

# **PREVALENCE OF GYNAECOLOGICAL DISEASE IN WOMEN WITH AN HMLH1 MUTATION IN THE NORTHERN CAPE PROVINCE**

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**SURVEY OF A POPULATION WITH  
LYNCH SYNDROME IN SOUTH AFRICA**



**FACULTY OF HEALTH SCIENCES**

**MASTERS OF MEDICINE DISSERTATION FOR THE UNIVERSITY OF CAPE TOWN**

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**DECLARATION BY APPLICANT**

I, Marlize Lerm, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university. I empower the university to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

The study was carried out while a registrar in the Department of Obstetrics and Gynaecology (O&G) at the University of Cape Town.

Signed by candidate

Signature removed

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KEYWORDS/ ABBREVIATIONS

BSO – Bilateral salpingo-oophorectomy

HIV – Human Immunodeficiency Virus

hMLH – Human mutL homologue

hMSH – Human mutS homologue

HNPCC – Hereditary Non Polyposis Colorectal Cancer

HREC – Human Research and Ethical Committee

GSH – Groote Schuur Hospital

ICG – International Collaborative Group

IUCD – Intra uterine contraceptive device

MMR – Mismatch repair

N - Normal

NHLS – National Health Laboratory Services

PMB – Postmenopausal bleeding

pMS2 – Subtype p of hMSH

TAH – Total abdominal hysterectomy

UCT – University of Cape Town

### ABSTRACT

*Title:* Prevalence of gynaecological disease in women with an hMLH1 mutation in the Northern Cape Province – Survey of a population with Lynch syndrome in South Africa

*Objective:* Lynch syndrome, previously called hereditary non-polyposis colorectal cancer (HNPCC), is one of the most common hereditary cancer syndromes with an association with gynaecological cancers. Members of affected families have an increased risk for colon cancer as well as extra colonic sites; in particular endometrial and ovarian cancer.

A cohort of patients with Lynch syndrome in the Northern Cape, South Africa has been identified and followed up.

According to recommendations by the International Collaborative Group on HNPCC (ICG-HNPCC); women affected with the gene mutation warrant full gynaecological assessment to exclude endometrial and ovarian cancer. Thus far the recommended screening has not been possible and the apparent prevalence of gynaecological cancer or premalignancies among this high risk group has not been established. The aim of this study was to determine the ~~actual~~ apparent prevalence of gynaecological pathology in this cohort of patients; by way of screening.

*Methods:* Women with a known gene mutation, or close relatives of affected family members, utilising the annual colorectal service in the Northern Cape, who fulfilled the inclusion criteria, were recruited to undergo gynaecological evaluation. The participants had a gynaecological examination which included a Papanicolaou smear, a pelvic ultrasound and endometrial sampling. The resultant data was captured on an Excel spread sheet and a descriptive analysis was done.

*Results:* In total 43 women were recruited, of which 18 were postmenopausal and 25 premenopausal. 35 of these women had a known hMLH1 gene mutation. The eight remaining women had either normal genotyping (n=7) or were awaiting molecular test results (n=1).

Only twenty-one of these participants agreed to endometrial sampling, in addition to pelvic ultrasound and gynaecological examination. Histological results were therefore available for the 21 participants. One patient was diagnosed with a grade-2 endometroid adenocarcinoma. No cases of endometrial hyperplasia were found.

Thirty pelvic ultrasound scans were performed. Of these, one patient had an enlarged adnexal mass. No cervical premalignancies were diagnosed on cervical smears, with one abnormal smear of atypical cells of unknown significance (ASCUS) diagnosed.

*Conclusion:* The apparent prevalence of gynaecological disease in this study population was lower than expected. We conclude however that this high risk group of women should still undergo regular gynaecological screening which should include history taking and clinical examination. Screening using routine endometrial sampling and pelvic ultrasound in asymptomatic women did not appear to be beneficial.

### CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

#### Background

Epidemiological studies have long recognised the association between a positive family history of a cancer and a susceptibility of other family members to the same cancer type. Approximately 10% of cancers arise as a result of a genetic predisposition, but less than 5% will arise as a result of a single highly penetrant cancer predisposition gene. These single gene disorders which are inherited are also termed cancer susceptibility syndromes, in which family members who carry the gene mutation are at high risk of developing various cancers. These family members tend to develop these cancers at a young age, compared to the general population [1].

The two most common hereditary syndromes associated with gynaecological cancer are hereditary breast and ovarian cancer syndrome (mutation in genes BRCA 1 and BRCA 2) and Lynch syndrome. Lynch syndrome, previously called Hereditary Non Polyposis Colorectal Cancer (HNPCC) is thought to be caused by a mutation in mismatch repair (MMR) genes. The four most common mutated gene subtypes in Lynch syndrome tumours are hMLH1, hMSH2, MSH6 and PMS2 [2].

#### Lynch syndrome

Lynch syndrome is one of the earliest recognised and most prevalent familial cancer syndromes which was formally described by Henry Lynch in 1966 [3]. The syndrome is defined as an inherited condition (autosomal dominant) involving defective DNA mismatch repair (MMR) system. The MMR genes repair base pair errors during cell division. A mutation in any of the MMR genes results in loss of function of the encoded protein. This change in the gene product leads to microsatellite instability (MSI) which refers to a molecular phenotype with alterations in repetitive DNA sequences. Lynch associated tumours can be assayed through immunohistochemistry to prove MSI. There are five known MutS genes (MSH2, MSH3, MSH4, MSH5 and MSH6) and four MutL genes (MLH1, PMS1, PMS2 and MLH3) in the human MMR system.

The syndrome has a worldwide population prevalence of approximately 1 in 600 to 1 in 3000 [4]. These affected families have an increased risk of colon as well as extra colonic cancers; in particular, endometrial cancer. Other extracolonic sites associated with the genetic defect

include: cancer of the ovary, urinary tract, hepatobiliary tract, brain, small bowel and pancreatic cancer.

Prior to molecular genetic testing, Lynch syndrome was diagnosed by an algorithm using clinical and family history criteria. This criterion is known as the Amsterdam criteria I and was developed by the ICG-HNPPC in 1991. This was amended to the Amsterdam criteria II in 1999 and includes the spectrum of extracolonic cancers (see appendices 6). There have been studies to suggest an increase in breast cancer as another extracolonic cancer [5] but currently, breast cancer is not included as part of the diagnostic criteria according to Amsterdam II.

With the discovery of microsatellite instability, diagnosing Lynch syndrome changed dramatically. Guidelines were developed to assess which tumours needed to be tested for MSI; these are known as the Bethesda guidelines and were revised in 2004. Once MSI has been proven, the patient may be offered further germline DNA testing to confirm the presence of Lynch syndrome [6].

### **Risk of endometrial and ovarian cancer**

Endometrial cancer is the second most common malignancy (after colon cancer) seen in females with Lynch syndrome, and appears to occur at an earlier age than sporadic cancer. The estimated cumulative risk is as high as 40% by the age of 70 years in mutation carriers and 15% for first degree relatives (without the genetic abnormality) compared to 3% in the general population [7]. Females with the genetic defect also have an up to nine time higher risk to develop ovarian cancer (8-12%) [8].

There have been numerous studies which have emphasized that woman with Lynch syndrome have an equal or even greater risk of endometrial cancer than colon cancer [9]. Although we do not screen for endometrial and ovarian cancer in the general population, the high prevalence of these cancers in female mutation carriers warrants screening. For these reasons gynaecological surveillance has been recommended by the International Collaborative Group on HNPCC (ICG-HPCC) for asymptomatic cases [10].

### **Endometrial cancer**

Endometrial cancer is the second most common gynaecological cancer in the general world population, second only to cervical cancer [11]. It has an even higher incidence in more developed countries being the 4<sup>th</sup> most common cancer.

