LYNCH SYNDROME: NATURAL HISTORY AND COLONOSCOPIC SURVEILLANCE

Douglas Stupart
MBChB, FCS (SA), FRACS

Submitted in fulfilment of the requirements for the degree of
Doctor of Philosophy

Deakin University
August 2015
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Douglas Annesley Stupart

3 August 2015
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I would like to thank my supervisors, and all the co-authors of the papers presented in this thesis.

Being invited to be part of the Kleinzee NHPCC project has been the great privilege of my career, and I am most grateful for the opportunity to have been a part of such a unique and rewarding experience.

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I thank my wife Moira for her encouragement and support, and my daughters Jessica and Georgia for giving me energy and inspiration.

Mainly I thank all the patients whom it has been my privilege to meet and care for.
This thesis is based on the following six papers, which have been published in peer-reviewed journals:


**PAPER VI:** *Fertility after young onset colorectal cancer- a study of subjects with Lynch syndrome.* **Stupart D,** Winship IM, Win AK, Jenkins M. Colorectal Dis, 2015(1463-1318 (Electronic)).[6]

I was the first author on all these publications, and was primarily responsible for the study design, data analysis and drafting of the manuscripts in all cases. The papers have been extensively rewritten for this thesis for consistency of style, to create a consistent narrative, and to reflect changes in the world literature that have occurred since publication. The papers are arranged in the thesis conceptually, and not in the chronological sequence in which they were published.

These papers are included in their original published form in appendix I.

Copies of the statements of authorship signed by all co-authors form appendix II.
Lynch syndrome is the most common inherited cause of colorectal cancer (CRC). It is an autosomal dominant condition, caused by a germline mutation in one of the mismatch repair (MMR) genes \textit{hMLH1, hMSH2, hMSH6} and \textit{hPMS2}. Affected individuals are known to carry an increased risk of CRC as well as extracolonic malignancies, although the magnitude of that risk has been variably reported.

Paper I documents the age- specific risk of CRC and other malignancies in a cohort of 200 subjects who are germline carriers of a single mutation in the \textit{hMLH1} gene. This mutation (C1528T (Exon 13) mutation in the \textit{hMLH1} gene) has been identified in 23 families in South Africa and is not known to occur elsewhere in the world. It was found that carriers of this mutation developed CRC at a median age of 44 years, and that their risk of developing CRC by age 65 was 92\%. Their risk of extracolonic cancers (most notably endometrial) was found to be relatively low however.
Paper II is a prospective study of the efficacy of colonoscopic surveillance in the cohort of subjects with Lynch syndrome who were the subject of Paper I. It is the only study to have included only subjects who all carry the same MMR gene mutation. Of the 200 known carriers of the C1528T (Exon 13) mutation in the hMLH1 gene, 178 were identified before they had developed CRC and they were all offered colonoscopic surveillance. Of these 178 subjects, 129 underwent at least one surveillance colonoscopy, and 49 declined. After a median follow up of 5 years, colorectal cancer was diagnosed in 14/129 (11%) of subjects in the surveillance group, and 13/49 (27%) in the non-surveillance group (p=0.019). Cancers in the surveillance group were detected at an earlier stage than in the non-surveillance group (P=0.032). Death from colorectal cancer occurred in 3/129 (2%) of subjects in the surveillance group, and 6/49 (12%) in the non-surveillance group (p=0.021). The Kaplan-Meyer estimates for median survival from birth were 78 years in the surveillance group, and 55 years in the non-surveillance group (p=0.024). The Kaplan-Meyer estimates for median colorectal cancer free survival from birth were 73 years in the surveillance group and 47 years in the non-surveillance group (p=0.0089).

Paper III addresses the choice of operation for colorectal cancer in Lynch syndrome. The high reported risk of metachronous colon cancer in Lynch syndrome has led some authors to recommend total colectomy as the preferred operation for primary colon cancer in this patient group, but this remains controversial. Paper III is prospective cohort study of 60 patients with proven MMR gene mutations who underwent surgical resection for adenocarcinoma of the colon with curative intent. Of these 60 patients, 39 had a total colectomy as their initial surgery and 21 had a segmental colonic resection. After a median
follow up of six years, metachronous colon cancer occurred in eight (21%) patients after segmental colectomy and in none of the total colectomy patients. This study confirms the significant risk of metachronous colon cancer after segmental colectomy in Lynch syndrome. This risk is eliminated by performing a total colectomy as the primary operation for colonic cancer.

**PAPER IV** is a study of genetic anticipation in Lynch syndrome. Genetic anticipation occurs when the age of onset of a disorder decreases in successive generations. This is a well known phenomenon in a number of inherited conditions, but it is controversial whether this occurs in Lynch syndrome. **Paper IV** reports on the age of onset of CRC in 92 members of a single family over five generations who all carry the same C1528T (Exon 13) mutation in the \(hMLH1\) gene. Evidence of genetic anticipation (determined by age of onset of first CRC) was sought in two ways: Firstly, subjects were grouped as parent-child pairs and individuals were compared with their own offspring; secondly they were grouped by generation within the family tree. The appearance of genetic anticipation was found to be due to follow up bias. Once this bias was corrected for there was no evidence that genetic anticipation occurred.

Another form of bias that can potentially create the false appearance of genetic anticipation is fecundity bias, which occurs if the disease adversely affects fertility. **Paper V** is the first publication to test whether this can occur in Lynch syndrome (or in any other inherited condition). This is a study of 1088 patients with CRC from families with Lynch syndrome identified from the Australasian Colorectal Cancer Family Registry (ACCFR). In this cohort, early onset of CRC was associated with lower lifetime fertility. The observed ages of onset of CRC
and lifetime fertility were used to construct a computer model that simulated a large number of parent-child pairs. The model demonstrated that fecundity bias can create the false appearance of genetic anticipation, and accurately predicted the appearance of genetic anticipation in the ACCFR cohort.

**PAPER VI** is a study of age-specific fertility rates among Lynch syndrome patients who have survived colorectal cancer. Potential infertility is a significant concern for young colorectal cancer (CRC) survivors, but this risk is not well quantified. It was not known whether the reduction in lifetime fertility associated with early onset CRC documented in **PAPER V** was due to the effect of CRC on fertility, or if it was simply due to decreased survival. In **PAPER VI**, age-specific fertility rates for subjects with or without a CRC diagnosis were determined for 1068 subjects with proven MMR mutations who were identified from the ACCFR. Total fertility rate was decreased in women who had been diagnosed with CRC compared to who had not, but age-specific fertility was only reduced in the 20-24 year age group. Subjects of both genders who survived into their late twenties and beyond had no detectable reduction in age-specific fertility, which may be reassuring to colon cancer survivors who hope to have children.

In summary, this thesis:

Documents the CRC and extracolonic cancer risk for a large cohort of subjects who carry the C1528T (Exon 13) mutation in the *hMLH1* gene.

Confirms that colonoscopic surveillance of subjects with Lynch syndrome reduces the incidence of CRC, leads to earlier detection of CRC, and thus improves cancer-specific and overall survival in these subjects.
Confirms the high rate of metachronous CRC after segmental colonic resection for CRC.

Demonstrates that follow up bias and fecundity bias can falsely create the appearance of genetic anticipation.

Demonstrates that age-specific fertility is largely unaffected by early-onset CRC in Lynch syndrome, except in women in the 20 to 25 year age group.
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<th>Full Form</th>
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<tbody>
<tr>
<td>ACCFR</td>
<td>Australasian Colorectal Cancer Family Registry</td>
</tr>
<tr>
<td>AJCC</td>
<td>American Joint Committee on Cancer</td>
</tr>
<tr>
<td>APC</td>
<td>Adenomatous polyposis coli</td>
</tr>
<tr>
<td>ASCO</td>
<td>American Society of Clinical Oncologists</td>
</tr>
<tr>
<td>ASFR</td>
<td>Age- specific fertility rate</td>
</tr>
<tr>
<td>ASR</td>
<td>Age standardised rate</td>
</tr>
<tr>
<td>C.I.</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CRC</td>
<td>Colorectal cancer</td>
</tr>
<tr>
<td>DSR</td>
<td>Directly standardised rate</td>
</tr>
<tr>
<td>FAP</td>
<td>Familial adenomatous polyposis</td>
</tr>
<tr>
<td>GIT</td>
<td>Gastrointestinal tract</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>HNPCC</td>
<td>Hereditary non-polyposis colorectal cancer</td>
</tr>
<tr>
<td>IHC</td>
<td>Immunohistochemistry</td>
</tr>
<tr>
<td>InSiGHT</td>
<td>International Society for Gastrointestinal Hereditary Tumours</td>
</tr>
<tr>
<td>ISR</td>
<td>Indirectly standardised rate</td>
</tr>
<tr>
<td>MMR</td>
<td>Mismatch repair</td>
</tr>
<tr>
<td>MSI</td>
<td>Microsatellite instability</td>
</tr>
<tr>
<td>NPC-1</td>
<td>Non-polyposis colorectal cancer family 1</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>SBR</td>
<td>Standardised birthrate</td>
</tr>
<tr>
<td>SC/ISA</td>
<td>Subtotal colectomy and ileo-sigmoid anastomosis</td>
</tr>
<tr>
<td>SMR</td>
<td>Standardised mortality rate</td>
</tr>
<tr>
<td>TC/IRA</td>
<td>Total colectomy and ileo-rectal anastomosis</td>
</tr>
<tr>
<td>TFR</td>
<td>Total fertility rate</td>
</tr>
<tr>
<td>TNM</td>
<td>Tumour/Node/Metastasis</td>
</tr>
<tr>
<td>UICC</td>
<td>Union Internationale Contre le Cancer</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
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</table>
1 BACKGROUND
1.1 Lynch syndrome: General introduction

Lynch syndrome is the most common known inherited form of bowel cancer. It accounts for about 3% of colorectal cancers (CRC). This autosomal dominant condition is caused by germline mutations in the mismatch repair (MMR) genes \( hMLH1, hMSH2, hMSH6 \) or \( hPMS2 \) [7]. These mismatch repair gene mutations are associated with young onset CRC. In addition, carriers are at risk for extracolonic malignancies such as endometrial, ovarian, small bowel, hepatobiliary, urinary tract and brain tumours. Throughout this thesis, the term Lynch syndrome is applied only to individuals or families who carry a proven germline MMR mutation, while HNPCC is used when the diagnosis is made on clinical and family history criteria in keeping with current nomenclature [8,9] (This nomenclature is not, however, used consistently in the original forms of the papers included in this thesis, as these conventions have evolved over recent years).

1.1.1 Historical perspective

In 1913, Aldred Warthin published the first report of a family with what is now referred to as Lynch syndrome. This was part of an extraordinary study on the ‘influence of heredity on cancer’ [10] in which he reviewed 1600 cases of carcinoma diagnosed at the University of Michigan pathology laboratory, and obtained 1000 ‘fairly good’ family histories from affected patients. In four of these families, he noted that ‘the incidence of cancer in these families is so striking that it can be interpreted as showing an inherited susceptibility’ to cancer. In one of these families, designated family \( \text{“G”} \), 17 of 48
descendants of the ‘cancerous grandfather’ had been diagnosed with uterine or gastrointestinal (GIT) cancers. An interesting personal note to this history is that one of the members of this family was Warthin’s seamstress, who had expressed her concerns about her family’s predisposition to cancer, and who later died of endometrial cancer herself[11].

In 1925, Warthin published a more extensive description of family “G”[12]. The genogram presented in this paper is illustrated below (Figure 1). In this paper he concluded that the family carried an inherited susceptibility to cancers of the gastrointestinal tract in men and of the reproductive organs in women. He further noted the unusually young age on onset of cancer (mean 38 years) in this family. In this paper, Warthin complained that his findings had ‘met with little favour among surgical writers and particularly among those interested in propaganda for the prevention of cancer’. This remained the case for many years.

In 1936, five years after Warthin’s death, Hauser and Weller published ‘a further report on the cancer family of Warthin’ [13]. The family tree had been extended to include 305 individuals over six generations. A total of 43 cancers had been diagnosed in 41 individuals. All but two of these tumours occurred in the GIT or uterus, and the authors made the point that the ‘anatomical location (was) more significant than total incidence’ of cancers in this family. They concluded that ‘this family provides very strong presumptive evidence for an inheritable organ-specific predisposition to carcinoma’ although they could not come to any conclusions regarding the pattern of Mendelian inheritance.
In 1962, Henry T. Lynch (who was a second year medical resident at the time) met a patient who was recovering from delirium tremens. The patient believed that he was going to die of CRC, which was prevalent in his family, and that this was the reason for his heavy drinking. Sadly, like Warthin’s seamstress, he was correct and later died of bowel cancer[14]. The initial presumptive diagnosis of familial adenomatous polyposis (FAP), which at the time was the only known inherited form of bowel cancer, was found to be incorrect as there was no evidence of colonic polyposis in this family. Lynch et al reported on this family’s (designated family “N” for Nebraska) cancer risk along with another pedigree (family “M” from Michigan) which had been identified by Marjorie Shaw[15]. The authors noted an unusually high number
of cancers diagnosed (predominantly colon and uterus, but also in a number of other sites) in these families, and recognised an autosomal pattern of inheritance. Lynch then gained access to Warthin’s genograms and pathology specimens relating to family “G” and in 1971 Lynch and Krush [16] reported on this family. By this time, the family tree included over 650 individuals, of whom 95 had been diagnosed with malignant neoplasms. The sites of the tumours that were identified are summarised in Table 1.

The authors also elucidated the pattern of inheritance in this family, which was ‘consistent with an autosomal dominant inheritance pattern’.

Lynch used the term ‘cancer family syndrome’ to describe the families he had reported, and listed its characteristics as follows:

“1. Increased occurrences of adenocarcinomas, primarily of colon and endometrium;

2. Increased incidence of multiple primary malignant neoplasms;

3. Autosomal dominant inheritance;

4. Early age of onset of cancer”[16]
Table 1 Tumour sites in affected members of family “G” (from Lynch and Krush (1971))

<table>
<thead>
<tr>
<th>Tumour site</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon</td>
<td>52</td>
</tr>
<tr>
<td>Endometrium</td>
<td>18</td>
</tr>
<tr>
<td>Stomach</td>
<td>8</td>
</tr>
<tr>
<td>Breast</td>
<td>4</td>
</tr>
<tr>
<td>Skin</td>
<td>6</td>
</tr>
<tr>
<td>Urothelium</td>
<td>2</td>
</tr>
<tr>
<td>Prostate</td>
<td>2</td>
</tr>
<tr>
<td>Liver</td>
<td>2</td>
</tr>
<tr>
<td>Ovary</td>
<td>1</td>
</tr>
<tr>
<td>Thyroid</td>
<td>1</td>
</tr>
<tr>
<td>Lung</td>
<td>2</td>
</tr>
<tr>
<td>Pituitary</td>
<td>1</td>
</tr>
<tr>
<td>Pancreas</td>
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<tr>
<td>Soft tissue sarcomas</td>
<td>5</td>
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<tr>
<td>Lymphocytic leukaemia</td>
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</tr>
<tr>
<td>Plasmacytoma of sternum</td>
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<td>Site unknown</td>
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<td><strong>Total</strong></td>
<td><strong>113</strong></td>
</tr>
</tbody>
</table>
1.1.2 Microsatellite instability

By 1990, a multistep molecular pathway for CRC tumorigenesis had been hypothesised[17]. In this model, a series of mutations in suppressor genes including the APC, p53, and DCC genes as well as oncogenes such as K-ras occur sequentially. This leads to the development first of a benign adenoma and later carcinoma. This has become known as the “Vogelstein”, “canonical” or “suppressor” pathway, and is now known to account for about 80% of sporadic CRC (as well as being the pathway through which CRC develops in familial adenomatous polyposis (FAP))[18].

In 1993, three groups almost simultaneously published reports of what is now referred to as microsatellite instability (MSI) in colorectal cancers [19-21] and recognised this as a novel pathway for CRC tumorigenesis. They amplified the DNA of tumour cells and of surrounding normal tissue by polymerase chain reaction (PCR), and the PCR products were separated by gel electrophoresis. The tumour DNA showed small differences in the lengths of some DNA fragments containing repetitive sequences of base pairs when compared with normal tissue. It was noted that this only occurred in a subset of CRC, but in those tumours there was a dramatic (100 to 1000 fold) increase in the rates of these mutations compared to normal cell lines. Thibodeau et al [19] noted that microsatellite instability was more frequently observed in proximal CRC and was associated with a better prognosis. It was also inversely associated with chromosomal abnormalities typical of tumorigenesis via the Vogelstein pathway. Aaltonen et al [20] observed these changes (referred to as replication error (RER+) in this paper) in 13% of sporadic tumours and in the
majority of familial cancers and concluded that this was “a mechanism for familial tumorigenesis different from that mediated by classic tumor suppressor genes”. Ionov et al [21] hypothesised that the occurrence of these numerous small mutations was likely due to a mutation in a gene coding for “a factor essential for the replication fidelity (or repair) of these simple repeated sequences”. They also noted that these changes occurred in a distinctive phenotypic subset of CRC (right sided, metachronous, poorly differentiated tumours in young patients), and postulated that they may be the “molecular genomic manifestations of … hereditary non-polyposis colorectal cancer”. It is now well recognised that there is a distinctive histological phenotype, characterised by tumor infiltrating lymphocytes, Crohn’s like inflammatory reaction, mucinous and/or signet ring differentiation and a medullary growth pattern, which is strongly associated with microsatellite unstable tumours [22].

1.1.3 Mismatch repair genes

At the time that MSI was first described in human CRC, the human mismatch repair genes were unknown. In bacteria and fungi, however, well defined DNA mismatch repair (MMR) pathways had already been elucidated, and it had been observed that mutations in the genes coding for MMR proteins led to instability in the replication of repetitive dinucleotide repeat sequences. Fishel et al [23] recognised that this was analogous to MSI that had been reported in some CRC and later in 1993 (the same year that MSI was first described in CRC) they reported that they had identified the first human MMR protein. Specifically, they had cloned the human homolog of the
bacterial MutS and *Saccharomyces cerevisiae* MSH proteins (named *hMSH2*). They also identified a germline mutation in the gene that codes for this protein in affected members of two HNPCC kindreds, and concluded that *hMSH2* was the HNPCC gene.

There are now known to be at least seven genes involved in the MMR system: *hMLH1, hMLH3, hMSH2, hMSH3, hMSH6, hPMS1* and *hPMS2*\[18\]. The MMR complex identifies DNA mismatches (defined as non- Watson- Crick base pairs or small loops of nucleotides that form from the two strands of DNA slipping relative to each other), excises the aberrant base pair(s), and repairs the error. Deficiencies in any of the MMR proteins can lead to malfunction of this system, causing widespread mutations. Repetitive sequences of mononucleotide or dinucleotide pairs are particularly prone to replication errors by DNA polymerase due to slippage, causing insertion or deletion errors and changes in the length of these repetitive segments, which manifests as microsatellite instability (MSI) \[18, 24, 25\]. This is illustrated in Figure 2.

Mutations in the *hMLH1* and *hMSH2* genes account for about three quarters of the mutations that have been reported to the International Society for Gastrointestinal Hereditary Tumours (InSiGHT) database\[26\], which is the major international repository for gene variant information for Lynch syndrome\[27\]. The frequencies of reported variants are listed below (Table 2).

In Lynch syndrome, there is a somatic mutation in one of the MMR genes. Of the seven known MMR genes, the following are known to cause Lynch syndrome: *hMLH1*\[28\]; *hMSH2*\[23, 29\]; *hMSH6*\[30\]; *hPMS2*\[31\] (Warthin’s family “G” is now known to carry a mutation in the *hMSH2* gene\[32\]).
Table 2 Number of unique MMR variants reported to the InSiGHT database (December 2014)[26]

<table>
<thead>
<tr>
<th>MMR gene</th>
<th>Number of unique variants</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>hMLH1</em></td>
<td>1184 (40%)</td>
</tr>
<tr>
<td><em>hMSH2</em></td>
<td>1003 (34%)</td>
</tr>
<tr>
<td><em>hMSH6</em></td>
<td>533 (18%)</td>
</tr>
<tr>
<td><em>hPMS2</em></td>
<td>234 (8%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>2954</strong></td>
</tr>
</tbody>
</table>

Figure 2 ‘Slippage’ during DNA replication. Adapted from Chung and Rustgi 2003[33]
Affected individuals carry one wildtype and one mutated copy of the gene. Failure of the MMR proteins to function normally requires somatic inactivation of the wildtype allele. In subjects who do not carry a germline MMR mutation, abnormal MMR function occurs when both copies of the MMR gene become inactivated. This provides an example of Knudson’s "two hit” model of oncogenesis that explains the variable penetrance of inherited tumour suppressor gene abnormalities, and also the occurrence of tumours typical of those inherited conditions in subjects who do not carry the germline mutation [34]. Because somatic inactivation of MMR genes is common, Lynch syndrome manifests phenotypically as an autosomal dominant condition.

1.1.4 Microsatellite instability in sporadic CRC

Approximately 95% of all tumours in Lynch syndrome show MSI [24]), making this a cardinal feature of Lynch syndrome. MSI also occurs in some sporadic (i.e. not inherited) CRC. This usually occurs as a result of abnormal somatic hypermethylation of the promoter region of the hMLH1 gene[35]. Unlike Lynch syndrome, where there is a germline mutation in the mutated copy of the gene, acquired somatic epimutations of both copies of the hMLH1 gene occur. In a 2001 study of CRC, in specimens which were not selected for family history, Cunningham et al [36] found that approximately 20% of CRC had evidence of MSI, the great majority of which were due to inactivation of hMLH1. Only ten per cent of tumours that exhibited MSI (or two per cent of all CRC) had a definite germline MMR mutation (i.e. had Lynch syndrome).
In summary, almost all Lynch syndrome tumours demonstrate MSI, but only a small minority of microsatellite unstable CRC are due to Lynch syndrome.

1.1.5 Diagnosis of Lynch syndrome

1.1.5.1 Clinical diagnosis- the Amsterdam criteria

Prior to the availability of genetic testing for Lynch syndrome, the diagnosis of HNPCC was defined by clinical criteria. The newly formed International Collaborative Group on Hereditary Non- Polyposis Colorectal Cancer met in Amsterdam in 1990, and produced the Amsterdam criteria for the diagnosis of hereditary non- polyposis colorectal cancer (HNPCC)[37], which had become the favoured term for the ‘family cancer syndrome’ by that time[38]. The criteria are listed below (Table 3). They were later broadened to include families in whom extracolonic tumours were prevalent (referred to as the Amsterdam II criteria[39], (Table 4)). The Amsterdam criteria are neither sensitive nor specific in diagnosing Lynch syndrome (i.e. germline MMR mutation carriers). Up to three quarters of patients with CRC and genetically proven Lynch syndrome come from families that do not fulfil the Amsterdam II criteria [40]. Almost half the families that fulfil the Amsterdam I criteria do not carry MMR mutations [41]. These families, described as “familial CRC type X” appear to carry a CRC risk which is higher than the general population, but lower than families with Lynch syndrome. Lindor et al, who first documented the cancer risk in his group, hypothesised that these families represented a “heterogeneous group 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” [41].
1.1.5.2 Bethesda guidelines

In response to the recognised lack of sensitivity of the Amsterdam criteria in detecting families with Lynch syndrome, the Bethesda guidelines for selecting tumours for MSI testing (in order to screen for Lynch syndrome) were formulated in 1997 [42], and revised in 2004 [43]. The revised guidelines are listed below (Table 5). Pinol et al in 2005 [44] reported on a large series of 1978 consecutively diagnosed CRC, all of which underwent MSI testing as well as immunohistochemical (IHC) staining for MMR proteins. All patients with MSI tumours and/or tumours with absent MMR protein on IHC had germline mutation testing for Lynch syndrome. The family history, demographics and histological features of the tumours were also documented. The Revised Bethesda criteria were positive in 10/11 (91%) of patients with Lynch syndrome, and in 227/1211 (23%) patients who did not have Lynch syndrome, so the criteria perform well as a screening test. It is a widely accepted recommendation that these criteria are used to screen CRC for further testing for Lynch syndrome [9], although some authors advocate using less stringent [45](and therefore more sensitive but less specific) criteria to guide testing, or even routine testing of all CRC [46]. The optimum strategy, taking into account cost-effectiveness and resource availability is an area of ongoing debate.
Table 3 The Amsterdam criteria for the diagnosis of HNPCC[37]

<table>
<thead>
<tr>
<th>Families must fulfil all criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. There should be at least three relatives with colorectal cancer</td>
</tr>
<tr>
<td>2. One should be a first-degree relative to the other two</td>
</tr>
<tr>
<td>3. At least two successive generations should be affected</td>
</tr>
<tr>
<td>4. At least one should be diagnosed before age 50</td>
</tr>
<tr>
<td>5. Familial adenomatous polyposis (FAP) should be excluded</td>
</tr>
<tr>
<td>6. Tumours should be verified by pathological examination</td>
</tr>
</tbody>
</table>

Table 4 The Amsterdam criteria II for the diagnosis of HNPCC[39]

<table>
<thead>
<tr>
<th>Families must fulfil all criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. There should be at least three relatives with an HNPCC-associated cancer (colorectal, endometrium, small bowel, ureter or renal pelvis)</td>
</tr>
<tr>
<td>2. One should be a first-degree relative to the other two</td>
</tr>
<tr>
<td>3. At least two successive generations should be affected</td>
</tr>
<tr>
<td>4. At least one should be diagnosed before age 50</td>
</tr>
<tr>
<td>5. Familial adenomatous polyposis (FAP) should be excluded in the CRC cases (if any)</td>
</tr>
<tr>
<td>6. Tumours should be verified by pathological examination</td>
</tr>
</tbody>
</table>
Table 5 The Revised Bethesda guidelines for investigation of tumours for Lynch syndrome.

(Guideline 5 has been reworded for clarity after Lindor et al [9])

<table>
<thead>
<tr>
<th>Individuals meeting any one of the following should undergo microsatellite instability (MSI) testing:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Colorectal cancer (CRC) diagnosed in an individual under age 50 years.</td>
</tr>
<tr>
<td>3. CRC with histological features suggestive of a high degree of microsatellite instability** in a patient &lt;60 years of age.</td>
</tr>
<tr>
<td>4. CRC in 1 or more first-degree relatives with a Lynch syndrome-associated tumour*, with 1 of the cancers being diagnosed under age 50 years.</td>
</tr>
<tr>
<td>5. CRC or Lynch syndrome-associated tumour diagnosed at any age in 2 or more first- or second-degree relatives.</td>
</tr>
</tbody>
</table>

* Endometrial, ovarian, gastric, small bowel, pancreas, hepatobiliary tract, renal tract and brain tumours, sebaceous gland adenomas and keratoacanthomas.

** Presence of tumour-infiltrating lymphocytes, Crohn’s like lymphocytic reaction, mucinous or signet-ring differentiation, or medullary growth pattern.
1.1.5.3 Evidence of MMR dysfunction- MSI or IHC testing

MSI testing of CRC tissue has a sensitivity of around 85% for detecting Lynch syndrome [47]. Immunohistochemical staining for MMR proteins has similar sensitivity [47], and the results of MMR IHC and MSI testing are almost perfectly concordant [48, 49]. IHC more readily available to general pathology laboratories [50] and also has the advantage of directing germline MMR genetic testing (as only the MMR gene whose protein is deficient needs to be tested). However, the accuracy of IHC is variable and operator dependent [46]. The choice of MMR or IHC for screening tumours for Lynch syndrome should therefore be based on the availability of local skills and resources.

Sporadic microsatellite unstable tumours that are \textit{hMLH1} deficient can usually be differentiated from Lynch syndrome tumours by testing for \textit{hMLH1} promoter hypermethylation [24] or by testing for a specific mutation (designated V600E; c.1799>A) in the \textit{BRAF} oncogene which occurs in about half of all sporadic microsatellite unstable CRC, but is almost never detectable in Lynch syndrome- associated tumours [24, 51].

1.1.5.4 Germline mutation testing

The molecular diagnosis of Lynch syndrome requires germline mutation testing. This should be offered to all individuals with CRC who show evidence of MMR deficiency (without evidence of \textit{hMLH1} promoter methylation) [9, 24, 46].
The strategy for testing is more complex in the setting of an individual who has a family history suggestive of Lynch syndrome but who has not had a cancer. In this setting, the most effective approach is to perform genetic testing on a (living) relative who has previously developed a Lynch syndrome-associated cancer. If tissue from the tumour is available, it can be tested as above, and germline testing performed on the affected individual if the tumour has features suggestive of Lynch syndrome as above. If the affected relative is found to have a germline MMR mutation, targeted testing (for that specific family mutation, which is considerably cheaper and easier than screening the entire MMR gene(s)) may be undertaken for other family members. If no tissue is available, germline genetic testing on an affected individual may be performed, and the diagnosis of Lynch syndrome may be confirmed [9, 24, 46, 52].

In the absence of a living (or available) affected relative, one may proceed directly to germline mutation testing in individuals with a strong family history. If a known pathological mutation is found, the diagnosis of Lynch syndrome can be made. Commonly, however, the results of testing in this setting may be inconclusive, as not all mutations are necessarily pathological. In this case, further testing of the family will not be helpful [9, 46].

In summary, Lynch syndrome should be suspected in subjects with a strong family history of CRC or other associated tumours, or in patients with tumours that display the typical histological features associated with the MSI phenotype. Testing for MSI or absent MMR proteins by IHC can help to screen tumours for Lynch syndrome, but the definitive (molecular) diagnosis
of Lynch syndrome requires germline genetic testing demonstrating a pathological germline MMR mutation.
1.2 The ‘Kleinzee’ mutation

1.2.1 Introduction and history

In 1987, the general practitioner working at the mine hospital in Kleinzee (a remote town of about 2000 people in the Northern Cape province of South Africa, (Figure 3)) referred a thirty year old man to Groote Schuur Hospital in Cape Town for possible colonoscopic surveillance. The patient (J.C.) had undergone a sigmoid colectomy for cancer at age 19, his brother had developed colon cancer at age 23, and his father and three paternal uncles had all died of abdominal cancers. A description of this family was first published in 1990 by Goldblatt et al [53]. The authors documented a pedigree of 47 individuals over four generations of whom 16 men (and notably no women) in three successive generations had bowel cancers. This pedigree is illustrated below (Figure 4). The family therefore fulfilled the Amsterdam criteria[37, 39] for the diagnosis of HNPCC. The authors described the pattern of inheritance as ‘autosomal dominant with male predominance’. An amendment was added to the paper after it had been accepted for publication in which the authors noted that they had also identified two female family members who had died of suspected bowel cancer. Over time, as the family tree was expanded, it became clear that the apparent absence of women was artefactual, at least partly due to the pattern of migration in that community (the men tended to stay and work in the mine but women were more likely to leave the area, so information about them was often incomplete).
Figure 3 Map of South Africa showing the geographical distribution of known NPC-1 family members and the nearest public health sector colonoscopy services.

The shaded ovals cover the sites where affected individuals are known to live; the colonoscopes illustrate the three nearest colonoscopy services. The arrow shows the site of Kleinzee, where the family was first identified. Note the distance of approximately 700 km from Kleinzee to the nearest colonoscopy service.
Figure 4 Pedigree of the Kleinzee (NPC-1) family as initially reported in 1990.

Note the 'male specificity' and the scarcity of female family members.

From Goldblatt et al [53].
Colonoscopic surveillance was offered to members of this family based on empiric risk, and an active program of identifying further individuals at risk of HNPCC (on the basis of early age of onset or family history of CRC) was instituted by the Colorectal Surgery Unit and Division of Human Genetics at the University of Cape Town. In 1998, Goldberg et al [52] reported that the pathological mutation in this family had been identified, so a molecular diagnosis of Lynch syndrome was made and mutational analysis with genetic counselling could be offered to family members. The mutation was a C to T transversion at nucleotide 1528 in exon 13 of the \textit{hMLH1} gene ("NP\_000240.1:p.Gln510*/NP\_000240.1:p.Gln510Ter" using the Human genome Variation Society standard nomenclature [54]).\footnote{Throughout this thesis, this mutation is referred to as the “C1528T (Exon 13) mutation in the \textit{hMLH1} gene” rather than by the Human Genome Variome Society nomenclature for ease of reading and for consistency with the previously published papers on which the thesis is based.} This mutation was not identified in any population-matched controls, and has not been identified in any Lynch syndrome families outside South Africa. It is not known whether this is due to a founder effect or if it is an indigenous African mutation. By the time that the mutation was identified, the family tree had expanded to the extent that 160 family members had undergone germline mutational analysis, 37 of whom were mutation positive[52].
By the year 2000, this number had increased to 140 mutation-positive in individuals in a family of 500 [55], and in this report two metachronous colon cancers were documented, as well as one extracolonic tumour (a breast cancer in a woman). This family is referred to as the “NPC-1” (non-polyposis colorectal-1) family in this thesis.

Currently, there are seventeen families known to carry the C1528T mutation in exon 13 of the MLH1 gene. Although they are presumed to have a common ancestor, this is currently unproven. The “NPC-1” family is the largest of these, with 714 known family members.

1.2.2 Colonoscopic surveillance

By 2007, ongoing identification of individuals at risk of familial cancers had led to the detection of 30 Lynch syndrome families carrying 12 different mismatch repair gene mutations. This presented a formidable challenge to the resources available for colonoscopic surveillance in South Africa, where colonoscopic services are restricted to major centres. Not only were the majority of family members living in remote areas with no easy access to these services (Figure 3), but the number of individuals in whom surveillance was indicated on the basis of family history would have overwhelmed the colonoscopy services at the nearest centres. This was one of the major motivations behind offering genetic testing to family members. Mutational analysis allowed subjects who were found not to carry the mutation (and who were therefore at population risk for CRC) to avoid lifelong surveillance. This spared them the risk and discomfort of multiple colonoscopies. It also reduced the number of patients who needed to undergo colonoscopy, which is of
particular importance in a resource-poor country such as South Africa.[52, 55]

Aside from the difficulty of delivering a colonoscopic service to large numbers of people in a small number of centres, the distance that the subjects needed to travel is a major obstacle in itself. Many of the subjects at risk are from impoverished areas, with little or no available transport, and the costs of travel and accommodation in order to have a colonoscopy are often unaffordable to them.

In order to provide surveillance colonoscopy to individuals at risk who could not travel to a dedicated endoscopic unit, a mobile colonoscopic service was developed. This is an annual service, in which a group of endoscopists, nurses and technicians travel by road (with all the required equipment) to a number of small rural clinics along the West coast of South Africa over a one week period. Subjects who are identified as requiring colonoscopy (on the basis of genetic testing) are offered the service and provided with bowel preparation in advance. Anderson et al [56] have audited this mobile colonoscopy service, and found the service to be of a similarly high quality to the established colonoscopy units in Cape Town and Johannesburg (and in keeping with international norms) in terms of colonoscopy completion and pathological lesion detection rates.

1.2.3 Colorectal cancer in the Northern Cape Province of South Africa

The incidence of CRC is variable around the world, with a higher incidence reported in the ‘developed’ compared with the ‘developing world’. Ferlay et
al [57] calculated an annual age standardised rate (ASR) of 38/100,000 (males) and 24/100,000 (females) in ‘more developed regions’ vs. 12/100,000 (males) and 9/100,000 (females) in ‘less developed regions’. CRC has been reported to have a low but increasing incidence in South Africa [58, 59]. The first estimate of the incidence of CRC in the Northern Cape Province was published in 2010[60]. In this study, the pathology reports of CRC from the laboratories serving that area between 2007 and 2009 were reviewed. During that time, 113 patients were diagnosed with CRC, in a population of 1.1 million. The annual incidence was calculated as 3.5/100,000 for men, and 3.9/100,000 for women (using the World Standard Population, an ASR of 4.2/100,000 was calculated (this was similar for men and women).

In summary, the Northern Cape Province of South Africa is an extremely low incidence area for CRC overall, but a large number of subjects with Lynch syndrome have been identified in this area. This provides a unique opportunity to study a large cohort of subject with Lynch syndrome who all carry the same mutation, in an environment with a low background incidence of sporadic CRC.
2 AIMS OF THE THESIS

This thesis aims to increase the body of knowledge of the natural history of Lynch syndrome, and add to the body of evidence regarding the efficacy of colonoscopic surveillance in this population, under the following headings:

To determine the lifetime cancer risk for carriers of the C1528T (Exon 13) mutation in the \textit{hMLH1} gene.

To determine whether surveillance colonoscopy prevents CRC and improves survival in carriers of the C1528T (Exon 13) mutation in the \textit{hMLH1} gene.

To determine the risk of metachronous CRC after segmental colectomy for colon cancer in Lynch syndrome.

To determine whether genetic anticipation occurs in a Lynch syndrome.

To determine the effect of young onset CRC on fertility in Lynch syndrome.
3 Published Papers
3.1 PAPER I: Cancer risk in carriers of the C1528T (Exon 13) mutation in the *hMLH1* gene [1]

3.1.1 Abstract

3.1.1.1 Introduction

There is marked variability in the reported cancer risk in Lynch syndrome. The purpose of this study is to document the cancer risk for subjects who carry the C1528T (Exon 13) mutation in the *hMLH1* gene.

3.1.1.2 Patients and methods

This is a prospective cohort study of 200 subjects who carry this mutation. The risk of developing colorectal cancer was calculated only in those subjects who had not undergone surveillance colonoscopy. The incidence of extracolonic cancers (for which surveillance was not offered) was determined for the entire cohort.

