis be the preferred treatment in young patients presenting with CRC, while segmental resection might be appropriate in older patients [Ramsoekh 2007; Vasen 2007b]. Whether patients undergo a subtotal colectomy or a segmental resection, the residual colon and rectum must be evaluated yearly. Ultimately, patient preference and issues related to compliance with follow-up surveillance will be major determinants in this decision.

There is limited data regarding the possible benefit of prophylactic colorectal surgery in patients with LS. Natarajan et al. [Natarajan 2010] found that none of the 8 patients who underwent prophylactic colectomy developed CRC during the study period. In a previous study, a decision analysis model was used to compare the efficacy of surveillance and
prophylactic colectomy in LS mutation carriers [Syngal 1998]. Compared to no intervention, surveillance led to an increased life expectancy of 13.5 years and prophylactic proctocolectomy led to an increased life expectancy of 15.6 years. The benefits of colectomy compared to surveillance decreased with increasing age and when health related quality of life was considered, surveillance led to the greatest quality adjusted life expectancy benefit. Prophylactic colectomy is not generally recommended due to decreased quality of life, but could be considered for patients who are unwilling to undergo regular colonoscopic screening.

### 2.7.2 Prophylactic Surgery for Gynecological Cancers

Lynch syndrome patients are at a high risk of developing endometrial cancer and a moderately increased risk of developing ovarian cancer, and as such, women with LS must decide between continued gynecological screening or prophylactic surgery. During the past several decades more effective and acceptable methods for CRC screening have become available, however, this is not the case for gynecological screening. The methods for endometrial screening are limited and ovarian screening techniques remain unsatisfactory. The diagnosis of interval endometrial and ovarian cancer is not uncommon and false-negative test results occur [Lynch 2007]. While data regarding the efficacy of gynecological screening is lacking, there is evidence of efficacy for prophylactic surgery [Lindor 2006; Vasen 2007b].
Schmeler et al. [Schmeler 2006] conducted a retrospective study to determine whether the risk of gynecologic cancers among women with LS was reduced after prophylactic surgery. Sixty-one mutation carriers who had previously undergone hysterectomy alone or with bilateral salpingo-oophorectomy (BSO) were matched with 210 mutation carriers who had not undergone hysterectomy. No endometrial cancers developed in the women who underwent hysterectomy compared with 69 endometrial cancers (33%) in the controls, however, three endometrial cancers were incidentally diagnosed at the time of prophylactic hysterectomy, two tumors were at stage I and one was at stage II. In addition, 47 mutation carriers who had undergone previous hysterectomy with BSO were matched with 223 carriers who had not undergone surgery. None of the women who had undergone surgery developed ovarian cancer, however, 12 (5%) of the controls developed ovarian cancer. This study showed a decrease in the incidence of gynecological cancers but did not address the effects of prophylactic surgery on survival and on deaths due to gynecological cancers. Further prospective studies with longer follow-up are needed to confirm the role of prophylactic surgery in preventing endometrial and ovarian cancer and in assessing the differences in survival between those who undergo prophylactic surgery and those who do not.

The disadvantages of prophylactic hysterectomy and BSO include surgical complications (most commonly, bleeding, infection, and injuries to the urinary tract and bowel) and premature menopause. In the study by Schmeler et al. [Schmeler 2006], surgical complications occurred in one of the 61 (1.6%) women who underwent prophylactic surgery.
surgery. This is consistent with complications rates of 1% - 9% reported in other studies [Goodno 1995; Kovac 2000].

Despite screening, occult cancers can be found at the time of prophylactic surgery [Schmeler 2006; Pistorius 2006]. At the time of surgery, the uterus and ovaries should be carefully assessed. The pathologist should be advised of the high risk of gynecological cancers and the specimens carefully examined intraoperatively, with frozen sections obtained if necessary. In addition, the surgeon should be prepared to complete staging operation in the case of occult carcinoma [Schmeler 2006].

Given the demonstrated benefit of prophylactic surgery and the lack of data on the efficacy of screening for gynecological cancers, risk-reducing surgery is the recommended option for women who have completed childbearing [Manchanda 2009]. Data derived from Markov modeling confirms that annual screening followed by prophylactic surgery at age 40 years as the most effective gynecologic cancer prevention strategy [Kwon 2008]. Other studies evaluating the cost-effectiveness of management strategies for women with LS using decision analytic models also support risk reducing surgery rather than annual gynecologic screening or annual gynecologic examinations [Chen 2007; Yang 2011]. In addition, women with LS are at an increased risk of synchronous and metachronous cancers, specifically, CRC and endometrial cancer or ovarian cancer [Lu 2005; Schmeler 2006]. It has therefore been suggested that women
who undergo surgery for CRC be offered concurrent prophylactic hysterectomy with BSO [Lindor 2006].

2.7.3 Chemotherapy

Chemotherapeutic regimes for CRC currently include 5FU with or without leucovorin, oxaliplatin and irinotecan [Ramsoekh 2007; Vasen 2007b]. These fluorouracil based agents have proven effective for the treatment of CRC and are considered the gold standard in adjuvant chemotherapy for bowel cancer [Vasen 2007a]. However, the effectiveness of these agents in LS are unknown. A few studies have reported on the efficacy of 5FU in MSI-H tumors but the results have been contradictory [Liang 2002; Fallik 2003; Ribic 2003; Carethers 2004; de Vos tot Nederveen Cappel 2004. Most studies showed that there was no benefit of 5FU based chemotherapy for MSI-H tumors, and in one study, there was even a trend towards a worse outcome for patients receiving 5FU chemotherapy compared to those not receiving treatment. However, one prospective non-randomized trial concluded that for stage IV sporadic CRC, patients with an MSI-H tumor who received 5FU plus leucovorin chemotherapy had a better survival [Liang 2002]. Due to the conflicting results found in these studies, prospective clinical trials are needed before definitive recommendations can be given regarding the effectiveness of chemotherapy in patients with MSI-H tumors [Vasen 2007a].
2.7.4 Chemoprevention in Lynch Syndrome

Data from observational studies and randomized clinical trials have demonstrated that the use of aspirin leads to a moderate reduction (20-30%) in the risk of sporadic colorectal adenomas and cancer [Baron 2003; Sandler 2003; Logan 2008]. A systematic review examining the benefits and harms of aspirin chemoprevention in average risk individuals found that the use of aspirin, especially if used in high doses for more than 10 years, reduces the incidence of colonic adenomas and CRCs [Dube 2007]. However, the data regarding a reduction in CRC incidence was inconsistent for cohort studies and randomized controlled trials. As well, the use of high dose aspirin increases the incidence of gastrointestinal complications, and as such, the possible harms should be considered. The use of resistant starch has also been suggested as a chemopreventative candidate for decreasing the risk of CRC [Vasen 2007a].

A recent randomized, placebo-controlled trial evaluated the use of aspirin and resistant starch in reducing the risk of adenoma and carcinoma in LS mutation carriers [Burn 2008]. They found no significant difference in neoplasia development between those who received aspirin compared to those who received a placebo (18.9% versus 19% respectively), nor did they find any significant difference between these groups with respect to the development of advanced neoplasia (7.4% vs. 9.9%, p=0.33). Similarly, no significant difference in neoplasia development was reported for those who received resistant starch compared to those who received placebo (18.7% versus 18.4 %, respectively). This study showed no benefit in terms of adenoma or CRC prevention in
LS patients due to the use of aspirin, resistant starch or both for up to 4 years. Longer-term follow-up may be required to fully evaluate the use of aspirin chemoprevention in LS.

The oral contraceptive pill has been shown to reduce the risk of both endometrial and ovarian cancer in the general population [Centers for Disease Control Cancer and Steroid Hormone Study 1983a, 1983b; Cancer and Steroid Hormone Study of the Centers for Disease Control and the National Institute of Child Health and Human Development 1987] and also to prevent ovarian cancer in carriers of BRCA1 and BRCA2 mutations [McLaughlin 2007]. However, there are currently no data addressing the efficacy of chemoprevention in reducing gynecologic cancers in LS.

2.8 NEWFOUNDLAND’S FOUNDER POPULATION

The province of Newfoundland and Labrador, on Canada’s east coast, has a unique island population that historically has been isolated from other parts of North America. Although the first documented discovery of Newfoundland was in 1497 by John Cabot, permanent settlement was not established until around 1610. The peak immigration to Newfoundland occurred during the period 1780-1830, and included mainly Protestant settlers from the south-west of England and Roman Catholic settlers from the south of Ireland [Mannion 1977].
By the mid-1830’s, immigration decreased and the population of Newfoundland was approximately 75,000. Thereafter, the population expanded rapidly to a peak of 580,000 in the mid 1980’s due to a high birth rate. Approximately 90% of the current population can trace their origins to the 20,000 to 30,000 original settlers [Mannion 1977].

In addition to geographic isolation, coastal communities (outports) remained small and isolated until recently due to lack of roads, segregation by religion and limited immigration. Descendants of the original settlers frequently only moved to nearby coves or offshore islands which kept related families together. As a result of the way Newfoundland was settled and expanded, Newfoundland’s population is relatively homogeneous and consists of a series of genetic isolates [Parfrey 2002; Spirio 1999]. Approximately 55-60% of the population live in communities of <2,500 people and 40% in communities of <1000 people [Rahman 2003; Woods 2005]. The current population of Newfoundland (~515,000) is a relatively new founder population (<20 generations) with a limited number of founders [Rahman 2003]. The Newfoundland population is influenced by founder effects and a high coefficient of kinship and is an ideal region for the investigation of genetic diseases due to large family size over the generations, close family ties to the ancestral communities, modest out-migration and a modern health care system [Parfrey 2002]. Of the 12 founder populations studied globally, Newfoundland has the greatest generalizability to other Caucasian populations [Service 2006].
2.8.1 Founder Mutations in Lynch Syndrome

Founder mutations are pathogenic mutations that are shared by apparently unrelated patients and are inherited from a common ancestor several or many generations previously. Founder mutations are characteristically introduced into a population due to a single mutation carrier, which over a period of generations, accounts for a large fraction of the total disease burden in a specific population. Several features of a population, such as isolation (absence of significant immigration), rapid population growth, bottlenecks in population size and chance, increase the probability of a founder mutation prospering [de la Chapelle 2004].

Several founder mutations in *MLH1* and *MSH2* have been detected for LS, and in many of these populations, accounts for a sizeable proportion of all LS cases. Two founder mutations in *MLH1* have been identified in the Finnish population and account for more than 60% of all disease-causing mutations identified in families with LS [Nystrom-Lahti 1995; Moisio 1996]. A third founder mutation in *MLH1* was identified in the Valais region of Switzerland [Hutter 1996; Lynch 2004]. Founder mutations in *MSH2* have been identified in the Ashkenazi Jews [Foulkes 2002], where it may account for as many as one third of all cases, and also in North Americans, where the mutation occurs with a high frequency in a large genetically heterogeneous population, spread over a wide geographic area [Lynch 2004].
One of the first mutations identified causing LS was an intron 5 splice site mutation in MSH2 [Leach 1993]. A large Newfoundland kindred (Family C) was critical to the original study that allowed LS to be mapped to the short arm of chromosome 2p [Peltomaki 1993]. This work later led to the identification of an MSH2 germline mutation in the 5' splice site of exon 5, resulting in deletion of exon 5 and a truncated protein [Liu 1994]. This point mutation (MSH2 c.942 +3A→T) is the most common LS-associated mutation in the world and accounts for approximately 10% of all LS mutations worldwide [Desai 2000; de la Chapelle 2004]. Based on haplotype analysis, this mutation arises de novo in most cases, but in Newfoundland, all carriers of this mutation share a common haplotype and a common geographic origin, indicating a founder effect [Froggatt 1999; Desai 2000].

2.9 MSH2 MUTATIONS IN NEWFOUNDLAND

As was previously stated, an intron 5 splice site mutation in the MSH2 MMR gene was the first LS-causing mutation to be identified in Newfoundland. This splice site mutation was first detected in a large Newfoundland family (Family C) and was later found to be widespread through a founder effect. All additional families were independently ascertained and found to share a common haplotype and also a common ancestor from the same geographic region as Family C [Froggatt 1999].

Two other mutations in MSH2 have also been identified in Newfoundland. An exon 8 deletion (c.1277-?_1386+?del) has been identified in several families and haplotype
analysis suggests that this mutation arose from a common founder. The third mutation is an exon 4-16 deletion that segregates in a very large family (Family 11) and includes >80 mutation carriers. All sibships with this mutation have been genealogically linked to a common founder.

2.10 OBJECTIVES

The main objectives of this thesis will be as follows:

1. To determine whether the cumulative risk of cancer and mortality in a group of \textit{MSH2} mutation carriers differs based on the specific mutation involved and whether gender differences in cancer and mortality outcomes is consistent across different mutations.

2. To compare the incidence of CRC and overall survival for \textit{MSH2} mutation carriers who entered a colonoscopic screening program to that expected, derived from the non-screened group, adjusting for gender and survivor bias.

3. To compare gynecological cancer incidence and overall survival for female \textit{MSH2} mutation carriers who received gynecological screening (case) and for matched controls, who were alive and disease-free at the age the case entered the screening program.
CHAPTER 3

The Phenotypic Expression of Three MSH2 Mutations in Large Newfoundland Families with Lynch Syndrome

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3.1 INTRODUCTION

Colorectal Cancer (CRC) is the third most common cancer; after breast and lung cancer in women and after prostate and lung in men [Canadian Cancer Society 2004]. One of the most significant risk factors for CRC is family history [Lynch 2003; National Cancer Institute 2005]. Lynch syndrome (LS), often called hereditary non-polyposis colorectal cancer (HNPCC), is the most common hereditary CRC syndrome and is responsible for approximately 1 to 3 percent of cases of CRCs [Ponz de Leon 1999; Peel 2000; Salovaara 2000; Cunningham 2001; Samowitz 2001; Katballe 2002; Olsson 2003; Pinol 2004; de la Chapelle 2005; Sarroca 2005; Zhang 2005]. However, in Newfoundland up to 47% of CRC patients come from high-risk and intermediate-risk families [Woods 2005].

Lynch syndrome is characterized by autosomal dominant inheritance, early age at onset of colorectal carcinoma, right-sided predominance, and increased incidence of synchronous and metachronous CRCs [Fitzgibbons 1987]. Additionally, extra-colonic cancers of the gastrointestinal tract, genitourinary tract, endometrium, ovaries, biliary tract, pancreas and brain are frequently observed [Mecklin 1991; Watson 1993; Aarnio 1995; Lynch 1997; Vasen 1999].

Lynch syndrome has been associated with germline mutations in several DNA mismatch repair (MMR) genes, including MSH2, MLH1, MSH6, and PMS2 [Syngal 2000; Wheeler 2000]. Ninety percent of LS cases are caused by mutations in either MLH1 or MSH2.
Extracolonic malignancies are more frequently associated with mutations in \textit{MSH2} than with mutations in \textit{MLH1} \cite{Rowley2005}.