According to Globocan 2012, endometrial cancer accounts for 4.8% (319 605) of the total new cancer cases and 2.1% (76 160) of the total cancer deaths worldwide [11]. The higher incidence in more developed countries can be attributed to the high rates of obesity and also to increased detection rates, with patients presenting with early disease. Risk factors for developing endometrial cancer are mainly associated with increased hormonal levels and this include a high BMI (body mass index), low parity, early menarche (<13 years old) and late menopause (>55). Protective factors include a later menarche, oral contraceptive use ( $\geq 1$  year) and a higher parity ( $\geq 1$ ). Most females diagnosed with endometrial cancer are over the age of 60.

Although this cancer has an excellent 5-year survival when detected early (70-90% of FIGO stage 1 and 2), one fifth of cases have a poor prognosis [12].

The proportion of hereditary endometrial cancer due to Lynch syndrome is estimated at 1.8% in the general population [13]. The average age for development of endometrial cancer in this population is estimated at 50, thus much earlier than in the general population [14]. The prognosis when diagnosed in the early stage of disease progression seems to be favourable with 5-year survival rate as high as 88% [15].

The majority of Lynch syndrome associated endometrial cancers are of endometrioid histology, similar to sporadic endometrial cancer. However, non-endometrioid subtypes, including uterine serous carcinoma, clear cell carcinoma and uterine malignant mixed müllerian tumors, have been reported in women with Lynch syndrome [15]. These histological subtypes have a poorer prognosis as seen in the general population.

### **Ovarian cancer**

In the general population ovarian cancer is the third most common gynaecologic malignancy in the developed world. In 2008, about 225,000 women around the world were diagnosed with ovarian cancer. Mortality was estimated at 140,000 from this disease [16].

In the developing world it has an incidence of 5 per 100,000 and a mortality rate of 3.1 per 100,000. Screening for ovarian cancer in the general population is not recommended as there is insufficient data to suggest benefit for widespread screening.

The lifetime risk of ovarian cancer in women with Lynch syndrome is estimated to be 8-12% compared with 1.5 percent in the general population [9]. These women also tend to develop ovarian cancer at a younger age than the general population (43 - 50 versus 60 years old) [17].

Histopathology and survival outcomes seem to be similar in women with Lynch-associated ovarian cancer and women with sporadic ovarian cancer. The most common histologic subtype seen in ovarian cancer associated with Lynch syndrome is epithelial serous, but endometrioid, clear cell and mucinous subtypes, have been reported [18].

The higher incidence of ovarian cancer in Lynch syndrome females may justify screening and recommendations for this high risk group have been developed by various centres around the world [19].

### **Recommended gynaecological surveillance in patients with Lynch syndrome**

With the high survival rate associated with endometrial cancer in Lynch syndrome patients, the role of gynaecological surveillance in asymptomatic women is unclear. The goal of monitoring in these women at high risk of endometrial cancer would be to diagnose cancer at an earlier stage and thus minimize the extent of treatment. Surveillance programs aimed at detecting colorectal cancer or precancerous lesions in people with Lynch syndrome have been highly successful in decreasing mortality and morbidity [20]. However there is paucity of data to suggest a benefit in gynaecological surveillance in asymptomatic women. Various prospective and retrospective studies have failed to show the effectiveness of gynaecologic surveillance procedures [21; 22]. The lifetime risk of endometrial and ovarian cancer is high in women with Lynch syndrome. Many of these women may not be aware of their risk or be able to recognise early symptoms of disease.

In the case of endometrial cancer many of affected women are premenopausal and might not seek medical attention in the event of irregular periods. Screening then appears to be justified and the ICG-HNPCC suggests annual or biannual screening from the age of 30 year old in asymptomatic women.

Thus far no randomised controlled trials have been done to prove whether gynaecological screening would be effective in diagnosing early endometrial or ovarian cancer in Lynch syndrome.