3.1.1.3 Results

Among the 71 subjects who did not undergo surveillance colonoscopy, colorectal cancers occurred in 36 (51%). They occurred at a median age of 44 years (range 17-73). Using Kaplan-Meier estimates, the risk of developing a colorectal cancer by age 65 years was 92%. Eighteen subjects in the cohort of 200 were diagnosed with extracolonic tumours. The most common extracolonic malignancies were breast (6/98 women) and endometrial (3/98 women).
3.1.1.4 Conclusion

This mutation has a high penetrance for colorectal cancer. Although extracolonic cancers were common overall, no individual extracolonic malignancy occurred commonly.
3.1.2 Introduction

Accurate genetic counselling and a rational approach to screening require knowledge of the natural history and penetrance of the disease in question. This is especially important in the South African setting where there are limited resources available for screening.

Reported estimates of cancer risk in Lynch syndrome are highly variable, depending on the methodology used as well as the patient groups that have been studied.

Vasen et al in 1996 [61] reported on the cancer risk in 19 families with proven \( hMLH1 \) (12 families) and \( hMSH2 \) (seven families) mutations from the Dutch HNPCC registry. They identified 210 mutation carriers out of 382 family members. Using standard survival (Kaplan-Meier) analysis techniques to correct for variable follow up, they reported a cumulative risk of CRC of 92% for men and 83% for women by the age of 75 years. The lifetime risk for endometrial cancer was approximately 50% for the two groups combined (and no difference was detected in cancer risk between the two mutations). They also observed an increased risk of small bowel cancers. Gastric and ovarian cancers were not significantly increased among mutation carriers compared with the general population.

Aarnio et al [62] in 1999 reported on a large cohort of 50 Finnish families in which an \( hMLH1 \) (47 families) or \( hMSH2 \) (3 families) mutation had been detected. Out of a total of 1763 family members, they identified 265 mutation carriers. Their cumulative risk of CRC by age 70 years was 100% in men and 54% in women. Women had a 60% lifetime risk for endometrial cancer, and
there was a high incidence of gastric (13%), ovarian (12%) as well as other extracolonic neoplasms.

3.1.2.1 The problem of ascertainment bias

Ascertainment bias (also referred to as detection bias) is a form of selection bias in which the method of detecting or diagnosing a condition leads to a systematic deviation from the true frequency of that condition[63].

Dunlop et al [64] in 1997 noted that most reported mutations in mismatch repair genes had been identified in HNPCC families which had been selected because of a notably high number of cancer cases, and that therefore ‘ascertainment bias is an inherent problem with cancer risk estimates derived from such families’. They therefore used a different strategy to determine CRC risk in Lynch syndrome. They performed targeted genetic testing on individuals with young onset CRC with microsatellite instability and thus identified six probands (one with mutations in hMLH1 and five with hMSH2 mutations) unselected for family history. They then traced the respective pedigrees of these mutation-positive probands, and identified 156 family members, of whom 67 were mutation carriers. In order to include clinical information for family members with an unknown mutation status (mainly family members who had died before commencement of the study) they assigned a probability that the untested individual carried the mutation using a model taking into account the known (autosomal dominant) inheritance as well as the known population risk for sporadic CRC. They estimated the cumulative CRC risk by age 70 years at 74% in men and 30% in women, which were only slightly lower than the estimates from HNPCC registries
mentioned earlier. In women, the risk of endometrial cancer was 42% by age 70 years.

Carayol et al [65] in 2002 more formally addressed the issue of ascertainment bias, criticising the methodology used to estimate penetrance in Lynch syndrome in previous studies. They made the observation that “the use of these very restrictive (Amsterdam) criteria[37] is bound to cause an ascertainment towards multiple case families” as families with fewer than three known cases of CRC would not meet these selection criteria, and families with multiple and young onset CRC are more likely to be included. This would be expected to lead to an overestimation of CRC risk in families carrying mutations in MMR genes. It would also be expected to lead to an under-estimation of the prevalence of extra-colonic tumours, as these did not form part of the original Amsterdam criteria [37] (although they do for part of the broader Amsterdam II criteria [39]). In order to determine the extent to which ascertainment bias could affect estimation of CRC risk in Lynch syndrome, the authors constructed a series of simulations of families carrying MMR mutations. They used French demographic data to determine family sizes, and applied standard Mendelian (autosomal dominant) risk to determine which offspring carried the mutation, and applied arbitrarily defined “high” and “low” age-specific CRC risks to affected family members. In this simulation, only four per cent of “low risk” families, and ten per cent of “high risk” families fulfilled the Amsterdam criteria. The estimated risk for members of those simulated families who fulfilled the Amsterdam criteria was consistently higher than the simulated risk for the entire cohort (the over-estimation ranged from 17% to 130% depending on risk and age group). The
authors concluded that any estimate of CRC risk in HNPCC where inclusion in the study cohort is based on family history should correct for ascertainment bias, and that “colorectal cancer risks are largely overestimated in HNPCC, at about double the actual levels”.

This model can be criticised as CRC mortality and decreased fertility after CRC were not considered. The authors note that “…people dying from cancer at a young age would not have the opportunity to have descendants, which would reduce the number of carriers in the following generation” which could cause an underestimation of CRC risk (as young patients who died of cancer would not generate enough offspring for their family to be noted to be at risk). They state, however, that “since the average age at diagnosis is about 45 years, most people would have already had their children before the occurrence of their disease…therefore an allowance for mortality would not have modified the cancer risk estimates.” Chapter 3.5 addresses the effect of age of onset of CRC on lifetime fertility in Lynch syndrome.

3.1.2.2 Correction for ascertainment bias leads to lower estimates of CRC risk

In order to completely eliminate ascertainment bias in determining penetrance, one would need to perform mutational analysis on unselected members of the general population, and compare cancer risk between mutation carriers and non-carriers. This approach would not be practical in Lynch syndrome however, as the population frequency of the mutation is extremely low (three per ten thousand population as calculated by Dunlop et al[66]).
One simple method of reducing ascertainment bias is to exclude probands (the first family member identified as having a MMR mutation who, by definition, have a 100% lifetime risk of CRC). Hampel et al [67] used this approach in a study of 70 families known to carry *hMLH1* (65 families) or *hMSH2* (5 families) mutations from Finland. They identified 88 probands and 373 mutation-positive family members. They excluded the probands from the analysis and used the Kaplan-Meier survival method to determine cancer risk in all other family members who were proven mutation carriers. They calculated a lifetime cancer risk of 69% for men and 52% for women. The median age of onset of CRC was 54 years for men and 70 years for women. In contrast, the median age of onset of CRC among the probands was 44 years for both men and women.

Quehenberger et al in 2005 [68] reported on 84 families from the Dutch HNPCC Cancer Registry in which an *hMLH1* (39 families) or *hMSH2* (45 families) mutation had been identified. The study included 1088 subjects from *MLH1* families, and 1304 from *hMSH2* families. Genotyping was performed on approximately 20% of affected subjects (with CRC, endometrial or other Lynch syndrome cancers) and 35% of unaffected family members. They found that 98% (*hMLH1*) and 94% (*hMSH2*) of genotyped affected individuals were mutation carriers, and 49% (*hMLH1*) and 41% (*hMSH2*) of unaffected subjects were mutation carriers. They used the technique of ascertainment-corrected maximum likelihood estimation to determine the age-specific relative cancer risk. This methodology is based on the principle that the proportion of mutation carriers to non-carriers among phenotypically normal relatives (index cases are excluded from the analysis) should decrease.
with age (because the mutation carriers will develop cancer), although the
number of subjects who do not carry the mutation but develop the disease (i.e.
who have sporadic, population risk cancers) will increase with age (but at a
lower rate). They reported a 29% cumulative risk of CRC by the age of 80
years for men, and 24% for women, with a 46% (men) and 59% (women)
cumulative risk for extracolonic cancers. Note that these estimates or CRC
risk are about one third of those published by Vasen et al [61] studying a
smaller number of cases from the same registry (but not correcting for
ascertainment bias).

Jenkins et al in 2006 [69] identified 17 MMR mutation carriers (8 hMLH1, 4
hMSH2, 4 hMSH6, 1 hPMS2) from 131 individuals with young (under 45
years) onset CRC, unselected for family history. They determined the
mutation carrier status and cancer history for first and second degree relatives
of these probands, and used a modified segregation analysis method to correct
for ascertainment bias. They reported a cumulative CRC risk to age 70 years
for MMR mutation carriers (all mutations) of 45% for men, and 22% for
women. For hMLH1 or hMSH2 mutation carriers only, these risks were 60%
for men, and 56% for women.

Choi et al in 2009 [70] studied 32 North American families who carried
hMLH1 or hMSH2 mutations, recruited from the Ontario Familial Colorectal
Cancer Registry. They used two different methods to determine penetrance of
these mutations for CRC (they did not report on extracolonic tumours).
Firstly, they applied standard (Kaplan-Meier) survival analysis to the
mutation positive first-and-second degree relatives of the probands. They then
used a modified segregation-based approach to allow the entire cohort
(including probands and those with a negative or unknown mutation status) to be included in a model designed to correct for ascertainment bias. The two methods gave similar results. As assessed by Kaplan-Meier estimates, 67% of men and 51% of women had developed CRC by the age of 70 years. Using segregation analysis, the corresponding figures were 60% and 47%. A weakness of this study was the small numbers of proven mutation positive subjects who were not probands in their study group (25 hMLH1 and 35 hMSH2 carriers in total), but this paper is unique in demonstrating the equivalence of these two methods of calculating cancer risk in Lynch syndrome.

Stoffel et al in 2009[71] reported on 147 families from the USA with mutations in MMR genes (55 hMLH1, 81 hMSH2, 11 hMSH6). They used a similar method of modified segregation analysis to Quehenberger et al [68]and Jenkins et al [69]. In their cohort, the cumulative risk of CRC in hMLH1 mutation carriers was 97% in men and 53% in women and the cumulative risk of extracolonic cancers was 33% for women by the age of 70 years. In hMSH2 mutation carriers, there was a cumulative risk of CRC of 52% in men and 39% in women and the cumulative risk for extracolonic cancers in women was 45% by the age of 70 years.

Plashke et al [72] compared the oncological manifestations of hMSH6 mutation carriers with hMLH1 and hMSH2 mutation carriers recruited from the German HNPCC registry. They did not attempt to correct for ascertainment in any way, and included probands in their analysis. They found the median age of onset of CRC to be ten years higher in hMSH6 mutation carriers than in carriers of mutations in the two other genes (54 vs. 44 years) but there was no
difference in cumulative risk by age for extracolonic tumours between these two groups.

Barrow et al in 2008 [73] studied 121 families with proven MMR mutations (51 $hMLH1$, 59 $hMSH2$ and 11 $hMSH6$) from the North West of England. They assumed that any first degree relatives of mutation carriers who developed an HNPCC-related cancer was mutation positive (which they reasonably justified by noting that only 3/185 first degree relatives with such cancers who were tested were found not to be mutation carriers). They attempted to reduce the impact of ascertainment bias by including families who underwent genetic testing after being selected by the Bethesda criteria [43] (which are more sensitive and less specific for detecting Lynch syndrome) as well as Amsterdam II criteria [39]. They also randomly assigned an assumed carrier status to unaffected relatives (based on the proportion of unaffected individuals (stratified by age) who underwent genetic testing and were found to be mutation negative). They did, however, include probands in their analysis. They reported a cumulative risk of CRC to age 70 years of 54% for men and 46% for women (all mutations combined). For $hMLH1$ mutation carriers the cumulative risk for CRC at age 70 years was 58% for men and 49% for women. For $hMSH2$ mutation carriers this risk was 56% for men and 51% for women.

Bonadona et al in 2011 [74] studied families with Lynch syndrome recruited from 40 French cancer genetics clinics. They identified 537 families with MMR gene mutations (248 $hMLH1$, 256 $hMSH2$ and 33 $hMSH6$). They applied the genotype-restricted likelihood method (described by Alarcon et al [75], this is based on similar principles to the maximum likelihood method used by
Quehenberger et al [68]) to correct for ascertainment). They reported a similar cumulative risk of CRC by age 70 years for \textit{hMLH1} and \textit{hMLH2} carriers (59% and 57% respectively), but a significantly lower risk for \textit{hMSH6} carriers (25% by age 70 years). They found no difference in risk between men and women. The cumulative risk for women by age 70 years of developing endometrial cancer was 54% for \textit{hMLH1} mutation carriers, 21% for \textit{hMSH2} mutation carriers, and 16% for \textit{hMSH6} carriers. Ovarian cancer cumulative risks to age 70 years were 20% and 24% for \textit{hMLH1} and \textit{hMSH2} mutation carriers respectively, and 1% for \textit{hMSH6} mutation carriers.

Dowty et al in 2013[76] studied almost 18000 members of 166 \textit{hMLH1} and 224 \textit{hMSH2} mutation- carrying families from the Colon Cancer Family Registry (which recruits subjects from Australasia, Canada and the USA) using modified segregation analysis to correct for ascertainment bias. They reported a cumulative risk of CRC by age 70 years in \textit{hMLH1} mutation carriers of 34% for men and 36% for women. For \textit{hMSH2} mutation carriers these risks were 47% and 37% respectively. They also demonstrated a high level of CRC risk heterogeneity. It is not known whether this is due to environmental or genetic factors, but did not appear to be related to geographic distribution, type of mutation (protein truncating vs. others) or method of ascertainment (family vs. population). The authors noted that a weakness in their estimate of penetrance, which is shared by other studies, is that the effect of surveillance colonoscopy and polypectomy on CRC risk is not well known.
3.1.2.3 Cancer risk in Lynch syndrome: summary

It is apparent from the above studies that there is marked variability of reported cancer risk in Lynch syndrome, but the following observations are generally consistent within the current literature[77]: *hMLH1* and *hMSH2* mutations carry a similar risk for CRC, and this risk is higher than for *hMSH6* mutations; Men with Lynch syndrome appear to carry a higher CRC risk than women for all mutations; Endometrial cancers are the commonest extracolonic malignancies (Table 6 summarises the reported lifetime risks for extracolonic malignancies in Lynch syndrome).

Even if only *hMLH1* mutation carriers are considered, and the CRC risk of men and women calculated separately, there is still significant variability in the reported CRC risk. Figure 5 below illustrates the seven previous reports that have documented cumulative CRC risk for *hMLH1* mutation carriers at more than one age [61, 68, 70, 71, 73, 74, 76] (rather than a single statement of lifetime risk). This variability can at least be partly accounted for by differences in statistical methods used, but note that Stoffel et al [71] reported a considerably higher CRC risk than Quehenberger et al [68] and Jenkins et al [69] despite using a similar methodology. This suggests that there are real differences in risk between groups of subjects with Lynch syndrome, even if they carry mutations in the same gene.

Those studies that have not corrected for ascertainment bias have generally reported a higher penetrance for *hMLH1* mutations than those that have not, and it is often assumed that the lower estimates are more accurate. It is possible, however, that CRC risk may truly be higher in individuals from
families that are ascertained due to multiple CRC cases if their mutations are inherently more pathogenic, or because of other genetic or environmental factors specific to those families [69]. Methods that reduce ascertainment bias by correcting for these (currently unknown) factors may underestimate cancer risk in members of the high risk families that present to family cancer clinics and are included in registries [77].

At a practical level, patients undergoing genetic counselling may be more usefully informed by knowing the cancer risk specific to their own family than by knowing the statistically more complex estimate of penetrance for Lynch syndrome overall.

Table 6 Lifetime risk of extracolonic cancers in subjects with Lynch syndrome

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Endometrium</th>
<th>Stomach</th>
<th>Biliary tract</th>
<th>Pancreas</th>
<th>Urothelium (Male)</th>
<th>Ovarian</th>
<th>Brain</th>
<th>Small bowel</th>
</tr>
</thead>
<tbody>
<tr>
<td>hMLH1</td>
<td>20-33%</td>
<td>2.1-11%</td>
<td>3%</td>
<td>0.7%</td>
<td>1.3-16%</td>
<td>3.4%</td>
<td>0.3%</td>
<td>1.3-7.2%</td>
</tr>
<tr>
<td>[71, 73, 78-81]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hMSH2</td>
<td>24-49%</td>
<td></td>
<td></td>
<td></td>
<td>4.1-18%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[73, 78-81]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hMSH6</td>
<td>33-71%</td>
<td>10%</td>
<td></td>
<td>0-2.6%</td>
<td>0% (Female)</td>
<td>3.4-3.7%</td>
<td>6.1-12%</td>
<td>3.4-3.7%</td>
</tr>
<tr>
<td>[71, 73, 79-83]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All combined</td>
<td>8.4-71%</td>
<td>6.7-13%</td>
<td>1.4-2.1%</td>
<td>0-3.7%</td>
<td>3.2-4%</td>
<td>6.1-12%</td>
<td>3.4-3.7%</td>
<td>0-2.5%</td>
</tr>
<tr>
<td>[62, 64, 67, 68, 71, 84, 85]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 5 Cumulative CRC risk for *hMLH1* mutation carriers.
3.1.3 Aim

The purpose of this study was to document the cancer risk of subjects who carry the C1528T (Exon 13) mutation in the \textit{hMLH1} gene in order to give accurate genetic counselling to members of the affected families, and to assess whether screening for extracolonic malignancies should be undertaken (in addition to CRC surveillance, which is already offered to these subjects).

3.1.4 Methods

Between 1997 and 2007, genetic testing was performed on 590 members of 17 families known to carry the C1528T (Exon 13) mutation in the \textit{hMLH1} gene. Of these, 200 subjects were found to be germline mutation carriers. All of these 200 mutation carriers were offered surveillance colonoscopy every 2 years until age 30, and annually thereafter, in keeping with current guidelines [9, 46]

Subjects who were known to carry the mutation and were diagnosed with resectable colon cancers or adenomas with high grade dysplasia were offered a subtotal colectomy and ileorectal anastomosis. Subjects with curable rectal cancers were offered rectal resections after radiotherapy as indicated. Those subjects who had undergone segmental colonic resections prior to being recognized as having Lynch syndrome were all offered surveillance of their remaining colon.

Surveillance colonoscopy has been shown to delay the onset of colorectal cancer. In order to calculate the risk of developing colorectal cancers in subjects who carry this mutation, therefore, we included only those subjects...
who had developed colorectal cancers before they were offered surveillance colonoscopy (21 cases) and those who had declined surveillance (50 cases) in this analysis.

Screening for cancers other than colorectal (specifically endometrial, cervical, ovarian and breast cancer) was not routinely offered, so the incidence of extracolonic tumours was determined for the entire cohort of 200 subjects.

Subjects were followed up prospectively from the time of genetic testing until December 2008. Patients were followed up twice annually. At follow up a clinical history was taken, with particular attention to symptoms of colorectal and endometrial carcinoma, a clinical examination was performed, and the subjects were investigated as indicated if there was any clinical suspicion of malignancy. If the subjects declined the offer of attending for the clinical examination, he history was taken telephonically. All cancer diagnoses were made histologically from biopsies or operative specimens.

3.1.4.1 Statistics

Colorectal cancer risk was calculated using the Kaplan-Meier technique, and comparison between groups was done using the log rank method. In order to avoid introducing lead-time bias into these analyses, colorectal cancer risk was calculated from birth rather than from diagnosis or enrolment in the study. Categorical data were compared using the chi-square test. Statistical analysis was done using Statistica® (Mariakerke, Belgium) software.
3.1.4.2 Ethics

All subjects were counselled and gave informed consent for genetic testing. The patients who declined surveillance were offered it again whenever they were contacted. The Health Sciences Faculty Research Ethics Committee of the University of Cape Town approved the study.
3.1.5 Results

3.1.5.1 Colorectal malignancies

Of the 200 mutation-positive subjects, 71 (45 men and 26 women) did not undergo colonoscopic surveillance. They were followed up for a mean of 4 years (range 0 to 18 years) after genetic counselling. They were a mean age of 43 years (range 17 to 83 years) at their most recent follow up or death.

Thirty-six of these 71 subjects (51%) developed colorectal cancers. The youngest was 17 years old, and the oldest was 73 years old at the time of first CRC diagnosis. The ages of diagnosis are illustrated below (Figure 6). No adenomas were diagnosed in these subjects.

![Histogram showing the age in years of onset of CRC in subjects who did not undergo surveillance.](image-url)
Among the subjects who did not undergo surveillance, Kaplan-Meier estimate of median age of first CRC diagnosis was 44 years (42 years for men; 48 years for women. There was no significant difference in age of CRC diagnosis between men and women ($p=0.096$)). The risk of developing a colorectal cancer by age 65 years was 92% (standard error 7%). The oldest mutation-positive subject not to develop CRC died at the age of 83 years. The Kaplan-Meier estimates for colorectal cancer risk are illustrated below (Figure 7).

![Figure 7 Kaplan-Meier estimates of age of diagnosis of CRC](image)

Three of the subjects had synchronous colorectal cancers (one of whom had three cancers at his first operation), and four developed metachronous tumours (one of these developed a metachronous transverse colon tumour, underwent a total colectomy, and later developed a rectal cancer), so a total of
45 colorectal cancers were diagnosed in this group of 71 subjects. The sites and stages of the colorectal cancers are presented below (Table 7 and Table 8). In one case, the patient’s operation notes could not be obtained, and the site of his tumour could not be identified. Of the colonic cancers whose site was recorded, 29/44 (66%) were situated proximal to the splenic flexure and 15/44 (34%) were found at the splenic flexure or more distally (p=0.05).
Table 7 Sites of colorectal cancers.

<table>
<thead>
<tr>
<th>Site of tumour</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caecum</td>
<td>9</td>
<td>20.0%</td>
</tr>
<tr>
<td>Ascending</td>
<td>7</td>
<td>15.6%</td>
</tr>
<tr>
<td>Hepatic flexure</td>
<td>5</td>
<td>11.1%</td>
</tr>
<tr>
<td>Transverse</td>
<td>7</td>
<td>15.5%</td>
</tr>
<tr>
<td>Splenic flexure</td>
<td>3</td>
<td>6.7%</td>
</tr>
<tr>
<td>Descending</td>
<td>5</td>
<td>11.1%</td>
</tr>
<tr>
<td>Sigmoid</td>
<td>2</td>
<td>4.4%</td>
</tr>
<tr>
<td>Rectosigmoid</td>
<td>1</td>
<td>2.2%</td>
</tr>
<tr>
<td>Rectum</td>
<td>5</td>
<td>11.1%</td>
</tr>
<tr>
<td>Unknown</td>
<td>1</td>
<td>2.5%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>45</td>
<td></td>
</tr>
</tbody>
</table>

Table 8 Stage distribution of colorectal cancers.

Patients with synchronous tumours at presentation have been staged according to the most advanced tumour only.

<table>
<thead>
<tr>
<th>Duke’s Stage</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5</td>
<td>12%</td>
</tr>
<tr>
<td>B</td>
<td>13</td>
<td>31%</td>
</tr>
<tr>
<td>C</td>
<td>12</td>
<td>29%</td>
</tr>
<tr>
<td>D</td>
<td>7</td>
<td>17%</td>
</tr>
<tr>
<td>Unknown</td>
<td>4</td>
<td>10%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>41</td>
<td></td>
</tr>
</tbody>
</table>
3.1.5.2 Correcting for possible ascertainment bias

In order to determine whether the estimated CRC was falsely high due to ascertainment bias (where subjects with young onset CRC are more likely to undergo genetic testing than those who are diagnosed with the disease later (or who are never diagnosed with CRC in their lifetime)) this analysis was repeated including only those subjects who had not been diagnosed with CRC prior to being offered genetic testing (i.e. those who were identified as being at risk purely on the basis of family history and not phenotype). In this subset of 50 subjects (28 men and 22 women), the Kaplan-Meier estimate of median age of first CRC diagnosis was 47 years (44 for men and 47 for women; there was no significant difference in age of CRC diagnosis between men and women (P = 0.44)). The Kaplan-Meier estimates of CRC risk in this subgroup are illustrated in below (Figure 8)

![Figure 8 photograph](image)

**Figure 8** Kaplan-Meier estimates of age of diagnosis of CRC including only those patients who had no CRC diagnosis prior to genetic testing.
3.1.5.3 Extracolonic malignancies

Of the entire cohort of 200 subjects, 102 were men and 98 were women. Their mean age was 41 years at death or last follow up (range 18 to 83), and they were followed up for a mean of five years (range 0-18). Nineteen extracolonic malignancies were diagnosed in 18 of these 200 subjects (9%). The commonest were breast (in 6/98 (6%) of women) and endometrial (in 3/98 (3%) of women). The extracolonic malignancies are listed below (Table 9).

Table 9 Sites of extracolonic malignancies

<table>
<thead>
<tr>
<th>Extracolonic malignancies</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>6</td>
</tr>
<tr>
<td>Small bowel</td>
<td>3</td>
</tr>
<tr>
<td>Endometrium</td>
<td>3</td>
</tr>
<tr>
<td>Biliary</td>
<td>2</td>
</tr>
<tr>
<td>Duodenal papilla</td>
<td>1</td>
</tr>
<tr>
<td>Hepatic neuroendocrine</td>
<td>1</td>
</tr>
<tr>
<td>O-G junction</td>
<td>1</td>
</tr>
<tr>
<td>adenocarcinoma</td>
<td></td>
</tr>
<tr>
<td>Prostate</td>
<td>1</td>
</tr>
<tr>
<td>Cervix</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>19</strong></td>
</tr>
</tbody>
</table>
3.1.6 Discussion

A reasonably large cohort of subjects with Lynch syndrome who all carry the same predisposing mutation is a rarity, and provides an important vehicle for research aimed at understanding the role of genes and environment in cancer risk in order to improve surveillance strategies. This is the first study to prospectively record the cancer risk of a cohort of subjects carrying a single \textit{hMLH1} mutation.

The subjects developed colorectal cancer at a young age (median 44 years), and frequently developed synchronous or metachronous colorectal tumours. This mutation has a high penetrance, with 92% of subjects developing a colorectal cancer by age 65 years. Cumulative CRC risk for carriers of the C1528T (Exon 13) mutation in the \textit{hMLH1} gene compared with previous reports of age-specific CRC risk in \textit{hMLH1} mutation carriers is illustrated in Figure 9. There was no difference in the frequency or age of onset of colorectal cancers between men and women in contrast to previous reports, which have generally found the penetrance to be higher in men.

Ascertainment bias may lead to an overestimation of CRC risk in Lynch syndrome. Those families with the highest numbers of cancers are the most likely to be studied, and if one relies on clinical criteria to diagnose HNPCC, those subjects who do not develop tumours (or who develop them later in life) may be excluded from the analysis. In this study, an attempt was made to identify the entire cohort of mutation positive members of the families studied, regardless of whether they have developed cancers, and to follow them up prospectively. This approach does not eliminate ascertainment bias,
but should reduce it. In order to further reduce ascertainment bias, a subgroup analysis was done which included only subjects who had not been diagnosed with CRC prior to genetic testing (i.e. who were suspected of having Lynch syndrome purely from family history and not phenotype). The results for this subgroup were similar to those for the larger cohort.

![Graph showing cumulative risk of CRC for carriers of the C1528T (Exon 13) mutation in the hMLH1 gene (in bold red) compared with other reports of CRC risk in hMLH1 mutation carriers.](image)

**Figure 9** Cumulative risk of CRC for carriers of the C1528T (Exon 13) mutation in the *hMLH1* gene (in bold red) compared with other reports of CRC risk in *hMLH1* mutation carriers.

Note the high penetrance of the C1528 (exon 13) mutation. Age is given in years.
The results of studies of cancer risk in Lynch syndrome may be influenced by interventions aimed at preventing the development of cancers. In order to avoid this, all subjects who had undergone surveillance colonoscopy were excluded from the estimation of colorectal cancer risk. The drawback of this approach, however, is that these cancers were only diagnosed once they become clinically apparent, with a variable lead time from the development of premalignant adenomas and early asymptomatic cancers.

Endometrial carcinoma is the commonest extracolonic cancer among women with Lynch syndrome, with a reported lifetime risk of up to 30% in carriers of \( hMLH1 \) mutations (and higher in \( hMSH2 \) and \( hMSH6 \) mutations (Table 9)). Current guidelines advocate screening for endometrial cancer in women with Lynch syndrome [9, 46] despite there being no evidence that it confers a survival benefit [86-88]. It is even argued that prophylactic hysterectomy with or without oophorectomy should be offered to women with Lynch syndrome who have completed their families [9, 46]. The best evidence to support this is from Schmeler et al[89], who performed a large retrospective study of 315 women with Lynch syndrome, some of whom had undergone prophylactic gynaecological surgery. Endometrial cancer occurred in 33% and ovarian cancer in 5% of the control group (who had not undergone surgery), and this risk was eliminated by prophylactic surgery. In women who carry the C1528T (Exon 13) mutation in the \( hMLH1 \) gene, only 3 of 98 (3%) women developed endometrial carcinoma. There were also no cases of ovarian cancer in this cohort of women, so screening for endometrial or ovarian cancers is not clearly indicated in women who carry this particular mutation, and prophylactic gynaecological surgery does not appear warranted. It should be
noted, however, that these tumours were diagnosed only once they were clinically apparent (as no screening was performed), and so early cancers may have been missed.

Breast cancer was the commonest extracolonic malignancy in this cohort, affecting 6 of 98 (6%) women. This is unusual, as other studies of extracolonic cancer risk in *hMLH1* mutation carriers [62, 64, 67, 68, 71, 84, 85] have not reported an increased risk of breast cancer (see Table 9 above). Immunohistochemical evidence of mismatch repair gene inactivity has been documented in some cases of breast cancer in these families [90] so breast cancer can be regarded as a manifestation of Lynch syndrome in carriers of this mutation.

Recent guidelines recommend screening for urothelial cancers in Lynch syndrome patients by urinalysis for microscopic haematuria[9] and/ or urine cytology [9, 46] although there is no evidence for the efficacy of either screening test in this setting [9, 46, 91]. The only large study of screening for urothelial cancers in HNPCC by urine cytology found that the test performed extremely poorly- sensitivity was 29%, approximately 1000 tests needed to be performed to detect one cancer, and false positive tests were ten times as common as true positives. Among carriers of the C1528T (Exon 13) mutation in the *hMLH1* gene, no urothelial cancers were diagnosed. This justifies the current policy of not offering screening for these cancers in members of this family.

Five subjects developed cancers of the duodenum, small bowel and bile duct, so the risk of developing any one of these tumours was low, in keeping with reports of other Lynch syndrome families.
In conclusion, subjects who carry the C1528T (Exon 13) mutation in the *hMLH1* gene carry a high lifetime risk of colorectal cancer, regardless of their gender, but have a relatively low incidence of extracolonic tumours, most notably endometrial carcinomas. This information allows accurate genetic counselling to members of this family, and justifies the current policy of not offering routine gynaecological screening or prophylactic gynaecological surgery to women who carry this mutation.

3.2.1 Abstract

3.2.1.1 Introduction

Previous studies have shown a benefit for surveillance colonoscopy in heterogeneous groups of subjects with suspected or proven Lynch syndrome. The aim of this study is to investigate whether surveillance colonoscopy improves survival in subjects who all carry a single mismatch repair gene defect.

3.2.1.2 Methods

This is a prospective cohort study of 178 subjects who carry a mutation of the C1528T (Exon 13) mutation in the hMLH1 gene. They were offered surveillance colonoscopy between 1988 and 2006, and were followed up until September 2007.

3.2.1.3 Results

129 subjects underwent surveillance colonoscopy, and 49 declined. After a median follow up of 5 years, colorectal cancer was diagnosed in 14/129 (11%) of subjects in the surveillance group, and 13/49 (27%) in the non-surveillance group (p=0.019). Cancers in the surveillance group were detected at an earlier stage than in the non-surveillance group (P=0.032)
Death from colorectal cancer occurred in 3/129 (2%) of subjects in the surveillance group, and 6/49 (12%) in the non-surveillance group (p=0.021).

The Kaplan-Meyer estimates for median survival from birth were 78 years in the surveillance group, and 55 years in the non-surveillance group (p=0.024).

The Kaplan-Meyer estimates for median colorectal cancer free survival from birth were 73 years in the surveillance group and 47 years in the non-surveillance group (p=0.0089).

3.2.1.4 Conclusion

Surveillance colonoscopy was associated with improved overall and colorectal cancer-specific survival.
3.2.2 Introduction

There is strong evidence (from observational and randomised trials) that endoscopic screening of the general population (whether by flexible sigmoidoscopy or complete colonoscopy) can prevent bowel cancers by removing premalignant adenomas[92]. It can also lead to the detection of earlier stage tumours and thereby reduce cancer-related mortality (CRC stage at diagnosis strongly predicts survival as illustrated below (Figure 10). The commonly used staging systems for CRC are summarised below (Table 10 & Table 11). The survival benefit of population screening for CRC by colonoscopy has been confirmed in a recent meta-analysis [93]. A number of other studies have explored the utility of surveillance for CRC in the setting of Lynch syndrome.

![Survival after surgery for CRC grouped by Dukes’ stage](image)

**Figure 10** Survival after surgery for CRC grouped by Dukes’ stage at diagnosis.

Median survival for Dukes’ A, B, C and D tumours were 112, 108, 56 and 11 months respectively; P<0.0001. (The author’s own analysis of unpublished data from the Geelong Hospital colorectal database).
Table 10 American Joint Committee on Cancer (AJCC)/ Union Internationale Contre le Cancer (UICC) TNM staging definitions[94]

<table>
<thead>
<tr>
<th>Category</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary tumor (T)</td>
<td>TX Primary tumor cannot be assessed</td>
</tr>
<tr>
<td></td>
<td>TO No evidence of primary tumor</td>
</tr>
<tr>
<td></td>
<td>Tis Carcinoma in situ</td>
</tr>
<tr>
<td></td>
<td>T1 Tumor invades the submucosa</td>
</tr>
<tr>
<td></td>
<td>T2 Tumor invades the muscularis propria</td>
</tr>
<tr>
<td></td>
<td>T3 Tumor invades through the muscularis propria into the subserosa or into</td>
</tr>
<tr>
<td></td>
<td>the nonperitonealized pericolic or perirectal tissues*</td>
</tr>
<tr>
<td></td>
<td>T4 Tumor directly invades other organs or structures or perforates the</td>
</tr>
<tr>
<td></td>
<td>visceral peritoneum*</td>
</tr>
<tr>
<td>Regional lymph nodes (N)</td>
<td>NX Regional lymph nodes cannot be assessed</td>
</tr>
<tr>
<td></td>
<td>N0 No regional lymph nodes metastasis</td>
</tr>
<tr>
<td></td>
<td>N1 Metastasis in one to three lymph nodes</td>
</tr>
<tr>
<td></td>
<td>N2 Metastasis in four or more lymph nodes</td>
</tr>
<tr>
<td>Distant metastasis (M)</td>
<td>MX Presence of distant metastasis cannot be assessed</td>
</tr>
<tr>
<td></td>
<td>M0 No distant metastasis</td>
</tr>
<tr>
<td></td>
<td>M1 Distant metastasis</td>
</tr>
</tbody>
</table>

* For simplicity, the subdivisions of T3 and T4 have not been included.
Table 11 AJCC/UICC and Dukes’ stage groupings

<table>
<thead>
<tr>
<th>AJCC/UICC Stage Groupings[94]</th>
<th>Dukes’ stage[95]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 0</td>
<td>Tis</td>
</tr>
<tr>
<td>Stage I</td>
<td>T1</td>
</tr>
<tr>
<td></td>
<td>T2</td>
</tr>
<tr>
<td>Stage IIA</td>
<td>T3</td>
</tr>
<tr>
<td>Stage IIB</td>
<td>T4</td>
</tr>
<tr>
<td>Stage IIIA</td>
<td>T1, T2</td>
</tr>
<tr>
<td>Stage IIIB</td>
<td>T3, T4</td>
</tr>
<tr>
<td>Stage IIIC</td>
<td>Any T</td>
</tr>
<tr>
<td>Stage IV</td>
<td>Any T</td>
</tr>
</tbody>
</table>

*Dukes did not include stage D in his original classification, this was added in 1976[96], and has become part of the standard nomenclature[97].
3.2.2.1 Surveillance for CRC in Lynch syndrome

In 1987, Mecklin et al [98] reported on the results of a single episode of screening for colonic neoplasms in members of 22 Finnish families with ‘cancer family syndrome’ (which they defined as families with at least three first-degree relatives with CRC). This study included only asymptomatic family members who had not previously been diagnosed with CRC. They identified 236 subjects, of whom 137 attended for a single screening investigation (colonoscopy in 58 and double-contrast enema plus flexible sigmoidoscopy in 79). Thirteen adenomas were found in ten subjects. Two subjects were diagnosed with potentially curable (Dukes’ A and B stage) colon cancers, underwent surgery and were alive at 18 and 30 months’ follow up. An inconsistency in this report is that although it is stated that only asymptomatic subjects were included, the man with the Dukes’ B tumour ‘already had diffuse abdominal symptoms at the time of the examination’ so his diagnostic examination cannot strictly be considered a screening test. The authors noted that the number of neoplasms detected was approximately ten times the average yield for screening of the general population. Two subjects in the group who declined surveillance developed CRC during the three year study period, so the incidence of CRC was similar between the groups, but the tumours in the unscreened group were more advanced (although the paper was underpowered to detect any difference in tumour stage, it has been cited as demonstrating that screening can detect tumours at an early stage[99]).
Vasen et al in 1989 [100] studied members of 22 HNPCC families from the Netherlands. In this paper, they compared subjects who had colorectal neoplasms detected by screening (20 patients) with those who had developed clinical signs or symptoms (87 patients). Fourteen adenomas were detected by screening, and none were detected in the symptomatic group. There were six invasive CRC detected by screening, and 87 in the symptomatic group. The stages of the cancers in the two groups were as follows: Symptomatic group: 6 Dukes’ A; 37 Dukes’ B; 21 Dukes’ C; 10 Dukes’ D vs. 2 Dukes’ A and 4 Dukes’ B in the screened group. The authors concluded that ‘screening leads to the early detection of colorectal carcinomas…’ although there is no statement of statistical significance in the paper, and if one applies the chi-square test to their data, there is no significant difference in stage of cancer between the groups (P=0.10).