Lynch syndrome shows incomplete penetrance (not all mutation carriers will develop a cancer) and variable expressivity (individuals develop different cancers at different ages). In individuals with an MMR mutation, the lifetime risk of developing CRC is about 80\% \cite{Watson1993, Aarnio1995, Peltomaki1997, Wheeler2000}. Endometrial and ovarian cancers occur in up to 60\% and 10\%, respectively, of female mutation carriers. Cancers such as gastric and urothelial cancers, also have a lifetime risk of about 10\%, whereas other cancers occur in smaller proportions of mutation carriers despite a marked increased relative risk (3 to 25 times the risk of the general population) for some of them, e.g., cancers of the small bowel and biliary system \cite{Vasen1996, Aarnio1999, Berends2001, Watson2001, Lynch2004, Rowley2005}.

The province of Newfoundland, Canada, has one of the most valuable populations for investigating autosomal dominant diseases \cite{Green2002, Parfrey2002, Hodgkinson2005}. Until very recently, little in or out migration had occurred since the founding immigrations from South East Ireland and South West England in the late 18\textsuperscript{th} and early 19\textsuperscript{th} centuries. Large families were the norm over successive generations and family members settled near the core community.
An extensive Newfoundland family was used in the original study showing linkage between hereditary CRC and a locus on chromosome 2p [Peltomaki 1993]. This later led to the identification of a germline mutation in MSH2, c.942+3A>T, in the 5' splice site of intron 5, resulting in an inframe deletion of exon 5 in the mRNA [Liu 1994, 1996]. This mutation was subsequently found in a total of 12 Newfoundland families. The phenotypic expression of this founder MSH2 mutation was assessed and male mutation carriers were found to be at an increased risk for CRC and death from cancer when compared to female mutation carriers [Green 2002]. Further analysis of high risk families in Newfoundland and Labrador has led to the identification of two different MSH2 mutations in six more families. These mutations include genomic deletions of exon 8 in five families and deletion of exons 4-16 in the sixth family. Tumors from these families do not express the MSH2 protein and exhibit microsatellite instability (MSI).

The association between genotype and phenotype is poorly understood despite the fact that a large number of predisposing mutations have been identified [Peltomaki 2001]. Whether there is a difference in cancer risk between carriers of the various MMR-gene mutations is unclear [Vasen 2001]. Based on current biochemical deduction, different mutations of the MSH2 and MLH1 genes may be predicted to have different functional consequences [Peltomaki 2001]. Consequently, assessment of the correlation between specific mutations (genotype) and their expression in family members (phenotype) is necessary [Terdiman 1999].
The current study was undertaken to determine whether the phenotypic expression of Lynch syndrome, caused by mutations in MSH2, is influenced by the specific mutation and whether the effect of gender on cancer and mortality outcomes is consistent across families with different mutations.

3.2 PATIENTS AND METHODS

More than 300 families with high or intermediate risk for hereditary CRC have been referred to the Medical Genetics Program of Newfoundland. Fifty-two of these families met either the Amsterdam I or Amsterdam II criteria. Of these, eighteen families have been confirmed as having an MSH2 mutation. Informed consent was obtained from all subjects or an appropriate proxy. Ethics approval was granted by the Human Investigations Committee of the Faculty of Medicine, Memorial University of Newfoundland, the Health Care Corporation of St. John’s and the Avalon Peninsula Health Board.

3.2.1 Mutation Detection and Genotyping

DNA from all available family members at 50% risk of inheriting an MSH2 mutation was prepared from whole blood using a simple salting-out method [Miller 1988]. The point mutation in the splice donor site of intron 5 in MSH2 (c.942+3A>T) was determined by restriction fragment analysis as described previously [Frogsatt 1995]. Exon deletions in MSH2 were detected by Multiplex Ligation-dependent Probe Amplification (MLPA) using genomic DNA [Schouten 2002]. MLPA, using the HNPCC probes (kit # SALSA
was conducted and analyzed according to the protocol provided by MRC-Holland (Amsterdam, Holland) on an ABI 377 Genetic Analyzer (Applied Biosystems, Foster City, CA). Genotyping, using microsatellite markers, for families segregating the exon 8 deletion, was performed on either an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA) or a Beckman Coulter CEQ 8000 Genetic Analysis System (Beckman Coulter Inc., Fullerton, CA). Marker positions on chromosome 2 were identified using the Genome Browser from the UCSC Genome Bioinformatics website (http://genome.ucsc.edu/). Primer sequences were obtained from the Human Genome Database (http://www.gdb.org/). PCR protocols are available upon request. Alleles were called blind to affection status.

### 3.2.2 Subjects

At risk individuals were further classified as mutation carriers if they met one of the following criteria: i) confirmation by mutation testing, ii) obligate carrier (in the line of descent and having offspring with a proven mutation), or iii) in the line of descent and presenting clinically with an HNPCC tumor < 50 years of age. Individuals falling into the first two categories were called “mutation positive” while those in the last category were referred to as “presumed positive”. Information on family history, age of onset of clinical manifestations, type of cancer, and cause of death was collected from mutation positive or presumed positive family members and confirmed by medical records.
The study group consisted of 290 individuals from 18 families who had inherited an \textit{MSH2} mutation (Table 3.1). Twelve families with 151 mutation carriers had the intron 5 splice site mutation (75 males and 76 females), five families with 74 mutation carriers had the exon 8 deletion (37 males and 37 females) and one family with 65 mutation carriers had the exon 4-16 deletion (31 males and 34 females). From these families, 235 individuals (81%) were found to be mutation positive (193 were confirmed by mutation testing to have an \textit{MSH2} mutation and 42 were labelled an obligate carrier), and the remaining 55 (19%) were considered presumed positive.

| Table 3.1 Characteristics of family members by type of \textit{MSH2} mutation |
|---------------------------------|-----------------|-----------------|-----------------|
|                                 | \textbf{Intron 5 Mutation} | \textbf{Exon 8 Deletion} | \textbf{Exon 4-16 Deletion} |
| \multicolumn{4}{c|}{N (%)} |
| \hline
| Gender                      | Males | Females | Males | Females | Males | Females |
| Males                      | 75 (50) | 37 (50) | 31 (48) | 34 (52) |
| Females                    | 76 (50) | 37 (50) |  |
| Year of Birth              |       |       |       |       |
| Before 1930                | 39 (26) | 18 (31) | 16 (25) |
| 1930-1969                  | 98 (65) | 36 (62) | 43 (66) |
| 1970 and After             | 14 (9) | 4 (7) | 6 (9) |
| Mutation Status            |       |       |       |       |
| Positive                   | 125 (83) | 60 (81) | 50 (77) |
| Presumed Positive          | 26 (17) | 14 (19) | 15 (23) |
| Parent Carrier             |       |       |       |       |
| Mother                     | 104 (69) | 22 (30) | 29 (45) |
| Father                     | 43 (28) | 43 (58) | 28 (43) |
| Unknown                    | 4 (3) | 9 (12) | 8 (12) |
| Cancer                     |       |       |       |       |
| Yes                        | 91 (60) | 51 (69) | 39 (60) |
| No                         | 60 (40) | 23 (31) | 26 (40) |
3.2.3 Statistical Analyses

Statistical analyses were done using version 11.5 of the Statistical Package for Social Sciences (SPSS). Chi-square tests were performed to determine if the distribution of specific cancer types (CRC, endometrial, ovarian, and transitional carcinoma of the ureter, bladder and renal pelvis) varied among the different mutation groups. Time to colorectal cancer, time to any first cancer, time to extracolonic HNPCC cancers (endometrial, ovarian, stomach and transitional cell carcinoma of the ureter, bladder and renal pelvis) and time to death were analyzed using the Kaplan Meier time-to-event analysis in mutation carriers. In the time-to-event analyses, mutation carriers were studied with respect to their risk of developing cancer from birth to death and lifetime risk was determined based on cumulative risk up to 70 years of age. Differences in survival curves for each mutation were tested for statistical significance using the log-rank test. Relative risk of developing each outcome was estimated for male and female mutation carriers using the Cox Regression Model. Similar analyses were run on mutation positive carriers only, to determine if the ascertainment bias inherent in our selection criteria had an impact on our overall conclusions. For time-to-event analyses, the number at risk is less than the study population totals due to missing data on ages at cancer, death and last follow-up.
3.3 RESULTS

3.3.1 Evidence for Founder Mutations

All the sibships with the exon 4-16 deletion were genealogically linked to a common founder. It was not possible to genealogically connect the five families segregating the exon 8 deletion but haplotype analysis (Table 3.2) strongly suggests that the mutation arose from a common founder in at least four of the five families. Since this is a frequently identified mutation, [Bapat 1999; Wang 2003; Thiffault 2004] it may have arisen independently in family R0134. However, the qter portion of the haplotype in this family is identical to that of the other four families, therefore it is possible that this mutation has occurred but once in the Newfoundland population. Genealogical study and haplotype analysis have shown that eleven of the twelve families segregating the intron 5 mutation likely have a common ancestor [Froeggatt 1999].

Table 3.2 Haplotype for mutation carriers in five families with an exon 8 deletion in MSH2. The marker order is pter-D2S119-0.1 Mb-D2S2298-1.0 Mb-D2S2174-0.9 Mb-D2S2240-0.5 Mb-D2S391-0.8 Mb-D2S2227-0.4 Mb-MSH2-1.5Mb-D2S1247-2.2 Mb-D2S123-qter.

<table>
<thead>
<tr>
<th></th>
<th>R0004</th>
<th>R0014</th>
<th>R0134</th>
<th>R0165</th>
<th>R0457</th>
</tr>
</thead>
<tbody>
<tr>
<td>D2S119</td>
<td>214</td>
<td>214</td>
<td>222</td>
<td>214</td>
<td>214/216</td>
</tr>
<tr>
<td>D2S2298</td>
<td>220</td>
<td>220</td>
<td>214</td>
<td>220</td>
<td>220</td>
</tr>
<tr>
<td>D2S2174</td>
<td>277</td>
<td>277</td>
<td>277</td>
<td>277</td>
<td>277</td>
</tr>
<tr>
<td>D2S2240</td>
<td>181</td>
<td>181</td>
<td>177/179</td>
<td>181</td>
<td>181</td>
</tr>
<tr>
<td>D2S391</td>
<td>143</td>
<td>143</td>
<td>147</td>
<td>143</td>
<td>143</td>
</tr>
<tr>
<td>D2S2227</td>
<td>214</td>
<td>214</td>
<td>206</td>
<td>214</td>
<td>214</td>
</tr>
<tr>
<td>D2S1247</td>
<td>308</td>
<td>308</td>
<td>308</td>
<td>308</td>
<td>308</td>
</tr>
<tr>
<td>D2S2739</td>
<td>242</td>
<td>242</td>
<td>242</td>
<td>242</td>
<td>242</td>
</tr>
<tr>
<td>D2S123</td>
<td>209/211</td>
<td>209/211</td>
<td>209/211</td>
<td>209/211</td>
<td>209/211</td>
</tr>
</tbody>
</table>

The marker order is pter-D2S119-0.1 Mb-D2S2298-1.0 Mb-D2S2174-0.9 Mb-D2S2240-0.5 Mb-D2S391-0.8 Mb-D2S2227-0.4 Mb-MSH2-1.5Mb-D2S1247-2.2 Mb-D2S123-qter
3.3.2 Comparison of Mutation Carriers

Table 3.3 shows the different types of first cancers and overall total cancers for all mutation carriers. CRC was the first manifestation of the disease in 54% of cases with the intron 5 splice site mutation, 59% of those with the exon 8 deletion and 72% of those with the exon 4-16 deletion. For females, a large proportion of first cancers were found to be cancers of the female reproductive system (endometrium, ovary and fallopian tube). Forty-nine percent of females with the intron 5 splice site mutation developed one of these cancers as did 33% of those with the exon 8 deletion and 44% of those with the exon 4-16 deletion. After exclusion of presumed positive individuals the distribution of cancers was quite similar to that for all carriers. CRC was still the most prominent first cancer and occurred in 51% of those with the intron 5 splice site mutation, 53% of those with the exon 8 deletion and 75% of those with the exon 4-16 deletion.
### Table 3.3 Types of first cancer and total overall cancers for each mutation N (%)  

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Intron 5 Mutation (151 carriers)</th>
<th>Exon 8 Deletion (74 carriers)</th>
<th>Exon 4-16 Deletion (65 carriers)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First cancer</td>
<td>Total cancers</td>
<td>First cancer</td>
</tr>
<tr>
<td>CRC</td>
<td>49 (54)</td>
<td>81 (49)</td>
<td>30 (59)</td>
</tr>
<tr>
<td><strong>Other HNPCC (Bethesda)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endometrial</td>
<td>14 (15)</td>
<td>22 (13)</td>
<td>6 (12)</td>
</tr>
<tr>
<td>Ovarian</td>
<td>8 (9)</td>
<td>12 (7)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Stomach</td>
<td>4 (5)</td>
<td>8 (5)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>-</td>
<td>1 (1)</td>
<td>-</td>
</tr>
<tr>
<td>Ureter-TCC</td>
<td>-</td>
<td>-</td>
<td>3 (6)</td>
</tr>
<tr>
<td>Renal-TCC</td>
<td>-</td>
<td>1 (1)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Bladder-TCC</td>
<td>-</td>
<td>3 (2)</td>
<td>-</td>
</tr>
<tr>
<td>Small bowel</td>
<td>1 (1)</td>
<td>4 (2)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Fallopian tube</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Non-HNPCC</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prostate</td>
<td>-</td>
<td>5 (3)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Skin(^a)</td>
<td>9 (10)</td>
<td>17 (10)</td>
<td>3 (6)</td>
</tr>
<tr>
<td>Brain</td>
<td>2 (2)</td>
<td>2 (1)</td>
<td>-</td>
</tr>
<tr>
<td>Breast(^b)</td>
<td>1 (1)</td>
<td>3 (2)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Cervix</td>
<td>2 (2)</td>
<td>2 (1)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Retropharyngeal</td>
<td>-</td>
<td>1 (1)</td>
<td>-</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GI</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Spinal Cord</td>
<td>-</td>
<td>1 (1)</td>
<td>-</td>
</tr>
<tr>
<td>Lung</td>
<td>-</td>
<td>-</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Liver</td>
<td>-</td>
<td>1 (1)</td>
<td>-</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>91(100)</strong></td>
<td><strong>165(100)</strong></td>
<td><strong>51(100)</strong></td>
</tr>
</tbody>
</table>

TCC, transitional cell cancer; GI, gastrointestinal

\(^a\)Many of these skin cancers are part of the Muir-Torre Syndrome (keratoacanthomas)

\(^b\)The higher rate of breast cancer in deletion 8 carriers seems likely to be linked to breast cancer in the family of a married-in individual and not the MSH2 mutation
Of the 151 mutation carriers with the intron 5 splice site mutation, 91 individuals (60.3%) developed at least one cancer. Forty-two of these individuals developed multiple cancers resulting in a total of 165 primary cancers for the 91 family members. Fifty-one individuals (68.9%) with the exon 8 deletion had at least one cancer and twenty-five of these went on to develop additional cancers. A total of 110 cancers were found among the 51 family members. For those with the exon 4-16 deletion, 39 family members (60%) were found to have at least one cancer. Eighteen individuals developed additional cancers resulting in a total of 72 cancers among the family members.