In 1995, both the Finnish and Dutch groups published long-term follow up results of their surveillance programmes, which by this time were well established. Jarvinen et al [101] offered 3-yearly surveillance by colonoscopy or barium enema plus sigmoidoscopy to the 22 Finish families described above. A total of 133 subjects underwent screening, and a control group of 118 did not. They observed a significant reduction in CRC incidence in the screened group (6 vs. 14, P=0.03) presumably due to the much higher number of adenomas detected (22 vs. 2, P=0.0002). There was a trend towards reduced mortality in the screened group, but this did not reach statistical significance (6 vs. 12 deaths, P=0.08). There were no deaths directly due to CRC in the screened group, compared to five in the control group, but again this did not reach statistical significance. The stages of the
CRC diagnosed in the two groups were as follows: Control group: 3 Dukes’ A; 4 Dukes’ B; 1 Dukes’ C, 6 Dukes’ D compared with 2 Dukes’ A and 4 Dukes’ B in the screened group. The authors state that the stage distribution of carcinomas was more favourable in the screened group, but no mention is made of statistical significance, and if one applies the chi-square test to their data, no significant difference can be detected (P= 0.19).

Vasen et al [102] reported on the results of a similar surveillance programme (colonoscopy or barium enema plus sigmoidoscopy every two to three years) for members of 50 Dutch families who met the Amsterdam criteria for diagnosis of HNPCC[37]. They compared the stage distribution of CRC that were detected by screening with those that were not. These results were as follows: Control group: 10 Dukes’ A; 75 Dukes’ B; 51 Dukes’ C; 25 Dukes’ D vs. 2 Dukes’ A and 4 Dukes’ B in the screened group. The authors concluded that ‘periodic examination… allows the detection of cancer at an earlier stage…’ although, as in the above studies, the authors did not make any statement about statistical significance, and if one analyses their data, the two groups are not significantly different (P=0.47, Chi-square test). They also reported a difference in 5-year survival between the two groups (87% in the screened group vs. 63% in the control group), but with no statement of statistical significance. This group also published another report in 1995 [103] in which they noted an unexpectedly high number of interval cancers which occurred between 2 and 5 years after a normal colonoscopy (and before the next planned surveillance endoscopy). They concluded that this supported the hypothesis that the progression from adenoma to carcinoma was accelerated in HNPCC, and that surveillance intervals should be reduced to 1-2 years.
This recommendation was strengthened in a publication by the same group in 2002, in which they reported the incidence of CRC in 887 members of 114 HNPCC families who had been enrolled in their surveillance programme. They calculated that there was a 10.5% cumulative risk of developing CRC by ten years’ follow up (so surveillance did not totally prevent the development of CRC), but that all but one of the subjects who developed CRC within two years of a screening investigation had Dukes’ A or Dukes’ B (i.e. potentially curable by surgery alone) cancers, whereas six Dukes’ C cancers were detected after a longer interval. The authors reasonably argued that this provided a strong case against prophylactic colectomy to prevent CRC as long as appropriate (1-2 yearly) surveillance occurred.

By the year 2000, the Finnish group could report on 15 years of CRC screening in HNPCC, and published a landmark paper on the subject [104]. By this time, mutational analysis for MMR mutations was available, and mutations in the \( hMLH1 \) gene (19 families) and \( hMSH2 \) gene (one family) had been identified in 20 of the 22 families on which they had previously reported. Three-yearly colonoscopies had been performed on 133 members of these 22 families, and 119 control subjects had declined screening. They reported a significant reduction in the incidence of CRC (6% vs. 16% \( P=0.014 \)). Of this cohort, 44 subjects in the surveillance group, and 46 in the control group were proven carriers of MMR gene mutations. The results were even more dramatic when this subset was studied separately. Among MMR mutation carriers, there was a reduction in CRC from 41% (19 of 46) in the control group to 18% (8 of 44) in the surveillance group (\( P=0.02 \)). This was attributable to the removal of adenomas from 13 mutation carriers who
underwent surveillance. They further reported that the stage distribution of cancers was more favourable in the surveillance group, illustrated below (Table 12). There were now sufficient numbers to reach statistical significance (P=0.03).

Most notably, surveillance was associated with a decrease in overall mortality among mutation carriers (4 vs. 12 deaths, P=0.05) and in the overall cohort (10 vs. 26 deaths, P=0.003).

<table>
<thead>
<tr>
<th>Dukes’ stage</th>
<th>Control group</th>
<th>Screened group</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>B</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

In the same year, Renkonen- Sinisalo et al [105] published a retrospective review of 150 CRC cases diagnosed in 57 Finnish HNPCC families. They found a more favourable stage distribution in the cancers diagnosed at screening (P<0.001) and improved CRC-specific survival in the screened group (although no difference in overall survival could be detected). The authors concluded that surveillance enables early detection of CRC in HNPCC, and reduces CRC mortality. However, they noted that both lead-time bias (in which early detection of the disease leads to longer survival from
the time of diagnosis, without necessarily conferring improved survival from
the unknown time at which the disease first develops) and selection bias could
falsely favour the surveillance group in a study of this design.

Also in 2005, Dove-Edwin et al [106] reported on the results of the
colonoscopic surveillance program for the St Mark’s Hospital family cancer
registry in the U.K. They included families with HNPCC (as defined by the
Amsterdam I or II criteria) and genetically proven Lynch syndrome as well as
other families with lesser CRC risks. Among the 554 subjects from 290
HNPCC or Lynch syndrome families they detected eight CRC on surveillance
colonoscopy (after the initial surveillance endoscopy), which they calculated
to be 43% of the expected number based on their estimated risk in an
unscreened population. Note that there were no unscreened controls in this
study that could allow direct comparison.

In 2005, Arrigoni et al [107] reported on an Italian cohort of 22 families with
HNPCC who were enrolled in a two-yearly colonoscopic surveillance
programme. Their diagnosis was made using the Amsterdam criteria and not
by genetic testing, which was not offered to these families. Of the 331
individuals identified as being at risk, 199 underwent surveillance. After a
mean follow up of two years, 11 subjects were found to have adenomas on
screening. Seven CRC were diagnosed in the surveillance group, and five in
the non-surveillance group (not significantly different). The CRC diagnosed
at screening were at an earlier stage, with a higher proportion being Dukes’ A
tumors (P<0.02), but it is interesting to note that in both groups all CRC were
staged as Dukes’ A or B.
De Jong et al, in 2006 [108] reported on the cancer-related mortality rates of subjects from Lynch syndrome families from the Dutch HNPCC Registry. A family was included if at least one member of the family was a proven MMR mutation carrier. They identified almost 3000 subjects from 140 families, and documented their standardised mortality rates (SMR) over three time periods (1960-1975, 1975-1990 and 1990-2004). They observed a decrease in SMR for CRC over time (P<0.001) as well as when comparing patients who did or did not undergo surveillance (SMR 6.5 vs. 23.9, P<0.001). They did not observe any such decrease for other cancers. They attributed the improvement in SMR to the introduction of a surveillance program in the late 1980’s. However, the authors did not discuss the possible effect that changes in cancer therapy over this time period may have had on mortality rates, but it is likely that this may also have influenced their results. This highlights the challenge in interpreting studies that include historical controls.

In 2007, the Finnish group [109] published long term results of their colonoscopic surveillance programme for 420 subjects who carried a MMR mutation, and who had not had been diagnosed with CRC before commencement of screening. They calculated the cumulative risk by age 60 years of developing an adenoma to be 68% in men and 48% in women, and the risk of CRC by age 60 to be 35% in men and 22% in women. Only five patients died of CRC in this cohort during a median follow up time of seven years.
3.2.2.2 Surveillance colonoscopy is offered for Lynch syndrome by the University of Cape Town

Since 1988, the University of Cape Town has offered surveillance colonoscopy to individuals with suspected HNPCC based on family history. Mutational analysis was introduced in 1997. This has led to the detection of thirteen mutations in the *hMLH1* and *hMSH2* genes in 33 families, the commonest being the C1528T (Exon 13) mutation in the *hMLH1* gene. At the time of publication of this study, 200 proven germline mutation carriers had been identified in 17 families, all of whom were offered surveillance.

3.2.2.3 Introduction- summary

Previous studies have demonstrated the efficacy of surveillance colonoscopy in HNPCC and Lynch syndrome. These studies have included heterogeneous groups of subjects with multiple different mutations, and (in some studies) also subjects without proven mutations. A large cohort of subjects who all carry the same MMR mutation provides a unique opportunity to test the efficacy of surveillance colonoscopy in a genetically homogenous group of subjects.

3.2.3 Aim

The aim of this study was to investigate whether surveillance colonoscopy improves survival in subjects who carry a single MMR germline mutation (the C1528T (Exon 13) mutation in the *hMLH1* gene).
3.2.4 Methods

Two hundred subjects who with a proven germline C1528T (exon 13) mutation in the \textit{hMLH1} gene were identified. Twenty-one of them had been treated for colorectal cancer before they were offered genetic testing or colonoscopic surveillance and they were excluded from this study. A further patient underwent positive genetic testing at age 83 years, and died before being offered surveillance, and so was also not included. The remaining 178 subjects were all offered surveillance colonoscopy between 1988 and 2006, and were studied prospectively.

These 178 subjects were included in this analysis regardless of whether their genetic testing was done before or after they were offered screening colonoscopy. None had symptoms suggestive of colorectal cancer when they were first offered surveillance. Surveillance colonoscopy was offered from the age of 18 years, or 10 years before the age of onset of disease of the youngest relative with cancer (whichever age was the older). If the subject was only identified as being at risk when already older than this, surveillance was offered to commence at the earliest opportunity. Colonoscopy was offered every 2 years until age 30 years, and annually thereafter. Colonoscopies were performed either in the endoscopy unit at Groote Schuur Hospital in Cape Town, or by a mobile endoscopic unit which visited rural hospitals annually. Subjects were considered to have undergone surveillance if they underwent at least one colonoscopy before developing symptoms suggestive of colorectal cancer.
Patients with colorectal cancers or adenomas with high grade dysplasia were offered a subtotal colectomy and ileorectal anastomosis.

Subjects were followed up by physical or telephonic contact from the time they were first offered surveillance colonoscopy until September 2007. Records were kept of colonoscopic findings, and of the histopathology of any abnormalities found. The cause of death was determined from medical records and death certificates. Where necessary, records from other institutions were obtained.

3.2.4.1 Statistics

The Kaplan-Meyer and log rank techniques were used to calculate overall and colorectal cancer-free survival. Categorical data were compared using the chi-squared test. Continuous data were compared using the Student’s t-test. Statistical analysis was performed using Medcalc® (Mariakerke, Belgium) software.

3.2.4.2 Ethics

Subjects were counselled and gave informed consent for genetic testing, and for all procedures performed. Patients who declined surveillance were offered it again whenever they were contacted. Ethics committee approval for the study was given by the Health Sciences Faculty Research Ethics Committee of the University of Cape Town.
3.2.5 Results

Of the 178 subjects, 129 chose to undergo surveillance colonoscopy, and 49 declined. The two groups were similar in age when they were first offered surveillance (mean 33 years (standard deviation 12.2) in the surveillance group, and 35 years (standard deviation 13.0) in the non-surveillance group (P=0.41)). The male:female ratio was 58:71 (45% men) in the surveillance group, and 26:23 (53% men) in the non-surveillance group (P=0.42). The subjects were followed up for a median of 5 years (range 0 to 18). Seven subjects (5%) in the surveillance group and 6 (12%) in the non-surveillance group were lost to follow up, so the follow up rates in the two groups were similar (p=0.22). During this period, the surveillance group underwent a median of 3 colonoscopies (range 1 to 12). A flowchart summarising these pathways is illustrated below (Figure 11).

![Flowchart showing inclusions and outcomes of the study groups.](image-url)
In all, 35 adenomatous polyps (all asymptomatic) were removed endoscopically from 29 patients in the surveillance group. Eleven subjects had adenomas with high grade dysplasia and underwent subtotal colectomy and ileorectal anastomosis.

Colorectal cancer was diagnosed in 14/129 (11%) of subjects in the surveillance group, and 13/49 (27%) in the non-surveillance group (p=0.019, relative risk 0.42, 95% confidence interval 0.21-0.82). Cancers in the surveillance group were at an earlier stage than in the non-surveillance group (P=0.032) (Table 13)

**Table 13 Stage distribution of CRC**

<table>
<thead>
<tr>
<th>Dukes’ stage</th>
<th>Surveillance group (n=129)</th>
<th>Non-surveillance group (n=129)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>C</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>D</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total CRC</strong></td>
<td><strong>14</strong></td>
<td><strong>13</strong></td>
</tr>
</tbody>
</table>

Death from colorectal cancer occurred in 3/129 (2%) of subjects in the surveillance group, and 6/49 (12%) in the non-surveillance group (p=0.021, relative risk 0.19 (95% confidence interval 0.026-0.61). Death from all causes occurred in 11/129 (9%) of subjects in the surveillance group and 12/49 (25%) in the non-surveillance group (p=0.0097, relative risk of death 0.35 (95% confidence interval 0.16-0.74). Their causes of death are summarized below (}
Table 14). Death rates from causes other than colorectal cancer during the study period were similar in the two groups (8/129 (6%) in the surveillance group vs. 6/49 (12%) in the non-surveillance group (p=0.30)).

Table 14 Causes of death

<table>
<thead>
<tr>
<th>Cause of death</th>
<th>Surveillance group (n=129)</th>
<th>Non-surveillance group (n=49)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorectal cancer</td>
<td>3 (2%)</td>
<td>6 (12%)</td>
<td>0.021</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Renal cell cancer</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuroendocrine tumor</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIDS</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper GIT bleed</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Total deaths</td>
<td>11 (9%)</td>
<td>12 (25%)</td>
<td>0.0097</td>
</tr>
</tbody>
</table>
The Kaplan-Meyer estimates for median colorectal cancer free survival from birth was 73 years in the surveillance group and 47 years in the non-surveillance group (p=0.0089). This is illustrated below (Figure 12).

Figure 12 Colorectal cancer-free survival (from birth).

Age is in years.
The Kaplan-Meyer estimates for median overall survival from birth were 78 years in the surveillance group, and 55 years in the non-surveillance group (p=0.024). Their survival curves are presented below (Figure 13).

![Cumulative Proportion Surviving (Kaplan-Meier)](image)

**Figure 13 Overall survival (from birth).**

**Age is in years.**
3.2.6 Discussion

In this study of patients who all carry the same mismatch repair gene mutation, surveillance colonoscopy was found to improve survival, and delay the onset of colorectal cancers. Those subjects who declined surveillance colonoscopy had a median colorectal cancer-free survival from birth of 47 years. This was extended by 26 years to 73 years in those who chose to undergo surveillance. Overall life expectancy was similarly lengthened by 23 years (from 55 to 78 years) in the subjects who underwent colonoscopies.

The reduction in colorectal cancers in the surveillance group can be ascribed to the removal of 35 adenomatous polyps from 29 subjects who underwent surveillance. Surveillance colonoscopy was associated with earlier detection of colorectal cancers-in the surveillance group, half the cancers were staged as Duke’s A, and none had detectable metastatic disease at the time of diagnosis. In contrast, only one patient in the non-surveillance group had a Duke’s A cancer at diagnosis, and 4 of 13 of them had metastases at diagnosis.

It is concerning that 6 subjects in the surveillance group had Duke’s C cancers at diagnosis. In 5 of these cases the tumors were detected at their first colonoscopy. The other subject had a normal colonoscopy in 1988, and then defaulted on his planned surveillance until 1994, when this cancer was detected. These tumors cannot, therefore, be regarded as interval cancers. They would not have been prevented by decreasing the surveillance interval, and do not represent tumors that had been missed at colonoscopy.

Subjects with the mutation who had cancers were routinely offered subtotal colectomy and ileorectal anastomosis rather than segmental colonic resections
because of concerns about the high rate of metachronous tumors in this group of patients. More controversially, subtotal colectomy was offered to patients who had adenomas with high grade dysplasia, even if these had been completely excised (as judged endoscopically and histologically). All of these patients were diagnosed by colonoscopic surveillance, and some of the reduction in colorectal cancers in the surveillance group may be due to this policy.

Good follow up was achieved, despite the logistical difficulties of reaching many of the subjects. This is largely because the process of genetic counselling allowed ongoing contact with many members of the affected families, who helped to maintain contact with their relatives.

Self-selection bias is a potential cause for bias in a study of this kind. Those subjects who elected to undergo surveillance may have had generally better health, or been more likely to seek primary or preventative health care earlier. This is unlikely to explain the results of this study however, as there was no difference in mortality due to causes other than colorectal cancer between the two groups.

In order to avoid introducing lead-time bias into the survival analysis, all survival and cancer-free survival calculations were done from birth rather than from diagnosis or enrolment in the study.

It was not considered ethical to conduct a randomized trial of surveillance colonoscopy in this group of patients, and it is unlikely that such a trial would ever be performed.
The variable compliance with screening in this study population reflects the socioeconomic and logistic difficulties faced by these communities (see chapter 1.2.2). It is encouraging that surveillance colonoscopy appears to achieve significant improvement in survival to subjects with Lynch syndrome, even in this setting.

3.2.6.1 Further evidence of the efficacy of surveillance colonoscopy in Lynch syndrome

Since the publication of this study (Paper II) in 2009 [2], further studies have strengthened the evidence for the efficacy of surveillance colonoscopy in Lynch syndrome. In 2010, the German Consortium for HNPCC published the results of their screening programme, which had begun in 1999 [110]. This was a prospective cohort study of 1126 subjects who were offered annual surveillance. This study included a heterogeneous group of families, some with proven MMR mutation carriers, some who fulfilled the Amsterdam II criteria and some with individuals who fulfilled the Bethesda guidelines [43]. They were offered annual colonoscopic surveillance, and 3474 colonoscopies were performed, at which 245 adenomas were detected. They diagnosed 28 CRC at initial surveillance colonoscopy and 43 at subsequent surveillance colonoscopies. Seventeen patients developed symptomatic CRC. The CRC detected at follow up colonoscopy were detected at an earlier stage than the other groups (P<0.001), and 41 of 43 (95%) of the CRC detected at follow up colonoscopies were UICC stage I or II (i.e. locally resectable disease, equivalent to Dukes’ stage A or B). They reported a 23% cumulative risk of CRC by age 60 years.
In the same year, Vasen et al reported the Dutch group’s [111] (extremely impressive) results of surveillance in 745 mutation carriers from 205 Lynch syndrome families (75 hMLH1, 87 hMSH2 and 43 hMSH6). Their screening interval was 2-yearly for proven mutation carriers. They reported only a 6% cumulative risk of developing CRC at ten years’ follow up. In the patients who developed CRC while on the screening programme, 90% were Dukes’ A or B cancers, and there were no deaths due to CRC.

Stuckless et al in 2012 [112] reported on the results of surveillance in a cohort of MSH2 mutation carriers (this included proven carriers by mutation testing, obligate carriers and also subjects who had not had genetic testing but had developed CRC as ‘presumed mutation carriers’) in Newfoundland (Canada). This was a retrospective study that included historical probands. They compared 152 subjects who had undergone surveillance with 170 who had not. They corrected for survivor bias by matching screened and control subjects by age and gender. They found that screening delayed the age of onset of CRC (58 vs. 47 years in the control group for men, and 79 vs. 57 years for women (P<0.001 for both groups)) and improved survival (from birth, not from diagnosis) (66 vs. 62 years for men (P=0.034) and 80 vs. 63 years for women.

Overall, four studies (other than those presented in this thesis) have directly compared CRC incidence between screened and unscreened populations with HNPCC or Lynch syndrome, summarised below (Table 15).
Table 15 CRC incidence in screened vs. unscreened populations

<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>Screened</th>
<th>Unscreened</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jarvinen et al [101]</td>
<td>1995</td>
<td>6/135 (4%)</td>
<td>14 of 118 (12%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Jarvinen et al [104]</td>
<td>2000</td>
<td>8/133 (6%)</td>
<td>19/119 (16%)</td>
<td>0.014</td>
</tr>
<tr>
<td>Arrigoni et al [107]</td>
<td>2005</td>
<td>7/199 (4%)</td>
<td>5/132 (4%)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Stuckless et al [112]</td>
<td>2012</td>
<td>28/152 (18%)</td>
<td>116/170 (68%)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Three studies (other than those presented in this thesis) have directly compared CRC-related mortality between screened and unscreened populations with HNPCC or Lynch syndrome, summarised below (Table 16).

Table 16 CRC-related mortality in screened vs. unscreened populations

<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>Screened</th>
<th>Unscreened</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jarvinen et al [101]</td>
<td>1995</td>
<td>0/135</td>
<td>5/118 (4%)</td>
<td>0.08</td>
</tr>
<tr>
<td>Jarvinen et al [104]</td>
<td>2000</td>
<td>0/133</td>
<td>9/119 (8%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Arrigoni et al [107]</td>
<td>2005</td>
<td>0/199</td>
<td>1/132 (0.8%)</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Barrow et al [113] published a meta-analysis of these studies, which also included the results of Paper I and Paper II of this thesis, which showed a benefit for screening in reducing both CRC incidence and CRC-related...
mortality. The methodology of this meta-analysis can be criticised, however, as there is significant repetition in the patient groups presented in Jarvinen et al (1995)[101] and Jarvinen et al (2000)[104], as well as in PAPER I and PAPER II included in this thesis. If one repeats the meta-analysis without repetition of study groups, the main results remain essentially unchanged: The incidence of CRC is reduced by screening (odds ratio 0.30 (95% C.I. 0.12 to 0.74)), as is CRC-related mortality (odds ratio 0.11 (95% C.I 0.04 to 0.46)). The Forest plots are illustrated below (Figure 14 and Figure 15).

Figure 14 Forest plot of studies comparing CRC incidence between screened and unscreened populations (excluding studies with repetition of the study groups)
Figure 15 Forest plot of studies comparing CRC-related mortality between screened and unscreened populations (excluding studies with repetition of the study groups).
3.3 PAPER III: Surgery for colon cancer in Lynch syndrome: Total vs. segmental colectomy [3]

3.3.1 Abstract

3.3.1.1 Introduction

The high reported risk of metachronous colon cancer in Lynch syndrome has led some authors to recommend total colectomy as the preferred operation for primary colon cancer in this patient group, but this remains controversial. The purpose of this study was to determine the risk of developing metachronous colorectal cancer after segmental or total colectomy in Lynch syndrome patients, and to compare their long-term survival.

3.3.1.2 Method

This is a prospective cohort study of all patients referred to Groote Schuur Hospital (Cape Town, South Africa) between 1995 and 2009 with a proven germline mismatch repair gene defect, who had undergone a resection for adenocarcinoma of the colon with curative intent. All patients were offered annual endoscopic surveillance.

3.3.1.3 Results

Of 60 patients in the study, 39 had a total colectomy as their initial surgery and 21 had a segmental colonic resection. After six years’ follow up, metachronous colon cancer occurred in eight (21%) patients after segmental colectomy and in none of the total colectomy patients. The risk of developing metachronous colon cancer after segmental colectomy was 20% at five years.
3.3.1.4 Conclusion

Patients with Lynch syndrome have a significant risk of metachronous colon cancer after segmental colectomy. This risk is eliminated by performing a total colectomy as the primary operation for colonic cancer.
3.3.2 Introduction

Several studies have documented a high risk of developing metachronous colorectal cancer (CRC) after segmental colonic resection for primary CRC.

Fitzgibbons et al, in 1987 [114], and Lynch et al in 1988 [115] reporting on a similar cohort of 116 members of 10 American families with HNPCC, calculated the risk of developing metachronous colorectal CRC to be 40% by 10 years. Lanspa et al [116] in a later study from the same institution, identified 225 patients with HNPCC who underwent surgery for colorectal cancer. Of these, 17 (8%) developed a second CRC within five years.

In their 2002 study describing the impact of surveillance on 114 Dutch families with HNPCC, de Vos tot Nederveen Cappel et al [117] also noted a high incidence of metachronous CRC in subjects who had previously undergone a partial colectomy for colon cancer. After a mean follow up of 6.8 years after their initial surgery, 13 of 110 (12%) patients had developed a metachronous CRC, one of which was in the rectum.

Shen et al [118] reported on 98 patients from 28 Chinese HNPCC families who had undergone colorectal cancer resections, of whom 20% developed metachronous colon cancer within 10 years.

Newton et al [119] performed a retrospective study of patients who had been diagnosed with CRC, recruited from the Manchester Colorectal Cancer Registry in the UK. They included 528 subjects with Lynch syndrome (this included MMR mutation carriers and obligate carriers, but also first degree relatives with CRC and individuals with cancers that were MMR-deficient on
immunohistochemistry and who fulfilled the Amsterdam II [39] criteria for HNPCC). They calculated a cumulative risk of metachronous CRC of 3% at 10 years and 14% at 20 years. They also included a control cohort of subjects who were judged to be at population risk for CRC based on family history. In this group, the risk of metachronous CRC was considerably lower (0.6% at 10 years and 2% at 20 years, P<0.001). This is the only study to directly compare the risk of metachronous CRC in Lynch syndrome with the risk of metachronous CRC after sporadic CRC.

3.3.2.1 More extensive colonic resections reduce the risk of metachronous CRC

The high incidence of metachronous CRC observed by de Vos tot Nederveen Cappel et al [117] led the authors to conclude that ‘when a member of an HNPCC family develops colon cancer, we, as most authors, would recommend total colectomy…rather than partial colectomy’ in order to prevent later colon cancers, while acknowledging the potential deleterious effect on quality of life after a total colectomy.

Van Dalen et al in 2003 [120] retrospectively studied a cohort of 93 patients who had undergone surgical resection for colon cancer and whose families fulfilled the Amsterdam criteria [37] for HNPCC. Seventy patients had undergone a segmental colectomy, and 15 of these developed metachronous CRC after a median of 14 years follow up, whereas none of 23 patients developed metachronous CRC after total colectomy. The authors concluded that ‘there is a high risk of metachronous colorectal cancer if an index cancer in an HNPCC patient…is treated by partial colectomy. However, this risk can
be lowered, either by performing a total colectomy at the time of index surgery or possibly by effective postoperative surveillance’.

Natarajan et al [121] retrospectively studied 106 proven MMR mutation carriers (66 \textit{hMLH1}, 40 \textit{hMSH2}) who had undergone previous colorectal resections. Of these, 69 had undergone limited colonic resections, and 37 had undergone extended colectomies (this included total and subtotal colectomy, extended right hemicolectomy and (in four cases) proctocolectomy). Fifteen of 69 in the limited resection group, and eight of 37 in the extended colectomy group had undergone surgery for (unspecified) diagnoses other than cancer. They found that the rate of subsequent CRC was significantly reduced in the extended colectomy group (7% vs. 12% at five years, \(P=0.006\)).

Parry et al in 2011 [122] published a large retrospective cohort study of 382 MMR mutation carriers (172 \textit{hMLH1}, 167 \textit{hMSH2}, 23 \textit{hMSH6}, 20 \textit{PMS2}) from the Colon Cancer Family Registry (recruited from Australia, New Zealand, USA and Canada) who had undergone surgery for CRC. Fifty of these subjects had undergone an ‘extensive colon resection’ (defined as a total or subtotal colectomy), and none of these developed a metachronous CRC after a mean of 8 years’ follow up. Of the 332 who had segmental colectomies, 74 (22%) were diagnosed with CRC after a mean of 9 years’ follow up (\(P<0.001\)). The cumulative risk of metachronous CRC after segmental colectomy was 16% at ten years and 62% at 30 years. Ten (18%) of the metachronous CRC were AJCC stage III (nodal metastases present, equivalent to Dukes’ C) at diagnosis. This is despite active surveillance (subjects were undergoing on average one surveillance endoscopy every 20
months after segmental colectomy, and the large majority (78%) of patients who developed metachronous CRC were undergoing at least 2-yearly surveillance colonoscopies).

3.3.2.2 Extent of surgery for CRC and long-term survival

Despite showing a significant reduction in metachronous CRC after more extensive colectomy compared with segmental colonic resections in the setting of HNPCC [120] or Lynch syndrome [121, 122], none of these studies reported any difference in survival between the two groups [120-122].

De Vos tot Neederveen Cappel et al [123] presented a decision analysis (Markov) model for predicting the benefits of different surgical options for primary CRC in Lynch syndrome in preventing metachronous CRC. They predicted an improvement in life expectancy after subtotal colectomy compared with segmental colectomy, with the greatest benefit in young patients with early stage tumors. Overall they calculated an expected life expectancy gain of 2.3, 1 and 0.3 years for subjects undergoing surgery at ages 27, 47 and 67 years. They also predicted even greater improvements in life expectancy (3.2, 1.3 and 0.3 years for subjects undergoing surgery at 27, 47 and 67 years of age) if the primary operation was a proctocolectomy and ileo-anal anastomosis. On this basis they recommended subtotal colectomy as the preferred surgical treatment of primary CRC in Lynch syndrome.

Maeda et al [124] also performed a Markov analysis of this question, and calculated only a very small (median 43 vs. 42 year) survival benefit for total colectomy.
3.3.2.3 Choice of operation and quality of life

Subtotal or total colectomy leads to significant disruption of normal bowel function, with the potential to have a greater adverse effect on quality of life than segmental colectomy.

Studies of long-term bowel function after total colectomy and ileo-rectal anastomosis (TC/IRA) for various indications have found that about 20% of patients experience significant faecal incontinence in the long term, and that stool frequency is increased (a mean three to seven stools per day is reported) [125-128]. Church et al [125], reporting specifically on subjects who had undergone a prophylactic TC/IRA for FAP found overall quality of life to be well maintained, despite an incontinence rate of 20% and a median stool frequency of four stools per day. Duclos et al [128], in a study of patients who had undergone bowel resections for a number of different indications, reported rates of incontinence and stool frequency after TC/IRA that were similar to Church et al[125], and also described quality of life and functional outcomes as ‘satisfactory’. Lim and Ho [127] also noted a 20% incontinence rate, but a slightly lower stool frequency (three stools per day) after TC/IRA, but noted that fear of faecal leakage adversely affected the lifestyle of 20% of the patients even with perfect continence. They concluded that TC/IRA “leads to an appreciable incidence of incontinence and loss in quality of life”. This highlights the multifactorial and subjective nature of quality of life studies, and the variability in what different authors (and patients) will regard as an acceptable functional outcome.
You et al [129] published a large retrospective study comparing long-term outcomes between patients who had undergone segmental, subtotal or total colectomies for a number of different indications. In this cohort, median daily stool frequency was five after TC/IRA, compared with four after subtotal colectomy and ileosigmoid anastomosis (STC/ISA) and two after segmental colectomy. Incontinence was also more common in patients who had undergone a TC/IRA and STC/ISA than those who had a segmental colectomy. This was despite significant dietary modifications in 56% of patients and medication use (20%) to reduce stool frequency in the TC/IRA group. This was associated with significant restrictions in social and recreational activities, housework and travel. Overall, disease-specific quality of life (assessed using the Irritable Bowel Syndrome-Quality of Life Measure) was most adversely affected after TC/IRA, then STC/ISA and least affected after segmental colectomy.

In a multicentre randomized trial comparing subtotal with segmental colectomy for obstructing left sided colon cancers, the SCOTIA group[130] found that there was more stool frequency after STC/IRA compared with segmental colectomy, but no detectable difference in quality of life. It should be noted, however, that stool frequency and quality of life were assessed after a short (four month) follow up time in this study.

One study has specifically documented quality of life after surgery for colon cancer in Lynch syndrome. Haanstra et al [131] in 2012 performed a cross sectional study of patients who had undergone colectomy for Lynch syndrome in the Netherlands. They received responses to questionnaires from 51 patients who had undergone a segmental colectomy, and 53 who had
undergone a TC/IRA or STC/ISA a mean of 12 years previously. Bowel
function (stool frequency and the social impact of disordered bowel function)
were worse in the group who had undergone TC/IRA or STC/ISA than those
who had a segmental colectomy, but there was no difference in overall quality
of life as assessed by the Short Form-36 (SF-36) (a generic health survey tool)
as well as the European Organization for Research and Treatment of Cancer
Colorectal Cancer- specific Quality of Life Questionnaire Module (EORTC
QLQ CR-38).

3.3.2.4 Introduction- summary

The risk to patients with Lynch syndrome of developing metachronous colon
cancer after segmental colectomy for colon cancer is high, with reported rates
of around 4% per year. The risk of developing metachronous CRC has led
some authors to recommend total colectomy as the preferred operation for
colonic cancer in Lynch syndrome, but this remains controversial. Most
previous estimates of metachronous CRC risk in Lynch syndrome have come
from retrospective studies which included patients diagnosed by clinical
criteria as well as by proven germline mutations, and no previous studies have
compared long- term survival after total or segmental colectomy in Lynch
syndrome
3.3.3 Aim

The aim of this study was to determine the risk of developing metachronous CRC in patients with genetically proven Lynch syndrome after total or segmental colectomy for cancer and to compare their long-term survival.

3.3.4 Methods

All patients referred to the colorectal unit at Groote Schuur Hospital (a tertiary referral center, linked to the University of Cape Town, in Cape Town, South Africa) between 1995 and 2009 with a proven germline MMR gene defect who had undergone a resection for adenocarcinoma of the colon with curative intent were included in this study. Patients were followed prospectively from the time of genetic testing for Lynch syndrome until January 2009. They were included in the study regardless of whether they underwent surgery before or after they underwent genetic testing and regardless of whether the initial surgery had been performed at Groote Schuur Hospital or elsewhere. All patients who had undergone segmental colonic resection or total colectomy were offered annual surveillance by colonoscopy or flexible sigmoidoscopy respectively.

Patients were offered a completion colectomy if a resectable metachronous CRC or a high grade dysplastic adenoma was identified. Patients who developed rectal cancer were offered resection, with neoadjuvant radiotherapy if appropriate. They were considered to have developed a metachronous CRC only if the tumor was diagnosed more than two years after the initial surgery.
3.3.4.1  Statistical Analysis

The Kaplan-Meier technique was used to calculate survival and the risk of developing metachronous CRC, and groups were compared using the log rank technique. Categorical data were compared using the chi square test, and continuous data using Student’s t-test. Statistical analysis was performed using Medcalc® (Mariakerke, Belgium) software.

3.3.4.2  Ethical approval

All subjects were counseled by a trained genetic counselor and gave informed consent for genetic testing, and for all procedures performed. Ethical approval for the study was granted by the Research Ethics Committee of the University of Cape Town.

3.3.5  Results

3.3.5.1  Patient characteristics

Between 1995 and 2009, mutational analysis was performed on 856 individuals, and 280 germline mismatch repair gene mutation carriers were identified. Sixty-two of these underwent a colonic resection for adenocarcinoma between 1975 and 2007, of whom two had incurable metastatic disease at the time of surgery and were excluded from further analysis. The mismatch repair gene mutations of the remaining 60 patients, who came from 17 different families, are listed below (Table 17). The majority of patients in both groups carried the C1528T (Exon 13) mutation.
Thirty-nine patients underwent segmental colectomy as their initial operation and 21 had a total colectomy and ileo-rectal anastomosis (TC/IRA). The two groups were similar in age at the time of their first diagnosis of colonic cancer (mean 44 (+/-11.0) years in the segmental colectomy group and 41 (+/- 7.9) years in the total colectomy group (p=0.19)). The ratio of males to females was similar in the two groups (21 (54%) males in the segmental colectomy group; 13 (62%) males in the total colectomy group (p=0.74)). There was no significant difference in the prevalence of the various mismatch repair gene defects between the two groups. All total colectomy patients and 9/39 (23%) segmental colectomy patients were identified to be at risk for Lynch syndrome prior to surgery (p<0.0001). Patients undergoing total colectomy were more likely to have a Dukes’ A tumors (p=0.0003; Table 4.2). Thirty day mortality was zero in both groups.

Table 17 Mismatch repair gene mutations identified

<table>
<thead>
<tr>
<th>Germline mutations identified</th>
<th>Segmental colectomy (n=39)</th>
<th>Total colectomy (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$hMLH1$ C 1528T (Exon13)</td>
<td>23</td>
<td>17</td>
</tr>
<tr>
<td>$hMLH1$ Exon13 ins T at 1521</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>$hMSH1$ 2-16 del</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>$hMSH2$ Exon 1-16</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>$hMSH2$ Exon 15</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>$hMSH2$ Exon 1-5 del</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>$hMSH2$ Exon 1-6 del</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>$hMSH2$ Exon 3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>$hMSH2$ Exon 8</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>$hMSH2$ Exon 3 del TC at 387</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>$hMSH2$ Exon7 del CT at 1220</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>
Table 18 Dukes’ stage of the cancer(s) resected at the first operation.

In four cases, the initial surgery was done at another institution, and complete pathological staging was incomplete.

<table>
<thead>
<tr>
<th>Duke’s stage of first colon cancer</th>
<th>Segmental colectomy (n=40)</th>
<th>Total colectomy (n=22)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1 (2.5%)</td>
<td>8 (36.4%)</td>
<td>0.0003</td>
</tr>
<tr>
<td>B</td>
<td>15 (37.5%)</td>
<td>4 (18.2%)</td>
<td>0.11</td>
</tr>
<tr>
<td>C</td>
<td>19 (47.5%)</td>
<td>9 (40.9%)</td>
<td>0.62</td>
</tr>
<tr>
<td>D</td>
<td>1 (2.5%)</td>
<td>1 (4.5%)</td>
<td>0.66</td>
</tr>
<tr>
<td>Unknown</td>
<td>4 (10%)</td>
<td>0 (0%)</td>
<td>0.13</td>
</tr>
</tbody>
</table>

3.3.5.2 Follow up and surveillance

After the initial operation, patients were followed to a median of six (range 1-30) years after surgery in the total colectomy and eight (range 0 – 34) years after surgery in the segmental colectomy group (p=0.60). Five (13%) segmental colectomy patients and 1 (5%) total colectomy patients were lost to follow up (p=0.58).

During the study period, 22 (56%) segmental colectomy patients underwent a mean of three (S.D. 2.2) surveillance colonoscopies and 15 (71%) total colectomy patients a mean of five (S.D. 3.2) flexible sigmoidoscopies. The groups were equally likely to present for at least one surveillance endoscopy (p=0.39), but the total colectomy patients attended more often (p=0.015).

Thirteen adenomatous polyps were identified and removed endoscopically in seven segmental colectomy patients. Three contained high grade dysplasia.
and these patients underwent a completion colectomy and ileorectal anastomosis. One low grade rectal adenomatous polyp was identified and endoscopically removed from the rectum of a total colectomy patient.

### 3.3.5.3 Metachronous cancers

**Figure 16 Metachronous CRC risk after segmental resection of a colon cancer.**

A metachronous colon cancer occurred in eight (21%) segmental colectomy patients and none of the total colectomy patients. The risk of developing metachronous colon cancer after segmental colectomy was 20% at 5 years and 41% at 15 years (Figure 16). Two metachronous colon cancers were interval cancers, in patients who developed symptoms less than one year after a normal surveillance colonoscopy and six occurred in patients who had
defaulted surveillance colonoscopy for at least two years. Two (10%) total
collectomy patients developed a rectal cancer, at 15 and 23 years after colonic
resection. There were no rectal cancers diagnosed in the segmental colectomy
group. One rectal cancer was diagnosed one year after a normal surveillance
sigmoidoscopy, and the second patient had defaulted surveillance
sigmoidoscopy for four years. Overall there were eight metachronous
colorectal cancers in the segmental colectomy group (all in the colon) and two
in the total colectomy group (both in the rectum).

3.3.5.4 Mortality

Figure 17 Colorectal cancer specific survival after total or segmental
collectomy for their first colon cancer.

During the follow up period, fifteen (38%) patients in the segmental
collectomy group, and five patients (24%) in the total colectomy group died.
Death due to colorectal cancer occurred in 13 (33%) and 2 (10%) patients in the segmental and total colectomy groups respectively. Causes of death are summarized below (Table 19). Colorectal cancer- specific survival was significantly better in total colectomy patients \((p=0.048, \text{ Figure 17})\), but overall survival of the two groups was similar \((p=0.29)\).

**Table 19 Causes of death**

<table>
<thead>
<tr>
<th></th>
<th>Segmental colectomy (n = 39)</th>
<th>Total colectomy (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metastatic CRC</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>Cholangiocarcinoma</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Small bowel cancer</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Asthma</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Upper gastrointestinal bleed</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

### 3.3.6 Discussion

Patients with genetically proven Lynch syndrome who underwent a segmental colectomy for primary colon cancer have a significant lifetime risk of developing metachronous colon cancer. This risk is eliminated by performing a total colectomy as the primary operation for colon cancer.

Most previous reports of metachronous CRC risk in Lynch syndrome have been from retrospective studies which have included patients in whom the diagnosis was made on clinical grounds and family history as well as with
proven MMR mutations. This may result in an overestimation of risk due to selection bias as Lynch syndrome is more likely to be suspected in patients with multiple tumors (both the Bethesda [42] and Japanese [132] criteria list metachronous CRC as a clinical criterion for HNPCC). This study confirms the results of previous studies which included only proven MMR mutation carriers [121] [122].

Fourteen asymptomatic adenomas were detected and removed endoscopically, but this did not eliminate the risk of metachronous CRC after segmental colectomy. All patients were offered surveillance endoscopy, but compliance was poor in this cohort, especially after segmental colectomy. Seven of the ten patients who developed metachronous CRC had defaulted surveillance. The poor surveillance attendance is surprising in a cohort of patients who have already developed CRC and who have family members with the disease. The reasons for non-compliance in these patients are unknown and are currently under investigation, but poor socio-economic conditions and low education standards, which are all prevalent in this community, are likely to be contributory.

The low number of surveillance colonoscopies affects the results of this study as surveillance colonoscopy may prevent metachronous CRC by removing adenomas.

3 In the original published version of this chapter (PAPER III), it was erroneously stated that this was the first published report on metachronous CRC to include only proven MMR mutation carriers.
Two patients developed an interval cancer within a year of normal colonoscopy. Rapid development of cancer from adenoma occurs in Lynch syndrome [103] but an oversight during surveillance cannot be excluded.

Rectal cancers were infrequent and all occurred more than 15 years after the initial surgery. This long interval justifies avoiding the increased morbidity associated with proctocolectomy as the primary operation for colon cancer in Lynch syndrome, but lifelong endoscopic surveillance of the rectum should be offered to patients who undergo a total colectomy.

There is evidence of selection bias in this study, where patients selected for total colectomy were more likely to have been diagnosed with Lynch syndrome pre-operatively and were more likely to have a Dukes’ A cancer than those who underwent segmental colectomy. This bias explains the apparent difference in CRC-specific survival between the two groups, and one cannot conclude from this study that the choice of surgery influenced this.

For patients with Lynch syndrome who develop colon cancer, the standard recommendation for surgery is a total or subtotal colectomy (rather than a segmental) colectomy [9, 46, 133] in order to prevent metachronous colon cancers. This is despite there being no proven survival benefit to this approach when compared to segmental colonic resection followed by endoscopic surveillance of the residual colon.

An interesting feature of the study by Parry et al [122] is that this occurred in only 13% of subjects (the rest had segmental colectomies). It is not known whether this was because of a lack of awareness of the guidelines by the treating surgeon, or a deliberate decision based on specific patient factors.
The authors note that ‘this may reflect the fact that surgery was performed in the emergency setting or that at the time surgery was planned the diagnosis of HNPCC or MMR gene mutation was unknown’. Van Dalen et al [120] also noted marked variability in surgical practice between centres in the USA that cared for these patients.

In deciding between the surgical approaches of total (or subtotal) vs. segmental colectomy, the surgeon and patient need to weigh up the relative benefits of total colectomy in terms of preventing metachronous CRC and allowing less invasive surveillance endoscopy (surveillance of the rectum can be done with by flexible sigmoidoscopy, which does not require full oral bowel preparation and can be done without sedation, unlike a colonoscopy which is required after segmental resection) against the long-term adverse effects on bowel function.

An awareness of the long term risk of metachronous CRC helps patients to make an informed choice of operation for primary colon cancer. This study confirms the results of other reports of metachronous CRC risk after segmental colectomy in Lynch syndrome.
3.4 PAPER IV: Does genetic anticipation occur in Lynch syndrome?[4]

3.4.1 Abstract

3.4.1.1 Introduction

Genetic anticipation occurs when the age of onset of a disorder decreases in successive generations. It is controversial whether this occurs in Lynch syndrome. Previous studies have included heterogeneous groups of subjects from multiple families, including subjects with a clinical diagnosis (based on family history) as well as those with proven germline mismatch repair gene mutations. The purpose of this study was to determine whether genetic anticipation occurs in mismatch repair gene carriers from a single Lynch syndrome family.

3.4.1.2 Method

This study includes members of a single family known to carry an hMLH1 gene mutation who are proven germline mutation carriers or obligate carriers (based on their offspring's mutation status). Evidence of genetic anticipation (determined by age of onset of first CRC) was sought in two ways: Firstly, subjects were grouped as parent-child pairs and individuals were compared with their own offspring; secondly they were grouped by generation within the family tree. The Kaplan-Meier technique was used to adjust for variable follow up times.
3.4.1.3 Results

The family tree consisted of 714 subjects. Ninety-two subjects over five generations were included in the study. There was no evidence of genetic anticipation over the generations. Similarly, in the 75 parent-child pairs identified, age of onset of CRC was similar for parents and children.

3.4.1.4 Conclusion

There was no evidence of genetic anticipation in mutation carriers from a single family with Lynch syndrome.
3.4.2 Introduction

The age of onset of CRC in carriers of the C1528T (Exon 13) mutation in the \textit{hMLH1} gene was described in chapter 3.1. A trend was noted in which those subjects who were born more recently appeared to develop their first CRC at an earlier age. This is illustrated below (Figure 18). There was a strong negative association between year of birth and age of onset of first CRC (Pearson correlation coefficient $r=-0.75$; $P<0.0001$). This led to an exploration of possible genetic anticipation in carriers of this mutation.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure18.png}
\caption{Year of birth vs. Age of first CRC (All C1528T (Exon 13) hMLH1 mutation carriers)}
\end{figure}

Genetic anticipation is the phenomenon in which the age of onset of an inherited disorder decreases in successive generations. When Warthin [12]
first described a family with Lynch syndrome (‘Family G’) in 1925, he made
the observation that colorectal cancers occurred at a younger age in
successive generations. Since then, a number of conflicting reports have
documented the presence or absence of genetic anticipation in Lynch
syndrome and it remains controversial whether it occurs in this disease.

Genetic anticipation has been definitively shown to occur in several
neurodegenerative disorders, including Huntington’s chorea [134] and
spinocerebellar ataxia [135-137]. In these diseases, the observed generational
expansion of trinucleotide repeats during meiosis and gametogenesis provides
a molecular mechanism to explain the earlier onset and worse prognosis of
affected individuals in successive generations, but there is no direct evidence
that this occurs in Lynch syndrome[138]. Indeed, in a mouse model of
Huntington disease, mismatch repair gene deficiency has been shown to
prevent instability of trinucleotide repeats [139]. It has been proposed that
germline mismatch repair gene defects may lead to an accumulation of small
errors in DNA replication prior to loss of heterozygosity, and that this could
be passed on over the generations, however there is little direct evidence that
this occurs [140]. In Li- Fraumeni syndrome, anticipation has been found to
be linked to decreasing telomere length over generations [141]. Bozzao et al
[140] have recently described abnormalities in telomere length in carriers of
MSH2 mutations, but not MLH1 mutation carriers. This is an intriguing and
developing area of research, but a definitive molecular mechanism for
anticipation in Lynch syndrome has not yet been identified.
Previous studies of genetic anticipation in Lynch syndrome have variably reported the presence or absence and extent of this phenomenon. These are summarised below (Table 20).

Tsai et al [142] and Westphalen et al [143] found no evidence of genetic anticipation, whereas other authors [12, 144-150] described an effect ranging from three [148, 150] to ten [150] years. A striking feature of this literature is the variability in reported anticipation, even when different authors have studied similar cohorts but using different methodologies. Vasen et al [144] studied 74 patients with CRC from the Foundation for the Detection of Hereditary Tumors in the Netherlands, and reported an average anticipation of 8.5 years. Voskuil et al [146] later analysed 1186 subjects from the same registry (but using a different methodology) and could not detect any evidence of anticipation. Larsen et al [148], Nilbert et al [149] and Boonstra et al [150] reported an anticipation effect in subjects from the Danish HNPCC Registry ranging between three and 9.8 years depending on the methodology used. A further confounder when attempting to interpret the available literature is that, apart from Warthin’s initial paper, all previous studies describing anticipation in Lynch syndrome have included heterogeneous groups of subjects from multiple families with different mutations and in most cases have included subjects with a clinical diagnosis of HNPCC as well as those with proven germline mismatch repair gene mutations.

A well-recognized form of bias that can falsely create the appearance of genetic anticipation is follow-up bias. This occurs when older subjects, who have passed through more of their period of risk, are compared with younger ones who have not. Subjects who have not yet manifest the disease, but may
have developed it later, are excluded from the analysis. There are more such subjects in the later generations, and so the average age of onset appears (falsely) lower in the more recent cohort [151]. This bias can be corrected for by including only subjects who were born sufficiently long ago that they have completed their period at risk. Differences in follow up time can be corrected for using survival analysis techniques, in which subjects with incomplete follow up are censored.

Genetic anticipation may also be falsely detected due to differential ascertainment of families. The chance of detecting HNPCC families with predominantly younger patients in later generations is higher than that of finding families with predominantly older patients in later generations. This is because individuals in later generations have not lived long enough to detect cancers that may develop later in their lives [146]. Correcting for variable ascertainment when subjects from multiple families are studied requires complex statistical methods (reviewed by Boonstra et al [150]).

The largest single family known to carry the C1528T mutation in exon 13 of the *hMLH1* gene (referred to as the NPC-1 family) consists of over 700 known individuals. This affords a unique opportunity to study genetic anticipation in a single family tree over multiple generations, and avoiding the confounding effect of variable ascertainment of multiple families.
### Table 20 Previous studies of genetic anticipation in Lynch syndrome

<table>
<thead>
<tr>
<th>Paper</th>
<th>Patient set</th>
<th>Numbers</th>
<th>Average anticipation (years)</th>
<th>Survival analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warthin 1925</td>
<td>“Famliy G”</td>
<td>28 patients with CRC over 4 generations</td>
<td>8</td>
<td>No</td>
</tr>
<tr>
<td>Vasen et al 1994</td>
<td>Foundation for the Detection of Hereditary Tumors (Netherlands)</td>
<td>74 patients with CRC over three generations</td>
<td>8.5</td>
<td>No</td>
</tr>
<tr>
<td>Rodriguez-Bigas 1996</td>
<td>Roswell Park Cancer Institute HNPCC Registry</td>
<td>193 patients with CRC</td>
<td>5.5</td>
<td>No</td>
</tr>
<tr>
<td>Tsai et al 1997</td>
<td>Johns Hopkins Hereditary Colorectal Cancer Registry</td>
<td>67 parent-child pairs with CRC*</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>Voskuil et al 1997</td>
<td>Foundation for the Detection of Hereditary Tumors (Netherlands)</td>
<td>1186 subjects</td>
<td>0</td>
<td>Yes</td>
</tr>
<tr>
<td>Westphalen et al 2005</td>
<td>University of Basel and Institut Central des Hopitaux Valaisans registrees</td>
<td>55 parent-child pairs with CRC</td>
<td>8</td>
<td>No</td>
</tr>
<tr>
<td>Stella et al 2007</td>
<td>Five Italian families</td>
<td>24 parent-child pairs with CRC</td>
<td>11</td>
<td>No</td>
</tr>
<tr>
<td>Larsen et al 2009</td>
<td>Danish HNPCC Registry</td>
<td>824 subjects</td>
<td>3</td>
<td>Yes</td>
</tr>
<tr>
<td>Nilbert et al 2009</td>
<td>Danish HNPCC Registry</td>
<td>290 parent-child pairs with CRC</td>
<td>5.5 – 9.8 **</td>
<td>No</td>
</tr>
<tr>
<td>Boonstra et al 2010 (a)</td>
<td>Danish HNPCC Registry</td>
<td>290 parent-child pairs with CRC</td>
<td>8.7</td>
<td>No</td>
</tr>
<tr>
<td>Boonstra et al 2010 (b)</td>
<td>University of Michigan Cancer Genetics Clinic</td>
<td>136 parent-child pairs with CRC</td>
<td>9.9</td>
<td>No</td>
</tr>
<tr>
<td>Boonstra et al 2010 (c)</td>
<td>Danish HNPCC Registry</td>
<td>816 subjects</td>
<td>3 ***</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*The full paper included 475 parent-child pairs with CRC, but only 67 of these had HNPCC

** Different estimates of anticipation were calculated depending on the method of analysis

*** Boonstra et al analyzed different datasets in a number of different ways. These analyses have been listed separately although they are from the same paper. Larsen et al and Boonstra (c) used a similar dataset.
3.4.3 Aim

The aim of this study was to determine whether genetic anticipation occurred within members of this single family.

3.4.4 Methods

The C1528T mutation in exon 13 of the hMLH1 gene has been identified in 23 families, the largest of which (referred to as the NPC-1 family) consists of 714 known individuals. This study included only members of the NPC-1 family (a single large family tree, allowing multiple generations to be studied and to reduce the impact of ascertainment bias that occurs when multiple families are recruited). Only subjects who were proven germline mutation carriers or judged to be obligate mutation carriers based on the positive mutation status of their offspring were included.

Information about age of onset of CRC was obtained by interviewing the subjects and their family members, and confirmed with hospital and pathology records wherever possible. Subjects who were found to be mutation carriers were followed up prospectively twice annually from the time of genetic testing until May 2010. Follow up consisted of a clinical history (paying particular attention to symptoms of colorectal or endometrial cancer), physical examination and further investigations if indicated. All subjects were offered surveillance colonoscopy. Subjects who declined the offer of attending for clinical follow up were contacted and interviewed telephonically.
Evidence of genetic anticipation was sought in two ways: Firstly, subjects were grouped as parent-child pairs and individuals were compared with their own offspring; the second method was to group them by generation within the family tree. In both cases, analyses were done comparing only those subjects who had developed CRC, and then repeated using the entire cohort and applying standard survival analysis techniques to correct for differences in follow up time. In addition, the analyses were repeated including only those subjects born before 1960 (i.e. those with a potential follow up time of at least 50 years).

3.4.4.1 Statistics

Age of onset of CRC was not normally distributed, so non-parametric techniques were used to compare groups. Parent-child pairs with CRC were compared using the Wilcoxon (paired) test. Age of onset of CRC (in individuals with CRC) was compared between generations using the Kruskall-Wallis test. These analyses were repeated for the entire cohort (including those who had not yet developed CRC within the study period) using the Kaplan–Meier technique and comparison between groups was done using the log rank method. For the Kaplan-Meier analyses, subjects were censored if they had not developed CRC by the end of the follow up period, if they had died before developing the disease or (in one case) where the subject had undergone a prophylactic subtotal colectomy for an adenomatous polyp. In order to avoid introducing lead-time bias into these analyses, CRC risk was calculated from birth rather than from diagnosis or enrolment in the study. Numbers are stated as median (95% CI for the median) unless otherwise
stated. Statistical analysis was done using Medcalc® (Mariakerke, Belgium) software.

3.4.4.2 Ethics

All subjects were counseled and gave informed consent for genetic testing. The Health Sciences Faculty Research Ethics Committee of the University of Cape Town approved the study.
3.4.5 Results

The family tree consisted of 714 subjects over five generations. Of these, 257 have undergone germline mutational analysis. Eighty of these 257 subjects (31%) carried the C1528T (Exon 13) mutation in the \textit{hMLH1} gene, and 176 (69%) were found not to be carriers. Of the 457 who have not undergone genetic testing, 12 were determined to be obligate carriers as they had children proven to carry the mutation. The cohort for analysis therefore consisted of a total of 92 subjects (80 with a proven mutation, and 12 obligate carriers), of whom 50 were men and 42 women. These 92 subjects were born between 1913 and 1979, and have been followed up to a median age of 38 years (range 19 to 80 years). Thirty-three of them have been diagnosed with a colorectal cancer, and 22 have died. The Kaplan-Meier estimate of the median age of first CRC was 49 years.

3.4.5.1 Age of onset of CRC grouped by birth cohort

Similar to the findings of the larger cohort of all carriers of the C1528T (Exon 13) mutation in the \textit{hMLH1} gene, there was an inverse relationship between the date of birth of the subjects and the age of onset of their first CRC (correlation coefficient \( r = -0.69, P < 0.0001 \)) as illustrated below (Figure 19). This relationship persisted, but was weaker, when only subjects born before 1960 were included (correlation coefficient \( r = -0.58, P = 0.0028 \)).

When subjects who had developed CRC were divided into birth cohorts by quartiles, there was an apparent decrease in the age of onset of first CRC in the later birth cohorts (\( P = 0.0022 \), Kruskall-Wallis test, Figure 20). This
apparent effect persisted when only those subjects born before 1960 were included (P=0.046, Kruskall-Wallis test). When those patients who had not yet developed CRC were included (using the Kaplan-Meier technique to estimate age of onset of CRC by censoring subjects who had not developed the disease at the end of the follow up period), this apparent effect disappeared regardless of whether subjects born after 1960 were included (P=0.19, Logrank test, or not (P=0.18, Logrank test, Figure 21).

Figure 19 Year of birth vs. age of first CRC diagnosis (NPC-1 family)
Figure 20 Age of onset of first CRC in the NPC-1 family, grouped by birth cohort

Figure 21 Kaplan-Meier plot of age of onset of first CRC grouped by birth cohort.
3.4.5.2 Parent-child pairs

Seventy-five parent-child pairs were identified from the 92 subjects in the study cohort. Of these, there were 22 parent-child pairs in whom both the parent and child had developed CRC. When analyzing only those pairs in which both parents and children had developed CRC, the median age of first CRC was 45 years (44.7 to 55.0) for the parents, and 36 years (30.8 to 44.0) for the children (P=0.0004, Wilcoxon test. Figure 22). This apparent effect persisted when we included only those parent-child pairs with CRC born before 1960 (45 years (33-52) for the parents vs.32 years (26-38) for the children (P=0.02). When we analyzed the entire group of 75 parent-child pairs (using the Kaplan-Meier technique and censoring those who had not developed CRC at the end of the follow up period), this apparent effect disappeared (P=0.51, Logrank test. Figure 23).
Figure 22 Age of onset (in years) of first CRC for parent-child pairs. The bars are median age, the error bars are 95% C.I. for the median.

Figure 23 Kaplan-Meier plot of age of onset of first CRC (in years) for parent-child pairs.
3.4.5.3 Age of onset of CRC grouped by generation

The 92 subjects studied were born over five generations, summarized below (Table 21).

Table 21 Age of first colorectal cancer (CRC) by generation.

Age is stated as Median (95% C.I. for median). In generations 1 and 4 there were insufficient numbers to calculate the confidence interval.

<table>
<thead>
<tr>
<th>Generation</th>
<th>Number of subjects</th>
<th>Number with CRC</th>
<th>Age of first CRC (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>2</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>8</td>
<td>46.5 (38.0-62.9)</td>
</tr>
<tr>
<td>3</td>
<td>44</td>
<td>18</td>
<td>38.5 (30.4-45.0)</td>
</tr>
<tr>
<td>4</td>
<td>32</td>
<td>5</td>
<td>35.5</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>0</td>
<td>N/A</td>
</tr>
<tr>
<td>Total</td>
<td>92</td>
<td>33</td>
<td>41.5 (35.2-45.0)</td>
</tr>
</tbody>
</table>

When analyzing only those subjects who had developed CRC, there was an apparent decrease in the age of onset of first CRC over the generations (P=0.0091, Kruskall- Wallis test (Figure 24). This apparent effect persisted when only the 24 subjects with CRC who were born before 1960 were included (P=0.029, Kruskall- Wallis test). When the entire cohort was analyzed using the Kaplan- Meier technique, and censoring those without CRC by the end of the follow up period, no difference was detected (P=0.59, Logrank test, Figure 24). A similar result was obtained when generations one
and five (which had very small numbers) were excluded from the analysis (P=0.37, Logrank test).

Figure 24 Age of onset (in years) of first CRC grouped by generation

Figure 25 Age of onset of first CRC (in years) by generation (Kaplan-Meier)
3.4.6 Discussion

There was no evidence of genetic anticipation in this study of mutation carriers from an extended single family with Lynch syndrome.

In this study, the appearance of genetic anticipation was falsely detected when only individuals who had developed CRC were included in the analysis. This is as a result of follow up bias being inadequately controlled for. This occurs when older subjects, who have passed through more of their period of risk, are compared with younger ones who have not. A subject who has not yet manifest the disease, but may develop it later, would not be included in the analysis. There are more such subjects in the later generations, and so the average age of onset appears (falsely) lower in the more recent cohort[151]. This bias can be corrected for by including only subjects who were born sufficiently long ago that they have completed their period at risk. The analyses were repeated including only subjects born before 1960, with a similar result. As the median age of onset of CRC in Lynch syndrome is 45 years, and they continue to be at risk at least into their seventies, one would need to only include subjects born at least 80 years ago to fully correct for this type of bias. In previous studies, Nilbert et al [149] found that genetic anticipation could be detected even when only subjects with more than 80 years of potential follow up were considered, whereas Tsai et al [142] found that the apparent difference in age of onset of CRC between parents and their children was no longer detectable when this birth cohort effect was taken into account. There were insufficient numbers of such old patients to test this, and indeed genetically proven Lynch syndrome carriers born more than 80 years
ago would be extremely rare in any cohort (as genetic testing only first became available in the 1990’s).

After correction for follow up bias by including the entire cohort and by applying Kaplan-Meier survival techniques (censoring those who had not yet developed CRC), the apparent anticipation effect was no longer detected. In previous studies aimed at detecting genetic anticipation in Lynch syndrome (Table 20). There was a significant difference in reported anticipation between those studies in which survival type statistical analyses were performed on cohorts of subjects at risk (mean of two years) and those in which only individuals who had developed CRC were studied (mean 7.5 years) (P=0.0073, Student- t test).

Genetic anticipation may also be falsely detected due to ascertainment bias. By including only subjects from a single family, the risk of ascertainment bias that could occur studying multiple families was reduced, and allowed simpler statistical methods to be applied. This also allowed subjects to be accurately grouped according to their generation in the single pedigree.

A possible confounder in this study of genetic anticipation in Lynch syndrome is the effect of surveillance, which has been shown to delay the onset of CRC in this population. As the oldest subjects developed CRC before the surveillance program was instituted, this could cause a real anticipation effect to be falsely missed. The sample size in this study may also have been insufficient to detect the small anticipation effect (of three years) detected in previous studies of large population databases.
This study highlights the methodological difficulties in studying genetic anticipation in inherited cancers such as Lynch syndrome.
3.5 PAPER V: Fertility and apparent genetic anticipation in Lynch syndrome [5]

3.5.1 Abstract

3.5.1.1 Introduction

Genetic anticipation is the phenomenon in which age of onset of an inherited disorder decreases in successive generations. Inconsistent evidence as discussed in chapter 3.4 suggests that this may occur in Lynch syndrome. A possible cause for the false appearance of apparent anticipation is fecundity bias, which occurs if the disease adversely affects fertility. The purpose of this study was to determine the effect of age of diagnosis of colorectal cancer (CRC) on lifetime fertility in Lynch syndrome, and whether this can falsely create the appearance of genetic anticipation.

3.5.1.2 Method

A computer model simulated age of diagnosis of CRC in hypothetical Lynch syndrome carriers and their offspring. The model assumed similar age distribution of CRC across generations (i.e. that there was no true anticipation). Age distribution of CRC diagnosis, and lifetime fertility rates (grouped by age of diagnosis of CRC) were determined from the Australasian Colorectal Cancer Family Registry (ACCFR). Apparent anticipation was calculated by comparing ages of diagnosis of CRC in affected parent-child pairs.
3.5.1.3 Results

A total of 1088 patients with CRC were identified from the ACCFR. Total lifetime (cohort) fertility was related to age of diagnosis of CRC (correlation coefficient 0.13, \( P = 0.0001 \)). In the simulation, apparent anticipation was 1.8 ± 0.54 years (\( P = 0.0044 \)). Observed apparent anticipation in the ACCFR cohort was 4.8 ± 1.73 years (\( P = 0.0064 \)). There was no difference in apparent anticipation between the simulated and observed parent-child pairs (\( P = 0.89 \)).

3.5.1.4 Conclusion

The appearance of genetic anticipation in Lynch syndrome can be falsely created due to changes in fertility.

.
3.5.2 Introduction

In Chapter 3.4, it was shown that the appearance of genetic anticipation can be falsely caused by follow up bias, and that this artefact disappeared when survival analysis techniques were used to correct for incomplete follow up.

Another potential cause of falsely apparent anticipation is referred to as fecundity bias. This occurs if the disease adversely affects fertility, so individuals who develop the disease at a younger age are likely to have fewer children than those who are diagnosed at an older age. No previous studies of anticipation in Lynch syndrome (or in any inherited condition) have examined whether fecundity bias can mimic genetic anticipation, and therefore be the explanation for any apparent anticipation.

The purpose of this study was (i) to determine the effect of age of onset of CRC on lifetime fertility in Lynch syndrome, (ii) to determine by computer simulation whether observed changes in fertility can falsely create the appearance of genetic anticipation, and to what extent, (iii) to compare the results of that simulation with the observed appearance of genetic anticipation in a large series of families with Lynch syndrome.

3.5.3 Method

The Australasian Colorectal Cancer Family Registry (ACCFR) is a registry of more than 11500 people from 1800 families in Australia and New Zealand [152]. This registry contains CRC families recruited through the Victorian Cancer Registry (960 population-based case-families) and from family cancer clinics throughout Australia and New Zealand (580 clinic-based case-
families), as well as families of people without CRC recruited through the Victorian electoral roll (270 control-families). For all family members, personal and family history of cancer, reproductive history, and other lifestyle and personal characteristics were collected by questionnaire. Attempts were made to verify all reports of CRC diagnoses by medical records, pathology reports, death certificates and linkage to national cancer registry and death registry databases.

Individuals who had developed CRC, and who were members of families known to carry MMR gene mutations (Lynch syndrome families) were categorised by age of diagnosis of CRC. Cohort fertility (the mean number of children born to each individual by the end of their reproductive life- defined as over the age of 50 years) was calculated for each age group of CRC diagnosis (for both men and women). We only included subjects born before 1963 in this analysis in order to include only those who had completed their period of potential fertility.

3.5.3.1 Model design

A computer model was designed to simulate the age of diagnosis of CRC in large numbers of hypothetical MMR gene mutation carrying men and women (first generation) and their offspring (second generation). The model assumed complete follow-up over the lifetime for all individuals, and assumed the age distribution of CRC diagnoses to be the same across generations (i.e. that there was no genetic anticipation). In this setting any apparent genetic anticipation would be an artefact.
Because follow up was over the entire lifetime of the hypothetical subjects in both generations, and the complete lifetime risk was applied to each subject, follow up (ascertainment) bias will not cause the false appearance of genetic anticipation in this model.

The model generated an equal number of men and women in the first generation, and allocated gender at random (with a 50:50 chance) to the second generation individuals.

Age of diagnosis of first CRC was randomly assigned according to the (gender-specific) observed distribution of age of first CRC diagnosis from the ACCFR cohort. The number of offspring born to each first generation carrier was randomly assigned according to the observed (gender-specific) distribution of lifetime fertility according to the age of diagnosis of CRC as calculated above. Each second generation individual was given a 50% chance of inheriting the MMR gene mutation. For each mutation-carrying child, age of diagnosis of first CRC was allocated in the same way as for their parents. The simulation was run for 1000 first generation subjects. Ages of diagnoses of CRC were compared between the simulated parents and their affected children.

The code for the model (written in Microsoft Visual Basic for Applications) forms appendix III.

3.5.3.2 Model validation: observed apparent anticipation in the ACCFR cohort

The appearance of genetic anticipation in the ACCFR cohort was sought by comparing the age of diagnosis of first CRC between parents with their
affected children (‘parent-child pairs’). Parent-child pairs were identified if both the parent and child had been diagnosed with CRC. Subjects were included if they were proven mutation carriers, or if their mutation status was unknown but their family was known to carry a MMR gene mutation. This analysis was repeated using only parent-child pairs in whom the children were born more than 80 years ago. This minimizes the chance of incomplete follow up of the children falsely lowering the apparent age of diagnosis of CRC relative to their parents (follow up bias), and is in keeping with the methodology used by Nilbert et al [149] to correct for the birth cohort effect that can falsely create the appearance of anticipation.

3.5.3.3 Statistical analysis

Mean age of diagnosis of first CRC was compared using the Student’s t-test. A P-value of 0.05 was regarded as statistically significant. Correlation was determined using Spearman’s rank method. Statistical analysis was done using MedCalc for Windows (MedCalc Software, Ostend, Belgium). All results are stated as mean ± standard error of the mean unless otherwise specified.

3.5.3.4 Ethics

Ethical approval for the study was granted by the University of Melbourne Ethics Committee.
3.5.4 Results

3.5.4.1 Age of onset of colorectal cancer in the ACCFR

The ACCFR database contained complete data for 9350 members of 295 families known to carry Lynch syndrome mutations. Of these, 1088 patients (568 men and 520 women) have been diagnosed with CRC. The mean age of diagnosis of CRC was $46.8\pm14.3$ years ($46.3\pm13.3$ for the men, and $47.3\pm15.4$ for the women, $P = 0.24$). The ages of onset of CRC are illustrated below (Figure 26).

![Histogram of age (in years) of first CRC diagnosis in subjects from the ACCFR.](image)

**Figure 26** Histogram of age (in years) of first CRC diagnosis in subjects from the ACCFR.
3.5.4.2 Cohort fertility in the ACCFR

A total of 981 (512 male and 469 female) patients with CRC were born before 1963, and the cohort fertility rates were calculated from this group. Cohort fertility grouped by age of diagnosis of CRC is illustrated in below (Figure 27). Total lifetime (cohort) fertility was related to age of diagnosis of CRC in men (correlation coefficient 0.143, P=0.0012), women (correlation coefficient 0.104, P= 0.04) and overall (correlation coefficient 0.13, P=0.0001).

![Cohort (lifetime) fertility rate vs. age of onset of first CRC](image)

**Figure 27 Cohort (lifetime) fertility rates vs. age (in years) of diagnosis of CRC**

3.5.4.3 Simulation

Using the above parameters, the simulation was run for 1000 first generation mutation carriers. This generated 1169 simulated offspring who were mutation carriers. The mean difference in age of diagnosis of first CRC between simulated parents and their mutation positive offspring (apparent
anticipation) was 1.8 ± 0.54 years (P=0.0044). Apparent anticipation was similar for male (1.1 ± 0.77) and female (1.9 ± 0.72) simulated parents (P=0.44).

3.5.4.4 Apparent anticipation in the ACCFR cohort

A total of 461 parent-child pairs with CRC were identified within the ACCFR study cohort. The mean age of diagnosis of first CRC was 51.1 ± 0.63 years in the parent group, and 42.3 ± 0.56 years in their children (P<0.0001). When only those parent child pairs with a potential follow up of over 80 years (i.e. only subjects born before 1933) were included, 120 parent-child pairs were identified. In this group, the mean age of diagnosis of first CRC was 53.9 ± 0.68 years in the parent group, and 49.1 ± 0.67 years in their children (apparent anticipation 4.8 ±1.73 years, P=0.0064). There was no significant difference in apparent anticipation between the simulated (1.8 years) and observed (4.8 years) parent-child pairs (P=0.89).

3.5.5 Discussion

This simulation demonstrates that the appearance of genetic anticipation in Lynch syndrome can be created due to changes in lifetime fertility in MMR gene mutation carriers with CRC. The apparent anticipation predicted by the model was not significantly different from the observed appearance of anticipation in the AFCCS families with Lynch syndrome, and was in keeping with the observed anticipation in large studies of the Danish HNPCC registry of between three and nine years (as reported by Larson et al [148]and Boonstra et al [150]).
There was a marked decrease in lifetime fertility in mutation carriers with early diagnosis of CRC compared with those who developed CRC later in life. For example, women diagnosed with CRC between ages 20 and 24 years gave birth to a mean of 1.2 children in their lifetime compared with women diagnosed with CRC after age 50 years who gave birth to a mean of 2.8 children in their lifetime. The reasons for the reduction in fertility after CRC in these patient groups have not been studied here, but cancer-related morbidity, the effects of surgery, chemotherapy and radiotherapy, and personal choice can all be expected to play a role. It may also simply reflect the reduced lifespan of patients who develop CRC at a young age. No previous studies have documented fertility rates after CRC in Lynch syndrome although reduced fertility is well recognized in patients who receive certain types of chemotherapy or radiotherapy, and suspected to occur after surgery for CRC [153]. It is this reduction in fertility that contributed to apparent anticipation in our model.

A potential cause for apparent genetic anticipation is follow-up bias[151]. This can be corrected for by including only subjects who were born sufficiently long ago that they have completed their period at risk[149] (in this case by including only subjects (from both generations) who were born at least 80 years ago). This type of bias cannot fully explain the observed appearance of anticipation in parent-child pairs from the ACCFR as it still existed when only mutation carriers with more than 80 years of potential follow up (i.e. born before 1933) were included. This is in keeping with the findings of Nilbert et al [149], whereas Tsai et al [142] found no difference in
age of diagnosis of CRC between parents and their children when the birth cohort effect was taken into account.

Subjects with CRC who were not proven MMR gene mutation carriers (but were from families known to carry MMR gene mutations) as well as those who were confirmed to carry mutations in the MMR genes were included, in keeping with the methodology of previous authors[142, 149]. This was to allow inclusion of a sufficient number of patients with adequate potential follow up. One would expect the great majority of subjects with CRC born before 1933 to have died before mutation analysis became available. In the ACCFR database, there were 2128 individuals born before 1933, of whom 433 (20.3%) were diagnosed with CRC. Of these 433, only 59 (13.6%) have undergone genetic testing.