3.3.3 Incidence of Cancer and Mortality

The lifetime risk of developing cancer at any site for all MSH2 mutation carriers was found to be 94% (Table 3.4, Figure 3.1). These predisposed individuals were found to have a high lifetime risk of developing CRC (75%), transitional cell carcinomas (TCCs) of the ureter, bladder and renal pelvis (32%), and stomach cancer (14%). In females, lifetime risk for endometrial cancer was 58% and for ovarian cancer was 21%. Twenty percent of mutation carriers developed CRC before 40 years of age and 6% of female mutation carriers developed endometrial cancer before 40 years of age. Median life expectancy was 63 years. Across all cancer types, lifetime risk of cancer development was quite similar for those with a confirmed mutation (Figure 3.2) compared to all mutation carriers.
Table 3.4 Cumulative risk of cancer development in *MSH2* mutation carriers

<table>
<thead>
<tr>
<th>Cumulative risk by age</th>
<th>Any first cancer</th>
<th>Colorectal cancer</th>
<th>Endometrial cancer</th>
<th>Ovarian cancer</th>
<th>Transitional cell cancers</th>
<th>Stomach cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 30</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>≤ 40</td>
<td>27</td>
<td>20</td>
<td>6</td>
<td>3</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>≤ 50</td>
<td>66</td>
<td>47</td>
<td>25</td>
<td>11</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>≤ 60</td>
<td>86</td>
<td>65</td>
<td>49</td>
<td>18</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>≤ 70</td>
<td>94</td>
<td>75</td>
<td>58</td>
<td>21</td>
<td>32</td>
<td>14</td>
</tr>
<tr>
<td>≤ 80</td>
<td>99</td>
<td>79</td>
<td>58</td>
<td>21</td>
<td>32</td>
<td>14</td>
</tr>
</tbody>
</table>

Mean 47 57 67 80 80 85
Median 46 51 63 NA NA NA
Events 176 119 40 16 20 12
# at risk 276 278 141 141 275 275

Figure 3.1 Cumulative risk of cancer development in all *MSH2* mutation carriers
Male carriers were found to have a significantly increased risk of developing any cancer (RR=1.4; 95% CI 1.0-1.9), of developing CRC (RR=2.9; 95% CI 2.0-4.2), and of death (RR=2.4; 95% CI 1.6-3.6) compared with female carriers (Table 3.5, Figure 3.3a-c).

Similar results were found for those considered mutation positive (Table 3.6). The risk of developing any first cancer (RR=1.5; 95% CI 1.0-2.1), CRC (RR=2.5; 95% CI 1.6-3.9) or death (RR=2.3; 95% CI 1.3-3.9) was significantly increased in male carriers compared to female carriers.
Table 3.5 Cumulative risk of cancer development or death for male and female mutation carriers

<table>
<thead>
<tr>
<th>Cumulative risk by age</th>
<th>Any first cancer</th>
<th>Colorectal cancer</th>
<th>Death</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td>≤ 30</td>
<td>9</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>≤ 40</td>
<td>36</td>
<td>18</td>
<td>32</td>
</tr>
<tr>
<td>≤ 50</td>
<td>71</td>
<td>60</td>
<td>65</td>
</tr>
<tr>
<td>≤ 60</td>
<td>85</td>
<td>87</td>
<td>81</td>
</tr>
<tr>
<td>≤ 70</td>
<td>95</td>
<td>94</td>
<td>88</td>
</tr>
<tr>
<td>≤ 80</td>
<td>98</td>
<td>98</td>
<td>88</td>
</tr>
</tbody>
</table>

Relative Risk (95% CI)
Mean 45 49 48 65 56 68
Median 45 48 46 66 58 69
Events 89 87 77 42 65 38
# at Risk 134 142 136 142 135 141

Figure 3.3a Cumulative risk of cancer development for male and female mutation carriers (RR=1.4; 95% CI 1.0-1.9).
Figure 3.3b Cumulative risk of CRC development for male and female mutation carriers (RR=2.9; 95% CI 2.0-4.2).

Figure 3.3c Cumulative risk of death for male and female mutation carriers (RR=2.4; 95% CI 1.6-3.6).
Table 3.6  Cumulative risk of cancer development or death for male and female mutation positive carriers

<table>
<thead>
<tr>
<th>Cumulative risk by age</th>
<th>Any first cancer</th>
<th>Colorectal cancer</th>
<th>Death</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td>≤ 30</td>
<td>6</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>≤ 40</td>
<td>33</td>
<td>16</td>
<td>31</td>
</tr>
<tr>
<td>≤ 50</td>
<td>72</td>
<td>57</td>
<td>65</td>
</tr>
<tr>
<td>≤ 60</td>
<td>79</td>
<td>84</td>
<td>72</td>
</tr>
<tr>
<td>≤ 70</td>
<td>90</td>
<td>92</td>
<td>76</td>
</tr>
<tr>
<td>≤ 80</td>
<td>95</td>
<td>98</td>
<td>76</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Relative Risk (95% CI)</th>
<th>Mean</th>
<th>Median</th>
<th>Events</th>
<th># at Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any first cancer</td>
<td>1.5</td>
<td>1.0-2.1</td>
<td>46</td>
<td>99</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>2.5</td>
<td>1.6-3.9</td>
<td>52</td>
<td>124</td>
</tr>
<tr>
<td>Death</td>
<td>2.3</td>
<td>1.3-3.9</td>
<td>62</td>
<td>101</td>
</tr>
</tbody>
</table>

3.3.3.1 Impact of Mutation

The cumulative probability of developing any first cancer, CRC, extracolonic cancers or death was compared for MSH2 mutation carriers with the intron 5 splice site mutation, the exon 8 deletion and the exon 4-16 deletion. The age-related risk of any of these events was not significantly different for the three MSH2 mutations. There was, however, an increased number of transitional cell cancers of the ureter, bladder, and renal pelvis in those with the exon 8 deletion. Fourteen percent of total cancers in exon 8 deletion carriers were transitional cell cancers compared to 11% in exon 4-16 deletion carriers and 3% in intron 5 splice site mutation carriers (Table 3.3). Ten individuals with the exon 8 deletion developed at least one transitional cell cancer compared to six individuals with the exon 4-16 deletion and four with the splice site mutation ($\chi^2 = 8.6; P=.013$). By age 70 years, 44% of those with the exon 8 deletion had developed one of these transitional...
cell cancers as compared to 31% of deletion 4-16 carriers and 16% of the splice site mutation carriers (Table 3.7), but the difference did not achieve statistical significance.

**Table 3.7 Cumulative risk of developing transitional cell cancers of the ureter, bladder and renal pelvis in MSH2 mutation carriers**

<table>
<thead>
<tr>
<th>Cumulative risk by age</th>
<th>Intron 5 mutation</th>
<th>Exon 8 deletion</th>
<th>Exon 4-16 deletion</th>
<th>All mutation carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 50</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>≤ 60</td>
<td>5</td>
<td>6</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>≤ 70</td>
<td>16</td>
<td>44</td>
<td>31</td>
<td>32</td>
</tr>
<tr>
<td>≤ 80</td>
<td>16</td>
<td>44</td>
<td>31</td>
<td>32</td>
</tr>
<tr>
<td>Mean</td>
<td>80</td>
<td>77</td>
<td>73</td>
<td>80</td>
</tr>
<tr>
<td>Median</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Events</td>
<td>4</td>
<td>10</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td># at Risk</td>
<td>151</td>
<td>59</td>
<td>65</td>
<td>255</td>
</tr>
</tbody>
</table>

**Relative Risk (95% CI)**

- Exon 8 deletion versus intron 5 mutation: 2.8 (0.9-9.1)
- Exon 4-16 deletion versus intron 5 mutation: 2.7 (0.8-9.5)
- Deletion carriers (exon 8 and exon 4-16) versus intron 5 mutation: 2.8 (0.9-8.3)

A second analysis compared those with the splice site mutation and those with a genomic deletion (exon 8 deletion and exon 4-16 deletion combined). The age-related risk of developing any first cancer, CRC or death was not significantly different between the two groups. There was, however, a significantly increased risk of ovarian cancer for family members with the splice site mutation (Table 3.8). Also, an elevated but not significant
risk for transitional cell cancers of the ureter, bladder and renal pelvis was found for deletion carriers.

Table 3.8 Cumulative Risk of Developing Ovarian Cancer in Female MSH2 Mutation Carriers

<table>
<thead>
<tr>
<th>Cumulative risk by age</th>
<th>Splice site mutation</th>
<th>Genomic deletion</th>
<th>All mutation carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 30</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>≤ 40</td>
<td>6.4</td>
<td>0.0</td>
<td>3.3</td>
</tr>
<tr>
<td>≤ 50</td>
<td>17.1</td>
<td>4.6</td>
<td>11.2</td>
</tr>
<tr>
<td>≤ 60</td>
<td>24.2</td>
<td>10.9</td>
<td>17.9</td>
</tr>
<tr>
<td>≤ 70</td>
<td>31.8</td>
<td>10.9</td>
<td>21.2</td>
</tr>
<tr>
<td>≤ 80</td>
<td>31.8</td>
<td>10.9</td>
<td>21.2</td>
</tr>
<tr>
<td>Mean</td>
<td>72.7</td>
<td>84.6</td>
<td>80.3</td>
</tr>
<tr>
<td>Median</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Events</td>
<td>12</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>Censored</td>
<td>64</td>
<td>61</td>
<td>125</td>
</tr>
</tbody>
</table>

Relative Risk (95% CI)
Splice site mutation versus genomic deletion  3.2 (1.0-10.1)

Similar results were found when the above analyses were repeated on those considered mutation positive only. Comparison of cumulative survival across the three MSH2 mutations showed that there was no significant difference in time to development of any first cancer, CRC or death. However, when comparing the cumulative risk of extracolonic cancers, a significant difference was seen in time to development of transitional cell cancers of the ureter, bladder, and renal pelvis. The relative risk was 5.0 (95% CI 1.1-23.3) for those with the exon 8 deletion compared to those with the intron 5 splice site mutation, and for those with the exon 4-16 deletion, the risk was 5.4 (95% CI
1.1-28.1) when compared to those with the splice site mutation. Comparing mutation positive individuals with a splice site mutation and those with a genomic deletion also led to similar results. Individuals with a genomic deletion were at a significantly increased risk for development of transitional cell cancers (RR=5.2; 95% CI 1.2-22.7) compared to those with the splice site mutation. Individuals with the splice site mutation, however, were found to have an increased risk for ovarian cancer compared to those with a genomic deletion but this did not achieve statistical significance (RR=2.6; 95% CI 0.8-8.2).

3.3.3.2 Impact of Gender

There was no significant gender difference between time-to-development of any first cancer for carriers of the splice site and carriers of the deletion 8 mutations (Table 3.9). However, for those with the exon 4-16 deletion, the relative risk of any cancer development was 2.5 (95% CI 1.3-5.0) in male carriers compared with female carriers.

Males were found to have a significantly increased risk of CRC development across all three MSH2 mutations (Table 3.9). For splice site mutation carriers, the risk of CRC in males compared to females was 2.7 (95% CI 1.6-4.7) with 77% of males versus 40% of females developing the CRC by age 60 years. The relative risk of CRC development was 2.2 (95% CI 1.1-4.7) for males versus females with the exon 8 deletion. Seventy-eight percent of males versus 45% of females developed CRC by 60 years. For those with the
exon 4-16 deletion, the risk of CRC in males compared to females was 4.3 (95% CI 1.9-9.5). By age 60 years, 89% of males compared to 58% of females had developed CRC.

Table 3.9 Cumulative risk of developing any 1st cancer, colorectal cancer or death in male and female mutation carriers

<table>
<thead>
<tr>
<th></th>
<th>Intron 5 mutation</th>
<th>Exon 8 deletion</th>
<th>Exon 4-16 deletion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td><strong>Any first cancer</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Events</td>
<td>44</td>
<td>47</td>
<td>22</td>
</tr>
<tr>
<td>Number at risk</td>
<td>75</td>
<td>76</td>
<td>28</td>
</tr>
<tr>
<td>Median age to 1st cancer</td>
<td>46</td>
<td>47</td>
<td>46</td>
</tr>
<tr>
<td>Relative risk</td>
<td>1.2</td>
<td></td>
<td>1.1</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.8 - 1.9</td>
<td></td>
<td>0.6 - 1.9</td>
</tr>
<tr>
<td><strong>Colorectal cancer</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Events</td>
<td>36</td>
<td>22</td>
<td>20</td>
</tr>
<tr>
<td>Number at risk</td>
<td>75</td>
<td>76</td>
<td>30</td>
</tr>
<tr>
<td>Median age to CRC</td>
<td>46</td>
<td>67</td>
<td>49</td>
</tr>
<tr>
<td>Relative risk</td>
<td>2.7</td>
<td></td>
<td>2.2</td>
</tr>
<tr>
<td>95% CI</td>
<td>1.6 - 4.7</td>
<td></td>
<td>1.1 - 4.7</td>
</tr>
<tr>
<td><strong>Death</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Events</td>
<td>31</td>
<td>22</td>
<td>20</td>
</tr>
<tr>
<td>Number at risk</td>
<td>75</td>
<td>76</td>
<td>29</td>
</tr>
<tr>
<td>Median survival</td>
<td>54</td>
<td>66</td>
<td>58</td>
</tr>
<tr>
<td>Relative risk</td>
<td>2.1</td>
<td></td>
<td>3.1</td>
</tr>
<tr>
<td>95% CI</td>
<td>1.2 - 3.7</td>
<td></td>
<td>1.3 - 7.3</td>
</tr>
</tbody>
</table>

*a* Compares males to females

All families showed a significant gender difference for death, with males being at a greater risk for death than females (Table 3.9). In splice site mutation carriers, the relative risk of death was 2.1 (95% CI 1.2-3.7) for males versus females. The median survival was 54 years for males versus 66 years for females. The relative risk of death was 3.1 (95% CI 1.3-7.3) for males with a deletion 8 mutation as compared to females.
with the same mutation, and the median survivals were 58 and 75 years, respectively. For those with an exon 4-16 deletion, the risk of death for males compared to females was 2.9 (95% CI 1.1-8.2). The median survivals were 63 years for males and 77 years for females.