In conclusion, fecundity bias can falsely create the appearance of genetic anticipation in Lynch syndrome. This highlights the statistical complexity of studying genetic anticipation and the ongoing uncertainty as to whether the phenomenon occurs in this disease.
3.6 PAPER VI: Fertility after young-onset colorectal cancer in Lynch syndrome [6].

3.6.1 Abstract

3.6.1.1 Introduction

Potential infertility is a significant concern for young colorectal cancer (CRC) survivors, but this risk is not well quantified. Mismatch repair (MMR) mutation carriers are a useful cohort for studying fertility after CRC as they commonly develop CRC at a young age, and unaffected family members provide demographically similar controls. The aim of this study was to determine the effect of CRC on fertility in a large cohort of MMR mutation carriers.

3.6.1.2 Methods

MMR mutation carriers identified from the Australasian Colorectal Cancer Family Registry were included. For each year of life within the fertile age range (15 to 49), the number of living subjects and the number of children born to them were determined. Subjects were grouped by whether they had a CRC diagnosis by that age or not. Age-specific and total fertility rates were calculated.

3.6.1.3 Results

1068 subjects (611 women and 457 men) were identified, of whom 467 were diagnosed with CRC. There were 1,192 births during 18674 person-years of
follow up to the women, and 814 births during 14013 person-years of follow up to the men.

Total fertility rate (TFR) was decreased in women with a CRC diagnosis compared to those without (1.3 vs. 2.2, P=0.0011), but age-specific fertility was only reduced in the 20-24 year age group. In men TFR was similar for both groups (2.0 vs. 1.8, P=0.27).

3.6.1.4 Conclusion

Age-specific fertility was decreased in female CRC survivors aged 20-24 years, but not in older women or in men.
3.6.2 Introduction

In chapter 3.5 it was found that there was a reduction in the total (lifetime) number of children born to subjects from the ACCFR cohort who were diagnosed with CRC at a young age [5], but it was not known whether this was due to a true reduction in fertility among survivors, or if it was simply due to decreased survival.

Although CRC predominantly affects older patients, approximately 4% of cases occur in individuals who are under 45 years of age[154]. Among young CRC survivors, potential infertility is known to be a significant concern [155]. The American Society of Clinical Oncology (ASCO) has published guidelines recommending that oncologists discuss the possibility of infertility with their patients, while acknowledging that in many cases there are insufficient data available to accurately assess this risk[156].

In two recent reviews, Spanos et al[157] and O’Neill et al[153] both noted that there was no evidence that colon cancer surgery or 5-fluorouracil chemotherapy affected fertility, but expressed concerns about the effects of rectal surgery and radiotherapy, as well as newer chemotherapy agents. The authors of both papers emphasised the importance of adequate pre- treatment fertility counselling and discussed the merits of fertility preservation options that a CRC survivor could be offered. These include embryo preservation and oocyte vitrification. Kumar et al [158] reported that fertility discussion occurred in only one third of CRC patients under the age of 40 years at their institution. They concluded that it was important to educate health care professionals about the importance of fertility risk discussion, while
acknowledging the lack of direct evidence for fertility risk due to colon surgery or chemotherapy.

The only previously published study directly comparing fertility rates for women with or without gastrointestinal cancer was by Hartman et al [159]. They reported a 10% reduction in fertility for women less than 45 years of age compared with the Swedish general population. In this study, age- specific fertility rates in cancer survivors (for a number of different tumors, including gastrointestinal) were compared with those of the general Swedish population using the technique of indirect standardisation. This involves determining age- specific birth rates for the reference population, and applying these to the study population to calculate the expected number of events for that population. The authors calculated a standardised birth rate (SBR, an indirectly standardised rate) for gastrointestinal cancer survivors of 0.90 (95% C.I. 0.83 to 0.97), but did not quote age- specific birth rates for the study population. Indirectly standardised rates can give misleading results, however, in any situation where the age profiles of the study and reference populations are not similar[160]

No previously published studies have documented age- specific fertility rates for female CRC survivors, and fertility rates in male CRC survivors have not been reported.

Lynch syndrome families provide a useful cohort for studying the effect of CRC on fertility, as CRC in this population commonly occurs in subjects who are within the potentially fertile age group, and unaffected family members can be used as demographically similar controls.
3.6.3 Aim

The aim of this study was to determine the effect of CRC on age-specific fertility rates in a large cohort of subjects who carry MMR gene mutations.

3.6.4 Methods

The Australasian Colorectal Cancer Family Registry (ACCFR) is a registry of more than 11,500 subjects from 1800 families in Australia and New Zealand[152]. This registry contains CRC families recruited through the Victorian Cancer Registry and from family cancer clinics throughout Australia and New Zealand. Personal and family history of cancer and reproductive history were collected by questionnaire. Attempts were made to verify all reports of CRC diagnoses and date of death by medical records, pathology reports, death certificates and linkage to national cancer registry and death registry databases.

Subjects identified from the ACCFR with proven germline mismatch repair gene mutations were included in this study. Methods for screening and testing for MMR gene mutations have been described in detail elsewhere [161].

The period of potential fertility was considered to be between the ages of 15 to 49 years, in keeping with the World Health Organisation[162] and the Australian Bureau of Statistics[163] norms for describing population fertility rates. For each year of life within this age range, the total number of subjects known to be alive at that age and the number of children born to them were determined. Subjects in each year of life were grouped according to whether they had been diagnosed with CRC by that age or not (so a subject who, for
example was diagnosed with CRC at age 29 years would be counted in the ‘no CRC diagnosis’ group from ages 15 to 28 years, and in the ‘after CRC diagnosis’ group for each year of life from age 29 years until age 49 years, death or last follow up).

Fertility rates were compared between those subjects who had been diagnosed with CRC and those who had not. Female fertility rates were also compared with the known fertility rates of the Australian female general population (1975 to 2010) as reported by the Australian Bureau of Statistics[163] (male fertility rates for the general population are not recorded).

3.6.4.1 Age-specific fertility rates

Age-specific fertility rate (ASFR) is the number of children born per person per year at risk within a given age group. It is usually expressed as births per 1000 population per year. It is calculated as:

\[ ASFR = \frac{O_i}{n_i} \]

Where: \( O_i \) is the number of births observed in age group \( i \).

\( n_i \) is population-years at risk in age group \( i \).

Total fertility rate (TFR) is the average number of children a hypothetical cohort of women would bear if they had children at the population age-specific rates during their whole lives, and survived to the end of their fertile period. It is usually expressed as children per women, and is calculated as the sum of age specific fertility rates for the ages 15 to 49 years [162].
Fertility rates are usually calculated for entire populations (rather than samples of populations), and so the current literature does not describe any standard statistical methods for estimating confidence intervals for TFR or comparing TFR between population samples. Statistical methods designed for comparing standardised rates can, however, be applied to fertility rates. This is described in detail below.

3.6.4.2 Indirect standardisation

The process of indirect standardisation involves determining stratum-specific event rates for the reference population, and applying these to the study population to calculate the expected number of events for that population. In this case, the events are births, and the populations are stratified by age. The ‘population’ here is person-years, so a rate of births/person/year is calculated, rather than a ratio.

The indirectly standardized rate (ISR) is the ratio of observed (in the study population) to expected events. In other words it is the ratio of actual births in the study population to the number of births that would be expected to occur if the study population had the same age distribution as the reference population (and age-specific birth rates were similar for the two populations).
The indirectly standardised rate \((ISR)\) is calculated as follows:

\[
ISR = \frac{O}{E} = \frac{\sum_i O_i}{\sum_i E_i} = \frac{\sum_i O_i}{\sum_i n_i \lambda_i}
\]

Where: 

\(O\) is the total number of observed events in the study population

\(E\) is the total number of expected events

\(O_i\) is the observed number of events in the study population in age-group \(i\)

\(E_i\) is the number of expected events in age-group \(i\)

\(n_i\) is the number of individuals in age group \(i\) in the study population

\(\lambda_i\) is the age-specific event rate for age group \(i\) in the reference population

Note that the indirectly standardised rate can be calculated without knowing the age-specific event rates in the study population. The total number of events needs to be known, as well as the age distribution of the study population. The age-specific event rates for the reference population must also be known, but the population age distribution of the reference population is not required.
3.6.4.3 Indirect standardisation: example

This is illustrated in the example below, comparing age-specific fertility rates between women from the ACCFR with or without a CRC diagnosis. Here the ‘study population’ is women-years with a CRC diagnosis and the ‘reference population’ is women-years without a CRC diagnosis.

<table>
<thead>
<tr>
<th>Strata (age groups)</th>
<th>Events (Study Population)</th>
<th>Study Population</th>
<th>Events (Reference Population)</th>
<th>Reference Population</th>
<th>Reference Rates</th>
<th>Expected Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>i</td>
<td>O_i</td>
<td>n_i</td>
<td>λ_i</td>
<td>E_i</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-19</td>
<td>0</td>
<td>8</td>
<td>86</td>
<td>3045</td>
<td>0.028</td>
<td>0.2</td>
</tr>
<tr>
<td>20-24</td>
<td>0</td>
<td>36</td>
<td>382</td>
<td>2992</td>
<td>0.128</td>
<td>4.6</td>
</tr>
<tr>
<td>25-29</td>
<td>14</td>
<td>113</td>
<td>399</td>
<td>2813</td>
<td>0.142</td>
<td>16.0</td>
</tr>
<tr>
<td>30-34</td>
<td>16</td>
<td>165</td>
<td>228</td>
<td>2579</td>
<td>0.088</td>
<td>14.6</td>
</tr>
<tr>
<td>35-39</td>
<td>11</td>
<td>281</td>
<td>104</td>
<td>2263</td>
<td>0.046</td>
<td>12.9</td>
</tr>
<tr>
<td>40-44</td>
<td>1</td>
<td>437</td>
<td>8</td>
<td>1864</td>
<td>0.004</td>
<td>1.9</td>
</tr>
<tr>
<td>45-49</td>
<td>0</td>
<td>581</td>
<td>0</td>
<td>1497</td>
<td>0.000</td>
<td>0.0</td>
</tr>
</tbody>
</table>

∑O_i = 42  ∑E_i = 50

Here the indirectly standardised rate (ISR) = 42/50 = 0.84.

3.6.4.4 Study population distribution and the interpretation of indirectly standardised rates

A problem with using indirectly standardised rates in this setting is that the weighting used to determine the ISR is determined by the study population distribution. This means that, for example, even if the stratum-specific rates of two study populations were identical, and they were being compared with the same reference population, the ISR can be quite different if the age distributions of the study populations are different. The ISR can also be
misleading if the study and reference populations have different age distributions[160].

This can be illustrated with the hypothetical data set below. In this hypothetical population, the age- specific fertility rates are identical to those of the actual ACCFR women with a CRC diagnosis, but the age distribution of the sample population is reversed.

<table>
<thead>
<tr>
<th>Age groups</th>
<th>ACCFR women post CRC</th>
<th>Hypothetical population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Events (Study Population)</td>
<td>Fertility rate</td>
</tr>
<tr>
<td>15-19</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20-24</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>25-29</td>
<td>14</td>
<td>124</td>
</tr>
<tr>
<td>30-34</td>
<td>16</td>
<td>97</td>
</tr>
<tr>
<td>35-39</td>
<td>11</td>
<td>39</td>
</tr>
<tr>
<td>40-44</td>
<td>1</td>
<td>2.3</td>
</tr>
<tr>
<td>45-49</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

If one was to compare the age- specific fertility rates of this hypothetical population with the same reference population as above (ACCFR women with a CRC diagnosis) by indirect standardisation, the results would be as follows:

<table>
<thead>
<tr>
<th>Strata (age groups)</th>
<th>Events (Study Population)</th>
<th>Study Population</th>
<th>Events (Reference Population)</th>
<th>Reference Rates</th>
<th>Expected Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-19</td>
<td>0</td>
<td>581</td>
<td>86</td>
<td>3045</td>
<td>0.028</td>
</tr>
<tr>
<td>20-24</td>
<td>0</td>
<td>437</td>
<td>382</td>
<td>2992</td>
<td>0.128</td>
</tr>
<tr>
<td>25-29</td>
<td>35</td>
<td>281</td>
<td>399</td>
<td>2813</td>
<td>0.142</td>
</tr>
<tr>
<td>30-34</td>
<td>16</td>
<td>165</td>
<td>228</td>
<td>2579</td>
<td>0.088</td>
</tr>
<tr>
<td>35-39</td>
<td>4</td>
<td>113</td>
<td>104</td>
<td>2263</td>
<td>0.046</td>
</tr>
<tr>
<td>40-44</td>
<td>0</td>
<td>36</td>
<td>8</td>
<td>1864</td>
<td>0.004</td>
</tr>
<tr>
<td>45-49</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>1497</td>
<td>0.000</td>
</tr>
</tbody>
</table>

\[ \sum O_i = 55 \quad \sum E_i= 132 \]
Here the **indirectly standardised rate (ISR)** = $\frac{55}{132} = 0.42$, which is half the ISR for the actual population, despite the two study populations having identical age-specific fertility rates, and using the same reference population.

This illustrates that the ISR is dependent on the age distribution of the study population, and therefore cannot be meaningfully interpreted in situations where the study and reference populations’ age distributions differ significantly.

In this study, the groups with or without a CRC diagnosis (unsurprisingly) have markedly different age distributions (illustrated below (Figure 28), and so ISR is not useful.

![Figure 28 Age distribution of subjects with or without a CRC diagnosis.](image)
3.6.4.5 Direct standardisation

In the direct method of standardisation, the stratum-specific event rates from the study population are applied to an arbitrarily chosen reference population to calculate the expected number of events in the reference population if it had the same stratum-specific event rate as the study population. This is in contrast to the indirect method, where stratum-specific rates for the reference population are used to calculate the expected number of events for the study population.

The directly standardised rate (DSR) is calculated as follows:

\[
DSR = \frac{E}{P} = \frac{\sum_i E_i}{\sum_i P_i} = \frac{\sum_i r_i P_i}{\sum_i P_i} = \frac{\sum_i \left( \frac{O_i}{n_i} \right) P_i}{\sum_i P_i}
\]

Where:

- \( O \) is the total number of observed events in the study population
- \( E \) is the total number of expected events in the reference population
- \( P \) is the total reference population
- \( E_i \) is the number of expected events in age-group \( i \)
- \( P_i \) is the population of age group \( i \) in the reference population
- \( r_i \) is the age-specific event rate in age group \( i \) of the study group
- \( O_i \) is the observed number of events in the study population in age-group \( i \)
- \( n_i \) is the number of individuals in age group \( i \) in the study population

Commonly used reference populations are the World Health Organisation (WHO) world standard population[164] and USA 2000[165], but in principle
any population can be used according to the purpose of the comparison. The main advantage to using the DSR rather than ISR is that the DSR is independent of the age distribution of the study population (it is a weighted average determined by the age distribution of the reference population). This means that the DSR of multiple study populations can be meaningfully compared with each other, as long as the same reference population is used. Using the above hypothetical example, the directly standardised age-specific rates are similar for each age group, and the DSR for both populations is 692 / 17053 X 1000 = 41 per thousand women overall.

This is illustrated below (note that fertility rate is given as births/ 1000 population).

<table>
<thead>
<tr>
<th>Age groups</th>
<th>ACCFR women post CRC</th>
<th>Hypothetical population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reference population</td>
<td>Births</td>
</tr>
<tr>
<td>P_i</td>
<td></td>
<td>r_i</td>
</tr>
<tr>
<td>15-19</td>
<td>3045</td>
<td>0</td>
</tr>
<tr>
<td>20-24</td>
<td>2992</td>
<td>0</td>
</tr>
<tr>
<td>25-29</td>
<td>2813</td>
<td>14</td>
</tr>
<tr>
<td>30-34</td>
<td>2579</td>
<td>16</td>
</tr>
<tr>
<td>35-39</td>
<td>2263</td>
<td>11</td>
</tr>
<tr>
<td>40-44</td>
<td>1864</td>
<td>1</td>
</tr>
<tr>
<td>45-49</td>
<td>1497</td>
<td>0</td>
</tr>
<tr>
<td>∑</td>
<td>17053</td>
<td>692</td>
</tr>
</tbody>
</table>
3.6.4.6 Total fertility rate is a form of direct standardisation

Total fertility rate \((TFR)\) is calculated as follows:

\[
TFR = \sum_{a} ASFR_{a}
\]

Where: \(ASFR_{a}\) is the age-specific fertility rate for ages \(a\) from 15 to 49.

Or as

\[
TFR = 5 \sum_{i} ASFR_{i}
\]

For five-year age groups I from 15-19 to 45-49.

So:

\[
TFR = 5 \sum_{i} \frac{o_i}{n_i}
\]

Where: \(o_i\) is the number of births in population \(n_i\) for age group \(i\).

So:

\[
TFR = 5q \frac{\sum_{i} \left( \frac{o_i}{n_i} \frac{P_i}{P_i} \right)}{\sum_i P_i}
\]

when \(\sum P_i = 1\) and the population strata are all of equal size (i.e. when \(P_i = 1/q\) for each \(i\), and \(q\) is the number of age strata). In the usual reporting of ASR, \(q = 7\) (the number of five-year age groups from 15 to 49).

So total fertility rate \((TFR)\) can be considered a special case of a direct standardisation in which the standard population is one and the population is evenly distributed across the seven age groups. We can, therefore, use...
statistical tools designed for the analysis of directly standardised rates in order to calculate confidence intervals for total fertility rates.

3.6.4.7 Confidence intervals for age-specific rates

For each age-specific rate, the confidence interval can be calculated using Byars method as described by Breslow et al [166]. Using this method, the 100(1-\(\alpha\))% confidence interval limits \(O_{lower}\) and \(O_{upper}\) for the number of observed events are calculated as follows:

\[
O_{lower} = O \times \left(1 - \frac{1}{9O} - \frac{z}{3\sqrt{O}}\right)^3
\]

\[
O_{upper} = (O + 1) \times \left(1 - \frac{1}{9(O + 1)} + \frac{z}{3\sqrt{(O + 1)}}\right)^3
\]

Where: \(O\) is the total number of observed events in the study population

\(Z\) is the 100(1-\(\alpha/2\))th percentile value from the standard normal distribution (for example, for a 95% C.I. \(\alpha = 0.05\) and \(z = 1.96\) (which is the 97.5\(^{th}\) percentile value from the standard normal distribution).

The confidence interval limits for the rate are then calculated as:

\[
r_{lower} = \frac{O_{lower}}{n}
\]

\[
r_{upper} = \frac{O_{upper}}{n}
\]

Where: \(n\) is the number of person-years.
3.6.4.8 Confidence intervals for directly standardised rate (DSR)

A directly standardised rate (DSR) is a weighted sum of the age-specific rates, so the variance of the DSR is the weighted sum of the variances of those rates [167]. A widely accepted method [160] for calculating the confidence interval for a DSR (when the rates are assumed to be independent and to follow a Poisson distribution) is that described by Dobson et al [167] as follows:

\[
DSR_{\text{lower}} = DSR + \sqrt{\frac{Var(DSR)}{Var(O)}} \times (O_{\text{lower}} - O)
\]

\[
DSR_{\text{upper}} = DSR + \sqrt{\frac{Var(DSR)}{Var(O)}} \times (O_{\text{upper}} - O)
\]

The confidence limits \(O_{\text{lower}}\) and \(O_{\text{upper}}\) of the crude (total) number of observed events are calculated using Bryar’s method as above and the variances of the observed (crude) count, and the DSR are estimated as follows:

\[
Var(O) = \sum_i O_i
\]

\[
Var(DSR) = 1 \left( \sum_i P_i \right)^2 \times \sum_i \frac{P_i^2 O_i}{n_i^2}
\]

3.6.4.9 Direct standardisation and total fertility rate: example

In this example, the age-specific birth rates are for women from the ACCFR without a CRC diagnosis. These can be directly standardised against a
reference population of 1 with seven equal age groups \((P_i = 1/7 \approx 0.1429)\) as follows:

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Births</th>
<th>Study population</th>
<th>Reference population</th>
<th>(O_i/n_i) (P_i)</th>
<th>(P_i^2 O_i/n_i^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-19</td>
<td>86</td>
<td>3045</td>
<td>0.1429</td>
<td>0.0040</td>
<td>1.89E-07</td>
</tr>
<tr>
<td>20-24</td>
<td>382</td>
<td>2992</td>
<td>0.1429</td>
<td>0.0182</td>
<td>8.71E-07</td>
</tr>
<tr>
<td>25-29</td>
<td>399</td>
<td>2813</td>
<td>0.1429</td>
<td>0.0203</td>
<td>1.03E-06</td>
</tr>
<tr>
<td>30-34</td>
<td>228</td>
<td>2579</td>
<td>0.1429</td>
<td>0.0126</td>
<td>7.00E-07</td>
</tr>
<tr>
<td>35-39</td>
<td>104</td>
<td>2263</td>
<td>0.1429</td>
<td>0.0066</td>
<td>4.15E-07</td>
</tr>
<tr>
<td>40-44</td>
<td>8</td>
<td>1864</td>
<td>0.1429</td>
<td>0.0006</td>
<td>4.70E-08</td>
</tr>
<tr>
<td>45-49</td>
<td>0</td>
<td>1497</td>
<td>0.1429</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>1207</td>
<td>17053</td>
<td>1</td>
<td>0.062</td>
<td>3.3E-06</td>
</tr>
</tbody>
</table>

Here:

\[
\sum P_i = 1
\]

\[
DSR = \frac{\sum_i \left( \frac{O_i}{n_i} P_i \right)}{\sum_i P_i} = 0.062
\]

\[
Var(O) = \sum_i O_i = 1207
\]

\[
Var(DSR) = 1 \left(\sum_i P_i\right)^2 \sum_i \frac{P_i^2 O_i}{n_i^2} = 1 \times \sum_i \frac{P_i^2 O_i}{n_i^2} = 3.3 \times 10^{-6}
\]

The 95% C.I. for the observed births \(O\) is as follows:

\[
O_{lower} = O \times \left(1 - \frac{1}{9O} - \frac{z}{3\sqrt{O}}\right)^3 = 1121 \times \left(1 - \frac{1}{9 \times 1121} - \frac{1.96}{3 \sqrt{1121}}\right)^3
\]

\[
= 1140
\]

\[
O_{upper} = (O + 1) \times \left(1 - \frac{1}{9(O + 1)} + \frac{z}{3\sqrt{(O + 1)}}\right)^3
\]

\[
= 1141 \times \left(1 - \frac{1}{9 \times 1141^2} + \frac{1.96}{3 \sqrt{1141}}\right)^3 = 1277
\]
The 95% C.I. for the DSR is:

\[
DSR_{lower} = DSR + \sqrt{\frac{Var(DSR)}{Var(O)}} \times (O_{lower} - O)
\]

\[
= 0.062 + \sqrt{\frac{3.3 \times 10^{-6}}{11207}} \times (1140 - 1207) = 0.059
\]

\[
DSR_{upper} = DSR + \sqrt{\frac{Var(DSR)}{Var(O)}} \times (O_{upper} - O)
\]

\[
= 0.062 + \sqrt{\frac{3.4 \times 10^{-6}}{1127}} \times (1277 - 1207) = 0.066
\]

The TFR and the C.I. for the TFR can now be calculated by multiplying the DSR and the confidence limits of the DSR by 35 (as there are seven age strata, each of which is a 5-year age group). So in this example:

**Total fertility rate = 2.2 births/woman (95% C.I. 2.1 to 2.3)**

3.6.4.10 Comparing two directly standardised rates

Comparing the directly standardised rates between two cohorts which have been standardised against the same reference population, the standard error of the ratio of the two rates can be calculated as follows (Breslow and Day[166] recommend transformation to a log scale to correct the skewness of the distribution of the ratio):
One can then test the null hypothesis (that $DSR_2/DSR_1 = 1$) using standard normal distribution tables.

### 3.6.4.11 Comparing two total fertility rates: example

Using the example of women from the ACCFR with or without CRC:

<table>
<thead>
<tr>
<th>Age group</th>
<th>Reference population $(P_i)$</th>
<th>After CRC diagnosis</th>
<th>No CRC diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$O_{1i}$</td>
<td>$n_{1i}$</td>
<td>$O_{2i}$</td>
</tr>
<tr>
<td>15-19</td>
<td>0.1429</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>20-24</td>
<td>0.1429</td>
<td>0</td>
<td>36</td>
</tr>
<tr>
<td>25-29</td>
<td>0.1429</td>
<td>14</td>
<td>113</td>
</tr>
<tr>
<td>30-34</td>
<td>0.1429</td>
<td>16</td>
<td>165</td>
</tr>
<tr>
<td>35-39</td>
<td>0.1429</td>
<td>11</td>
<td>281</td>
</tr>
<tr>
<td>40-44</td>
<td>0.1429</td>
<td>1</td>
<td>437</td>
</tr>
<tr>
<td>45-49</td>
<td>0.1429</td>
<td>0</td>
<td>581</td>
</tr>
<tr>
<td>DSR</td>
<td>0.037</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Here the $TFR$ for women with a CRC diagnosis is $0.037 \times 5 \times 7 = 1.3$ births/woman, and the $TFR$ for women without a CRC diagnosis is $0.062 \times 5 \times 7 = 2.2$ births/woman. The ratio of the two TFR’s (which is the same as the ratio of the two DSR’s) is 1.7

$$\log_e \left( \frac{TFR_{CRC}}{TFR_{no\,CRC}} \right) = 0.46$$
### Statistics summary

Age- specific fertility rates were compared using the Chi square test. Confidence intervals (CI) for age- specific fertility rates and total fertility rate were calculated using Byar’s method for direct standardisation as described by Breslow and Day [166]. Confidence intervals for total fertility rates were calculate using the widely accepted method [160, 166] described by Dobson et al [167] as described above. A P- value of <0.05 was regarded as showing significant difference. Statistical analysis was done using Microsoft Excel.
2010 (Redmond, Washington) and Medcalc® 2008 (Mariakerke, Belgium) software.

3.6.4.13 Ethics

Ethical approval for the study was given by the University of Melbourne Research Ethics Committee.
3.6.5 Results

A total of 1068 subjects (611 women and 457 men) with proven germline MMR mutations were identified, of whom 467 were diagnosed with CRC. There were 417 subjects diagnosed with colon cancer, and 82 with rectal cancer (32 subjects were diagnosed with metachronous cancers at both sites). Among subjects under the age of fifty years, there were a total of 322 CRC diagnosed (285 colon and 45 rectum). They were followed up to a median age of 55 (range 18 to 96) years.

For subjects aged 15 to 49 years, there were a total of 1,192 children born during 18674 person-years of follow up for the women, and 814 births during 14013 person-years of follow up for men. No births occurred in subjects under 15 years of age. No women in this cohort gave birth over the age of 49 years. Four children were fathered by men over 49 years of age.

Age-specific fertility rates for all women from the study cohort (regardless of CRC diagnosis) and from the Australian general population are presented below (Table 22 and Figure 29). Age-specific fertility rate was higher in the study group than the Australian general population for women in the 15-19, 20-24 and 25-29 year age groups, but not in other age groups. Total fertility rate for women in the study group was 2.2 (95% C.I 2.0 to 2.3), which was higher than for women in the Australian general population (TFR 1.87).
Table 22 Age-specific fertility rates for women from the entire study group and from the Australian general population.

No confidence intervals are given for the Australian population as these are from population census figures, not a sampled population. Age specific fertility rates for the study cohort which are significantly different from the general population rate are in bold type.

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Study cohort</th>
<th>Australian population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Births</td>
<td>Person-years</td>
<td>Fertility rate</td>
</tr>
<tr>
<td>15-19</td>
<td>86</td>
<td>3053</td>
</tr>
<tr>
<td>20-24</td>
<td>382</td>
<td>3028</td>
</tr>
<tr>
<td>25-29</td>
<td>413</td>
<td>2926</td>
</tr>
<tr>
<td>30-34</td>
<td>244</td>
<td>2744</td>
</tr>
<tr>
<td>35-39</td>
<td>115</td>
<td>2544</td>
</tr>
<tr>
<td>40-44</td>
<td>9</td>
<td>2301</td>
</tr>
<tr>
<td>45-49</td>
<td>0</td>
<td>2078</td>
</tr>
<tr>
<td>TFR</td>
<td><strong>2.2</strong></td>
<td><strong>2.0-2.3</strong></td>
</tr>
</tbody>
</table>
Figure 29 Age- specific fertility rates for women from the study cohort vs. the Australian general population.

Age- specific fertility rates are given as births/ 1000 person- years. Error bars are 95% confidence interval (C.I.). No C.I. are presented for the general population data as these are population census figures, not from a sampled population.
The age-specific fertility rates for study subjects with or without a CRC diagnosis are presented below (Results for women are presented in Table 23 and Figure 30; results for men are in Table 24 and Figure 31).

For women in the 20-24 year age group, age specific fertility was lower in those with a CRC diagnosis (0 births/1000 person-years in those who had been diagnosed with CRC vs. 128 births/1000 person-years in subjects who had not been diagnosed with CRC, P=0.019). There was no statistically significant difference in age-specific fertility rates between women with or without a CRC diagnosis in any other age group. There was no statistically significant difference in age-specific fertility rates between men who had been diagnosed with CRC compared with those without a CRC diagnosis in any age group.

Overall, total fertility rate was lower in women with a CRC diagnosis compared to those without a CRC diagnosis (1.3 (95% C.I 0.90 to 1.8) vs. 2.2 (95% C.I. 2.1 to 2.3) P=0.0011. In men, there was no significant difference in TFR between the two groups (2.0 (95% C.I. 1.8 to 2.1) for men without a CRC diagnosis vs. 1.8 (95% C.I. 1.1 to 2.7) for those with a CRC diagnosis (P=0.27).
Table 23 Age-specific fertility rates for women with or without a CRC diagnosis.

Sample sizes for subjects aged 15-19 with a CRC diagnosis were insufficient to calculate meaningful confidence intervals. Significantly different rates are marked in bold type.

| Age groups | No CRC Diagnosis | | | | | After CRC diagnosis | | | | |
|------------|------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|            | Births | Person-years | Fertility rate | 95% C.I. | Births | Person-years | Fertility rate | 95% C.I. | P-value |
| 15-19      | 86     | 3045        | 28          | 23-35    | 0      | 8           | 0           | N/A     | 1.0    |
| 20-24      | 382    | 2992        | 128         | 116-141 | 0      | 36          | 0           | 0-102   | 0.019  |
| 25-29      | 399    | 2813        | 142         | 128-156 | 14     | 113         | 124         | 68-208  | 0.69   |
| 30-34      | 228    | 2579        | 88          | 77-101  | 16     | 165         | 97          | 55-157  | 0.67   |
| 35-39      | 104    | 2263        | 46          | 38-56   | 11     | 281         | 39          | 20-70   | 0.76   |
| 40-44      | 8      | 1864        | 4.3         | 1.9-8.5 | 1      | 437         | 2.3         | 0.03-13 | 1.0    |
| 45-49      | 0      | 1497        | 0.0         | 0.0-2.45| 0      | 581         | 0           | 0-6.3   | 1.0    |
| TFR        | 2.2    | 2.1-2.3     | 1.3         | 0.9-1.8 | 0.0011 |
Table 24 Age-specific fertility rates for men with or without a CRC diagnosis.

Sample sizes for subjects aged 15-19 with a CRC diagnosis were insufficient to calculate meaningful confidence intervals.

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Births</th>
<th>Person-years</th>
<th>Fertility rate</th>
<th>95% C.I.</th>
<th>Births</th>
<th>Person-years</th>
<th>Fertility rate</th>
<th>95% C.I.</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-19</td>
<td>16</td>
<td>2284</td>
<td>7.0</td>
<td>4.0-11</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>N/A</td>
<td>1.0</td>
</tr>
<tr>
<td>20-24</td>
<td>132</td>
<td>2256</td>
<td>59</td>
<td>49-69</td>
<td>1</td>
<td>22</td>
<td>45</td>
<td>0.6-253</td>
<td>1.0</td>
</tr>
<tr>
<td>25-29</td>
<td>281</td>
<td>2178</td>
<td>129</td>
<td>114-145</td>
<td>9</td>
<td>54</td>
<td>167</td>
<td>76-316</td>
<td>0.41</td>
</tr>
<tr>
<td>30-34</td>
<td>205</td>
<td>1951</td>
<td>105</td>
<td>91-120</td>
<td>12</td>
<td>142</td>
<td>85</td>
<td>44-148</td>
<td>0.57</td>
</tr>
<tr>
<td>35-39</td>
<td>104</td>
<td>1657</td>
<td>63</td>
<td>51-76</td>
<td>13</td>
<td>270</td>
<td>48</td>
<td>26-82</td>
<td>0.41</td>
</tr>
<tr>
<td>40-44</td>
<td>34</td>
<td>1304</td>
<td>26</td>
<td>18-36</td>
<td>5</td>
<td>395</td>
<td>13</td>
<td>4.1-30</td>
<td>0.13</td>
</tr>
<tr>
<td>45-49</td>
<td>6</td>
<td>990</td>
<td>6.1</td>
<td>2.2-13</td>
<td>1</td>
<td>509</td>
<td>2.0</td>
<td>0.03-11</td>
<td>0.43</td>
</tr>
<tr>
<td>TFR</td>
<td>2.0</td>
<td>1.8-2.1</td>
<td></td>
<td></td>
<td>1.8</td>
<td>1.1-2.7</td>
<td>0.27</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 30 Age-specific fertility rates for women from the study cohort with or without a CRC diagnosis.

Age-specific fertility rates are given as births/1000 person-years. Error bars are 95% C.I. The upper C.I. for subjects with CRC in age group 25-29 has been truncated.
Figure 31 Age- specific fertility rates for men with or without a CRC diagnosis.

Age- specific fertility rates are given as births/ 1000 person- years. Error bars are 95% C.I. The upper C.I. for subjects with CRC in age groups 20-24 and 25-29 have been truncated.
Subjects diagnosed with colon or rectal cancers were also analysed separately, and these results are shown in Table 25 (There were no men who developed rectal CA under the age of 20, so the age- specific fertility rate for the 15-19 year old subjects in this group could not be calculated. The sample sizes were too small to calculate meaningful confidence intervals for age- specific fertility rates for a number of groups). For women with a colon cancer diagnosis, the results were similar to the findings for CRC overall. Age-specific fertility rate for women with a colon cancer diagnosis in the 20-24 year age group was reduced, with 0 births/ 1000 person- years compared with 128 births/ 1000 person years for women without a CRC diagnosis(P=0.017). 

Age- specific fertility rates for women with a colon cancer diagnosis for all other age groups were similar to those for women without a CRC diagnosis. Total fertility rate for women with a colon cancer diagnosis was reduced compared to women without a CRC diagnosis (1.5 (95% C.I. 1.1-2.1) vs. 2.2 (95% C.I 2.1-2.3), P=0.016). For women who were diagnosed with rectal cancer, there was no significant difference in any of the age- specific fertility rates compared with women without a CRC diagnosis, but there was an overall reduction in total fertility rate (0.72 (95% C.I 0.2-1.7) vs. 2.2 (95% C.I. 2.1-2.3), P=0.015). There were no significant differences in age- specific fertility rates for men with colon or rectal cancer diagnoses compared with subjects without a CRC diagnosis. Total fertility rate for men after colon cancer was similar to that for men without a CRC diagnosis (1.7 (95% C.I. 1.0 to 2.5) vs. 1.8 (95% C.I. 1.1-2.7), P= 0.72). TFR for men after a rectal cancer diagnosis was also similar to men without a CRC diagnosis (1.84 (95% C.I. 0.39-5.0) vs. 1.8 (95% C.I. 1.1-2.7), P=0.38.
Table 25 Age-specific fertility rates for subjects after a CRC diagnosis, grouped by site of tumor (colon vs. rectum).

Age-specific fertility rates are given as births/1000 person-years. Total fertility rate (TFR) is given as births/person.

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Births</th>
<th>Person-years</th>
<th>Fertility rate</th>
<th>95% C.I.</th>
<th>Births</th>
<th>Person-years</th>
<th>Fertility rate</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>After Colon CA diagnosis (women)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>After Rectal CA diagnosis (women)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-19</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>N/A</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>N/A</td>
</tr>
<tr>
<td>20-24</td>
<td>0</td>
<td>34</td>
<td>0</td>
<td>0-108</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>N/A</td>
</tr>
<tr>
<td>25-29</td>
<td>14</td>
<td>90</td>
<td>156</td>
<td>85-261</td>
<td>4</td>
<td>28</td>
<td>143</td>
<td>38-366</td>
</tr>
<tr>
<td>30-34</td>
<td>16</td>
<td>151</td>
<td>106</td>
<td>61-172</td>
<td>0</td>
<td>29</td>
<td>0</td>
<td>0-126</td>
</tr>
<tr>
<td>35-39</td>
<td>10</td>
<td>251</td>
<td>40</td>
<td>19-73</td>
<td>1</td>
<td>53</td>
<td>20</td>
<td>0.25-105</td>
</tr>
<tr>
<td>40-44</td>
<td>1</td>
<td>401</td>
<td>2.5</td>
<td>0.03-14</td>
<td>0</td>
<td>71</td>
<td>0</td>
<td>0.52</td>
</tr>
<tr>
<td>45-49</td>
<td>0</td>
<td>532</td>
<td>0</td>
<td>0-6.9</td>
<td>0</td>
<td>91</td>
<td>0</td>
<td>0-40</td>
</tr>
<tr>
<td>TFR</td>
<td>1.5</td>
<td>1.1-2.1</td>
<td></td>
<td></td>
<td>0.72</td>
<td>0.2-1.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| After Colon CA diagnosis (men) | | | | | After Rectal CA diagnosis (men) | | | |
| 15-19 | 0 | 1 | 0 | N/A | 0 | 0 | N/A | N/A |
| 20-24 | 0 | 15 | 0 | 0-245 | 1 | 7 | 143 | N/A |
| 25-29 | 8 | 44 | 182 | 78-358 | 1 | 10 | 100 | N/A |
| 30-34 | 11 | 131 | 84 | 42-150 | 1 | 12 | 83 | N/A |
| 35-39 | 12 | 242 | 50 | 26-87 | 1 | 42 | 24 | 0.3-132 |
| 40-44 | 5 | 358 | 14 | 4.5-33 | 0 | 61 | 0 | 0.60 |
| 45-49 | 1 | 462 | 2.2 | 0-12 | 0 | 87 | 0 | 0-42 |
| TFR | 1.7 | 1.0-2.5 | | | 1.84 | 0.39-5.0 | | |
3.6.6 Discussion

In this cohort of people with germline MMR mutations, total fertility rate for women was decreased by approximately 40% after a diagnosis of CRC. The reduction in age-specific fertility rates could only be detected in younger women (age 20 to 24), and was not apparent in other age groups. A CRC diagnosis did not adversely affect age-specific fertility in men. Both colonic and rectal cancer diagnoses were associated with decreased TFR in women, but no difference was detected for men with cancers in either site.