The previous analyses were also performed on mutation positive individuals only (Table 3.10). Across all 3 MSH2 mutations, the age-related risks for development of any first cancer were similar to the gender differences found for all mutation carriers. This was not true for time to CRC development or death. Only those with the splice site mutation and the exon 4-16 deletion showed a significant gender difference for CRC development, with males being at a greater risk than females. Also, only male carriers with the exon 8 deletion were found to be at a significantly increased risk for death when compared to their female family members.

3.4 DISCUSSION

The penetrance of MMR gene mutations (lifetime cancer risk) has proven difficult to determine in LS cases and could be heavily dependent on the source of the reference sample [Carayol 2002; Hampel 2005b; Quehenberger 2005]. The results of our study cannot be directly compared to other penetrance studies, due to differences in methods of ascertainment, geographical variations in environmental factors, possible genetic modifiers and allelic heterogeneity, yet there are consistent findings.
Table 3.10 Cumulative risk of developing any 1st cancer, colorectal cancer or death in male and female mutation positive carriers

<table>
<thead>
<tr>
<th></th>
<th>Intron 5 mutation</th>
<th>Exon 8 deletion</th>
<th>Exon 4-16 deletion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td>Any first cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Events</td>
<td>25</td>
<td>40</td>
<td>14</td>
</tr>
<tr>
<td>Number at risk</td>
<td>56</td>
<td>69</td>
<td>20</td>
</tr>
<tr>
<td>Median age to 1st cancer</td>
<td>45</td>
<td>48</td>
<td>46</td>
</tr>
<tr>
<td>Relative risk(^a)</td>
<td>1.2</td>
<td>1.0</td>
<td>4.1</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.7 – 2.0</td>
<td>0.5 – 2.1</td>
<td>1.6 – 10.3</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Events</td>
<td>21</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>Number at risk</td>
<td>56</td>
<td>69</td>
<td>22</td>
</tr>
<tr>
<td>Median age to CRC</td>
<td>46</td>
<td>67</td>
<td>50</td>
</tr>
<tr>
<td>Relative risk(^a)</td>
<td>2.3</td>
<td>1.8</td>
<td>5.4</td>
</tr>
<tr>
<td>95% CI</td>
<td>1.2 – 4.2</td>
<td>0.8 – 4.2</td>
<td>2.0 – 14.8</td>
</tr>
<tr>
<td>Death</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Events</td>
<td>13</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>Number at risk</td>
<td>56</td>
<td>69</td>
<td>21</td>
</tr>
<tr>
<td>Median survival</td>
<td>68</td>
<td>68</td>
<td>58</td>
</tr>
<tr>
<td>Relative risk(^a)</td>
<td>2.0</td>
<td>3.2</td>
<td>3.3</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.9 – 4.3</td>
<td>1.1 – 9.0</td>
<td>0.7 – 16.2</td>
</tr>
</tbody>
</table>

\(^a\) Compares males to females

Our lifetime CRC (75%) and endometrial cancer (58%) risk estimates are very similar to those reported in the literature. Most of these published risk estimates are derived from families collected at HNPCC registries and vary between 65-85% for CRC [Aarnio 1995, 1999; Vasen 1996, 2001] and 40-60% for endometrial cancer [Aarnio 1995, 1999; Vasen 1996; Dunlop 1997]. Similarly, our increased lifetime risks for ovarian, stomach, and transitional cell carcinomas of the ureter, bladder and renal pelvis are consistent with results from other published studies. In many cases however, our risk estimates are even higher than those reported especially for transitional cell cancers [Aarnio 1995, 1999;
We also found that CRC risk was higher in male mutation carriers than in female mutation carriers. This sex difference in CRC risk is in agreement with that reported by other investigations [Dunlop 1997; Aarnio 1999; Froggatt 1999; Vasen 2001] and is perhaps due to sex-linked modifier genes. Also, our findings for carriers of the intron 5 splice site mutation are consistent with those published previously. This mutation is one of the most frequently occurring mutations in HNPCC families worldwide [Liu 1994, 1996; Froggatt 1995, 1999; Miyaki 1995; Pensotti 1997; Bai 1999; Chan 1999; Wang 1999; Desai 2000; Fidalgo 2000] with CRC being the most common cancer site, followed by endometrial and ovarian cancers for females [Miyaki 1995; Froggatt 1999]. Other infrequent cancer sites include skin and stomach cancer [Froggatt 1999]. Our penetrance estimates (up to 60 years of age) for any first cancer (89%), CRC (58%), endometrial cancer (53%) and our intersex differences in the lifetime risk of CRC (77% male vs. 40% female; \( P < .001 \)) are consistent with that reported elsewhere [Froggatt 1999].

Studies have shown that there may be variation in the tumor spectrum depending on the specific MMR gene and mutation involved and the position of the mutation may be more important than the type [Aarnio 1999]. Therefore, it is possible that the intron 5 splice site mutation may have a functional consequence different than the genomic deletions,[Peltomaki 2001] although the \( MSH2 \) protein was not detectable on immunoassay for any of the three mutations.
An important consideration when performing genotype-phenotype studies is the underlying genetic background of the mutations. When trying to elucidate the phenotypic consequences of a particular mutation, studying a group of individuals with a very similar genetic background is preferred – as is the case when carriers of a specific mutation are members of the same extended family. We have demonstrated from genealogical study and haplotype analysis that each of the three \( MSH2 \) mutations identified in Newfoundland likely arose from a common ancestor.

The lifetime probabilities of developing CRC were similar for all three mutations, as were the age-and gender-related risks. Transitional cell cancers of the urinary tract comprised a significantly lower proportion of cancers in carriers of the splice site mutation than in carriers of the genomic deletions. In addition, the incidence of ovarian cancer was higher in carriers of the splice site mutation. These observations may imply that the phenotypic expression of extracolonic cancers differs according to the type of \( MSH2 \) mutation, but care needs to be taken with this interpretation because of several limitations in this study.

Although we have studied 290 mutation carriers, the number of extracolonic cancers is relatively small. In addition to small event rates, the likelihood of finding a false positive is quite high in this study because of multiple comparisons. Finally, the time-to-event analyses (Kaplan Meier and Cox Regression) do not take account of the possibility that clustering of events could occur in families that may be unrelated to the \( MSH2 \) mutations.
For example, there may be other genetic variants in the family causing the development of these cancers, or lifestyle and environmental issues may be influencing the similarity of cancers found in these families.

Another limitation is that carrier status was not confirmed in all family members by mutation testing. While the definition of an obligate carrier is reasonable, the genetic status of those at 50% risk who developed an HNPCC cancer before 50 years of age is less definitive. Kindreds identified in this manner, through family history, will inherently have a large number of CRC cases, and estimates of penetrance obtained in this manner may not be relevant to mutation carriers in the general population. Recent studies have suggested that current risk estimates for colorectal and endometrial cancers may be largely overestimated due to ascertainment bias introduced in the selection of families [Carayol 2002; Hampel 2005b; Quehenberger 2005]. However, after exclusion of presumed positive carriers, we found that our primary conclusions were the same: time to first CRC, first HNPCC cancer and death were similar in families with different mutations; type of mutation may have an impact on development of extracolonic cancers; and male gender was associated with a worse outcome.

In this study three very large families, with three different mutations, have been ascertained in the same way. Confounding by environmental factors is likely to have been small because the families live in the same geographic area and have a similar cultural background. Clinical screening programs have been established, and to date, 1450 at risk
individuals have had at least one screening colonoscopy, and more than 70% of these have had multiple colonoscopies. The number of CRC and other cancer cases that this program has prevented will be investigated in a future study.

In conclusion, the cumulative risk of developing cancer by 70 years is 94% in carriers of these \textit{MSH2} mutations. The age- and gender- related risks of CRC and death are similar for the exon 5 splice site mutation, the exon 4-16 deletion and the exon 8 deletion. There is evidence to suggest that transitional cell cancers of the urinary tract occur less frequently, and ovarian cancer in women occurs more frequently in carriers of the exon 5 splice site mutation than in carriers with these genomic deletions.
CHAPTER 4

Impact of Colonoscopic Screening in Male and Female Lynch Syndrome Carriers with an MSH2 Mutation

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4.1 INTRODUCTION

Lynch syndrome (LS), often called hereditary nonpolyposis colorectal cancer (HNPCC), is the most common hereditary colon cancer syndrome and accounts for 2-5% of all colorectal cancer (CRC) cases [Aaltonen 1998; de la Chapelle 2005; Hampel 2005a; Lynch 2009a]. This autosomal dominant disorder is characterized by early onset CRC, right-sided predominance, increased incidence of synchronous and metachronous CRCs, increased risk of other LS-associated malignancies [Mecklin 1991; Watson 1993; Aarnio 1995, 1999; Lynch 2008a, 2009a], and is caused by germline mutations in DNA mismatch repair (MMR) genes [Wheeler 2000; Lynch 2003].

The lifetime risk of developing CRC is about 70-80% in individuals with an MMR mutation [Vasen 1996; Aarnio 1999; Lynch 2000; Johnson 2006; Stuckless 2007; Barrow 2008] and is influenced by sex, method of ascertainment and the MMR genes involved. The reported lifetime risks for CRC range from 27-100% in males and 22-83% in females [Vasen 1996, 2001; Aarnio 1999; Hampel 2005b; Quehenberger 2005; Stuckless 2007; Barrow 2008; Ramsoekh 2009]. In Newfoundland MSH2 mutation carriers, females had a 39% lifetime risk of CRC compared with 85% in males, but females had a substantial risk of gynecologic cancers [Kopciuk 2009].

Screening with colonoscopy can potentially prevent the great majority of CRCs by detection and removal of adenomas, the precursors of most CRCs [Fletcher 2008]. Presently, screening for CRC in LS requires complete colonoscopy to the cecum every 1
to 2 years starting at ages 20 to 25 years, or 10 years younger than the youngest age of the person diagnosed in the family [Lynch 2000, 2008a; Silva 2005; Lindor 2006].

In longitudinal studies of screening effectiveness, survivor bias favours screening when compared with those not screened. Furthermore, the phenotypic expression of MMR mutations is different in males and females [Green 2002; Stuckless 2007; Kopciuk 2009], and higher enrollment and compliance with screening programs may also differ between males and females. A prior cohort study evaluated colonoscopic screening in LS and found that screening every 3 years decreased the CRC rate by 62% and decreased overall mortality by about 65% [Jarvinen 2000]. A more recent study showed that the median age at onset of CRC was delayed by more than 20 years in the screened group [Stupart 2009]. However, no analysis by sex or adjustment for survivor bias was undertaken in these studies.

This study was undertaken to determine the effectiveness of colonoscopic screening in male and female LS carriers of three different, phenotypically similar, MSH2 founder mutations. To correct for survivor bias we compared actual outcomes in the screened group with expected outcomes derived from the non-screened group, matching for age of entry to screening. Additionally, we determined compliance with screening recommendations and the relationship of the screening interval to CRC development in a subset of MSH2 mutation carriers.
4.2 MATERIALS AND METHODS

Families with a known \textit{MSH2} mutation were initially identified through the Provincial Medical Genetics Program or the Department of Surgery at Grenfell Hospital, St. Anthony. Pedigrees were extended over time and the phenotype reported [Green 2002; Stuckless 2007]. Family members at 50\% risk of inheriting a mutation were recommended to enter screening prior to discovery of the mutation, and carriers were recommended to do so following discovery of the mutation. Informed consent was obtained from all eligible subjects or an appropriate proxy. Individuals were considered eligible for the study if they were at 50\% \textit{a priori} risk to be mutation carriers and were included if they met one of the following criteria:

1. Confirmation by mutation testing (\textit{Proven mutation carrier});

2. In the line of descent and having offspring with a proven mutation (\textit{Obligate mutation carrier});

3. In the line of descent and presenting clinically with a LS tumour $< 50$ years of age (\textit{Presumed mutation carrier}).

4.2.1 Eligible Participants vs. Study Participants

On the basis of the above criteria, 387 individuals born after 1910 were considered to be eligible participants. Thirteen of these subjects declined to participate, 39 were lost to follow-up and 13 were excluded because they did not return their consent form or because records were never received. Thus study subjects comprised 322 of 387 (83\%) eligible individuals (148 males, 174 females) belonging to 18 LS families. Nine families
had an intron 5 splice site mutation (c.942+3A/T), eight families had an exon 8 deletion (c.1277-?_1386+?del) and one family had an exon 4-16 deletion (c.646-?_2802+?del) (13). In total, 216 were proven mutation carriers, 50 were obligate mutation carriers and 56 were considered presumed carriers.

Screening for LS began in the late 1980s and initially included only colonoscopy every 1-2 years starting at age 20-25 years. Of the 322 study participants, 152 (47%) entered a CRC screening program and the remaining 170 (53%) did not. The majority of those not screened had either had a prior CRC, and thus were not eligible as the study is limited to screening for first CRC, or died prior to implementation of the screening program. Individuals were considered to have entered a screening program if they had at least one colonoscopy prior to any symptoms suggestive of colorectal cancer. Median follow-up from entry into screening to death or last follow-up was 9 years in males and 11 years in females.

To address compliance with screening recommendations, all colonoscopic information available for study subjects with an intron 5 splice site mutation and an exon 8 deletion were reviewed. Complete colonoscopy information following initial screening colonoscopy was not available for those with an exon 4-16 deletion. All mutation carriers who had at least two colonoscopic examinations performed after 1 January 1994 were included. Eleven mutation carriers were excluded because they had only one colonoscopy
performed. Study subjects were considered compliant if all screening colonoscopies were performed within 2 years of the previous examination.

### 4.2.2 Data Collection

Medical records were reviewed and demographic data, dates and results of genetic testing, dates and results of various screening tests, dates and details of CRC, and mortality data were collected from 2006 to 2009.

### 4.2.3 Statistical Analysis

Survivor bias was shown by comparing the age of entry to screening in males and females with the age of onset of CRC in the non-screened group (Figure 4.1a-b). Twenty eight percent of males screened entered the program after age 40 years, whereas by age 40, approximately 50% in the non-screened group had already developed CRC. Comparable percentages for the females were 40% and 16%. To overcome this survivor bias in males, we compared the time to CRC and survival in the screened group to that expected from the non-screened group, where the expected outcome for each individual who entered a screening program was determined by calculating the median time-to-event in non-screened individuals who were the same age and gender, alive and disease-free, at the time the individual entered the screening program. For example, the expected survival for a 40 year old male who entered screening was the median survival of all males who survived to 40 years and were disease-free in the non-screened group. As survivor bias was less in females, and lifetime risk of CRC is lower than in males.
[Kopciuk 2009], we compared time to CRC in screened females with that in the non-screened group.