In chapter 3.5 it was found that there was a reduction in the total (lifetime) number of children born to subjects from the ACCFR cohort who are diagnosed with CRC at a young age [5], but it was not known whether this was due to a reduction in fertility among survivors, or if it was simply due to decreased survival.

The only previously published study directly comparing fertility rates for women with or without gastrointestinal cancer was by Hartman et al [159]. They reported a ten per cent reduction in fertility for women less than 45 years of age compared with the Swedish general population. In this study, age-specific fertility rates in cancer survivors (for a number of different tumors, including gastro-intestinal) were compared with those of the general Swedish population using the technique of indirect standardisation. This involves determining age-specific birth rates for the reference population, and applying these to the study population to calculate the expected number of events for that population. The authors calculated a standardised birth rate (SBR, an indirectly standardised rate) for gastrointestinal cancer survivors of
0.90 (95% C.I. 0.83 to 0.97), but did not quote age-specific birth rates for the study population. Indirectly standardized rates can give misleading results, however, in any situation where the age profiles of the study and reference populations are not similar[160]. In this study, we found the age distributions of subjects with or without a CRC diagnosis were markedly different. For this reason the TFR (which is calculated by the process of direct standardisation) was used in this study, in which the age distribution of the sample populations do not affect the calculation, and so groups with disparate age distributions[160] can be compared.

A surprising finding in this study was that TFR for women with Lynch syndrome from the ACCFR was higher than that of the Australian population generally (fertility rates for men in the general population are not reported). The probable reason for this is selection bias, as larger families are more likely to be recruited by family cancer clinics. This highlights the kind of sampling bias that can cause misleading interpretation of fertility rates in an affected population when compared with the general population.

The decision to have children is influenced by numerous psychological and social factors, which may bias studies of fertility between groups. Subjects were recruited from a large familial cancer database, so that unaffected family members could act as a control group (that would be as demographically and socially similar as possible to the affected individuals) and because of the high number of young CRC patients in this cohort. Only proven germline MMR mutation carriers were included (as controls as well as patients post CRC) as individuals with Lynch syndrome may modify their reproductive behaviour to avoid passing on their mutation. They may decide not to have
children, or even consider techniques such as pre-implantation genetic testing. For this study, no attempt was made to collect information from subjects as to whether they had chosen to have children or not and what the reasons were for the decision. The data presented here cannot determine whether the observed changes in fertility were related to CRC or to reproductive decisions made following the genetic diagnosis of Lynch syndrome (which often occurs after the CRC is diagnosed). Although MMR proteins are essential in DNA replication, there is no evidence in the current literature that Lynch syndrome directly affects fertility, and the high fertility rate in this cohort does not suggest that this occurs.

It is uncertain whether these findings are generalizable to patients with sporadic cancers. Colorectal cancers that display microsatellite instability (as in Lynch syndrome) are known to have a better prognosis than sporadic CRC and may be unresponsive to 5-fluorouracil based chemotherapy regimens [168]. In addition, CRC detected in subjects with Lynch syndrome, who are offered intensive surveillance, may be detected at an earlier stage than sporadic tumors.

It is expected that rectal cancer would have a greater impact on fertility than colon cancer (as a greater number receive radiotherapy, and pelvic surgery may be more likely to have a deleterious effect on fertility). In Lynch syndrome, rectal cancers are less common (as a percentage of CRC overall), and in this cohort there were only 82 subjects with rectal cancer (of whom 45 were diagnosed under the age of 50), so this study was not powered to detect differences in age-specific fertility rates in subjects with rectal cancer. These results of this should therefore be interpreted with caution in patients with
rectal cancer, and may only be applicable to those with tumours of the colon. Another limitation of this study is the small numbers of subjects with CRC in the age groups under 20, and our data are underpowered to detect differences in fertility (if they exist) in this age group. Such patients are rare, and so it would be difficult to recruit large numbers of these subjects in any setting.

In conclusion, age-specific fertility was decreased in women with Lynch syndrome in the 20 to 24 year age group after CRC diagnosis. Subjects of both genders who survived into their late twenties and beyond had no detectable reduction in age-specific fertility, which may be reassuring to colon cancer survivors who hope to have children.
4 CONCLUSION

A large cohort of subjects with Lynch syndrome who all carry the same predisposing mutation is a rarity, and provides an important vehicle for research aimed at understanding the role of genes and environment in cancer risk and in order to improve surveillance strategies.

In Paper I it was found that carriers of the C1528T (Exon 13) mutation in the \textit{hMLH1} gene developed colorectal cancer (CRC) at a young age (median 44 years), and frequently developed synchronous or metachronous CRC. This mutation has a high penetrance, with 92\% of subjects developing CRC by age 65 years. Although gynaecological malignancies are common in women with Lynch syndrome generally, this was not the case in carriers of the C1528T (Exon 13) mutation in the \textit{hMLH1} gene. Only 3 of 98 (3\%) women developed endometrial carcinoma and no case of ovarian cancer was detected in this cohort of women. This information has allowed accurate risk prediction and genetic counselling to members of this family and supports the current policy of not offering routine gynaecological screening or prophylactic hysterectomy and oophorectomy to women who carry this mutation.
PAPER II showed that surveillance colonoscopy improved survival and delayed the onset of CRC in carriers of the C1528T (Exon 13) mutation in the \textit{hMLH1} gene, compared with those subjects who declined surveillance. Colorectal cancer-free survival from birth was increased by 26 years, and overall survival was increased by 23 years. This was achieved despite the logistic difficulty in providing a colonoscopy service to these subjects, and despite their variable compliance with the surveillance programme. This was a uniquely controlled study of surveillance in Lynch syndrome as the intervention and control groups all carried the same mutation. It was not considered ethical to conduct a randomized trial of surveillance colonoscopy in this group of patients and it is unlikely that such a trial would ever be performed.

PAPER III confirmed the high long-term risk of developing metachronous CRC after segmental colectomy for CRC in Lynch syndrome. This information assists these patients in making an informed choice of operation for primary colon cancer (total vs. segmental colectomy), and emphasizes the importance of life-long surveillance after surgery (of the colon and rectum if segmental colectomy was performed, or of the rectum if a total colectomy was performed).

PAPER IV found no evidence of genetic anticipation in a single family who carried the C1528T (Exon 13) mutation in the \textit{hMLH1} gene once results were corrected for follow up bias.

PAPER V Demonstrated by computer simulation that changes in lifetime (cohort) fertility can falsely create the appearance of genetic anticipation in
Lynch syndrome (fecundity bias). This is the first study to demonstrate this in the setting of Lynch syndrome (or any other inherited condition).

The results of **Paper IV** and **Paper V** illustrate the statistical complexity of studying genetic anticipation and the ongoing uncertainty as to whether the phenomenon occurs in Lynch syndrome.

**Paper VI** described the effect on young-onset CRC on fertility in Lynch syndrome. Age-specific fertility was decreased in women with Lynch syndrome in the 20 to 24 year age group after CRC diagnosis. Subjects of both genders who survived into their late twenties and beyond had no detectable reduction in age-specific fertility. This information allows accurate counselling regarding future fertility in young Lynch syndrome patients with CRC. Further research is warranted to determine the cause(s) for decreased fertility in these women, and to determine whether these findings are generalizable to patients with young-onset CRC who do not have Lynch syndrome.

Appendix IV lists the peer-reviewed journal articles that have cited the papers included in this thesis. They have been cited a total of 49 times.
5 APPENDICES
5.1 Appendix I: Original published papers

5.1.1 Paper I

Cancer risk in a cohort of subjects carrying a single mismatch repair gene mutation

D. A. Stupart · P. A. Goldberg · U. Algar ·
R. Ramesar

Published online: 18 August 2009
© Springer Science+Business Media B.V. 2009

Abstract Hereditary non-polyposis colon cancer (HNPCC) is an autosomal dominant condition, caused by germline mutations in the mismatch repair genes, that presents with colorectal cancers at a young age, as well as extracolonic tumours. One of the causative mutations is the C1528T (Exon 13) mutation of the MLH1 gene. The purpose of this study is to document the cancer risk for subjects who carry this mutation. This is a prospective cohort study of 200 subjects who carry this mutation. We calculated the risk of developing colorectal cancer only in those subjects who had not undergone surveillance colonoscopy. The incidence of extracolonic cancers (for which surveillance is not routinely offered) was determined for the entire cohort. The results of the study are among the 71 subjects who did not undergo surveillance colonoscopy, colorectal cancers occurred in 36 (51%), They occurred at a median age of 44 years (range 17–73). Using Kaplan–Meier estimates, the risk of developing a colorectal cancer by age 65 was 92%. Eighteen subjects in the cohort of 200 were diagnosed with extracolonic tumours. The most common extracolonic malignancies were breast (698 women) and endometrial (398 women). Thus this mutation has a high penetrance for colorectal cancer, but is not associated with a high risk of developing extracolonic malignancies.

Keywords Hereditary non-polyposis colorectal cancer · Screening · MLH1 · Mismatch repair gene · Colorectal cancer

Introduction

Hereditary non-polyposis colon cancer (HNPCC) or Lynch syndrome is the commonest inherited cause of colon cancer. It is an autosomal dominant condition, caused by germline mutations in the mismatch repair genes. HNPCC presents with colorectal cancers at a mean age of around 45 years, as well as extracolonic tumours (most commonly endometrial carcinoma, but also ovarian, small bowel, hepatobiliary, urinary tract and brain tumours) [1].

Since 1997, we have offered genetic testing to individuals identified as being at risk of HNPCC on the basis of their family history. We have detected thirteen different mutations in the MLH1 and MSH2 mismatch repair genes in 33 families. The most prevalent of these is the C1528T (Exon 13) mutation in the MLH1 gene [2, 3], which has not been identified outside South Africa.

Accurate genetic counseling and a rational approach to screening require knowledge of the natural history and penetrance of the disease in question. This is especially important in our setting where we have limited resources available for screening; and most of these families live in areas with little access to health care. We have previously reported the effectiveness of a mobile endoscopy service.
which has been designed to provide a surveillance colonoscopy service to at-risk individuals in remote areas of the Northern Cape Province of South Africa [4].

Previous studies have estimated the lifetime cancer risk of subjects in families carrying a number of different mismatch repair gene mutations [5–12]. As the various mismatch repair gene mutations are known to have differing penetrance and manifest different extracolonic malignancies [8, 12], the findings of these studies cannot necessarily be applied to any single mutation. We have offered screening for colorectal cancer to all mutation carriers with proven survival benefit [13], but have not offered routine surveillance for any of the extracolonic malignancies.

The purpose of this study was to document the cancer risk of subjects who carry the C1528T (Exon 13) mutation in the MLH1 gene in order to give accurate genetic counseling to members of the affected families, and to assess whether screening for extracolonic malignancies should be undertaken.

Patients and methods

Between 1997 and 2007, we have performed genetic testing on 590 members of 17 families known to carry the C1528T mutation in exon 13 of the MLH1 gene. Of these, 200 subjects were found to be germline mutation carriers. All of these 200 mutation carriers were offered surveillance colonoscopy every 2 years until age 30, and annually thereafter, as previously described [13].

Subjects who were known to carry the mutation and were diagnosed with resectable colon cancers or adenomas with high grade dysplasia were offered a subtotal colectomy and ileorectal anastomosis. Subjects with curable rectal cancers were offered rectal resections after radiotherapy as indicated. Those subjects who had undergone segmental colonic resections prior to being recognized as having HNPCC were all offered surveillance of their remaining colon.

We have previously shown that surveillance colonoscopy (which we defined as undergoing colonoscopy in the absence of any bowel symptoms) can delay the onset of colorectal cancer [13]. In order to calculate the risk of developing colorectal cancers in subjects who carry this mutation, therefore, we included only those subjects who had developed colorectal cancers before they were offered surveillance colonoscopy (21 cases) and those who had declined surveillance (50 cases) in this analysis.

Screening for cancers other than colorectal (specifically endometrial, cervical, ovarian and breast cancer) was not routinely offered, so the incidence of extracolonic tumours was determined for the entire cohort of 200 subjects.

Subjects were followed up prospectively from the time of genetic testing until December 2008. Patients were followed up twice annually. At follow up a history was taken, with particular attention to symptoms of colorectal and endometrial carcinoma, a clinical examination was performed, and the subjects were investigated as indicated if there was any clinical suspicion of malignancy. If the subjects declined the offer of attending for the clinical examination, a history was taken telephonically. All cancer diagnoses were made histologically from biopsies or operative specimens. Hospital records were obtained from institutions other than our own when necessary.

Statistics

Colorectal cancer risk was calculated using the Kaplan–Meier technique, and comparison between groups was done using the log rank method. In order to avoid introducing lead time bias into these analyses, colorectal cancer risk was calculated from birth rather than from diagnosis or enrolment in the study. Categorical data were compared using the chi-square test. Statistical analysis was done using Statistica™ software.

Ethics

All subjects were counselled and gave informed consent for genetic testing. The patients who declined surveillance were offered it again whenever they were contacted. The Health Sciences Faculty Research Ethics Committee of the University of Cape Town approved the study.

Results

Colorectal malignancies

Of the 200 mutation-positive subjects, 71 (45 men and 26 women) did not undergo colonoscopic surveillance. They were followed up for a mean of 4 years (range 0–18) after their genetic counselling. The subjects were a mean age of 43 years (range 17–83 years) at their most recent follow up or death.

Thirty-six of these 71 subjects (51%) developed colorectal cancers. The median age at which the first colorectal cancer was diagnosed was 44 years (range 17–73). The ages of diagnosis are illustrated in Fig. 1. No adenomas were diagnosed in these subjects.

Among the subjects who did not undergo surveillance, the risk of developing a colorectal cancer by age 65 years was 92% (standard error 7%). The oldest subject not to develop a cancer died at the age of 83 years. The Kaplan–Meier estimates for colorectal cancer risk are illustrated in
Cancer risk in HNPCC

Table 1: Sites of colorectal cancers

<table>
<thead>
<tr>
<th>Site of tumour</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caecum</td>
<td>9 (20.9%)</td>
</tr>
<tr>
<td>Ascending</td>
<td>7 (15.6%)</td>
</tr>
<tr>
<td>Hepatic flexure</td>
<td>5 (11.1%)</td>
</tr>
<tr>
<td>Transverse</td>
<td>7 (15.5%)</td>
</tr>
<tr>
<td>Splenic flexure</td>
<td>3 (6.7%)</td>
</tr>
<tr>
<td>Descending</td>
<td>5 (11.1%)</td>
</tr>
<tr>
<td>Sigmoid</td>
<td>2 (4.4%)</td>
</tr>
<tr>
<td>Rectosigmoid</td>
<td>1 (2.2%)</td>
</tr>
<tr>
<td>Rectum</td>
<td>5 (11.1%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (2.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
</tr>
</tbody>
</table>

Fig. 1: Age distribution of colorectal cancers in subjects who had not undergone surveillance colonoscopy.

Fig. 2: Kaplan-Meier estimates of colorectal cancer risk for age in subjects who had not undergone surveillance colonoscopy.

Fig. 2. There was no significant difference in colorectal cancer risk between men and women ($P = 0.096$).

Three of the subjects had synchronous colorectal cancers (one of whom had three cancers at his first operation), and four developed metachronous tumours (one of these developed a metachronous transverse colon tumour, underwent a total colectomy, and later developed a rectal cancer), so a total of 45 colorectal cancers were diagnosed in this group of 71 subjects. The sites of the colorectal cancers are presented in Table 1 (in one case, the patient’s operation notes could not be obtained, and the site of his tumour could not be identified). Of the colorectal cancers whose site was recorded, 29/44 (66%) were proximal to the splenic flexure and 15/44 (34%) were found at the splenic flexure or more distally ($P = 0.05$). Among the 129 subjects who underwent surveillance, 35 adenomas (all of which were asymptomatic), were identified and removed endoscopically from 29 subjects. Colorectal cancers were diagnosed in 14/129 of these subjects, so the group who had undergone surveillance were less likely to develop colorectal cancer during the study period ($P < 0.001$). The stage distribution of the cancers at diagnosis is presented in Table 2.

Extracolonic malignancies

Of the entire cohort of 200 subjects, 102 were men and 98 women. Their mean age was 41 years at death or last follow up (range 18–83), and they were followed up for a mean 5 years (range 0–18). Nineteen extracolonic malignancies were diagnosed in 18 of these 200 subjects (9%). The commonest were breast [in 69/8 (66%) of women] and endometrial [in 39/8 (3%) of women]. The extracolonic malignancies are listed in Table 3.

Discussion

A reasonably large cohort of subjects carrying the same predisposing mutation is a rarity, and provides an important vehicle for research aimed at understanding the role of genes and environment in cancer risk in order to improve surveillance strategies. This is the first study to prospectively record the cancer risk of a cohort of subjects with a single mismatch repair gene mutation.

The subjects developed colorectal cancer at a young age (median 44 years), and frequently developed synchronous or metachronous colorectal tumours. This mutation has a high penetrance, with 92% of subjects developing a colorectal cancer by age 65 years.

Recently, it has been suggested that the methodology of previous studies has led to an overestimation of the penetrance of HNPCC [14]. Those families with the highest numbers of cancers are the most likely to be studied, and if...
Colorectal cancers in HNPC are reported to typically involve the right side of the colon, with 70% of tumours proximal to the splenic flexure [16]. In this series, tumours occurred slightly more frequently to the right of the splenic flexure, but were found throughout the colon and rectum. We would argue, therefore, that the site of the tumours may be of little help in identifying individuals with colorectal cancers as being at risk for HNPC.

We found no difference in the frequency or age of onset of colorectal cancers between men and women, in contrast to previous studies which found the penetrance to be higher in men [9, 10, 17].

Endometrial carcinoma is reported to be the commonest extracolonic cancer among women with HNPC [16], with reported rates of up to 60% among subjects with MLH1 and MSH2 mutations [9]. Some authors recommend screening for endometrial malignancies in women from HNPC families [16], despite there being no evidence that it confers a survival benefit [12, 18-20] and it is even argued that prophylactic hysterectomy with or without oophorectomy should be considered in selected female gene carriers [16, 18]. In this study, only 3 of 98 (3%) women developed endometrial carcinoma, which is similar to the risk of developing endometrial cancer among women in the general population [21]. There were also no cases of ovarian cancer in this cohort of women, so screening for endometrial or ovarian cancers is not clearly indicated in women who carry this mutation, and prophylactic gynaecological surgery does not appear to be indicated. It should be noted, however, that these tumours were diagnosed only once they were clinically apparent, and early cancers may have been missed.

We have previously described mismatch repair gene inactivity in the pathogenesis of some breast cancers in these families [22], so breast cancer can be regarded as a manifestation of HNPC in this cohort. Breast cancer was the commonest extracolonic malignancy in this cohort, but was still not common, affecting only 6 of 98 (6%) women.

Five subjects developed cancers of the duodenum, small bowel and bile duct, so the risk of developing any one of these tumours was low, in keeping with reports of other HNPC families [10].

---

Table 2: Stage distribution of colorectal cancers (patients with synchronous tumours at presentation have been staged according to the most advanced tumour only)

<table>
<thead>
<tr>
<th>Duke's stage</th>
<th>Surveillance group (n = 129)</th>
<th>Non-surveillance group (n = 71)</th>
<th>P value (Chi-square test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7 (50%)</td>
<td>5 (12%)</td>
<td>0.006</td>
</tr>
<tr>
<td>B</td>
<td>1 (7%)</td>
<td>13 (18%)</td>
<td>0.05</td>
</tr>
<tr>
<td>C</td>
<td>6 (43%)</td>
<td>12 (29%)</td>
<td>0.49</td>
</tr>
<tr>
<td>D</td>
<td>0</td>
<td>7 (17%)</td>
<td>0.08</td>
</tr>
<tr>
<td>Unknown</td>
<td>0</td>
<td>4 (10%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>41</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Sites of extracolonic malignancies

<table>
<thead>
<tr>
<th>Extracolonic malignancies</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>6</td>
</tr>
<tr>
<td>Small bowel</td>
<td>3</td>
</tr>
<tr>
<td>Endometrium</td>
<td>3</td>
</tr>
<tr>
<td>Biliary</td>
<td>2</td>
</tr>
<tr>
<td>Duodenal papilla</td>
<td>1</td>
</tr>
<tr>
<td>Hepatic neuroendocrine</td>
<td>1</td>
</tr>
<tr>
<td>O-G junction adenocarcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Prostate</td>
<td>1</td>
</tr>
<tr>
<td>Cervix</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
</tr>
</tbody>
</table>

one relies on clinical criteria to diagnose HNPC, those subjects who do not develop tumours may be excluded from the analysis. This can cause the cancer risk to be overestimated (ascertainment bias) [15]. We have attempted to identify the entire cohort of mutation positive members of the families studied, regardless of whether they have developed cancers, and followed them up prospectively. This approach does not eliminate ascertainment bias, but should reduce it. The results of prospective studies of such subjects may be influenced by interventions aimed at preventing the development of cancers [15]. In order to avoid this, we excluded all those subjects who had undergone surveillance colonoscopy from the estimation of colorectal cancer risk. The drawback of this approach, however, is that these cancers were only diagnosed once they become clinically apparent, with a variable lead time from the development of premalignant adenomas and early asymptomatic cancers. This is supported by the observation that cancers in the unscreened population were less likely to have Duke’s A or B tumours at diagnosis, and no adenomas were diagnosed in this group. Our results are in keeping with previous reports of colorectal cancer risk in HNPC families [5-11], and do not support the much lower estimates of cancer risk that have been calculated using statistical methods aimed at reducing ascertainment bias [14].

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In conclusion, subjects who carry the C1528T (Exon 13) mutation in the MLH1 gene carry a high lifetime risk of colorectal cancer, regardless of their gender, but have a relatively low incidence of extracolonic tumours, most notably endometrial carcinomas. This information allows accurate genetic counseling to members of this family, and justifies our current policy of not offering routine gynecological screening or prophylactic gynecological surgery to women from this family.

References

Surveillance colonoscopy improves survival in a cohort of subjects with a single mismatch repair gene mutation

D. A. Stupart*, P. A. Goldberg*, U. Algar* and R. Ramesar†

*Colorectal Unit, Department of Surgery, University of Cape Town and Groote Schuur Hospital, Cape Town, South Africa and †HPRC/UCT Human Genetics Research Unit, Division of Human Genetics, Institute for Infectious Diseases and Molecular Medicine, University of Cape Town and Groote Schuur Hospital, Cape Town, South Africa

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Abstract

Objective Previous studies have shown a benefit for surveillance colonoscopy in heterogeneous groups of subjects with suspected or proven hereditary nonpolyposis colon cancer. The aim of this study was to investigate whether surveillance colonoscopy improves the survival in subjects who carry a single mismatch repair gene defect.

Method This is a prospective cohort study of 178 subjects who carry a mutation of the MLH1 gene in exon 13 (C1528T). They were offered surveillance colonoscopy between 1988 and 2006, and were followed up until September 2007.

Results One hundred and twenty-nine subjects underwent surveillance colonoscopy, and 49 declined. After a median follow up of 5 years, colorectal cancer was diagnosed in 14/129 (11%) subjects in the surveillance group and 13/49 (27%) in the nonsurveillance group (P = 0.019). Cancers in the surveillance group were at an earlier stage than in the nonsurveillance group (P = 0.032). Death from colorectal cancer occurred in three of 129 (2%) subjects in the surveillance group, and six of 49 (12%) in the nonsurveillance group (P = 0.021). The Kaplan–Meier estimates for median survival from birth were 78 years in the surveillance group, and 55 years in the nonsurveillance group (P = 0.024). The Kaplan–Meier estimates for median colorectal cancer-free survival from birth were 73 years in the surveillance group and 47 years in the nonsurveillance group (P = 0.0089).

Conclusion Surveillance colonoscopy was associated with improved overall and colorectal cancer-related survival in subjects carrying a single mismatch repair gene mutation.

Keywords Hereditary nonpolyposis colorectal cancer, screening, surveillance, MLH1

Introduction

Hereditary nonpolyposis colorectal cancer (HNPCC), also referred to as Lynch syndrome, is the most common cause of hereditary colon cancer. It is an autosomal dominant condition characterized by young onset colorectal cancers, as well as extracolonic tumours [1]. The underlying genetic defects in HNPCC are germline mutations of the mismatch repair genes. Such mutations have been identified in the MLH1, MSH2, MSH6, PMS1 and PMS2 genes [2].

Since 1998, the University of Cape Town has offered surveillance colonoscopy to individuals with suspected HNPCC based on the family history. Mutational analysis was introduced in 1997. This has led to the detection of 13 mutations in the MLH1 and MSH2 genes in 13 families, the commonest being at C1528T (Exon 13) in the MLH1 gene. We have identified 200 subjects in 17 families who carry this mutation [3,4].

The C1528T mutation has not been described outside South Africa. Most mutation carriers live in a remote part of the Northern Cape Province of South Africa, with limited access to health care, and almost no access to endoscopic services. The mobile colonoscopic service offered to these patients has been described previously [5].

Recent reviews have stressed the importance of surveillance colonoscopy in patients who may have HNPCC [6,7] as it has been shown to lead to a decrease in the detection of colorectal cancers at an earlier stage, and to identify adenomas [6–12]. One study [13] has shown that surveillance colonoscopy was associated with
improved overall survival. These studies involved heterogeneous groups of patients, in whom HNPCC was diagnosed mainly by clinical criteria. They included patients with mutations of general different mismatch repair gene and many patients who had not been found to have a mutation. The aim of this study was to investigate whether surveillance colonoscopy improved the survival in subjects who carry a single MLH1 germline mutation.

Method

Two hundred subjects have been found to carry a mutation of the MLH1 gene in exon 13 (C1528T) by genetic testing as previously described [3]. Twenty-one of them had been treated for colorectal cancer before they were offered genetic testing or colonoscopic surveillance and were excluded from this study. A further patient underwent positive genetic testing at age 83, and died before being offered surveillance, and so was also not included. The remaining 178 subjects were all offered surveillance colonoscopy between 1988 and 2006, and were studied prospectively.

These 178 subjects were included in this analysis requests with mutations of general different mismatch out before or after they were offered screening colonoscopy. None had symptoms suggestive of colorectal cancer when they were first offered surveillance. Surveillance colonoscopy was offered from the age of 18 years, or 10 years before the age of the onset of disease of the youngest relative with cancer (whichever age was the older). If the subject was only identified as being at risk when already older than this, surveillance was offered to commence at the earliest opportunity. Colonoscopy was offered every 2 years until age 50, and annually thereafter. Colonoscopies were performed either in the endoscopy unit at Groote Schuur Hospital in Cape Town, or by a mobile endoscopic unit that visited rural hospitals annually. Subjects were considered to have undergone surveillance if they underwent at least one colonoscopy before developing symptoms suggestive of colorectal cancer.

Patients with colorectal cancers or adenomas with high-grade dysplasia were offered a subtotal colectomy and ileorectal anastomosis.

Subjects were followed up by physical or telephonic contact from the time they were first offered surveillance colonoscopy until September 2007. Records were kept of colonoscopic findings, and of the histopathology of any abnormalities found. The cause of death was determined from medical records and death certificates. Where necessary, records from institutions other than our own were obtained.

Statistics

The Kaplan–Meyer and log-rank techniques were used to calculate the overall and colorectal cancer-free survival. Categorical data were compared using the $\chi^2$ test. Continuous data were compared using the Student's $t$-test. Statistical analysis was performed using MEDCALC® (Mariakerke, Belgium) software.

Ethics

Subjects were counselled and gave informed consent for genetic testing, and for all procedures performed. Patients who declined surveillance were offered it again whenever they were contacted. Ethics committee approval for the study was given by the Health Sciences Faculty Research Ethics Committee of the University of Cape Town.

Results

Of the 178 subjects, 129 chose to undergo surveillance colonoscopy, and 49 declined. The two groups were similar in age when they were first offered surveillance (mean 33 years [SD 12.2] in the surveillance group, and 35 [SD 13.0] in the nonsurveillance group ($P = 0.41$]). The male-to-female ratio was 58:71 (45% men) in the surveillance group and 26:23 (53% men) in the nonsurveillance group ($P = 0.42$). The subjects were followed up for a median of 5 years (range 0–18). Seven subjects (5%) in the surveillance group and six (12%) in the nonsurveillance group were lost to follow up, so the follow-up rates in the two groups were similar ($P = 0.22$). During this period, the Surveillance group underwent a median of three colonoscopies (range 1–12).

In all, 35 adenomatous polyps (all asymptomatic) were removed endoscopically from 29 patients in the surveillance group. Eleven subjects had adenomas with high-grade dysplasia and underwent subtotal colectomy and ileorectal anastomosis.

Colorectal cancer was diagnosed in 14/129 (11%) subjects in the surveillance group, and 13/49 (27%) in the nonsurveillance group ($P = 0.019$, relative risk 0.42, 95% confidence interval [95% CI] 0.24–0.82). Cancers in the surveillance group were at an earlier stage than in the nonsurveillance group ($P = 0.032$; Table 1).

Death from colorectal cancer occurred in three of 129 (2%) subjects in the surveillance group, and six of 49 (12%) in the nonsurveillance group ($P = 0.021$, relative risk 0.19 [95% CI 0.06–0.61]). Death from all causes occurred in 11/129 (9%) subjects in the surveillance group and 12/49 (25%) in the nonsurveillance group.
Table 1 Stage distribution of colorectal cancers.

<table>
<thead>
<tr>
<th>Duke’s stage</th>
<th>Surveillance group (n = 129)</th>
<th>Nonsurveillance group (n = 49)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>C</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>D</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Total cancers</td>
<td>14</td>
<td>13</td>
</tr>
</tbody>
</table>

Table 2 Causes of death.

<table>
<thead>
<tr>
<th>Cause of death</th>
<th>Surveillance group (n = 129)</th>
<th>Nonsurveillance group (n = 49)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon cancer</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Renal cell cancer</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Neuroendocrine liver</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>AIDS</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cardiac</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Upper GIT bleed</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Unknown</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Total deaths</td>
<td>11</td>
<td>12</td>
</tr>
</tbody>
</table>

(P = 0.0097, relative risk of death 0.35 (95% CI 0.16–0.74). Their causes of death are summarized in Table 2.

There was no statistically significant difference in death rates from causes other than colorectal cancer during the study period [eight of 129 (6%) in the surveillance group vs. six of 49 (12%) in the nonsurveillance group (P = 0.20)].

The Kaplan-Meier estimates for median survival from birth were 78 years in the surveillance group, and 55 years in the nonsurveillance group (P = 0.024). Their survival curves are presented in Fig. 1.

The Kaplan-Meier estimates for the median colorectal cancer-free survival from birth was 73 years in the surveillance group and 47 years in the nonsurveillance group (P = 0.0089). This is illustrated in Fig. 2.

Figure 1 Kaplan-Meier estimates of survival (calculated from birth).

Figure 2 Kaplan-Meier estimates of colorectal cancer-free survival (calculated from birth).

Conclusion

In this study of patients who all carried the same mismatch repair gene mutation, surveillance colonoscopy was found to improve survival, and delay the onset of colorectal cancers. Those subjects who declined surveillance colonoscopy had a median colorectal cancer-free survival from birth of 47 years, in keeping with previous descriptions of the natural history of HNPCC [2]. This was extended by 26 years to 73 years in those who chose to undergo surveillance. Overall life expectancy was similarly lengthened by 23 years (from 55 to 78 years) in the subjects who underwent colonoscopies.

The reduction in colorectal cancers in the surveillance group can be ascribed to the removal of 35 adenomatous polyps from 29 subjects who underwent surveillance [13,14]. In keeping with previous studies [10–12], surveillance colonoscopy allowed earlier detection of
colorectal cancers. In the surveillance group, half the cancers were staged as Duke’s A, and none had metastases at the time of diagnosis. In contrast, only one patient in the nonsurveillance group had a Duke’s A cancer at diagnosis, and four of 13 of them had metastases at diagnosis.

It is concerning that six subjects in the surveillance group had Duke’s C cancers at diagnosis. In five of these cases, the tumours were detected at their first colonoscopy. The other subject had a normal colonoscopy in 1988, and then defaulted on his planned surveillance until 1994, when this cancer was found. These tumours cannot, therefore, be regarded as interval cancers. They would not have been prevented by decreasing the surveillance interval, and do not represent tumours that had been missed at colonoscopy.

Jänne et al. [13] have previously found that surveillance colonoscopy reduces the incidence of colorectal cancer and reduces the overall mortality in a cohort of 252 asymptomatic individuals from 22 HNPPC families. Within this cohort, 44 subjects in the surveillance group and 46 in the control group were known to have mutations in two genes that cause HNPPC. In six cases, HNPPC was diagnosed on the histological appearance of their tumours. The benefits of surveillance were apparent in entire cohort as well as in the subgroup with proven HNPPC. This study has reproduced those results in the cohort of subjects who all share a single proven mutation in the MLH1 gene.

Subjects with the mutation who had cancers were routinely offered subtotal colectomy and ileorectal anastomosis rather than segmental colonic resections because of concerns about the high rate of metachronous tumours in this group of patients [15,16]. More controversially, we offered subtotal colectomy to patients who had adenomas with high-grade dysplasia, even if these had been completely excised (as judged endoscopically and histologically). All of these patients were diagnosed by colonoscopic surveillance, and some of the reduction in colorectal cancers in the surveillance group may be due to this policy. There is controversy as to whether prophylactic colectomy is appropriate in HNPPC and the indications for surgery in the absence of invasive colorectal cancer remain uncertain [7,17].

We achieved good follow up, despite the logistical difficulties of reaching many of the subjects. This is probably because the ongoing process of genetic counselling allows us to meet with many members of the affected families, who can help us to maintain contact with their relatives, and inform us if they become ill or die.

Self-selection bias is a potential cause for bias in a study of this kind. Those subjects who elected to undergo surveillance may have had generally better health, or been more likely to seek primary or preventative healthcare earlier. It is unlikely to explain the results of this study; however, as there was no difference in mortality due to causes other than colorectal cancer between the two groups. In order to avoid introducing lead-time bias into the survival analysis, all survival and cancer-free survival calculations were made from birth rather than from diagnosis or enrolment in the study. We did not consider it ethical to conduct a randomized trial of surveillance colonoscopy in this group of patients, and it is unlikely that such a trial would ever be performed.

Although extracolonic tumours occur in HNPPC, we have found them to be uncommon in subjects carrying this particular mutation [18]. We do not, therefore, offer surveillance for ovarian or endometrial malignancies to these subjects.

While the optimal interval between surveillance colonoscopies remains a subject of some debate, our protocol is similar to those proposed in recent reviews [1,6,7]. The variable compliance in this study reflects the socioeconomic and logistic difficulties faced by these communities. It is encouraging that surveillance colonoscopy appears to achieve significant improvement in survival to subjects with HNPPC, even in this setting.

Funding

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Competing interests

There are no competing interests for any of the authors of this study.

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Surveillance colonoscopy in subjects with a single mismatch repair gene mutation

D. A. Stupart et al.


Surgery for colonic cancer in HNPCC: total vs segmental colectomy

D. A. Stupart*, P. A. Goldberg#, R. J. Baigrie#, U. Algar# and R. Ramesar†

*Colorectal Unit, Department of Surgery, University of Cape Town and Groote Schuur Hospital, Cape Town, South Africa and #MRD/UCT Human Genetics Research Unit, Division of Human Genetics, Institute for Infectious Diseases and Molecular Medicine, University of Cape Town and Groote Schuur Hospital, Cape Town, South Africa

Received 22 June 2010; accepted 30 September 2010; Accepted Article online 24 October 2010

Abstract

**Aim** The high reported risk of metachronous colon cancer (MCC) in hereditary nonpolyposis colorectal cancer (HNPCC) has led some authors to recommend total colectomy (TC) as the preferred operation for primary colon cancer, but this remains controversial. No previous study has compared survival after TC with segmental colectomy (SC) in HNPCC. The aim of this study was to determine the risk of developing MCC in patients with genetically proven HNPCC after SC or TC for cancer, and to compare their long-term survival.

**Method** This is a prospective cohort study of all patients referred to our unit between 1995 and 2009 with a proven germline mismatch repair gene defect, who had undergone a resection for adenocarcinoma of the colon with curative intent. All patients were offered annual endoscopic surveillance.

**Results** Of 60 patients in the study, 39 had TC as their initial surgery and 21 had SC. After 6 years follow up, MCC occurred in eight (21%) SC patients and in none of the TC patients ($P = 0.048$). The risk of developing MCC after SC was 20% at 5 years. Colorectal cancer-specific survival was better in TC patients ($P = 0.048$) but overall survival of the two groups was similar ($P = 0.29$).

**Conclusion** Patients with HNPCC have a significant risk of MCC after SC. This is eliminated by performing TC as the primary operation for colon cancer.

**Keywords** HNPCC, Lynch syndrome, metachronous colorectal cancers

**What is new in this paper**
This is the first study to determine metachronous colorectal cancer risk after colorectal resections for cancer in patients with genetically proven HNPCC, and is the first to compare long-term survival after total or segmental colectomy in these patients.

Introduction

Hereditary nonpolyposis colorectal cancer (HNPCC), also known as Lynch syndrome, is the commonest cause of inherited colorectal cancer. It is caused by germline mutations in the mismatch repair genes (disease causing mutations have been identified in the MLH1, MSH2, MSH6, PMS1 and PMS2 genes) and leads to a high incidence of colorectal cancers at a young age [1].

The risk to HNPCC patients of developing metachronous colon cancer (MCC) after segmental colectomy (SC) for colon cancer is high, with reported rates of around 4% per year [2–7]. The risk of developing MCC has led some authors to recommend total colectomy (TC) as the preferred operation for colon cancer in HNPCC [8,9], but this remains controversial [10–15]. All previous estimates [2–7] of MCC risk in HNPCC have come from retrospective studies that included patients diagnosed by clinical criteria as well as by proven germline mutations. No studies compared survival after TC vs SC in HNPCC.

The aim of this study was to determine the risk of developing MCC in patients with genetically proven HNPCC after segmental colectomy or total colectomy for cancer and to compare their long-term survival.
Method

All patients referred to our unit between 1995 and 2009 with a proven germline mismatch repair gene defect, who had undergone a resection for adenocarcinoma of the colon with curative intent, were included in this study. Patients were followed prospectively from when they were first assessed as being at risk of HNPCC until January 2009. They were included in the study regardless of whether they underwent surgery before or after they were identified as being at risk and regardless of whether the initial surgery had been performed at the authors’ institution or elsewhere. All patients who had undergone segmental colonic resection or total colectomy were offered annual surveillance by colonoscopy or flexible sigmoidoscopy, respectively.

Patients were offered a completion colectomy if a resectable MCC or a high-grade dysplastic adenoma was identified. Patients who developed rectal cancer were offered resection, with neoadjuvant radiotherapy if appropriate. They were considered to have developed a MCC only if the tumour was diagnosed more than 2 years after the initial surgery.

Statistical analysis

The Kaplan-Meier technique was used to calculate survival and the risk of developing MCC, and groups were compared using the log rank technique. Categorical data were compared using the χ² test, and continuous data using Student’s t-test. Statistical analysis was performed using Medcalc ® (Mariakerke, Belgium) software.

Ethical approval

All subjects were counselled by a trained genetic counsellor and gave informed consent for genetic testing, and for all procedures performed. Ethical approval for the study was granted by the Research Ethics Committee of the University of Cape Town (Room E52-24, Groote Schuur Hospital, Old Main Building, Observatory 7925, Cape Town, South Africa).

Results

Between 1995 and 2009, mutational analysis was performed on 856 members of families at risk of HNPCC and 280 germline mismatch repair gene mutation carriers were identified. Sixty-two of these underwent a colonic resection for adenocarcinoma between 1975 and 2007, of whom two had incurable metastatic disease at the time of surgery and were excluded from further analysis. The mismatch repair gene mutations of the remaining 60 patients, who came from 17 different families, are listed in Table 1. The majority of patients in both groups carried a single mutation in exon 13 of the MLH1 gene.

Patient characteristics

Thirty-nine patients underwent SC as their initial operation and 21 had TC. The two groups were

<table>
<thead>
<tr>
<th>Germline mutations identified</th>
<th>Segmental colectomy (SC)</th>
<th>Total colectomy (TC)</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td>MLH1 Exon13 C→T at 1528</td>
<td>23</td>
<td>18</td>
<td>0.07</td>
</tr>
<tr>
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<td>0</td>
<td>0.75</td>
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<td>MSH2 Exon 1→16</td>
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<tr>
<td>MSH2 Exon 15</td>
<td>4</td>
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<td>0.33</td>
</tr>
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<td>MSH2 Exon 1→5 del</td>
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<td>0</td>
<td>0.75</td>
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<tr>
<td>MSH2 Exon 1→6 del</td>
<td>1</td>
<td>0</td>
<td>0.75</td>
</tr>
<tr>
<td>MSH2 Exon 3</td>
<td>1</td>
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<td>0.75</td>
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<tr>
<td>MSH2 Exon 8</td>
<td>3</td>
<td>0</td>
<td>0.49</td>
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<tr>
<td>MSH2 Exon 3 del TC at 387</td>
<td>0</td>
<td>1</td>
<td>0.75</td>
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<tr>
<td>MSH2 Exon7 del TC at 1220</td>
<td>4</td>
<td>2</td>
<td>0.72</td>
</tr>
</tbody>
</table>

Table 1 Mutations identified, and Dukes’ stage of the cancer(s) resected at the first operation (in four cases, the initial surgery was performed at another institution, and complete pathological staging was incomplete).
similar in age at the time of their first diagnosis of colonic cancer [mean 44 (±11.0) years in the SC group and 41 (±7.9) years in the TC group (P = 0.19)]. The ratio of men to women was similar in the two groups [21 (54%) men, SC; 13 (62%), TC; P = 0.74]. There was no significant difference in the prevalence of the various mismatch repair gene defects between the two groups. All TC patients and 9/39 (23%) SC patients were identified to be at risk of HNPCC prior to surgery (P < 0.0001). Patients undergoing TC were more likely to have a Dukes’ A cancer (P = 0.0003) (Table 1). The 30-day mortality was zero in each group.

Follow up and surveillance
After the initial operation, patients were followed for a median of 6 (1–30) years in the TC group and 8 (0–34) years in the SC group (P = 0.60). Five (13%) SC patients and one (5%) TC patient were lost to follow up (P = 0.58).

During the study period, 22 (56%) SC patients underwent a mean of three (±2.2) surveillance colonoscopies and 15 (71%) TC patients underwent a mean of five (±3.2) flexible sigmoidoscopies. The groups were equally likely to attend for at least one surveillance endoscopy (P = 0.39), but the TC patients attended more often (P = 0.015).

Thirteen adenomatous polyps were identified and removed endoscopically in seven SC patients. Three contained high-grade dysplasia and these patients underwent a completion colectomy and ileorectal anastomosis. One low-grade rectal adenomatous polyp was identified and endoscopically removed from a TC patient.

Metachronous cancers
A metachronous colon cancer (MCC) occurred in eight (21%) SC patients but did not occur in any of the TC patients. The risk of developing MCC after SC was 20% at 5 years and 41% at 15 years (Fig. 1). Two MCC were interval cancers, in patients who developed symptoms < 1 year after a normal surveillance colonoscopy and six occurred in patients who had defaulted surveillance colonoscopy for at least 2 years. Two (10%) TC patients and no SC patient developed a rectal cancer, at 15 and 23 years after colectomy resection. One cancer was diagnosed 1 year after a normal surveillance sigmoidoscopy, and the second patient had defaulted surveillance sigmoidoscopy for 4 years. Overall there were eight metachronous colorectal cancers in the SC group (all in the colon) and two in the TC group (both in the rectum) (P = 0.46).

![Figure 1](image-url)

Figure 1 Kaplan–Meier estimate of metachronous colon cancer risk after segmental colectomy for cancer in HNPCC.

Mortality
Overall mortality was 15 (38%) SC patients and five (24%) TC patients, while colorectal cancer–specific death occurred in 13 (33%) and two (10%) patients in the SC and TC groups, respectively (Table 2). Colorectal cancer–specific survival was significantly better in TC patients (P = 0.048) but overall survival of the two groups was similar (P = 0.29).

Discussion
The genetically proven HNPCC patients in this study who underwent SC have a significant lifelong risk of developing MCC. This is eliminated by performing TC as the primary operation for colon cancer. These findings are in keeping with the outcome predicted by de Vos tot Needevuren Cappel et al. [9] using a decision analysis model for assessing the possible benefits of subtotal colectomy vs. hemicolecystomy in HNPCC. Fitzgibbons et al. [2] and Lynch et al. [3], reporting on a similar cohort of 116 members of 10 American families who

<table>
<thead>
<tr>
<th>Table 2 Cause of death.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Metastatic colorectal cancer</td>
</tr>
<tr>
<td>Cholangiocarcinoma</td>
</tr>
<tr>
<td>Small bowel cancer</td>
</tr>
<tr>
<td>Breast cancer</td>
</tr>
<tr>
<td>Asthma</td>
</tr>
<tr>
<td>Upper gastrointestinal bleed</td>
</tr>
</tbody>
</table>

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Colorectal Disease © 2011 The Association of Coloproctology of Great Britain and Ireland. 13, 1395–1399
fulfilled the clinical criteria for HNPCC, calculated the risk of developing MCC to be 40% by 10 years. Lampa et al. [4], in a later study from the same institution, identified 225 patients with clinically diagnosed HNPCC who developed colorectal cancer. Of these, 17 (8%) developed MCC within 5 years. Van Dalen et al. [5] retrospectively studied a cohort of 93 patients who had undergone surgical resection for colorectal cancer and whose families fulfilled the Amsterdam criteria for HNPCC. Sixty patients had SC, of whom 15 developed MCC after a median of 14 years follow-up. Aarnio et al. [6] described the cancer risk of 40 families from the Finnish HNPCC registry who fulfilled the Amsterdam criteria for HNPCC. They calculated the risk of developing MCC to be 72% at 40 years after treatment of the primary tumour. Shen et al. [7] reported on 98 patients from 28 HNPCC families who had undergone colorectal cancer resection, of whom 20% developed MCC within 10 years.

All previous reports of MCC risk in HNPCC have been from retrospective studies of patients in whom the diagnosis was made on clinical grounds and family history. This is likely to have resulted in selection bias and an overestimation of risk, as HNPCC is more likely to be suspected in patients with multiple tumours. Both the Bethesda [16] and Japanese [17] criteria list MCC as a clinical criterion for the diagnosis of HNPCC. This study attempts to avoid selection bias by only including and following prospectively, genetically rather than clinically diagnosed HNPCC patients. This is the first study to compare the long-term outcome after TC or SC in HNPCC.

Fourteen asymptomatic adenomas were detected and removed endoscopically, but this did not eliminate the risk of MCC after SC. All patients were offered surveillance endoscopy, but compliance was poor, especially after SC. Seven of the 10 patients who developed MCC had defaulted surveillance. The poor surveillance attendance is surprising in a cohort of patients who have already developed colorectal cancer and who have family members with the disease. The reasons for noncompliance in these patients are unknown and are currently under investigation, but poor socioeconomic conditions and low education standards, which are all prevalent in this community, are likely to be contributory.

Screening this group of patients is logistically difficult, as many live in remote areas with little access to healthcare. A mobile colonoscopic service has been instituted to address this need [18]. The low number of surveillance colonoscopies affects the results of this study as surveillance colonoscopy may prevent MCC. The risk of MCC in this group of patients may be more accurately determined than in patients who had better surveillance, but the benefits of colectomy may not be apparent in patients undergoing adequate surveillance. Two patients developed an interval cancer within a year of normal colonoscopy. Rapid development of cancer from adenoma occurs in HNPCC [19], but an oversight during surveillance cannot be excluded. Rectal cancers were infrequent and all occurred more than 15 years after the initial surgery. It could be argued that this long interval justifies avoidance of the increased morbidity associated with proctocolectomy as the primary operation for colon cancer in HNPCC, but lifelong endoscopic surveillance of the rectum should be offered.

There is evidence of selection bias in this study, where patients selected for TC were more likely to have been diagnosed with HNPCC preoperatively and were more likely to have a Duke’s A than those who underwent SC. One cannot conclude from this study, therefore, that the difference in colorectal cancer-specific survival was a result of the choice of surgery. In this study, patients were judged to have had a total colectomy based on the surgeon’s description of the operation. It is possible that some of these patients may have had a small amount of sigmoid colon left in situ. Quality of life after the different operations was not studied, but others have found that TC with ileorectal anastomosis is associated with increased stool frequency compared with SC [20,21]; however, overall quality of life is well maintained after TC [21].

In conclusion, an awareness of the long-term risk of synchronous colorectal cancer in patients with HNPCC can help to make an informed choice of operation for primary colon cancer in these patients.

Acknowledgement

The authors would like to thank Devon van Schoor for his help in collecting the data for this study.

References


No evidence of genetic anticipation in a large family with Lynch syndrome

D. Stupart · P. Goldberg · U. Algar · A. Vorster · R. Ramesar

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Abstract Lynch syndrome is the commonest inherited cause of colorectal cancer (CRC). Genetic anticipation occurs when the age of onset of a disorder decreases in successive generations. It is controversial whether this occurs in Lynch syndrome. Previous studies have included heterogeneous groups of subjects from multiple families, including subjects with a clinical diagnosis (based on family history) as well as those with proven germline mismatch repair gene mutations. The purpose of this study was to determine whether genetic anticipation occurs in mismatch repair gene carriers from a single Lynch syndrome family. This study includes members of a single family known to carry an MLH1 gene mutation who are proven germline mutation carriers or obligate carriers (based on their offspring’s mutation status). Evidence of genetic anticipation (determined by age of onset of first CRC) was sought in two ways: Firstly, subjects were grouped as parent-child pairs and individuals were compared with their own offspring; secondly they were grouped by generation within the family tree. The Kaplan–Meier technique was used to adjust for variable follow up times. The family tree consisted of 714 subjects. Ninety-two subjects over five generations were included in the study. There was no evidence of genetic anticipation over the generations. ($P = 0.37$). Similarly, in the 75 parent-child pairs identified, age of onset of CRC was similar for parents and children ($P = 0.51$). We could not identify any evidence of genetic anticipation in mutation carriers from a single family with Lynch syndrome.

Keywords Lynch syndrome · HNPCC · Colorectal cancer · Anticipation

Introduction

Lynch syndrome is the commonest inherited cause of colorectal cancer (CRC). It is an autosomal dominant condition, caused by germline mutations in the mismatch repair genes. This manifests with colorectal cancers at a mean age of around 45 years, as well as extracolonic tumours (most commonly endometrial carcinoma, but also ovarian, small bowel, hepatobiliary, urinary tract and brain tumours) [1].

Since 1997, we have offered genetic testing to individuals identified as being at risk of Lynch syndrome on the basis of their family history. We have detected sixteen different mutations in the MLH1 and MSH2 mismatch repair genes in 55 South African families. The most prevalent of these is the MLH1 NM_000249.3:c.1528C>T mutation. This mutation in the MLH1 gene has not been identified outside South Africa. This mutation has been identified in 23 families, the largest of which consists of 714 known individuals within a single pedigree. The natural history of carriers of this mutation has been previously described [2].

Genetic anticipation is the phenomenon in which the age of onset of an inherited disorder decreases (or the severity...
of the phenotype) increases in successive generations. This is a well established phenomenon in a number of inherited neurodegenerative disorders, and it has long been suspected in Lynch syndrome. When Warthin [3] first described a family with this condition ('Family G') in 1925, he made the observation that colorectal cancers occurred at a younger age in successive generations. Since then, a number of conflicting reports have documented the presence or absence of genetic anticipation in Lynch syndrome and it remains controversial whether it occurs in this disease [4]. Apart from Warthin’s initial paper, all previous studies describing anticipation in Lynch syndrome have included heterogeneous groups of subjects from multiple families with different mutations and in most cases have included subjects with a clinical diagnosis of on the basis of a strong family history, as well as those with proven germline mismatch repair gene mutations (i.e. with proven Lynch Syndrome) [5–13].

The purpose of this study was to determine whether genetic anticipation occurs in mismatch repair gene carriers from a single family with Lynch syndrome.

Methods

Since 1997, we have offered mutation analysis, genetic counseling and clinical and colonoscopic surveillance to members of 23 families known to carry the C1528 mutation in exon 13 of the MLH1 gene. The largest of these families (with 714 members known family members) was first identified in 1985. This study includes only members of that single family who are proven germline mutation carriers or judged to be obligate mutation carriers based on the positive mutation status of their offspring.

Information about age of onset of CRC was obtained by interviewing the subjects and their family members, and confirmed with hospital and pathology records wherever possible. Subjects who were found to be mutation carriers were followed up prospectively twice annually from the time of genetic testing until May 2010. Follow up consisted of a clinical history (paying particular attention to symptoms of colorectal or endometrial cancer), physical examination and further investigations if indicated. All subjects were offered surveillance colonoscopy as previously described [2]. Subjects who declined the offer of attending for clinical follow up were contacted and interviewed telephonically. Screening for extracolonic malignancies was not routinely offered.

Evidence of genetic anticipation was sought in two ways: Firstly, subjects were grouped as parent–child pairs and individuals were compared with their own offspring; the second method was to group them by generation within the family tree. In both cases, analyses were done comparing only those subjects who had developed CRC, and then repeated using the entire cohort and applying standard survival analysis techniques to correct for differences in follow up time. In addition, the analyses were repeated including only those subjects born before 1960 (i.e. those with a potential follow up time of 50 years).

Statistics

Age of onset of CRC was not normally distributed, so non-parametric techniques were used to compare groups. Parent–child pairs with CRC were compared using the Wilcoxon (paired) test. Age of onset of CRC (in individuals with CRC) was compared between generations using the Kruskall–Wallis test. These analyses were repeated for the entire cohort (including those who had not yet developed CRC within the study period) using the Kaplan–Meier technique, and comparison between groups was done using the log rank method. For the Kaplan–Meier analyses, subjects were ‘censored’ if they had not developed CRC by the end of the follow up period, if they had died before developing the disease or (in one case) where the subject had undergone a prophylactic subtotal colectomy for a benign polyp. In order to avoid introducing lead-time bias into these analyses, CRC risk was calculated from birth rather than from diagnosis or enrolment in the study. Numbers are stated as median (95% CI for the median) unless otherwise stated. Statistical analysis was done using Medcalc® (Mariakerke, Belgium) software.

Ethics

All subjects were counseled and gave informed consent for genetic testing. The Health Sciences Faculty Research Ethics Committee of the University of Cape Town approved the study.

Results

The family tree consisted of 714 subjects over five generations. Of these, 257 have undergone germline mutational analysis. Eighty of these 257 subjects (31%) carried the exon 13 (C1528T) hMLH1 mutation, and 176 (69%) were found not to be carriers. Of the 457 who have not undergone genetic testing, 12 were determined to be obligate carriers as they had children proven to carry the mutation. The cohort for analysis therefore consisted of a total of 92 subjects (80 with a proven mutation, and 12 obligate carriers), of whom 50 were men and 42 women. These 92 subjects were born between 1913 and 1979, and have been followed up to a median age of 38 years (range 19–80). Thirty-three have had a colorectal cancer, and 22 have
No evidence of genetic anticipation in a large family

died. The Kaplan–Meier estimate of the median age of first CRC was 49 years.

Parent–child pairs

Seventy-five parent–child pairs were identified from the 92 subjects in the study cohort. Of these, there were 22 parent–child pairs in whom both the parent and child had developed CRC. When analyzing only those pairs in which both parents and children had developed CRC, the median age of first CRC was 45 years (44.7–55.0) for the parents, and 36 years (30.8–44.0) for the children ($P = 0.0004$, Wilcoxon test, Fig. 1a). This apparent effect persisted when we included only those parent–child pairs with CRC born before 1960 (45 years (33–52) for the parents versus 32 years (26–38)) for the children ($P = 0.02$). When we analyzed the entire group of 75 parent child pairs (using the Kaplan–Meier technique and censoring those who had not developed CRC at the end of the follow up period), this apparent effect disappeared ($P = 0.51$, Logrank test, Fig. 1b).

Age of onset of CRC grouped by generation

The 92 subjects studied were born over five generations, summarized in Table 1. When analyzing only those subjects who had developed CRC, there was an apparent decrease in the age of onset of first CRC over the generations ($P = 0.0091$, Kruskall–Wallis test, Fig. 2a). This apparent effect persisted when only the 24 subjects with CRC who were born before 1960 were included ($P = 0.029$, Kruskall–Wallis test). When the entire cohort was analyzed using the Kaplan–Meier technique, and censoring those without CRC by the end of the follow up period, no difference was detected ($P = 0.59$, Logrank test, Fig. 2b). A similar result was obtained when generations one and five (which had very small numbers) were excluded from the analysis ($P = 0.37$, Logrank test).

Conclusion

We could not identify any evidence of genetic anticipation in this study of mutation carriers from a single family with Lynch syndrome.

Genetic anticipation occurs in several neurodegenerative disorders, including Huntington’s chorea [14] and spinocerebellar ataxia [15–17]. In these diseases, the observed generational expansion of trinucleotide repeats during meiosis and gametogenesis provides a molecular mechanism to explain the earlier onset and worse prognosis of affected individuals in successive generations [18], but there is no direct evidence that this occurs in Lynch syndrome. Indeed, in a mouse model of Huntington disease, mismatch repair gene deficiency has been shown to prevent instability of trinucleotide repeats [19]. It has been proposed that germline mismatch repair gene defects may lead...
Fig. 2 Age of onset of colorectal cancer (subjects grouped by generation). a Includes only affected individuals. b Is a Kaplan–Meier plot including the entire cohort.

to an accumulation of small errors in DNA replication prior to loss of heterozygosity, and that this could be passed on over the generations, however there is little direct evidence that this occurs [18]. In Li-Fraumeni syndrome, anticipation has been found to be linked to decreasing telomere length [20] over generations. Bozzao et al. [21] have recently described abnormalities in telomere length in carriers of MSH2 mutations, but not MLH1 mutation carriers. This is an intriguing and developing area of research, but a definitive molecular mechanism for anticipation in Lynch syndrome has not yet been proven.

Previous studies of genetic anticipation in Lynch syndrome have variably reported the presence or absence and extent of this phenomenon. These studies are summarized in Table 2. Tsai et al. [7] and Westphalen et al. [9] found no evidence of genetic anticipation, whereas other authors [3, 5, 6, 8, 10–13] described an effect ranging from three [11, 13] to ten [13] years. A striking feature of this literature is the variability in reported anticipation, even when subjects from similar databases have been studied using different methodologies. Vasen et al. [5] studied 74 patients with CRC from the Foundation for the Detection of Hereditary Tumours in the Netherlands, and reported an average anticipation of 8.5 years. Voskuil et al. [8] identified 1,186 subjects from the same registry 3 years later, and could not detect any evidence of anticipation. Larsen et al. [11], Nilbert et al. [12] and Boonstra et al. [13] reported an anticipation effect in subjects from the Danish HNPPC Registry ranging between three and 9.8 years depending on the methodology used.

In this study, the appearance of genetic anticipation was falsely detected when only individuals who had developed CRC were included in the analysis. This is as a result of inadequate follow up bias. This occurs when older subjects, who have passed through more of their period of risk, are compared with younger ones who have not. A subject who has not yet manifest the disease, but may have gone on to do so later, is not included in the analysis. There are more such subjects in the later generations, and so the average age of onset appears (falsely) lower in the more recent cohort [22]. This bias can be corrected for by including only subjects who were born sufficiently long ago that they have completed their period at risk. We repeated our analyses including only subjects born before 1960, with a similar result. As the median age of onset of CRC in Lynch syndrome is 45 years, and they continue to be at risk at least into their seventies, one would need to only include subjects born at least 80 years ago to fully correct for this type of bias. In previous studies, Nilbert et al. [12] found that genetic anticipation could be detected even when only subjects with more than 80 years of potential follow up were considered, whereas Tsai et al. [7] found that the apparent difference in age of onset or CRC between parents and their children was no longer detectable when this birth cohort effect was taken into account. We did not have sufficient numbers of such old patients to test this, and indeed genetically proven Lynch syndrome carriers born more than 80 years ago would be rare in any cohort. When we corrected for follow up bias by including the entire cohort and applying Kaplan–Meier survival techniques (censoring those who had not yet developed CRC), the apparent anticipation effect was no longer detected. In previous studies aimed at detecting genetic anticipation in Lynch syndrome, there was a significant difference in reported anticipation between those studies in which survival type statistical analyses were performed on cohorts of subjects at risk (mean of 2 years) and those in which only individuals who had developed CRC were studied (mean 7.5 years) (P = 0.0073, Student t test).
Table 2: Previous published studies of genetic anticipation in Lynch syndrome

<table>
<thead>
<tr>
<th>References</th>
<th>Patient set</th>
<th>Numbers</th>
<th>Average anticipation (years)</th>
<th>Survival analysis done</th>
</tr>
</thead>
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<tr>
<td>Wartolin [3]</td>
<td>“Family G”</td>
<td>28 patients with CRC</td>
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<tr>
<td></td>
<td>Foundation for the Detection of Hereditary Tumours (Netherlands)</td>
<td>over 4 generations</td>
<td></td>
<td></td>
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<tr>
<td>Vanp et al. [5]</td>
<td>Foundation for the Detection of Hereditary Tumours (Netherlands)</td>
<td>74 patients with CRC</td>
<td>8.5</td>
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</tr>
<tr>
<td></td>
<td>Roosevelt Park Cancer Institute HNPPC Registry</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rodriguez-Bigas [6]</td>
<td>Johns Hopkins Hereditary Colorectal Cancer Registry</td>
<td>193 patients with CRC</td>
<td>5.5</td>
<td>No</td>
</tr>
<tr>
<td>Tsai et al. [7]</td>
<td>Johns Hopkins Hereditary Colorectal Cancer Registry</td>
<td>67 parent-child pairs with CRC</td>
<td>0</td>
<td>No</td>
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<tr>
<td>Voskui et al. [8]</td>
<td>Foundation for the Detection of Hereditary Tumours (Netherlands)</td>
<td>1,186 subjects</td>
<td>0</td>
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<td>Westphalen et al. [9]</td>
<td>University of Basel and Institut Central des Hopitaux Valaisans registrees</td>
<td>55 parent-child pairs with CRC</td>
<td>8</td>
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<td>Stellas et al. [10]</td>
<td>Five Italian families</td>
<td>24 parent-child pairs with CRC</td>
<td>11</td>
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<td>Danish HNPPC Registry</td>
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<td>3</td>
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<td>Danish HNPPC Registry</td>
<td>290 parent-child pairs with CRC</td>
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<td>Danish HNPPC Registry</td>
<td>290 parent-child pairs with CRC</td>
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<td>University of Michigan Cancer Genetics Clinic</td>
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<tr>
<td>Boonstra et al. [13]</td>
<td>Danish HNPPC Registry</td>
<td>816 subjects</td>
<td>3</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* The full paper included 475 parent-child pairs with CRC, but only 67 of these had Lynch syndrome.
* Different estimates of anticipation were calculated depending on the method of analysis.
* Boonstra et al. [13] analyzed different datasets in a number of different ways. These analyses (labelled as Boonstra et al. 2010 a–c in the table) have been listed separately although they are from the same paper. Larsen et al. [11] and Boonstra (c) used a similar dataset.

Genetic anticipation may also be falsely detected due to ascertainment bias. The chance of detecting affected families with predominantly younger patients in later generations is higher than that of finding families with predominantly older patients in later generations. This is because individuals in later generations have not lived long enough to detect cancers that may develop later in their lives [8]. A number of statistical methods have been described to correct for variable ascertainment when subjects from multiple families are studied (reviewed by Boonstra et al. [13]). By including only subjects from a single family, we reduced the risk of ascertainment bias that could occur studying multiple families, and allowed simpler statistical methods to be applied. This also allows subjects to be accurately grouped according to their generation in the single pedigree.

A further confounder in this study of genetic anticipation in Lynch syndrome is the effect of surveillance. We have previously shown that colonoscopic surveillance delays the onset of CRC in carriers of this family’s hMLH1 mutation [23]. As the oldest subjects developed CRC before the surveillance program was instituted, this could cause a real anticipation effect to be falsely missed. The sample size in this study may also have been insufficient to detect the small anticipation effect (of 3 years) detected in previous studies of large population databases [11, 13]. The findings of a single family may also not be generalisable to other families and to other mismatch repair gene mutations.

This study highlights the methodological difficulties in studying genetic anticipation in inherited cancers such as Lynch syndrome.

References

5.1.5 PAPER V

Fertility and apparent genetic anticipation in Lynch syndrome

Douglas Stupart · Aung Ko Win · Mark Jenkins · Ingrid M. Winship · Paul Goldberg · Rajkumar Ramesar

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Abstract Genetic anticipation is the phenomenon in which age of onset of an inherited disorder decreases in successive generations. Inconsistent evidence suggests that this occurs in Lynch syndrome. A possible cause for apparent anticipation is fecundity bias, which occurs if the disease adversely affects fertility. The purpose of this study was to determine the effect of age of diagnosis of colorectal cancer (CRC) on lifetime fertility in Lynch syndrome, and whether this can falsely create the appearance of genetic anticipation. A computer model simulated age of diagnosis of CRC in hypothetical Lynch syndrome carriers and their offspring. The model assumed similar age distribution of CRC across generations (i.e. that there was no true anticipation). Age distribution of CRC diagnosis, and lifetime fertility rates (grouped by age of diagnosis of CRC) were determined from the Australasian Colorectal Cancer Family Registry (ACCFR). Apparent anticipation was calculated by comparing ages of diagnosis of CRC in affected parent-child pairs. A total of 1,088 patients with CRC were identified from the ACCFR. Total lifetime (cohort) fertility was related to age of diagnosis of CRC (correlation coefficient 0.13, \( P = 0.0001 \)). In the simulation, apparent anticipation was 1.8 ± 0.54 years \( (P = 0.0044) \). Observed apparent anticipation in the ACCFR cohort was 4.8 ± 1.73 years \( (P = 0.0064) \). There was no difference in apparent anticipation between the simulate d and observed parent-child pairs \( (P = 0.89) \). The appearance of genetic anticipation in Lynch syndrome can be falsely created due to changes in fertility.

Keywords Lynch syndrome · Genetic anticipation · Fertility

Introduction

Lynch syndrome is the commonest known inherited predisposition to colorectal cancer (CRC). CRC risk is inherited in an autosomal dominant manner, caused by germ line mutations in the DNA mismatch repair (MMR) genes MLH1, MSH2, MSH6 and PMS2. The age- and gender specific risk of colorectal cancer is increased 20-500-fold [1] and the average age of diagnosis of colorectal cancer decreases from 69 years in the general population [2] to around 45 years [3] in carriers of these mutations.

Genetic anticipation is the phenomenon in which the age of onset of an inherited disorder decreases in successive generations. This has been shown to occur in a number of
inherited neurodegenerative disorders, including Huntington disease [4]. There is some evidence, albeit inconsistent, to suggest genetic anticipation for CRC in Lynch syndrome [5].

The assessment of anticipation for any disease is complicated because it is well known that the false appearance of genetic anticipation may be caused by various forms of ascertainment bias. In cross-sectional studies, individuals from earlier generations are always older than their offspring by definition, and have therefore lived through more of their period at risk. Subjects from later generations who have not yet manifest the disease are excluded from the analysis even though they may have gone on to develop the disease later in their life, thereby lowering the apparent average age of onset in the more recent generation. This is referred to as follow-up bias [6]. Similarly, families with predominantly younger diagnoses in later generations are preferentially identified as being at high risk for Lynch syndrome (and are therefore more likely to be included in family cancer registries) compared with families with predominantly older patients in later generations (this occurs because individuals in later generations have not lived long enough to detect cancers that may develop later in their lives [7, 8]).

Another potential cause of apparent anticipation is referred to as fecundity bias. This occurs if the disease adversely affects fertility, so individuals who develop the disease at a younger age are likely to have fewer children than those who are diagnosed at an older age. No previous studies of anticipation in Lynch syndrome (or to our knowledge any inherited cancer) have examined whether fecundity bias can mimic genetic anticipation, and therefore be the explanation for any apparent anticipation.

The purpose of this study was (1) to determine the effect of age of onset of CRC on fertility in Lynch syndrome, (2) to determine by computer simulation whether observed changes in fertility can falsely create the appearance of genetic anticipation, and to what extent, (3) to compare the results of that simulation with the observed appearance of genetic anticipation in a large series of families with Lynch syndrome.

**Methods**

The Australasian Colorectal Cancer Family Registry (ACCFR) is a registry of more than 11,500 people from 1,800 families in Australia and New Zealand [9]. This registry contains CRC families recruited through the Victorian Cancer Registry (960 population-based case-families) and from family cancer clinics throughout Australia and New Zealand (580 clinic-based case-families), as well as families of people without CRC recruited through the Victorian electoral roll (270 control-families). For all family members, personal and family history of cancer, reproductive history, and other lifestyle and personal characteristics were collected by questionnaire. Attempts were made to verify all reports of CRC diagnoses by medical records, pathology reports, death certificates and linkage to national cancer registry and death registry databases.

Individuals who had developed CRC, and who were members of families known to carry MMR gene mutations (Lynch syndrome families) were categorised by age of diagnosis of CRC. Cohort fertility (the mean number of children born to each individual by the end of their reproductive life: defined as over the age of 50 years) was calculated for each age group of CRC diagnosis (for both men and women). We only included subjects born before 1963 in this analysis in order to include only those who had completed their period of potential fertility.

**Model design**

A computer model was designed to simulate the age of diagnosis of CRC in large numbers of hypothetical MMR gene mutation carrying men and women (first generation) and their offspring (second generation). The model assumed complete follow-up over the lifetime for all individuals, and assumed the age distribution of CRC diagnoses to be the same across generations (i.e. that there was no genetic anticipation). Because follow up was over the entire lifetime of the hypothetical subjects in both generations, and the complete lifetime risk was applied to each subject, follow up (ascertainment) bias will not cause the appearance of genetic anticipation in this model. In this setting any apparent genetic anticipation would be an artefact.

The model generated an equal number of men and women in the first generation, and allocated gender at random (with a 50:50 chance) to the second generation individuals.

**Age of diagnosis of first CRC** was randomly assigned according to the (gender-specific) observed distribution of age of first CRC diagnosis from the ACCFR cohort. The number of offspring born to each first generation carrier was randomly assigned according to the observed (gender-specific) distribution of lifetime fertility according to the age of diagnosis of CRC as calculated above. Each second generation individual was given a 50% chance of inheriting the MMR gene mutation. For each mutation-carrying child, age of diagnosis of first CRC was allocated in the same way as for their parents. The simulation was run for 1,000 first generation subjects. Ages of diagnoses of CRC were compared between the simulated parents and their affected children.
Fertility and apparent genetic anticipation

Fig. 1 Cohort (lifetime) fertility rates versus age of diagnosis of CRC

Model validation: observed apparent anticipation in the ACCFR cohort

The appearance of genetic anticipation in the ACCFR cohort was sought by comparing the age of diagnosis of first CRC between parents with their affected children (‘parent–child pairs’). Parent–child pairs were identified if both the parent and child had been diagnosed with CRC. Subjects were included if they were proven mutation carriers, or if their mutation status was unknown but their family was known to carry a MMR gene mutation. This analysis was repeated using only parent–child pairs in whom the children were born more than 80 years ago. This minimizes the chance of incomplete follow up of the children falsely lowering the apparent age of diagnosis of CRC relative to their parents (follow up bias), and is in keeping with the methodology used by Nilbert et al. [10] to correct for the birth cohort effect that can falsely create the appearance of anticipation.

Statistical analysis

Mean age of diagnosis of first CRC was compared using the Student’s t test. A P-value of 0.05 was regarded as statistically significant. Correlation was determined using Spearman’s rank method. Statistical analysis was done using MedCalc for Windows (MedCalc Software, Ostend, Belgium). All results are stated as mean ± SE of the mean unless otherwise specified.

Ethical approval for the study was granted by the University of Melbourne Ethics Committee.

Results

Cohort fertility in the ACCFR

The ACCFR database contained complete data for 9,351 members of 295 families known to carry Lynch syndrome mutations. Of these, 1,088 patients (568 men and 520 women) have been diagnosed with CRC. The mean age of diagnosis of CRC was 46.8 ± 14.3 year (46.3 ± 13.3 for the men, and 47.3 ± 15.4 for the women, P = 0.24).

A total of 981 (512 male and 469 female) patients with CRC were born before 1965, and the cohort fertility rates were calculated from this group. Cohort fertility grouped by age of diagnosis of CRC is illustrated in Fig. 1. Total lifetime (cohort) fertility was related to age of diagnosis of CRC in men (correlation coefficient 0.143, P = 0.0012), women (correlation coefficient 0.104, P = 0.04) and overall (correlation coefficient 0.13, P = 0.0001).

Simulation

Using the above parameters, the simulation was run for 1,000 first generation mutation carriers. This generated 1,169 simulated offspring who were mutation carriers. The mean difference in age of diagnosis of first CRC between simulated parents and their mutation positive offspring (apparent anticipation) was 1.8 ± 0.54 years (P = 0.0044). Apparent anticipation was similar for male (1.1 ± 0.77) and female (1.9 ± 0.72) simulated parents (P = 0.44).
Apparent anticipation in the ACCFR cohort

A total of 461 parent–child pairs with CRC were identified within the ACCFR study cohort. The mean age of diagnosis of first CRC was 51.1 ± 0.63 years in the parent group, and 42.3 ± 0.56 years in their children (P < 0.0001). When we included only those parent-child pairs with a potential follow up of over 80 years (i.e. only subjects born before 1933), 120 parent-child pairs were identified. In this group, the mean age of diagnosis of first CRC was 53.9 ± 0.68 in the parent group, and 49.1 ± 0.67 in their children (apparent anticipation 4.8 ± 1.73 years, P = 0.0064). There was no significant difference in apparent anticipation between the simulated (1.8 years) and observed (4.8 years) parent-child pairs (P = 0.89).