Statistical analyses were done using Version 15.0 of the Statistical Package for Social Sciences (SPSS). Cumulative incidences of CRC and death (all-cause mortality) were calculated using the Kaplan-Meier time-to-event analysis, and the significance of the difference between the groups was tested by the log rank test. Calculation of expected outcomes from the non-screened group for each screened individual assumed that the outcome would occur at a particular median age. This assumption is reasonable for CRC in men where penetrance is very high, and also for death in men and women, but may be less reasonable for CRC in women where the lifetime risk is lower. Therefore, the CRC comparison to expected outcomes was not performed for females. Median age at CRC diagnosis and median survival were based on Kaplan-Meier estimates and accounted for censored cases. Relative risk of developing each outcome was estimated for the screened group compared to that expected (derived from the non-screened group) using the Cox Regression Model.
Figure 4.1a-b Age at entry into colorectal (CRC) screening compared with age at diagnosis of CRC in non-screened group by gender [(a) males, (b) females].
4.2.4 Ethics

Ethics approval was granted by the Human Investigations Committee of the Faculty of Medicine, Memorial University of Newfoundland.

4.3 RESULTS

Significantly more females than males entered screening (56% vs. 36%; $\chi^2 = 12.6; P < .001$), with median age at screening being 36 and 38 years, respectively, for male and female mutation carriers. As anticipated, a predominance of the non-screened group was born between 1910 and 1950 (Table 4.1). Of 69 males born after 1950, 46 (67%) entered screening, and of 87 females born after 1950, 70 (80%) did so ($\chi^2 = 3.84; P = .05$).

4.3.1 Outcomes in Non-screened Group

In males, 79% developed at least one CRC with median age at CRC diagnosis being 41 years, and in females, 55% developed CRC with median being 57 years. In males, median survival was 52 years, and 35 of 69 deaths (51%) were due to CRC. In females, median survival was 63 years, and 15 of 50 deaths (30%) were caused by CRC.
Table 4.1 Clinical characteristics of screened and non-screened group by gender.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th></th>
<th>Females</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Screened n (%)</td>
<td>Non-screened n (%)</td>
<td>p-value</td>
<td>Screened n (%)</td>
</tr>
<tr>
<td><strong>Year of birth</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1910-1950</td>
<td>8 (15)</td>
<td>67 (74)</td>
<td>0.000</td>
<td>28 (29)</td>
</tr>
<tr>
<td>After 1950</td>
<td>46 (85)</td>
<td>23 (26)</td>
<td></td>
<td>70 (71)</td>
</tr>
<tr>
<td><strong>Mutation Status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proven mutation carrier</td>
<td>50 (92)</td>
<td>37 (39)</td>
<td>0.000</td>
<td>96 (98)</td>
</tr>
<tr>
<td>Obligate mutation carrier</td>
<td>2 (4)</td>
<td>26 (28)</td>
<td></td>
<td>1 (1)</td>
</tr>
<tr>
<td>Presumed mutation carrier</td>
<td>2 (4)</td>
<td>31 (33)</td>
<td></td>
<td>1 (1)</td>
</tr>
<tr>
<td><strong>Colorectal cancer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>14 (26)</td>
<td>74 (79)</td>
<td>0.000</td>
<td>14 (14)</td>
</tr>
<tr>
<td>No</td>
<td>40 (74)</td>
<td>20 (21)</td>
<td></td>
<td>84 (86)</td>
</tr>
<tr>
<td><strong>Dead</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>4 (7)</td>
<td>69 (73)</td>
<td>0.000</td>
<td>7 (7)</td>
</tr>
<tr>
<td>No</td>
<td>50 (93)</td>
<td>25 (27)</td>
<td></td>
<td>91 (93)</td>
</tr>
<tr>
<td><strong>MSH2 mutation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intron 5 splice site</td>
<td>30 (56)</td>
<td>54 (57)</td>
<td>0.967</td>
<td>59 (60)</td>
</tr>
<tr>
<td>Exon 8 deletion</td>
<td>14 (26)</td>
<td>24 (26)</td>
<td></td>
<td>17 (17)</td>
</tr>
<tr>
<td>Exon 4-16 deletion</td>
<td>10 (18)</td>
<td>16 (17)</td>
<td></td>
<td>22 (23)</td>
</tr>
</tbody>
</table>

*Those with missing date of births were excluded

4.3.2 Comparison of Outcomes after Screening to those Expected

For screened males, median age to CRC was 58 years. Compared to the expected median age of 47 years derived from the non-screened group, screened males had significantly lower risks for CRC (RR=0.29; 95% CI 0.16-0.53) (Figure 4.2a). In screened females, median age to CRC was 79 years, substantially better than median age in the non-screened group (RR=0.29; 95% CI 0.16-0.53) (Figure 4.2b).
Median survival in males who entered screening was 66 years. Compared to the expected median survival of 62 years for males, those who entered screening had better survival (RR=0.38; 95% CI 0.13-1.0) (Figure 4.2c). For females, median survival in the screened group was 80 years compared to the expected median survival of 63 years (RR=0.19; 95% CI 0.085-0.44) (Figure 4.2d).
Figure 4.2a. Lifetime risk of colorectal cancer (CRC) in screened male group compared with expected from non-screened males; b. Lifetime risk of CRC in screened female group compared with non-screened females.
Figure 4.2c-d. Lifetime risk of death in screened group compared with expected from non-screened group by gender [(c) males, (d) females]
4.3.3 Compliance with Screening Recommendations

Forty one males and 68 females in 16 families with the intron 5 splice site mutation and the exon 8 deletion had at least two colonoscopic examinations performed (Figure 4.3). Eighteen males (44%) and 28 females (41%) were compliant with colonoscopy every 1-2 years.

![Diagram showing the compliance and colorectal cancer outcomes of mutation carriers.]

Figure 4.3 Colorectal cancer (CRC) outcomes in mutation carriers with at least two colonoscopic examinations performed.
Eleven of 41 males (27%) developed an interval CRC, with median time from last screening colonoscopy to diagnosis being 1.7 years. Four individuals had a screening colonoscopy 1-2 years prior to their cancer diagnosis and four had screening within one year of their diagnosis (Table 4.2). Ten of 68 females (15%) developed an interval CRC, with median time from previous colonoscopy to diagnosis being 2.1 years. Four individuals had a screening colonoscopy 1-2 years prior to their diagnosis and one was diagnosed at a screening interval less than one year (Table 4.2).
Table 4.2 Characteristics of screened mutation carriers with an interval cancer.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age at CRC</th>
<th>Location</th>
<th>Stage (TNM)</th>
<th>Stage (Dukes)</th>
<th>Compliant</th>
<th>Interval (years)</th>
<th>Full colonoscopy at previous exam</th>
<th>Result of previous examination</th>
<th>Dead</th>
<th>Cause of Death</th>
</tr>
</thead>
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<td>Males</td>
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<td>II</td>
<td>B</td>
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<td>3.9</td>
<td>Yes</td>
<td>Normal</td>
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<tr>
<td></td>
<td>53</td>
<td>Proximal</td>
<td>I</td>
<td>A</td>
<td>No</td>
<td>1.8</td>
<td>Yes</td>
<td>Normal</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>Proximal</td>
<td>III</td>
<td>C</td>
<td>Yes</td>
<td>0.70</td>
<td>Yes</td>
<td>Normal</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>Distal</td>
<td>0</td>
<td>-</td>
<td>No</td>
<td>1.7</td>
<td>Yes</td>
<td>Normal</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td></td>
<td>46</td>
<td>Proximal</td>
<td>I</td>
<td>A</td>
<td>Yes</td>
<td>0.96</td>
<td>Yes</td>
<td>Normal</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td></td>
<td>58</td>
<td>Distal</td>
<td>I</td>
<td>A</td>
<td>Yes</td>
<td>1.8</td>
<td>Yes</td>
<td>Normal</td>
<td>No</td>
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<tr>
<td></td>
<td>52</td>
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<td>II</td>
<td>B</td>
<td>No</td>
<td>3.9</td>
<td>Yes</td>
<td>TA</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td></td>
<td>43</td>
<td>Proximal</td>
<td>0</td>
<td>-</td>
<td>Yes</td>
<td>0.62</td>
<td>Yes</td>
<td>Normal</td>
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</tr>
<tr>
<td></td>
<td>38</td>
<td>Rectal</td>
<td>III</td>
<td>C</td>
<td>Yes</td>
<td>1.4</td>
<td>Yes</td>
<td>2 HP</td>
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<td>CRC</td>
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<tr>
<td></td>
<td>43</td>
<td>Rectal</td>
<td>I</td>
<td>A</td>
<td>No</td>
<td>2.7</td>
<td>Yes</td>
<td>TA &amp; 7 HP</td>
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<tr>
<td></td>
<td>47</td>
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<td>II</td>
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<td>Females</td>
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<td>B</td>
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<tr>
<td></td>
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<td></td>
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<td>1.1</td>
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<tr>
<td></td>
<td>79</td>
<td>Distal</td>
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<td>B</td>
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<td>Stomach Ca</td>
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<td>III</td>
<td>C</td>
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</tr>
<tr>
<td></td>
<td>43</td>
<td>Distal</td>
<td>I</td>
<td>A</td>
<td>Yes</td>
<td>1.1</td>
<td>Yes</td>
<td>TA</td>
<td>No</td>
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</tr>
<tr>
<td></td>
<td>35</td>
<td>Distal</td>
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<td>-</td>
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<td>2.5</td>
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<td></td>
<td>64</td>
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<td>I</td>
<td>A</td>
<td>No</td>
<td>1.2</td>
<td>Yes</td>
<td>UP</td>
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<td>C</td>
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</tr>
<tr>
<td></td>
<td>39^e</td>
<td>Proximal</td>
<td>I</td>
<td>A</td>
<td>-</td>
<td>0.0</td>
<td>-</td>
<td>-</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>
CRC, colorectal cancer; HP, hyperplastic polyp; TA, tubular adenoma; TNM, tumor, node, metastasis; UP, polyp with unknown histology

\(^a\) Compliant with current screening recommendations of colonoscopy every 1-2 years

\(^b\) Time from last colonoscopy to CRC development (years)

\(^c\) Individual had a double primary at diagnosis

\(^d\) Investigative colonoscopy: individual presented with fatigue and shortness of breath (had 2 prior colonoscopies 1 year apart that were normal)

\(^e\) Individual had CRC diagnosed on initial screening colonoscopy
4.4 DISCUSSION

This is the first study to separately examine colonoscopy screening outcomes in male and female LS mutation carriers and to adjust for survivor bias. In a group of genetically homogeneous families, it appears that colonoscopic screening prevented CRC and delayed the age at onset of CRC by more than 10 years for both male and female mutation carriers. It was associated with a 4-year improvement in life expectancy for males and a substantial improvement in life expectancy of more than 15 years for females. Further work on the impact of gynecological screening is ongoing to determine its contribution to the significant survival benefit found in female mutation carriers.

In those born after 1950, men participated in screening less frequently than women. In a qualitative study, we have identified personal, health care provider and health care system factors as dominant issues in managing LS properly [Watkins 2011]. These factors may have a bigger influence on men than women, as barriers to effective screening behaviour.

In the screened group, CRCs continued to occur even in those who were compliant with colonoscopy every 1-2 years. Only 44% of males and 41% of females had all colonoscopies within 2 years of the previous examination. Twenty-one individuals developed an interval CRC, of whom eight had a colonoscopy 1-2 years prior to their diagnosis. Similar results were reported from other studies of colonoscopic screening in LS mutation carriers: 13 of 34 interval CRCs were diagnosed within 2 years of a previously normal screening colonoscopy [de Jong 2006a] and 16 of 33 CRCs in another
study [Vasen 2010]. Given the high incidence of CRC and the accelerated adenoma-carcinoma sequence in LS [de Jong 2004b], annual screening colonoscopies may be appropriate for this group.

In our study, five compliant individuals developed CRC within 1 year of their previous colonoscopy. In another study, 19 of 43 interval cancers detected by screening were preceded by a normal colonoscopic exam within the recommended interval of 12 months [Engel 2010]. This must question the quality of the colonoscopic examination as these tumours were likely to have been missed lesions. Studies have shown that the adenoma miss rate in LS carriers with conventional colonoscopy is more than 50%, and that many of these missed lesions are small, flat adenomas [Hurlestone 2005; Lecomte 2005; East 2008; Stoffel 2008]. Given the accelerated carcinogenesis in LS, improved detection of small lesions may be very important. Comparison of back-to-back examinations, in which standard colonoscopy was followed by chromoendoscopy, chromoendoscopy substantially increased the number of adenomas detected in individuals with LS [Hurlestone 2005; Lecomte 2005; Stoffel 2008], as did standard colonoscopy with intensive inspection (lasting > 20 minutes) [Stoffel 2008], narrow band imaging [East 2008] and autofluorescence endoscopy [Ramsoekh 2010]. It is likely that prevention of CRC in LS will be more effective with better colonoscopic imaging technologies.

Several limitations may be associated with cohort studies of this type. We have shown that direct comparison of screening outcomes with outcomes in the non-screened group

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favours the screening intervention, as only survivors without CRC can enter the screening program. In fact, median survival in all the non-screened males was 52 years, whereas expected survival in the non-screened males derived from those matched by the age of entry to the screening program was 62 years. Furthermore, the bias associated with the majority of the non-screened group being historical controls also favours the screening intervention, as does volunteer bias, as subjects who agreed to undergo colonoscopic screening may have been in better health than those who were not screened. This was a retrospective study, and compliance rates may be underestimated if colonoscopy reports were missed. Finally, median follow-up was 9 years in men and 11 years in women, probably not long enough to evaluate the long-term outcomes of screening, particularly as the number who survived longer than 60 years in the screened group was small.

In conclusion, the colonoscopic screening program in MSH2 mutation carriers reduced the incidence of CRC and improved survival, but potential still exists to obtain better outcomes by improving compliance, reducing the colonoscopy screening interval to 1 year, and enhancing the quality of colonoscopy examination.
CHAPTER 5

Impact of Gynecological Screening in Lynch Syndrome

Carriers with an MSH2 Mutation

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5.1 INTRODUCTION

Lynch syndrome (LS) is an autosomal dominant syndrome caused by germline mutations in DNA mismatch repair (MMR) genes [Lynch 2009a]. For female mutation carriers, the lifetime risk of developing endometrial cancer is estimated to be 30-70%, which equals or exceeds their risk of colorectal cancer (CRC) [Hendricks 2004; Hampel 2005b; Stuckless 2007; Barrow 2009], and may vary depending on the MMR gene involved [Hendricks 2004; Senter 2008; Barrow 2009; Ramsoekh 2009]. Lifetime risk estimates for ovarian cancer vary from 6% to 20% [Hampel 2005b; Stuckless 2007; Barrow 2009].