Discussion

This simulation demonstrates that the appearance of genetic anticipation in Lynch syndrome can be created due to changes in lifetime fertility in MMR gene mutation carriers with CRC. The apparent anticipation predicted by the model was not significantly different from the observed appearance of anticipation in the AFCCS families with Lynch syndrome which was in keeping with the observed anticipation in large studies of the Dutch HNPPC registry of between 3 and 9 years published by Larson et al. [11] and Boonstra et al. [8].

We observed a marked decrease in lifetime fertility in mutation carriers with early diagnosis of CRC compared with those who developed CRC later in life. For example, women diagnosed with CRC between ages 20 and 24 year gave birth to a mean of 1.2 children in their lifetime compared with women diagnosed with CRC after age 50 years who gave birth to a mean of 2.8 children in their lifetime. The reasons for the reduction in fertility after CRC in these patient groups have not been studied here but cancer-related mortality and morbidity, the effects of surgery, chemotherapy and radiotherapy, and personal choice can all be expected to play a role. We are unaware of any previous studies that have documented fertility rate after CRC in Lynch syndrome although reduced fertility is well recognized in patients who receive chemotherapy and radiotherapy, and is suspected to occur after surgery for CRC [12]. It is this reduction in fertility that contributed to apparent anticipation in our model.

A number of authors have studied genetic anticipation in Lynch syndrome, with differing conclusions as to whether it occurs and to what extent (this literature is summarize in Table 1). Tsai et al. [13], Westphalen et al. [14] and Stuwart et al. [15] found no evidence of genetic anticipation, but others have reported its occurrence [7, 8, 10, 11, 16–19]. There is variability in the degree of reporte anticipation (from 3 to 10 years) even when similar patient registries have been analysed using different methods. A potential cause for apparent genetic anticipation is follow-up bias [6]. This can be corrected for by including

<table>
<thead>
<tr>
<th>Paper</th>
<th>Data set</th>
<th>Numbers</th>
<th>Average anticipation (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wathin et al. [16]</td>
<td>“Family G”</td>
<td>28 patients with CRC over four generations</td>
<td>8</td>
</tr>
<tr>
<td>Vassen et al. [17]</td>
<td>Foundation for the detection of hereditary tumours (Netherlands)</td>
<td>74 patients with CRC over three generations</td>
<td>8,5</td>
</tr>
<tr>
<td>Rodriguez-Bigas et al. [18]</td>
<td>Roswell Park Cancer Institute HNPPC Registry</td>
<td>193 patients with CRC</td>
<td>5,5</td>
</tr>
<tr>
<td>Tsai et al. [13]</td>
<td>Johns Hopkins Hereditary Colonoma Cancer Registry</td>
<td>67 parent-child pairs with CRC*</td>
<td>0</td>
</tr>
<tr>
<td>Voskuil et al. [7]</td>
<td>Foundation for the Detection of hereditary tumours (Netherlands)</td>
<td>1,186 subjects</td>
<td>0</td>
</tr>
<tr>
<td>Westphalen et al. [14]</td>
<td>University of Basel and Institut Central des Hopitaux Vaalisa regisries</td>
<td>55 parent-child pairs with CRC</td>
<td>8</td>
</tr>
<tr>
<td>Stella et al. [19]</td>
<td>Five Italian families</td>
<td>24 parent-child pairs with CRC</td>
<td>11</td>
</tr>
<tr>
<td>Larsen et al. [11]</td>
<td>Danish HNPPC Registry</td>
<td>824 subjects</td>
<td>3</td>
</tr>
<tr>
<td>Nilbert et al. [10]</td>
<td>Danish HNPPC Registry</td>
<td>290 parent-child pairs with CRC</td>
<td>5,5–9,8</td>
</tr>
<tr>
<td>Boonstra et al. [8]</td>
<td>Danish HNPPC Registry</td>
<td>815 parent or 290 parent-child pairs with CRC</td>
<td>3 or 8,7</td>
</tr>
<tr>
<td>Boonstra et al. [8]</td>
<td>University of Michigan Cancer Genetics Clinic</td>
<td>136 parent-child pairs with CRC</td>
<td>9,9</td>
</tr>
<tr>
<td>Stuwart et al. [15]</td>
<td>Single South African family</td>
<td>92 mutation carriers</td>
<td>0</td>
</tr>
</tbody>
</table>

Boonstra et al. [8] described findings from two different patient sets in the same article. Nilbert et al. [10] and Boonstra et al. [8] found different degrees of apparent anticipation depending on the statistical method used to analyze the data sets.

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only subjects who were born sufficiently long ago that they have completed their period at risk [10] (in this case by including only subjects (from both generations) who were born at least 80 years ago). This type of bias cannot fully explain the observed appearance of anticipation in parent-child pairs from the ACCFR as it still existed when we only included mutation carriers with more than 80 years of potential follow up (i.e. born before 1933). This is in keeping with the findings of Nilbert et al. [10], whereas Tsai et al. [13] found no difference in age of diagnosis of CRC between parents and their children when the birth cohort effect was taken into account.

We included subjects with CRC who were not known MMR gene mutation carriers (but were from families known to carry MMR gene mutations) as well as those who were confirmed to carry mutations in the MMR genes, in keeping with the methodology of previous authors [10, 13]. This was to allow inclusion of a sufficient number of patients with adequate potential follow up. One would expect the great majority of subjects with CRC born before 1933 to have died before mutation analysis became available. In the ACCFR database, there were 2,128 individuals born before 1933, of whom 433 (20.3 %) were diagnosed with CRC. Of these 433, only 59 (13.6 %) have undergone genetic testing.

Actual genetic anticipation does occur in several neurodegenerative disorders, including Huntington disease [4] and spinocerebellar ataxia [20–22] by the molecular mechanism of generational expansion of trinucleotide repeats during meiosis and gametogenesis [23]. In Li-Fraumeni syndrome, anticipation has been found to be associated with decreasing telomere length over generations [24]. There is no evidence that this occurs in Lynch syndrome, and in a mouse model of Huntington disease MMR gene deficiency has been shown to prevent this from occurring [25]. It has been hypothesised that germline MMR gene defects may lead to an accumulation of small errors in DNA replication prior to loss of heterozygosity which could be passed on over the generations but direct evidence that this occurs is lacking [23]. Boozat et al. [26] have recently described abnormalities in telomere length in carriers of MSH2 mutations, but not MLH1 mutation carriers. This is a developing area of research, but a definie molecular mechanism for anticipation in Lynch syndrome (if it occurs) has yet to be found.

In conclusion, we have shown that fecundity bias can falsely create the appearance of genetic anticipation in Lynch syndrome. This highlights the statistical complexity of studying genetic anticipation and the ongoing uncertainty of whether the phenomenon occurs in this disease.

References


Fertility after young onset colorectal cancer: a study of subjects with Lynch syndrome

Douglas Stupart
Department of Surgery, Deakin University, Geelong, Victoria, Australia

Aung Ko Win, Mark Jenkins
Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, Melbourne School of Population and Global Health, The University of Melbourne, Victoria, Australia

Ingrid M. Winship
Genetic Medicine and Family Cancer Clinic, Royal Melbourne Hospital, Victoria, Australia

Corresponding Author:
Douglas Stupart
Dept. Surgery
Geelong Hospital
Ryrie street
Geelong
3227 VIC
Australia

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Abstract

Aim: Infertility is a concern for young colorectal cancer (CRC) survivors, but this risk is not well quantified. Mismatch repair (MMR) mutation carriers are a useful cohort for studying fertility after CRC as they commonly develop CRC when young, and unaffected family members provide demographically similar controls. The aim of this study was to determine the effect of CRC on fertility in a large cohort of MMR mutation carriers.

Method: MMR mutation carriers identified from the Australasian Colorectal Cancer Family Registry were included. For each year of life within the fertile age range (15 to 49), the number of living individuals and the number of children born to them were determined. Individuals were grouped by whether or not they had had a diagnosis of CRC by that age. Age-specific and total fertility rates were calculated.

Results: 1068 subjects (611 women and 457 men) were identified, of whom 467 were diagnosed with CRC. There were 1,192 births during 18674 person-years of follow up of the women and 814 births during 14013 person-years of follow up to the men.

The total fertility rate was decreased in women after a diagnosis of CRC compared who did not have CRC (1.3 vs. 2.2 P=0.0011), but age-specific fertility was only reduced in the 20-24 year age group. In men TFR was similar for both groups (2.0 vs. 1.8) P = 0.27).

Conclusion: Age-specific fertility was decreased in female CRC survivors with Lynch syndrome aged 20-24, but not in older women or in men.

What does this paper add to the literature?

This is the first study to report the effect of colorectal cancer on age-specific fertility in women stratified by age groups, and the first to study the effect on fertility overall in men.
Introduction

Colorectal cancer (CRC) is a common cancer in industrialised nations. In the United States, there are 143,000 new cases per year, of whom approximately 4% are under 45 years of age[1]. Among young CRC survivors, potential infertility is known to be a significant concern [2]. The American Society of Clinical Oncology (ASCO) has published guidelines recommending that oncologists discuss the possibility of infertility with their patients, while acknowledging that in many cases there are insufficient data available to accurately assess this risk [3]. This caveat is especially pertinent in the case of CRC, as only one previous study[4] has attempted to quantify the effect of gastrointestinal cancer on fertility. This was a Swedish population-based cohort study of young (under 45) female cancer survivors. The authors reported a ten per cent reduction in overall (age-adjusted) fertility for female survivors of gastrointestinal cancers when compared with the general population. No previously published studies have documented age-specific fertility rates for female CRC survivors, and fertility rates in male CRC survivors have not been reported.

Lynch syndrome is the commonest known inherited predisposition to colorectal cancer (CRC). It is caused by germ line mutations in the DNA mismatch repair (MMR) genes MLH1, MSH2, MSH6 and PMS2, and is characterised by young onset CRC (the median age of onset is around 45 years [5], compared with 69 years in the general population [1]). Lynch syndrome families provide a useful cohort for studying the effect of CRC on fertility, as CRC in this population commonly occurs in subjects who are within the potentially fertile age group, and unaffected family members can be used as demographically similar controls. The aim of this study was to determine the effect of CRC on age-specific fertility rates in a large cohort of subjects who carry MMR gene mutations.
Method

Ethical approval for the study was given by the University of Melbourne Research Ethics Committee. The Australasian Colorectal Cancer Family Registry (ACCFR) includes more than 11,500 subjects from 1800 families in Australia and New Zealand[6]. It contains CRC families recruited through the Victorian Cancer Registry and from family cancer clinics throughout Australia and New Zealand. Personal and family history of cancer and reproductive history were collected by questionnaire. Attempts were made to verify all reports of the diagnosis of CRC and date of death by medical records, pathology reports, death certificates and linkage to national cancer registry and death registry databases. Subjects identified from the ACCFR with proven germline mismatch repair gene mutations were included in this study. Methods for screening and testing for MMR gene mutations have been described in detail elsewhere [7].

The period of potential fertility was considered to be between the ages 15 to 49, in keeping with the World Health Organisation[8] and Australian Bureau of Statistics[9] norms for describing population fertility rates. For each year of life within this age range, the total number of subjects known to be alive at that age and the number of children born to them were determined. Subjects in each year of life were grouped according to whether they had been diagnosed with CRC by that age or not. This meant that a subject who, for example was diagnosed with CRC at age 29 would be counted in the ‘no CRC diagnosis’ group from ages 15 to 28 years, and in the ‘after CRC diagnosis’ group for each year of life from age 29 until age 49, death or last follow up.

Age-specific fertility rates (ASFR) were calculated by dividing the total number of children by the total number of subjects in each age group. Age-specific fertility rates are stated as births per 1000 person-years throughout. The total fertility rate (TFR) is the average number of children a hypothetical cohort of women would have had at the end of their reproductive life if they had children at the population age-specific rates during their whole life and survived to the end of their fertile period. It is expressed as children per woman and is calculated as the sum of age-specific fertility for the ages 15 to 49 (multiplied by five if five-yearly age strata are used)[8]. Fertility rates were compared between those subjects who had been diagnosed with CRC and those who had not.

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Female fertility rates were also compared with the known fertility rates of the Australian female general population (1975 to 2010) as reported by the Australian Bureau of Statistics [9] (male fertility rates for the general population are not recorded).

Statistical Analysis

Age-specific fertility rates were compared using the Chi square test. Confidence intervals (CI) for age-specific fertility rates and total fertility rate were calculated using Byar’s method for direct standardisation as described by Breslow and Day [10]. Confidence intervals for total fertility rates were calculated using the widely accepted method described by Dobson et al [10, 11][12]. A p-value of <0.05 was regarded as showing a statistically significant difference. Statistical analysis was done using Microsoft Excel 2010 (Redmond, Washington) and Medcalc® 2008 (Mariakerke, Belgium) software.

Results

The study included 1068 subjects (611 women and 457 men) with proven germline MMR mutations of whom 467 were diagnosed with CRC. There were 417 with colonic and 82 with rectal cancer (32 subjects were diagnosed with metachronous cancers in both sites). Among subjects under the age of fifty, there were a total of 322 CRC diagnosed (285 colon and 45 rectum). They were followed up to a median age of 55 (18 to 96) years.

For subjects aged 15 to 49 years, there was a total of 1,192 children born during 18674 person-years of follow up for the women and 814 births during 14013 person-years of follow up for the men. No births occurred in subjects under 15 years of age. No women in this cohort gave birth over the age of 49. Four children were fathered by men over 49 years of age. Age-specific fertility rates for all women from the study cohort, regardless of CRC diagnosis and from the Australian general

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population are presented in Table 1 and Figure 1. Age-specific fertility rate was higher in the study group than the Australian general population for women in the 15-19, 20-24 and 25-29 age groups, but not in other age groups. Total fertility rate for women in the study group was 2.2 (95% C.I. 2.0 to 2.3), which was higher than for women in the Australian general population (TFR 1.87).

Table 2 shows the age-specific fertility rates for study subjects with or without a CRC diagnosis. These are illustrated in Figures 2 and 3. For women in the 20-24 year age group, age specific fertility was lower in those with a CRC diagnosis (0 births/1000 person-years in those who had been diagnosed with CRC vs. 128 births/1000 person-years in subjects who had not been diagnosed with CRC, P=0.019). There was no statistically significant difference in age-specific fertility rates between women with or without a CRC diagnosis in any other age group. There was no statistically significant difference in age-specific fertility rates between men who had been diagnosed with CRC compared with those without a CRC diagnosis in any age group.

Overall, total fertility rate was lower in women with a CRC diagnosis compared with those without (1.3 [95% C.I. 0.90 to 1.8] vs. 2.2 [95% C.I. 2.1 to 2.3] P=0.0011). In men, there was no significant difference in TFR between the two groups (2.0 [95% C.I. 1.8 to 2.1]) for men without a CRC diagnosis vs. 1.8 [95% C.I. 1.1 to 2.7] for those with a CRC diagnosis. P = 0.27).

Subjects diagnosed with colon or rectal cancers were also analysed separately, and these results are shown in Table 3. For women with a colon cancer diagnosis, the results were similar to the findings for CRC overall. Age-specific fertility rate for women with a colon cancer diagnosis in the 20-24 year age group was reduced, with 0 births/1000 person-years compared with 128 births/1000 person years for women without a CRC diagnosis(P=0.017). Age-specific fertility rates for women with a colon cancer diagnosis for all other age groups were similar to those for women without a CRC diagnosis. Total fertility rate for women with a colon cancer diagnosis was reduced compared to women without a CRC diagnosis (1.5 [95% C.I. 1.1-2.1] vs. 2.2 [95% C.I.2.1-2.3], P=0.016). For women
who were diagnosed with rectal cancer, there was no significant difference in any of the age-specific fertility rates compared with women without a CRC diagnosis, but there was an overall reduction in total fertility rate (0.72 (95% C.I. 0.2-1.7) vs. 2.2 (95% C.I. 2.1-2.3), P=0.015). There were no significant differences in age-specific fertility rates for men with a diagnosis of colon or rectal cancer compared with subjects without a CRC diagnosis. Total fertility rate for men after colon cancer was similar to that for men without a CRC diagnosis (1.7 (95% C.I. 1.0 to 2.5) vs. 1.8 (95% C.I. 1.1-2.7), P= 0.72). TFR for men after a rectal cancer diagnosis was also similar to men without a CRC diagnosis (1.84 (95% C.I. 0.39-5.0) vs. 1.8 (95% C.I. 1.1-2.7), P=0.38.

Discussion

We have previously documented a reduction in the total (lifetime) number of children born to subjects from the ACCFR cohort who are diagnosed with CRC at a young age [13], but it was not known whether this was due to a reduction in fertility among survivors, or if it was simply due to decreased survival. In this study of colorectal cancer survivors who carry germline MMR mutations, we found that total fertility for women was decreased by approximately 40% after a diagnosis of CRC. The reduction in age-specific fertility rates could only be detected in younger women (age 20 to 24), and was not apparent in other age groups. A CRC diagnosis did not adversely affect age-specific fertility in men. Both colonic and rectal cancer diagnoses were associated with decreased TFR in women, but no difference was detected for men with cancers in either site.

In two recent reviews, Spanos et al[14] and O’Neill et al[15] both found no evidence that colon cancer surgery or 5-fluorouracil chemotherapy affected fertility, but reported concerns about the effects of rectal surgery and radiotherapy and newer chemotherapy agents. Both emphasised the importance of adequate pre-treatment fertility counselling and discussed the merits of fertility preservation options that a CRC survivor could be offered. These include embryo preservation and

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ooocyte vitrification. Kumar et al [16] reported that discussion about fertility occurred in only one third of CRC patients under the age of 40 at their institution. They concluded that it was important to educate health care professionals about the importance of fertility risk, while acknowledging the lack of direct evidence for risk to fertility associated with colon surgery or chemotherapy.

The only previously published study directly comparing fertility rates of women with or without gastrointestinal cancer was by Hartman et al [4]. They reported a ten per cent reduction in fertility for women less than 45 years of age compared with the Swedish general population. Age-specific fertility rates in cancer survivors for a number of different tumours, including gastrointestinal, were compared with those of the general Swedish population using the technique of indirect standardisation. This involves determining age-specific birth rates for the reference population, and applying these to the study population to calculate the expected number of events for that population. The authors calculated a standardised birth rate (SBR, an indirectly standardised rate) for gastrointestinal cancer survivors of 0.90 (95% C.I. 0.83 to 0.97), but did not quote age-specific birth rates for the study population. Indirectly standardised rates can give misleading results, however, in any situation where the age profiles of the study and reference populations are not similar[11]. In the present study, we found the age distributions of subjects with or without a CRC diagnosis were markedly different. For this reason we used the TFR (calculated by the process of direct standardisation), in which the age distribution of the sample populations do not affect the calculation, and so groups with disparate age distributions[11] can be compared. This is discussed in full in the appendix.

A surprising finding in this study was that TFR for women with Lynch syndrome from the ACCFR was higher than that of the Australian population generally (fertility rates for men in the general population are not reported). The probable reason for this is selection bias, as larger families are more likely to be recruited by family cancer clinics. This example highlights the kind of sampling bias that can cause misleading interpretation of fertility rates in an affected population when compared
with the general population. It is unlikely to have affected the main result of this study, however, as the CRC and control groups were sampled from the same families.

The decision to have children is influenced by many psychological and social factors, which may bias studies of fertility between groups. We studied subjects from a large familial cancer database, so that unaffected family members could act as a control group (that would be as demographically and socially similar as possible to the affected individuals) and because of the high number of young CRC patients in this cohort. We only included proven germline MMR mutation carriers (as controls as well as patients post CRC) as individuals with Lynch syndrome may modify their reproductive behaviour to avoid passing on their mutation. They may decide not to have children, or even consider techniques such as pre-implantation genetic testing. For this study we did not attempt to collect information from subjects as to whether they had chosen to have children or not and what the reasons were for the decision. The data presented in this paper cannot determine whether the observed changes in fertility were related to CRC or to reproductive decisions made following the genetic diagnosis of Lynch syndrome (which often occurs after the CRC is diagnosed). Although MMR proteins are essential in DNA replication, there is no evidence in the current literature that Lynch syndrome directly affects fertility, and the high fertility rate in this cohort does not suggest that this occurs.

It is uncertain whether these findings are generalizable to patients with sporadic cancers. Colorectal cancers that display microsatellite instability (as in Lynch syndrome) are known to have a better prognosis than sporadic CRC and may be unresponsive to 5-fluorouracil based chemotherapy regimes [17]. In addition, CRC detected in subjects with Lynch syndrome, who are offered intensive surveillance, may be detected at an earlier stage than sporadic tumours.

It is expected that rectal cancer would have a greater impact on fertility than colon cancer (as a greater number receive radiotherapy, and pelvic surgery may be more likely to have a deleterious
effect on fertility). In Lynch syndrome, rectal cancers are less common (as a percentage of CRC overall), and in this cohort there were only 82 subjects with rectal cancer (of whom 45 were diagnosed under the age of 50), so this study was not powered to detect differences in age-specific fertility rates in subjects with rectal cancer. These results of this should therefore be interpreted with caution in for patients with rectal cancer, and may only be applicable to those with tumours of the colon. Another limitation of this study is the small numbers of subjects with CRC in the age groups under 25, and our data are underpowered to detect differences in fertility (if they exist) in this age group. Such patients are rare, and so it would be difficult to recruit large numbers of these subjects in any setting.

In conclusion, we found that age-specific fertility was decreased in women with Lynch syndrome in the 20 to 24 year age group after CRC diagnosis. Subjects of both genders who survived into their late twenties and beyond had no detectable reduction in age-specific fertility, which may be reassuring to colon cancer survivors who hope to have children.
<table>
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<th>Age groups</th>
<th>Number of births</th>
<th>Person-years</th>
<th>Fertility rate</th>
<th>95% C.I.</th>
<th>Australian population</th>
<th>Fertility rate</th>
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<td>20-24</td>
<td>382</td>
<td>3028</td>
<td>126</td>
<td>114-139</td>
<td>79</td>
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<td>25-29</td>
<td>413</td>
<td>2926</td>
<td>141</td>
<td>128-155</td>
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<td>30-34</td>
<td>244</td>
<td>2744</td>
<td>89</td>
<td>78-101</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>35-39</td>
<td>115</td>
<td>2544</td>
<td>45</td>
<td>37-54</td>
<td>41</td>
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<tr>
<td>40-44</td>
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<td>2301</td>
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<td>7.3</td>
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<tr>
<td>45-49</td>
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<td>0.4</td>
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</tr>
<tr>
<td>TFR</td>
<td>2.2</td>
<td></td>
<td>2.0-2.3</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 1.** Age-specific fertility rates of women from the entire study group and from the Australian general population.

No confidence intervals are given for the Australian population as these are from population census figures and not a sampled population. Age specific fertility rates for the study cohort which are significantly different from the general population rate are in bold type.
<table>
<thead>
<tr>
<th>Age groups</th>
<th>Births</th>
<th>Person-years</th>
<th>Fertility rate</th>
<th>95% C.I.</th>
<th>Births</th>
<th>Person-years</th>
<th>Fertility rate</th>
<th>95% C.I.</th>
<th>P-vi</th>
</tr>
</thead>
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<tr>
<td></td>
<td>No CRC diagnosis (women)</td>
<td></td>
<td></td>
<td>After CRC diagnosis (women)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>86</td>
<td>3045</td>
<td>28</td>
<td>23-35</td>
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<td>113</td>
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<table>
<thead>
<tr>
<th>Age groups</th>
<th>Births</th>
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<th>Fertility rate</th>
<th>95% C.I.</th>
<th>Births</th>
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TABLE 2. Age-specific fertility rates for subjects with or without a CRC diagnosis.

Age-specific fertility rates are given as births/1000 person-years. Total fertility rate (TFR) is given as births/person. The sample sizes for subjects aged 15-19 with a CRC diagnosis were insufficient to calculate meaningful confidence intervals. Significantly different rates are marked in bold type.

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<th>Person-years</th>
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<th>Fertility rate</th>
<th>95% C.I.</th>
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<th>Fertility rate</th>
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**TABLE 3.** Age-specific fertility rates of subjects after a diagnosis of CRC, grouped by site of tumour (colon vs. rectum).

Age-specific fertility rates are given as births/1000 person-years. Total fertility rate (TFR) is given as births/person. There were no men who developed rectal CA under the age of 20, so the age-specific fertility rate for the 15-19 year old subjects in this group could not be calculated. The sample sizes were too small to calculate meaningful confidence intervals for age-specific fertility rates for a number of groups.
Figure 1. Age-specific fertility rates for women from the study cohort vs. the Australian general population.

Error bars are 95% confidence interval (C.I.). No C.I. are presented for the general population data as these are population census figures, not from a sampled population.

FIGURE 2. Age-specific fertility rates for women from the study cohort with or without a CRC diagnosis.

Age-specific fertility rates are given as births/1000 person-years. Error bars are 95% C.I. The upper C.I. for subjects with CRC in age group 25-29 has been truncated.

FIGURE 3. Age-specific fertility rates for men with or without a CRC diagnosis.

Age-specific fertility rates are given as births/1000 person-years. Error bars are 95% C.I. The upper C.I. for subjects with CRC in age groups 20-24 and 25-29 have been truncated.

References


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Age-specific fertility rates - women

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5.2 Appendix II: Statements of authorship

5.2.1 PAPER I

AUTHORSHIP STATEMENT

1. Details of publication and executive author

Title of Publication: "Nanomaterials as carriers for the delivery of anti-cancer drugs."
Journal: Advanced Materials, Volume 28, Issue 18, Pages 5599-5605

2. Inclusion of publication in a thesis

Is this research included in a thesis for a higher degree by research (HDR) thesis? Yes
If yes, please indicate Section 3 if this goes straight to Section 4.

3. HDR thesis author's declaration

Name of HDR thesis author: Jane Doe
Institution: University of Melbourne

4. Description of all author contributions

- Jane Doe: Conceptualization, Methodology, Data curation, Writing - Original Draft, Supervision, Project administration
- John Smith: Validation, Formal analysis, Investigation, Resources, Writing - Review and Editing, Visualization
- Emily Johnson: Resources, Writing - Review and Editing, Supervision
- Michael Brown: Visualization

This work was supported by the Australian Government, within the scope of its investment in Australian Research. If the contribution of the author is included in part of an HDR thesis, it may be included in the thesis and the publication.

Sincerely,
Jane Doe
University of Melbourne
5.2.3 PAPER III

AUTHORSHIP STATEMENT

1. Details of publication and executive author

Title of publication
Publication details
Surgery for idiopathic scoliosis in 10 year olds vs. surgical instability
Co-authorship, U.K. 2011

Name of executive author
School of Health Sciences, Bangor University
College address and phone
Email or phone

2. Inclusion of publication in a thesis

Is it intended to include the publication in a higher degree by research (HDR) thesis? Yes
If yes, please complete part below: If No, go straight to Section 4.

3. HDR thesis author’s declaration

Name of HDR thesis author (if different from above, if the same, "As above")
School or Institute/Division (if based at Durham)
Thesis title
As above
Lunatic mixture: Human Errors and Colloquial Warwick

Before the thesis was completed, there were no contributing authors to this author’s contribution to the publication. The names of all contributors to the submission of the abstract, the design of methodology or design of experimental system, methodological procedures, in particular, the interpretation of the results were not derived from the research. The publication has been approved by all the authors. The contribution of the author’s name is an appropriate description of my contribution to this paper, and the contributions of other authors are described as described below.

4. Description of all author contributions

Name and affiliation of author
Contribution (e.g., conception of the project, design of methodology or experiment, preparation of data, collection, analysis, drafting the manuscript, revising the manuscript for important intellectual content, etc.)
I. Kneen, Division of Human Services, University of Cape Town
Surgical planning, surgical technique

F. Goldberg, Division of Human Services, University of Cape Town
Responsible for the inclusion of prospective collation of data for the subjects who were included in the study. Also involved in data collection.

L. Alger, Division of Human Services, University of Cape Town
Responsible for the data collection.

5. Author biosketches

I agree to be named as a new author contributor to this work.

Name and affiliation of new author
Contribution
Signature* and date

*In each author or co-author is unwilling to submit a contribution, the statement of authorship is that of level of contribution. Level of contribution must be ranked as substantial or minor, and the reasons for their unwillingness provided. There is no evidence to support that the shared intellectual object being named as an author.

6. Data sources

The original data for this project was collected in the following locations. The researchers will be in an appropriate institutional setting. If the research is conducted in a clinical research setting and data are shown outside the peer-reviewed literature, the data will be shared with the research participant.

7. Other contributing declarations

I agree to be named as a new author contributor to this work.

Name and affiliation of new author
Contribution
Signature* and date

8. Other author declarations

I agree to be named as a new author contributor to this work.

9. Other author declarations

I agree to be named as a new author contributor to this work.

This form must be retained by the executive author, with the school or institution to which they are based.

If the publication is to be included as part of an HDR thesis, a copy of this form must be included in the thesis with the publication.
5.2.4 Paper IV

Authorship Statement

1. Details of publication and execution author

Title of Publication
Publication details
No address of paper publication for the paper
Title of execution author
Editorial details
Phone numbers

2. Introduction of publication is a thesis

If your work is a thesis, please indicate this on the title page of the thesis.

3. MMH thesis author's declaration

Name of MMH thesis author of different schools of the same

School/Institution/Degree obtained from what

Degree obtained

4. Description of all author contributions

Name and collection of all authors
Contributions to the research, contributions to the preparation of the manuscript, any

5. Author declarations

I hereby declare that none of the authors of the work, and co-authors

6. Other contributor declarations

I agree to be named as a non-author contributor to this work.

7. Data storage

The original date for this project is stored in the following locations. The locations are

This form must be retained by the executive author, within the school or institute in which they are based.

Signature

Date

This form must be included in this thesis with the publications.
Authors' Declaration

I declare that the named co-authors have made a significant contribution to this work. The work has been reviewed by an independent expert in the field.

Date: [insert date]

[signature]
[Name of author]

5. Author Declarations

I declare that the named co-authors have made a significant contribution to this work. The work has been reviewed by an independent expert in the field.

Date: [insert date]

[signature]
[Name of author]

6. Other contributor declarations

I declare that the named co-authors have made a significant contribution to this work. The work has been reviewed by an independent expert in the field.

Date: [insert date]

[signature]
[Name of author]
5.3 Appendix III: Code (Visual Basic for Applications) of the model used for simulating Lynch syndrome parent-child pairs with CRC

Option Explicit

Sub ParentChildPairs()
Dim AffectedChildren(1 To 15000) As Integer, NumberOfParents As Integer
Dim ChildGender(1 To 15000, 1 To 20) As Integer, Counter As Integer
Dim i As Integer, n As Integer, AgeCRCParent(1 To 15000) As Integer,
AgeCRCChild(1 To 15000, 1 To 20) As Integer, Mendel As Double
Dim NumberOfChildren(1 To 15000) As Integer
Dim CRC_age_group(1 To 15000) As Integer

'AgeCRCChild (parent identifier, child number of that parent)
'Will use odd numbers for mothers, even numbers for fathers

Worksheets("results sheet").Columns("A:CA").Clear
Worksheets("parent child pairs").Columns("A:AA").Clear
Worksheets("children").Columns("A:AA").Clear

NumberOfParents = Worksheets("Variable Input").Range("G7")

For i = 1 To NumberOfParents Step 2 'assigns age of first CRC for mothers
    n = Int(1000 * Rnd) + 1
    AgeCRCParent(i) = Worksheets("1000 first cancers female").Cells(n + 1, 2)
    Worksheets("Results sheet").Cells(i, 2) = AgeCRCParent(i)
    If AgeCRCParent(i) > 50 Then CRC_age_group(i) = 1 Else If AgeCRCParent(i) > 45 Then CRC_age_group(i) = 2 Else If AgeCRCParent(i) > 40 Then CRC_age_group(i) = 3 Else: If AgeCRCParent(i) > 35 Then CRC_age_group(i) = 4 Else: If AgeCRCParent(i) > 30 Then CRC_age_group(i) = 5 Else: If AgeCRCParent(i) > 25 Then CRC_age_group(i) = 6 Else: If AgeCRCParent(i) > 20 Then CRC_age_group(i) = 7 Else: CRC_age_group(i) = 8
    Worksheets("Results sheet").Cells(i, 3) = CRC_age_group(i)

End Sub
Next i

For i = 2 To NumberOfParents Step 2 'assigns age of first CRC for fathers
    n = Int(1000 * Rnd) + 1
    AgeCRCParent(i) = Worksheets("1000 first cancers male").Cells(n + 1, 2)
    Worksheets("Results sheet").Cells(i, 2) = AgeCRCParent(i)
    If AgeCRCParent(i) > 50 Then CRC_age_group(i) = 1 Else If AgeCRCParent(i) > 45
        Then CRC_age_group(i) = 2 Else If AgeCRCParent(i) > 40 Then CRC_age_group(i) = 3
        Else: If AgeCRCParent(i) > 35 Then CRC_age_group(i) = 4 Else: If AgeCRCParent(i) > 30
            Then CRC_age_group(i) = 5 Else: If AgeCRCParent(i) > 25 Then CRC_age_group(i) = 6
            Else: If AgeCRCParent(i) > 20 Then CRC_age_group(i) = 7 Else: CRC_age_group(i) = 8
    Worksheets("Results sheet").Cells(i, 3) = CRC_age_group(i)
Next i

For i = 1 To NumberOfParents Step 2 'assigns number of children for mothers
    n = Int(500 * Rnd) + 1
    NumberOfChildren(i) = Worksheets("Female fertility vs age of CA").Cells(n + 1,
    CRC_age_group(i))
    Worksheets("Results sheet").Cells(i, 4) = NumberOfChildren(i)
Next i

For i = 2 To NumberOfParents Step 2 'assigns number of children for fathers
    n = Int(500 * Rnd) + 1
    NumberOfChildren(i) = Worksheets("Male fertility vs age of CA").Cells(n + 1,
    CRC_age_group(i))
    Worksheets("Results sheet").Cells(i, 4) = NumberOfChildren(i)
Next i

For i = 1 To NumberOfParents 'Assigns number of mutation positive children
    AffectedChildren(i) = 0
    For n = 1 To NumberOfChildren(i)
        Mendel = Rnd
        If Mendel > 0.5 Then AffectedChildren(i) = AffectedChildren(i) + 1
    Next n
    Worksheets("Results sheet").Cells(i, 5) = AffectedChildren(i)
Next i
For $i = 1$ To NumberOfParents 'Assigns gender to mutation positive children
  For $n = 1$ To AffectedChildren(i)
    ChildGender(i, n) = 1 'male
    Mendel = Rnd
    If Mendel > 0.5 Then ChildGender(i, n) = 2 'female
  Worksheets("Children").Cells(i, n) = ChildGender(i, n)
  Next n
  Next i

For $i = 1$ To NumberOfParents 'Assigns age of first CRC to mutation positive children
  For $n = 1$ To AffectedChildren(i)
    Mendel = Int(Rnd * 1000) + 1
    If ChildGender(i, n) = 1 Then AgeCRCChild(i, n) = Worksheets("1000 first cancers male").Cells(Mendel + 1, 2)
    If ChildGender(i, n) = 2 Then AgeCRCChild(i, n) = Worksheets("1000 first cancers male").Cells(Mendel + 1, 2)
    Worksheets("Children").Cells(i, n + 15) = AgeCRCChild(i, n)
  Next n
  Next i

Counter = 1
For $i = 1$ To NumberOfParents
  If AffectedChildren(i) > 0 Then
    For $n = 1$ To AffectedChildren(i)
      Counter = Counter + 1
      Worksheets("Parent child pairs").Cells(Counter, 1) = AgeCRCParent(i)
      Worksheets("Parent child pairs").Cells(Counter, 3) = AgeCRCChild(i, n)
      Worksheets("Parent child pairs").Cells(Counter, 4) = AgeCRCParent(i) - AgeCRCChild(i, n)
      If $i / 2 <> $Int(i / 2) Then 'mothers
        Worksheets("Parent child pairs").Cells(Counter, 14) = AgeCRCParent(i)
        Worksheets("Parent child pairs").Cells(Counter, 15) = AgeCRCChild(i, n)
      Else 'fathers
        Worksheets("Parent child pairs").Cells(Counter, 11) = AgeCRCParent(i)
      End If
    Next n
  End If
Next i
Worksheets("Parent child pairs").Cells(Counter, 12) = AgeCRCChild(i, n) - AgeCRCParent(i) - AgeCRCChild(i, n)

End If

Next n

End If

Next i

End Sub
5.4 Appendix IV: Citations

This lists peer-reviewed journal articles that have cited the papers included in this thesis. It does not include textbook chapters, policy documents etc., and excludes citations by papers which I have co-authored.

5.4.1 Paper I

5.4.2 Paper II


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5.4.3 Paper III


5.4.4 Paper IV

6 REFERENCES


130. group, T.S.s., Single-stage treatment for malignant left-sided colonic obstruction: a prospective randomized clinical trial comparing subtotal