Current recommendations for gynecologic screening in LS require endometrial biopsy, transvaginal ultrasound (TVU) and CA125 testing, every 1-2 years starting at ages 30-35 years [Lindor 2006; Schmeler 2008; Lynch 2009a; Meyer 2009; Auranen 2011]. The benefit of screening for gynecological cancers in LS, however, has not yet been proven and there is also no consensus on the optimal screening tests to be performed [Manchanda 2009; Auranen 2011]. Endometrial cancer screening may have limited benefit because of early detection of disease due to postmenopausal vaginal bleeding and high survival rates following diagnosis. Screening for ovarian cancer in other high-risk groups has not led to early cancer detection using TVU and CA125 testing or improved survival [Evans 2009].

We recently evaluated colonoscopic screening in male and female LS mutation carriers with three different founder MSH2 mutations [Stuckless 2012], between which there was
little phenotypic difference [Stuckless 2007]. After adjustment for survivor bias, we found that colonoscopic screening was associated with a 4 year improvement in life expectancy for males and a substantial improvement of more than 15 years for females [Stuckless 2012]. However, the contribution of screening for gynecological cancers in female mutation carriers to this improvement was unknown. This study was undertaken to compare the incidence of endometrial and ovarian cancer and overall survival in carriers who entered a screening program for gynecologic cancer and in matched controls who were not screened.

5.2 MATERIALS AND METHODS

Seventeen families with one of three known MSH2 founder mutations were identified through the Provincial Medical Genetics Program or the Department of Surgery at Grenfell Hospital, St. Anthony. Eight families had an intron 5 splice site mutation (c.942+3A/T), eight an exon 8 deletion (c.1277-?_1386+?del) and one an exon 4-16 deletion (c.646-?_2802+?del) [Stuckless 2012]. In the early 1990s, all family members at 50% risk of inheriting a mutation were recommended to enter screening prior to discovery of the mutation, and carriers were recommended to do so following discovery of the mutation. All female mutation carriers, born after 1910, were identified and informed consent was obtained from all eligible subjects or an appropriate proxy. Individuals were considered eligible for the study if they were at 50% a priori risk to be mutation carriers and fulfilled one of the following criteria:
1. confirmation by mutation testing (*Proven mutation carrier*);

2. in the line of descent and having offspring with a proven mutation (*Obligate mutation carrier*);

3. in the line of descent and presenting clinically with a LS tumour < 50 years of age (*Presumed mutation carrier*).

### 5.2.1 Eligible participants vs. Study participants

Based on the above criteria, 204 females were considered to be eligible participants. Thirty (15%) subjects were excluded because they either declined to take part in the study (n=7), their records were never received (n=9), or they were lost to follow-up (n=14). Of the 174 included in the study, 129 were proven mutation carriers, 22 were obligate mutation carriers and 23 were presumed mutation carriers.

Individuals were considered to have entered a gynecological screening program if they had at least one gynecological examination (TVU, endometrial biopsy or CA 125 test) prior to any symptoms suggestive of endometrial or ovarian cancer. Screening for endometrial cancer was carried out using TVU and endometrial biopsy and screening for ovarian cancer was performed using TVU and CA 125 testing.
5.2.2 Data Collection

Dates and results of genetic testing, screening tests, cancer onset and death were collected between September 2006 and January 2010. FIGO stage was also collected for cases and matched controls.

5.2.3 Statistical Analysis

Survivor bias is inherent in screening studies of this type as only those who are alive and disease-free can enter a screening program (Figure 5.1). To overcome this bias, we compared time to event outcomes in the screened group (case) to matched controls in the non-screened group, taking into account age at entry into the screening program. One control was randomly selected for each case from all non-screened individuals who were alive and disease free, at the same age as the case at entry into the screening program.

Cumulative incidences of gynecological cancer and all-cause mortality were calculated using the Kaplan-Meier time-to-event analysis, and the significance of the difference between groups was tested by the log rank test. Individuals were censored at date of gynecological cancer, hysterectomy and bilateral oophorectomy, death or last follow-up.
5.2.4 Ethics

Ethics approval was granted by the Human Investigations Committee of the Faculty of Medicine, Memorial University of Newfoundland.

5.3 RESULTS

Fifty four of 174 (31%) female MSH2 mutation carriers had at least one gynecological screening exam and 120 did not. Median age at screening was 36 years with 61% of females screened by the age of 40 years. Thirty nine percent of females were screened after 40 years by which time 12% of the non-screened group had already developed
gynecological cancer (Figure 5.1). Median follow-up from entry into gynecological screening to death or last follow-up was 8.5 years.

5.3.1 Non-Screened Group Outcomes

Fifty-five of 120 (46%) women in the non-screened group developed a gynecological cancer (Table 5.1), with median age to gynecological cancer being 60 years (Figure 5.1). There were 54 deaths with 11 due to endometrial cancer and five to ovarian cancer. Mean survival was 66 years. Four of the 11 women who died of their endometrial cancer died within one year of their diagnosis, and eight within the first three years. Three of the five women who died of ovarian cancer died within one year of their diagnosis. Six of the 11 deaths from endometrial cancer occurred before 1990 as did two of the five deaths from ovarian cancer.
# Table 5.1 Clinical characteristics of female MSH2 mutation carriers by gynecological screening status

<table>
<thead>
<tr>
<th></th>
<th>Screened group (cases)</th>
<th>Non-screened group</th>
<th>Matched controls</th>
<th>P-value (cases vs. controls)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td><strong>Year of birth</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1910-1950</td>
<td>8 (15)</td>
<td>76 (65)</td>
<td>44 (81)</td>
<td>0.000</td>
</tr>
<tr>
<td>After 1950</td>
<td>46 (85)</td>
<td>41 (35)</td>
<td>10 (19)</td>
<td></td>
</tr>
<tr>
<td><strong>Mutation status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proven mutation carrier</td>
<td>53 (98)</td>
<td>76 (64)</td>
<td>36 (67)</td>
<td>0.000</td>
</tr>
<tr>
<td>Obligate mutation carrier</td>
<td>0 (0)</td>
<td>22 (18)</td>
<td>10 (18)</td>
<td></td>
</tr>
<tr>
<td>Presumed mutation carrier</td>
<td>1 (2)</td>
<td>22 (18)</td>
<td>8 (15)</td>
<td></td>
</tr>
<tr>
<td><strong>Gynecological cancer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>14 (26)</td>
<td>55 (46)</td>
<td>25 (46)</td>
<td>0.028</td>
</tr>
<tr>
<td>No</td>
<td>40 (74)</td>
<td>65 (54)</td>
<td>29 (54)</td>
<td></td>
</tr>
<tr>
<td><strong>FIGO stage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Localized (Stage I &amp; II)</td>
<td>11 (92)</td>
<td>-</td>
<td>12 (71)</td>
<td>0.168</td>
</tr>
<tr>
<td>Advanced (Stage III &amp; IV)</td>
<td>1 (9)</td>
<td>-</td>
<td>5 (29)</td>
<td></td>
</tr>
<tr>
<td><strong>Endometrial cancer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>9 (17)</td>
<td>44 (37)</td>
<td>20 (37)</td>
<td>0.017</td>
</tr>
<tr>
<td>No</td>
<td>45 (83)</td>
<td>76 (63)</td>
<td>34 (63)</td>
<td></td>
</tr>
<tr>
<td><strong>Ovarian cancer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>6 (11)</td>
<td>16 (13)</td>
<td>6 (11)</td>
<td>1.000</td>
</tr>
<tr>
<td>No</td>
<td>48 (89)</td>
<td>104 (87)</td>
<td>48 (89)</td>
<td></td>
</tr>
<tr>
<td><strong>Colorectal cancer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>8 (15)</td>
<td>48 (40)</td>
<td>24 (44)</td>
<td>0.001</td>
</tr>
<tr>
<td>No</td>
<td>46 (85)</td>
<td>72 (60)</td>
<td>30 (56)</td>
<td></td>
</tr>
<tr>
<td><strong>Gynecological surgery</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hysterectomy with BSO</td>
<td>8 (15)</td>
<td>14 (12)</td>
<td>6 (11)</td>
<td>0.246</td>
</tr>
<tr>
<td>Hysterectomy with RSO</td>
<td>0 (0)</td>
<td>2 (2)</td>
<td>2 (4)</td>
<td></td>
</tr>
<tr>
<td>Hysterectomy with LSO</td>
<td>0 (0)</td>
<td>1 (1)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Hysterectomy</td>
<td>4 (7)</td>
<td>9 (8)</td>
<td>7 (13)</td>
<td></td>
</tr>
<tr>
<td><strong>Dead (all-cause mortality)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3 (6)</td>
<td>54 (45)</td>
<td>29 (54)</td>
<td>0.000</td>
</tr>
<tr>
<td>No</td>
<td>51 (94)</td>
<td>66 (55)</td>
<td>25 (46)</td>
<td></td>
</tr>
<tr>
<td><strong>Death due to gynec cancer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2 (67)</td>
<td>16 (30)</td>
<td>6 (21)</td>
<td>0.080</td>
</tr>
<tr>
<td>No</td>
<td>1 (33)</td>
<td>38 (70)</td>
<td>23 (79)</td>
<td></td>
</tr>
</tbody>
</table>

BSO, bilateral salpingo-oophorectomy; RSO/LSO, right/left salpingo-oophorectomy

*a* Those with missing date of births were excluded

*b* Those with both an endometrial and ovarian cancer were counted only once

*c* FIGO stage for gynecological cancers (endometrial and ovarian cancers combined); Those with missing FIGO stage were excluded

*d* Those who had gynecological surgery performed prophylactically or for non-malignant conditions
5.3.2 Screened Group Outcomes

Fourteen of 54 (26%) women in the screened group developed at least one gynecological cancer (Table 5.1). Nine females had endometrial cancer diagnosed, five of which were within one year of a prior negative screening test and two were at initial screening test. Of the nine endometrial tumors, seven were localized cancers (all Stage I), one was at an advanced stage (stage III), and staging was unavailable for one (Table 5.2). No deaths were due to endometrial cancer. Six females had ovarian cancer detected, three of which were diagnosed within one year of a prior negative screening test. All four ovarian tumors with available staging information were localized tumors (Stage I/II). However, of the three deaths in the screened group, two were due to ovarian cancer. One died within 1 year of her ovarian cancer diagnosis and the other within two years (Table 5.2).

5.3.3 Comparison of Outcomes After Screening to Matched Controls

Of the 54 randomly selected matched controls, 25 (46%) developed at least one gynecological cancer (Table 5.1). Median age to gynecologic cancer in the screened group versus the control group was not significantly different (54 years versus 56 years; P=.50) (Figure 5.2a). In the control group, 12 of 17 (71%) had localized tumors (six stage I, six stage II) and five were at an advanced stage (two stage III, three stage IV). Staging was unavailable in eight. Although 92% of cancers diagnosed in the screened group were localized compared to 71% in the control group, this difference did not achieve statistical significance (Table 5.1).
Table 5.2 Characteristics of screened mutation carriers who developed endometrial or ovarian cancer.

<table>
<thead>
<tr>
<th>Study ID</th>
<th>Age at cancer</th>
<th>Stage</th>
<th>Screen detected</th>
<th>Type of last screening exam</th>
<th>Interval (years)</th>
<th>Result of previous exam</th>
<th>Follow-up (years)</th>
<th>Dead</th>
<th>Cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endometrial Cancer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>152e</td>
<td>50</td>
<td>1a</td>
<td>No</td>
<td>TVU</td>
<td>0.98</td>
<td>Normal</td>
<td>0.92</td>
<td>Yes</td>
<td>Ovarian Ca</td>
</tr>
<tr>
<td>186f</td>
<td>47</td>
<td>3a</td>
<td>Yes</td>
<td>TVU</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>46</td>
<td>1a</td>
<td>Yes</td>
<td>TVU/Endo Biopsy</td>
<td>5.76</td>
<td>Normal</td>
<td>7.84</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>74</td>
<td>54</td>
<td>1a</td>
<td>Yes</td>
<td>TVU/Endo Biopsy</td>
<td>1.00</td>
<td>Abnormal</td>
<td>12.93</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>203</td>
<td>48</td>
<td>1b</td>
<td>No</td>
<td>TVU</td>
<td>0.47</td>
<td>Normal</td>
<td>6.36</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>224</td>
<td>53</td>
<td>N/A</td>
<td>No</td>
<td>Endo Biopsy</td>
<td>0.87</td>
<td>Normal</td>
<td>0.55</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>590f</td>
<td>37</td>
<td>1a</td>
<td>Yes</td>
<td>TVU</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>723</td>
<td>40</td>
<td>1a</td>
<td>No</td>
<td>TVU</td>
<td>0.12</td>
<td>Normal</td>
<td>8.86</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>735</td>
<td>41</td>
<td>1a</td>
<td>Yes</td>
<td>Endo Biopsy</td>
<td>1.04</td>
<td>Normal</td>
<td>4.09</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td><strong>Ovarian Cancer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>152e</td>
<td>50</td>
<td>2c</td>
<td>No</td>
<td>TVU</td>
<td>0.98</td>
<td>Normal</td>
<td>0.92</td>
<td>Yes</td>
<td>Ovarian Ca</td>
</tr>
<tr>
<td>29</td>
<td>50</td>
<td>2c</td>
<td>Yes</td>
<td>TVU</td>
<td>0.94</td>
<td>Normal</td>
<td>8.36</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>507</td>
<td>45</td>
<td>1a</td>
<td>No</td>
<td>Endo Biopsy</td>
<td>1.56</td>
<td>Normal</td>
<td>3.80</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>518</td>
<td>38</td>
<td>2b</td>
<td>No</td>
<td>TVU</td>
<td>1.06</td>
<td>Abnormal</td>
<td>5.83</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>761</td>
<td>37</td>
<td>N/A</td>
<td>No</td>
<td>Endo Biopsy</td>
<td>0.46</td>
<td>Normal</td>
<td>1.12</td>
<td>Yes</td>
<td>Ovarian Ca</td>
</tr>
<tr>
<td>PID=205</td>
<td>82</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>0.69</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

TVU, transvaginal ultrasound; N/A, not available

*Cancer was detected on a screening exam

*bLast screening exam (TVU, Endometrial biopsy or CA125) prior to cancer diagnosis

*cTime from last screening exam to cancer development (years)

*dFollow up is from cancer diagnosis to last follow-up or death

*eIndividual had a double primary at diagnosis (endometrial and ovarian cancer)

*fIndividual had endometrial cancer diagnosed on initial screening exam

*gTVU and endometrial biopsy show possible endometrial hyperplasia

*hTVU showed cyst in right ovary
Figure 5.2a-b  a. Lifetime risk of gynecological cancer in screened group compared to matched controls in the non-screened group; b. Lifetime risk of death in screened group compared to matched controls in the non-screened group
There were 29 deaths in the matched control group, six of which were due to a gynecological cancer (Table 5.1). Mean survival was substantially better in the screened group compared to matched controls but did not achieve statistical significance (79 years versus 69 years; \( P = .11 \)) (Figure 5.2b). Three of six deaths in the matched control group were due to an endometrial cancer and three were due to an ovarian cancer. Of those who died due to endometrial cancer, one had stage IIA disease, one had stage IIIB disease and one was of unknown stage. For those who died due to ovarian cancer, one had stage I disease, one had stage IV disease and one was of unknown stage.

5.3.4 Impact of Endometrial and Ovarian Cancer Screening

For the endometrial screened group, median age to endometrial cancer was 54 years compared to 57 years in matched controls (\( P = .77 \)) (Figure 5.3a). In the ovarian screened group, three cancers were detected, all by age 50 years, with no difference in the cumulative incidence of ovarian cancer in the two groups (Figure 5.3b).
Figure 5.3a-b a. Lifetime risk of endometrial cancer in screened group compared to matched controls in the non-screened group; b. Lifetime risk of ovarian cancer in screened group compared to matched controls in the non-screened group.
5.3.5 Impact of Colonoscopic Screening

Fifty one of 54 (94%) women in the gynecological screened group also entered colonoscopic screening. However, 47 of 120 (39%) women in the non-screened group had colonoscopic screening but could not enter gynecologic screening. This was due to the fact that these women had already had either a gynecologic cancer (n=15), a hysterectomy with or without ovaries removed due to a non-malignant condition (n=21), or were less than 30 years of age at last follow-up and had not yet reached the start age for gynecological screening (n=6). In five women the reason was unknown. For those who received both gynecologic and colonoscopic screening, median age to CRC was significantly higher (82 years versus 60 years; \( P=.009 \)) and mean survival was substantially better (78 years versus 67 years; \( P=.08 \)) than that in matched controls without any screening.

5.4 DISCUSSION

Despite the substantially increased risk of endometrial and ovarian cancer for female \( MSH2 \) mutation carriers, gynecological cancer screening did not result in earlier diagnosis of gynecologic cancer. This may be due to the fact that gynecological cancers in LS appear to present at an early stage regardless of screening status [Boks 2002; Crijnen 2005; Grindedal 2010]. Interval cancers were also detected despite screening. Eight of 15 gynecological cancers were diagnosed within one year of prior screening suggesting that the methods for gynecologic screening were limited.
In the total non-screened group of female *MSH2* mutation carriers, gynecologic cancer occurred frequently and both endometrial and ovarian cancer contributed to the poor survival. Cancer was likely diagnosed at a later stage because eight of 11 endometrial cancer deaths occurred within three years of diagnosis and three of five ovarian cancer deaths occurred within one year of diagnosis. However, in the matched control group, of the four gynecological related deaths with available staging, two deaths occurred after diagnosis of a localized tumor and two occurred in those diagnosed at an advanced stage. This is similar to a study that found that 50% of women with an MMR mutation who died within the first three years of their ovarian cancer diagnosis had stage I or II disease [Grindedal 2010].

None of the nine women in the screened group diagnosed with endometrial cancer died but two deaths at a young age were attributable to ovarian cancer. Given the fact that these two women died quickly after their diagnosis and that in both women the cancer was diagnosed within a year of prior screening, the option of risk-reducing hysterectomy and bilateral salpingo-oophorectomy should be recommended for women who have completed childbearing [Schmeler 2006]. In our study, if women had undergone prophylactic surgery at the age of 40 years, over 70% of gynecological cancers might have been prevented. Compared to annual gynecologic screening and annual examination prophylactic surgery is the most cost-effective strategy, regardless of the starting age of any of the three strategies [Chen 2007; Kwon 2008; Yang 2011]. However, as the age at first screening increases, the cost-effectiveness advantage rapidly diminishes and
therefore women are recommended to have prophylactic surgery as early as possible, particularly after completion of child-bearing.

Being part of a surveillance (screening) program, not the actual gynecologic screening procedures themselves, likely contributed to the longer life expectancy in those who had gynecological screening. In particular, colonoscopic screening prevented CRC and contributed at least in part to the improved survival in the screened group [Stuckless 2012].

Our study is limited by its retrospective nature and its use of historical controls in the non-screened group. Prior to 1990, cancer risk in these families may not have been well established, which likely predisposed to later stage of cancer at diagnosis. Lack of proper nutrition, health care and management of advanced disease may have contributed to poor outcomes. However, the use of historical controls should lead to bias in favor of screening in studies of this type. Survivor bias also favors screening and we have adjusted for this bias by selecting appropriately age matched controls. Although we report on relatively small numbers and short follow-up, this paper suggests that screening for gynecologic cancer did not contribute to the virtual normalization of life expectancy in screened female carriers. Future studies with longer follow-up and more patients may be necessary to evaluate the long-term outcomes of screening. Gynecologic screening of women in their fourth decade and those who chose not to have prophylactic gynecologic
surgery requires further evaluation, particularly as there was a trend towards diagnosis of gynecologic cancer at an earlier stage in the screened group.

In conclusion, screening for gynecological cancer in female LS mutation carriers did not result in earlier diagnosis of gynecologic cancer and interval cancers occurred. The occurrence of both ovarian cancer deaths at a young age in screened patients and gynecological cancer deaths despite early stage disease in matched controls suggests that prophylactic hysterectomy and bilateral salpingo-oophorectomy should be performed in women who have completed childbearing.
CHAPTER 6

Discussion
6.1 INTRODUCTION

Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths in Canada. Of the common cancers, CRC has the highest potential for prevention through detection and removal of adenomas by screening colonoscopy [Fletcher 2008]. Detection of CRCs at an earlier stage and more effective treatments can improve morbidity and mortality. Therefore it is important that those at high risk for CRC be identified so that they can receive appropriate colonoscopic screening. Newfoundland has the highest incidence of CRC and highest rate of CRC death in Canada, and the highest rate of familial CRC in the world. In Newfoundland, approximately 30% of incident CRC cases have at least one first degree relative affected with CRC [Green 2007]. Four percent of these incident cases have monogenic disease of known etiology, such as Lynch syndrome (LS), 8% have a high risk family history but no known genetic cause of CRC has been identified, and 18% have familial CRC but do not meet the high risk criteria for known hereditary CRC syndromes. Those with high risk family histories for CRC have a lifetime cancer risk of 40-50% or more and those with at least one first or second degree relative with CRC have a predicted lifetime risk of CRC of approximately 20%. Therefore, accurate assessment of familial risk is necessary and allows for directed colonoscopic screening.

6.2 IMPACT OF SCREENING IN MSH2 MUTATION CARRIERS

Among those with high risk family histories, LS is the most common hereditary CRC syndrome. Due to the young age at onset and high penetrance of CRC in LS families and the significantly increased risk of gynecological cancers for female mutation carriers,
early identification of mutation carriers is necessary to prevent and reduce cancer-related morbidity and mortality associated with this disease.

In chapter three we looked at the phenotypic expression of MSH2 mutation carriers in Newfoundland and found that male mutation carriers had a significantly increased lifetime risk of CRC compared to female mutation carriers. This gender difference was consistent across the three different founder MSH2 mutations reported in our study. Female mutation carriers were also found to have a high lifetime risk of developing both endometrial and ovarian cancer. Our significantly increased risks for CRC and gynecological cancer are consistent with published reports of high lifetime risk of CRC and increased extracolonic cancer incidence for MSH2 mutation carriers [Vasen 1996; Dunlop 1997; Aarnio 1999; Vasen 2001; Hampel 2005b; Barrow 2008, 2009; Ramsoekh 2009].

Identification of LS mutation carriers and an accurate description of the natural history and penetrance of disease are necessary to provide a rational and directed screening program. Individuals found to have LS will require intensive surveillance whereas those without the mutation will not. In chapter 4 we evaluated the impact of colonoscopic screening in male and female MSH2 mutation carriers, adjusting for survivor bias. As discussed in chapter 4, colonoscopic screening was found to significantly reduce the risk and delay the age of CRC onset for both male and female mutation carriers. More importantly, colonoscopic screening was associated with a significant improvement in
life expectancy for males and a substantial improvement of more than 15 years for female mutation carriers. Our study supports the previous work by Jarvinen et al. [Jarvinen 2000] and Stupart et al. [Stupart 2009] of a reduced risk of CRC and improved survival for LS mutation carriers who receive colonoscopic screening, and also provides novel information regarding the benefit of colonoscopic screening for male and female mutation carriers separately.

Although colonoscopic screening has been shown to reduce the risk of CRC development, CRCs are still occurring in individuals compliant with the screening recommendation for LS of colonoscopy every 1 to 2 years. In our study, 13 of 21 individuals who developed an interval CRC had a screening colonoscopy within two years of their CRC diagnosis (Chapter 4). This is consistent with results from other published studies that report interval CRCs in LS mutation carriers within 2 years of a previously normal colonoscopic exam [de Jong 2006a; Vasen 2010]. Given the accelerated carcinogenesis in LS and the relatively high incidence of CRC in screened subjects, it has been proposed that the screening interval be shortened to one year [Lynch 2008a; Engel 2010]. To identify the most optimal screening interval, future prospective large-scale controlled trials are needed to compare cancer and mortality outcomes in those with 1 to 2 year screening intervals and those with 1 year intervals.

Colorectal cancer screening in LS is generally performed using conventional colonoscopy, however, studies have shown that the adenoma miss rate is more than 50%
in this group and that many of these missed lesions are small, flat adenomas [Hurlestone 2005; Lecomte 2005; East 2008; Stoffel 2008]. In our study, five compliant individuals developed CRC within one year of a previously normal screening colonoscopic examination (Chapter 4). Other studies have found that over 40% of interval CRCs detected by screening were diagnosed within 12 months of a normal screening colonoscopy [Engel 2010]. Given the accelerated adenoma to carcinoma sequence in LS mutation carriers, these tumors were likely missed lesions. Chromoendoscopy [Hurlestone 2005; Lecomte 2005; East 2008], standard colonoscopy with intensive inspection (lasting > 20 minutes) [East 2008], narrow band imaging [Stoffel 2008] and autofluorescence endoscopy [Ramsoekh 2010] have all been shown to improve adenoma detection in LS patients. Future studies comparing each of these imaging techniques are needed to determine which is the most effective for CRC screening in LS.

As was stated earlier, endometrial cancer screening has limited benefit and is not performed in the general population for a number of reasons: low prevalence of disease; early detection of disease due to the occurrence of postmenopausal vaginal bleeding; and high survival rates [Schmeler 2008]. Similarly, screening for ovarian cancer is not performed due to the low prevalence of disease and the lack of good screening modalities for early cancer detection [Schmeler 2008; Buys 2011]. However, due to the high gynecologic cancer risk in LS mutation carriers, gynecological cancer screening is recommended even though the benefit of screening for this population has not been proven.
In chapter 5 we evaluated the impact of gynecological screening in female $MSH2$ mutation carriers. We found that gynecological screening did not lead to earlier stage detection nor did it prevent interval gynecological cancers from occurring. The apparent inefficiency of gynecological screening in LS is likely due to both inadequate screening methods for early cancer detection [Schmeler 2008; Buys 2011] and the diagnosis of early stage endometrial and ovarian cancers in LS carriers regardless of screening status [Boks 2002; Crijnen 2005; Grindedal 2010]. Given the lack of data on the efficacy of screening for endometrial and ovarian cancers and the demonstrated benefit of prophylactic gynecologic surgery, it has been proposed that prophylactic hysterectomy and bilateral salpingo-oophorectomy (BSO) be performed in female LS carriers who have completed child-bearing [Schmeler 2006; Manchanda 2009]. As we stated earlier, over 70% of gynecological cancers likely would have been prevented in our study if women had underwent prophylactic hysterectomy and BSO after child-bearing.

Outcomes regarding prophylactic gynecological surgery are not well studied. Differences in survival and disease-specific survival between women who undergo prophylactic gynecological surgery and those who do not are needed to assess the long-term effects of prophylactic surgery. There are also disadvantages to having prophylactic hysterectomy and BSO and these include surgical complications (most commonly, bleeding, infection, and injuries to the urinary tract and bowel) in a small number of individuals and premature menopause [Schmeler 2006]. Patients should be made fully aware of the limitations of endometrial and ovarian screening in LS and the possible surgical risks of
prophylactic surgery so that they can make an informed decision about the most appropriate management strategy for them. They must also take into consideration that at least 40% of female mutation carriers will never develop an endometrial cancer and 80-90% may never develop ovarian cancer. Larger studies with longer follow-up are needed to fully evaluate the efficacy of gynecologic screening compared to prophylactic surgery in reducing gynecological cancer incidence and disease-specific mortality for female LS carriers. As well, evaluation of the factors influencing the decision to undergo prophylactic surgery and the impact of surgical menopause on quality of life, offer significant research opportunities.

6.3 STUDY LIMITATIONS

Bias of some degree will always be present in genetic epidemiological studies. It can affect all the major parts of an investigation, such as selection of subjects, performance of the maneuver, measurement of the outcome, data collection, data analysis, data interpretation, and even reporting the findings, leading to erroneous conclusions [Stuckless 2009]. These inherent limitations are often compounded by lack of power due to the reality of small sample sizes when studying genetic diseases. In chapters 3, 4 and 5, a brief summary of some of the potential limitations were addressed in the discussion sections of each respective chapter. I will expand on these limitations here.

In chapter 3 we looked at the lifetime risks of colorectal, endometrial and other extracolonic cancers in a group of \textit{MSH2} mutation carriers. Kindreds were initially
identified by family history and met either the Amsterdam I or II criteria. However, as was stated earlier these criteria miss a substantial proportion of LS cases as they inherently select out a subgroup of families with a large number of CRC cases and a more severe phenotype. Smaller families with variable phenotypes are missed using these criteria and this can lead to ascertainment bias. This type of bias is introduced when the individuals selected for study are not representative of all cases in the population [Delgado-Rodríguez 2004; Stuckless 2009]. Therefore, our risk estimates in chapter 3 are likely to be overestimated due to use of multiple case families with earlier cancer onset and may not be generalizable to other LS carriers with an MSH2 mutation.

Competing risks bias may have been an issue with this study as well. Often times in medical research, a patient may experience an event, other than the one of interest, which alters their risk of experiencing the actual event of interest. Such an event is referred to as a competing risk event [Satagopan 2004; Stuckless 2009]. MSH2 mutation carriers are at risk of developing a number of cancers, such as CRC, endometrial, ovarian and stomach cancer. In our study, for example, those who died early due to stomach cancer would no longer be at risk for CRC. In this situation, stomach cancer would be a competing risk event and it may cause the risk estimates for CRC to be overestimated.

Another limitation relates to the fact that index cases were included in the study and this may have overestimated the lifetime cancer risk estimates. However, on the other hand, our penetrance estimates did not take into account screening status. It was likely that
some individuals were receiving colonoscopic screening, and as such, the risk estimates were likely to be underestimated. Screening status may also have been a potential confounder as the number of individuals receiving colonoscopic screening likely differed between the three mutation groups. Age and gender are also obvious potential confounders in practically all studies. No adjustment for these potential confounders was made when we compared the three specific mutations and this may have impacted our risk estimates.

In chapters 4 and 5 we looked at the impact of colonoscopic screening and gynecological screening, respectively, on cancer incidence and overall survival. Most studies dealing with treatment effectiveness are best answered by means of randomized controlled trials. However, due to the high risk of cancer development in LS mutation carriers it is unethical to randomly allocate mutation carriers to "screening" or "no screening", and as such, identification of an unbiased control group to compare outcomes of screening in LS families is difficult. As was stated earlier, our screening studies were limited by their retrospective nature and the use of an historical control group. However, due to time constraints and the lack of potential non-screened subjects in the present and future, the use of historical data was necessary. Historical controls are likely to have poorer outcomes due to lack of disease awareness in the past by both physicians and family members. As well, possible confounders such as improvements in general nutrition, healthcare and management of advanced disease can impact these studies. This type of bias favors the screening intervention and may overestimate the benefit of screening.
Retrospective studies also rely partly on patient recollection of information and although efforts were made to confirm diagnoses, incomplete information for earlier records and the non-confirmation of early cancers may have impacted our study.

Survivor bias, volunteer bias and compliance bias are also common in screening studies of this type. Survivor bias was a potential limitation in our screening studies as only those who were alive and disease-free could enter a screening program. Patients who died early of disease and did not receive screening likely differed in disease severity from those who received screening. Efforts were made to minimize the effect of this bias through our specific definition of controls in these studies. Volunteer bias, also referred to as self-selection bias, is related to the fact that those who volunteer for study are likely different from those who refuse participation [Hartman 2002, Sica 2006, Stuckless 2009]. Volunteers tend to be better educated, healthier and lead better lifestyles than those who do not choose to enter screening. Both these biases can lead to an incorrect assumption that the screening intervention favorably affects outcome when in fact it may be that disease severity is responsible for the observed difference between the screened and non-screened groups. These biases may have overestimated the effectiveness of screening in our studies. Compliance bias on the other hand can underestimate the effectiveness of screening. This bias occurs when differences in subject adherence to the planned screening protocol affects the study outcomes [Hartman 2002; Delgado-Rodríguez 2004; Stuckless 2009]. Patients who do not follow protocol guidelines may have worse outcomes than compliant patients leading to an apparent decrease in the effectiveness of
screening. This bias may also be compounded by physician compliance as well as the availability of screening procedures. If physicians do not provide appropriately scheduled appointments for patients or if wait times prevent timely access to screening procedures, the effectiveness of screening will again be underestimated. These issues likely played a role in our screening studies.

As was stated earlier, longer follow-up is needed to fully evaluate the long-term outcomes of these screening studies. As well, due to small event rates for the gynecological screening study, our study may have lacked the power to detect a significant difference in outcome between the screened and non-screened groups if there had actually been one. Endometrial and ovarian cancers are biologically different and behave differently when it comes to morbidity and mortality associated with their development. Larger studies with more events are needed to evaluate the effectiveness of endometrial and ovarian cancer screening separately.

Two other biases related to screening studies, but not addressed in chapters 4 and 5, are lead-time bias and length-time bias. Lead-time bias refers to the interval of time between diagnosis of cancer by screening and usual clinical detection due to symptoms. Lead-time, therefore, is the amount of time that diagnosis was advanced as a result of screening [Herman 2002]. It can lead you to incorrectly conclude that screening prolongs life, when in fact, it simply extends the period of time for which cancer is observed. We tried to avoid introducing lead-time bias into our analyses by calculating survival from birth
rather than from diagnosis or enrolment in the study. Length-time bias refers to the fact that slower growing tumors (those with more favorable prognoses) exist for a longer period of time in the preclinical phase and are therefore more likely to be detected by screening. Faster growing tumors (more aggressive cancers), by contrast, exist for a shorter period of time in the preclinical phase and are more likely to be detected by symptoms between screening sessions [Herman 2002]. This bias can lead you to conclude that screening is beneficial when, in fact, observed differences in mortality rates resulted from detection of less severe disease in the screened group and diagnosis of more fatal disease in those with symptoms.

6.4 BARRIERS TO SCREENING IN MSH2 MUTATION CARRIERS

The effectiveness of colonoscopic screening in reducing the incidence of CRC and improving mortality in LS is well supported [Jarvinen 2000; Stupart 2009; Stuckless 2012]. However, despite this proven benefit, many high risk individuals are still not receiving the appropriate care, with adherence rates for colonoscopic screening varying widely [Hadley 2004; Halbert 2004; Bleiker 2005; Wagner 2005]. Although the benefit of gynecological screening in LS is controversial, screening guidelines do recommend transvaginal ultrasound (TVU), endometrial biopsy and CA 125 testing. As with colonoscopic screening, the uptake for these gynecological screening exams is suboptimal [Wagner 2005; Collins 2007; Hadley 2008].
To improve adherence to screening recommendations, it is important to understand the barriers that prevent appropriate clinical management. In the general population, factors related to compliance with CRC screening include physician recommendations, perceived benefits from screening, age, having relatives with CRC, perceived susceptibility to CRC and coherence of screening [Collins 2005]. Studies of preventive behaviors in high-risk populations suggest that the main predictors of screening uptake are genetic test results and recommendations from health care providers [Hadley 2004; Halbert 2004; Collins 2005]. Another study of high risk individuals found that barriers to colonoscopic screening included the embarrassing nature of the procedure itself, fear that a tumor would be detected during screening, the discomfort associated with the procedure and the absence of symptoms or complaints [Bleiker 2005]. In the United States, concerns about losing health insurance coverage and increased costs of health care have also been shown to be significant barriers to colon screening [Lynch 1993]. In regards to gynecological cancers, adherence to screening may be related to women’s perceived risk of extracolonic cancers and physician’s knowledge of the gynecological cancer risk and screening recommendations for female LS mutation carriers [Hadley 2008]. Belief in the efficacy of gynecological screening procedures and a family history of endometrial and/or ovarian cancer may also impact the uptake of gynecological screening [Collins 2007].

In chapter 4 we found that less than 50% of individuals in our study entered a colonoscopic screening program, and of those who did, less than 50% were compliant with colonoscopy every 1-2 years. Males were also less likely than females to enter a
colonoscopic screening program. In chapter 5, we found that less than one-third of females in our study had entered a gynecological screening program. Although many individuals in the non-screened groups were historical controls and were either deceased or had developed cancer prior to development of the screening program in Newfoundland, there were individuals who were not participating in the full scope of the program and/or not adhering to the recommended screening intervals.

A recent study of these MSH2 mutation carriers looked at the factors influencing decisions about disease management post genetic testing [Watkins 2011]. Although most participants seemed to be well-informed about LS, have accurate cancer risk perceptions and understand the benefits of regular screening, there were several personal, provider and health care system barriers to effective disease management. On a personal level, individual’s worries/concerns about potential test results/prophylactic interventions, frequency and type of screening required, preparation for and past experiences with screening examinations and scheduling issues, all increased the burden of disease management and sometimes became a deterrent to continuation or adherence. Practical issues such as financial status, family responsibilities and employment history, also interfere with one’s willingness and ability to follow recommended screening protocols. The perceived knowledge and skills of health care providers about the natural history of LS and their thoroughness of family history taking and physical examinations were key factors impacting regular screening and disease management. Health care provider’s familiarity with their family cancer history, completeness of medical care and quality of
communications improved adherence with screening and treatment protocols. Health care system challenges, such as gaining timely access to needed services, ineffective coordination of diagnostic, treatment and specialists' appointments, and lack of follow-up, enhance the burden of disease and cause some individuals to distance themselves from the screening process. Recognition and prevention of the barriers to effective disease management is necessary for improved adherence to screening recommendations which in turn can lead to improved morbidity and mortality.

6.5 BENEFIT OF THIS STUDY AND FUTURE DIRECTIONS

Of the major cancers, CRC has the greatest opportunity for screening to have a major impact, both socially and economically. Colonoscopy can prevent cancer by removal of pre-malignant polyps or result in earlier stage diagnosis of CRC which is easier and less costly to treat. Colonoscopic screening in the general population has been shown to be cost-effective with savings in treatment outweighing the cost of screening [Ginsberg 2010]. An efficient screening program is necessary to improve the health outcomes of families at risk of CRC development.

As was stated earlier, Newfoundland has the highest incidence of CRC in Canada and the highest incidence of familial CRC in the world. Of those with familial CRC, over 30% have high risk family histories and includes those with LS and familial colorectal cancer type X (FCCTX). These families have a high lifetime risk of developing CRC and require ongoing colonoscopic screening. Our research indicates the benefit of screening in these
high risk families with an *MSH2* mutation and the need for adequate and timely colonoscopic examinations (Chapter 4). The high risk of gynecologic malignancy in LS also has important clinical implications. Our research indicates that gynecologic screening did not result in earlier gynecologic detection nor did it prevent gynecologic cancers from developing or ovarian cancer deaths from occurring (Chapter 5). For this high risk group of women, prophylactic hysterectomy with BSO may be the best method of endometrial and ovarian cancer prevention.

Our research findings have helped in the development of a novel community-based screening program targeted at family members of incident CRC and gynecologic patients, stratified by cancer risk. Selectively targeting colonoscopic screening to those at high risk of developing CRC seems logical but no such program has previously existed. This health research project is the first of its type in Canada and pro-actively identifies families of CRC patients to provide a directed and coordinated screening program based on their level of cancer risk.

All CRC patients in Newfoundland will be offered this new community-based clinical screening service. A 3-generation pedigree will be developed for each CRC patient and family history of all cancers will be collected. A multi-step custom-designed algorithm which incorporates family history and other features, such as pathology manifestations and molecular testing, will be used to identify families at increased risk of CRC. Those at highest risk will have their DNA tested against a panel of all CRC-associated mutations.
currently identified in the Newfoundland population (currently 18 mutations specific for either LS, polyposis syndromes or MUTYH). Clinical screening guidelines for each level of risk and tumor pattern have been developed based on results of prior screening and barriers to adherence and effective screening have been identified so that the most efficient and targeted surveillance program for CRC and also gynecological cancers can be developed.

The objective of this research screening program is to reduce the incidence and overall cost of managing CRC and gynecological cancer in Newfoundland. Families with the highest risk of developing CRC (families with well-documented mutations) will receive the most intensive screening protocols because the likelihood of preventing cancer is very high as evidenced by our study of MSH2 mutation carriers (Chapter 4). Those at lowest risk (no cancer history and no features characteristic of inherited cancers) will receive the least intensive screening protocol because their likelihood of cancer detection will be very low. For those at intermediate risk, the objective is to discover the genes and environmental factors that influence CRC development in this group, so that accurate risk assessment can be determined and the appropriate screening protocol can be initiated.

Due to the high risk of gynecologic malignancy in cancer predisposition syndromes, identification of a high risk group can allow targeted prevention through prophylactic surgery. By targeting screening and management resources, cancers will be prevented and more lives will be saved, and this can have a large and immediate impact on both the social and economic burden of CRC and gynecologic cancer in Newfoundland.
This thesis is part of a larger project addressing the impact and evaluation of CRC screening on the burden of CRC in Newfoundland. Future work addressing the cost-effectiveness of CRC screening in LS is planned and will use our findings for *MSH2* mutation carriers discussed in chapter 4. Determination of the impact of screening in FCCTX families and other high risk families of unknown genetic etiology is also planned and will use the same methodology as that used in chapter 4 for LS. As well, the costs and outcomes of the community-based screening strategy in Newfoundland will be compared to Ontario’s population screening program, where people with a family history of CRC can be referred by their doctor for colonoscopy. If our proactive targeted screening program leads to a more effective and economical use of screening resources compared to a generic population-based screening strategy, the screening intervention in Newfoundland may lead to improvements in the efficiency of surveillance for CRC in all of Canada.
CHAPTER 7

Summary & Recommendations
7.1 SUMMARY

Lynch syndrome (LS) is the most common hereditary colorectal cancer (CRC) syndrome, however, lack of specific diagnostic features makes diagnosis of LS difficult.

Identification of LS mutation carriers is very important as these individuals are at a substantially increased risk of CRC and endometrial cancer and also an increased risk of other extracolonic cancers. CRC develops at a young age and early detection is necessary for improved survival. Morbidity and mortality can be substantially improved in LS carriers due to early detection and prevention of CRCs by colonoscopic screening.

Colonoscopic screening has been shown to reduce the incidence of CRC, delay the age of CRC onset and improve survival. The value of gynecological screening, however, is still controversial and does not appear to lead to earlier stage detection or prevention of gynecologic cancers. It has been proposed that prophylactic hysterectomy with bilateral salpingo-oophorectomy (BSO) may be the most appropriate management option for female LS carriers and should be performed after childbearing is complete or at the time of CRC surgery for females who develop early onset CRC. Not all LS mutation carriers adhere to the recommended screening and treatment protocols and are therefore at a significantly increased risk of cancer-related morbidity and mortality. Identification of the barriers to screening and disease management are therefore necessary to ensure that individuals are receiving appropriate care. In conclusion, knowledge of the natural history and molecular genetics of LS are necessary for targeted screening and management strategies and ultimately cancer prevention, and emphasis should be placed upon barriers to effective disease management.
7.2 IMPLICATIONS

- Colonoscopic screening was shown to dramatically reduce cancer and cancer-related mortality in LS mutation carriers. Therefore, targeted screening programs aimed at those who are at high risk of CRC are necessary.

- Fewer men than women were participating in colonoscopic screening, and as such, special efforts are necessary to enroll these men.

- Compliance with the current screening recommendations of colonoscopy every 1-2 years did not completely prevent the development of CRC. Future studies are needed to evaluate whether a screening interval of one year can further reduce the number of CRCs diagnosed in LS mutation carriers.

- Interval CRCs were detected within one year of a previously “normal” colonoscopic examination and this must question the quality of the colonoscopic exam. Future work is needed to determine the most effective colonoscopic imaging techniques for detection of precancerous lesions.

- Prophylactic hysterectomy and salpingo-oophorectomy for gynecological cancers in high risk populations has been shown to be cost-effective. In our study, gynecological screening did not result in earlier stage detection and interval cancers occurred, suggesting that gynecological screening may be ineffective for women with L.S.
However, due to the limitations of our study and our small event rates, future studies are needed to address whether prophylactic surgery, as opposed to gynecological screening, should be recommended for these women.

A coordinated system of care and follow-up for screening and treatment of L.S mutation carriers is necessary for appropriate disease management.
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