Screening techniques aimed at evaluation of the endometrium, include transvaginal ultrasound and endometrial sampling or biopsy. The value of ultrasonography alone in evaluating the endometrium in patients with Lynch syndrome would be limited as 25% of endometrial cancers occur in premenopausal women [23]. Biopsy of the endometrium improves the sensitivity of the screening technique [24]. The screening recommendation states that women with a known genetic mutation or who are at risk based on a genetic mutation in her family should be offered annual endometrial biopsy starting at age 30 to 35 years [19].

Transvaginal ultrasound may also assist in evaluating ovarian morphology and is recommended annually. Studies focussing on surveillance for ovarian cancer in high risk population groups have unfortunately not been very successful in detecting early ovarian cancer. As this disease usually presents late and has a poor outcome once symptomatic, gynaecological screening by way of transvaginal ultrasound in these at risk females is still advised.

Tumour markers may play a role in screening for ovarian cancer in Lynch syndrome patients. Measuring the serum concentration of the CA-125 glycoprotein antigen could indicate ovarian cancer in about 50 percent of women with early disease and over 80 percent of women with advanced ovarian cancer in the general population [25]. However the specificity of this screening test is low because serum levels may fluctuate in healthy women according to their menstrual cycle. It can also be raised in a number of other benign or non-benign gynaecological and medical diseases making this test not the ideal screening test.

Combining transvaginal ultrasound and CA125 levels might improve the specificity and some centres advise this combination 6 monthly or annually [26].

### **The Northern Cape study population**

In the mid-1980s a family in a rural part of the Northern Cape was identified with most members diagnosed with colorectal cancer. The family was investigated and found to have Lynch syndrome with a mutation in the hMLH 1 gene. This was the first familial case of colorectal cancer to be described in South Africa [27]. Subsequently more than 1500 people, most of them living in the Northern Cape province of South Africa, have been recognised as having a high risk for Lynch syndrome based on their individual family tree [27]. These families are mostly of mixed race ancestry and Afrikaans speaking. The Northern Cape is the largest province in the country but with the smallest population size of 1,145 861 people [28]. The majority (75%) of the population lives in small towns, with minimal access to health care facilities.

For the last 20 years these patients diagnosed with Lynch syndrome have and still are receiving annual screening for colon cancer by a mobile unit travelling from Groote Schuur Hospital in Cape Town to the four temporary screening sites.

### **Aim of our study**

Due to poor socio economic circumstances and few resources available to this group of patients, the recommended gynaecological screening thus far has not been possible. The apparent prevalence of gynaecological cancers or pre-cancerous lesions, particularly of endometrial and ovarian origin, among this high risk group has not been established. In addition, the risk factors present in this study population associated with gynaecological disease will also be defined.

The main aim of the survey was to determine the prevalence of gynaecological pathology in patients with Lynch syndrome included in our study and specifically to screen for endometrial and ovarian cancer. Although cervical cancer is not included in the extracolonic tumour spectrum in patients with Lynch syndrome, screening for this cancer in this population with limited resources is crucial. Cervical cancer is the second most prevalent cancer (after breast) in Southern Africa and other developing countries, and a gynaecological risk profile analysis would be incomplete without this type of screening.

The data obtained by gynaecological screening of these women with the mutation will prove valuable to determine future gynaecological screening requirement and management.

## **CHAPTER 2: METHODS**

### **Study design**

This descriptive / observational study aimed to assess the gynaecological health status of this high risk group.

### **Aim**

To evaluate the prevalence of gynaecological cancerous and pre-cancerous lesions in patients with Lynch syndrome included in our study with particular emphasis on endometrial and ovarian cancers.

### **Objectives were**

1. To determine the prevalence of endometrial cancer
2. To determine the prevalence of ovarian cancer
3. To screen for cervical abnormalities
4. To define risk factors associated with gynaecological disease

### **Ethical considerations**

Participation was entirely voluntary and patients were required to give written informed consent prior to the interviews (see Appendix A).

Ethical approval was acquired from the Research Ethics Committee of the University of Cape Town (REC REF 3435/5454, see Appendix B). Females known with the hMLH1 gene mutation were identified from the database within the Division of Human Genetics at UCT.

### **Setting**

This study was conducted amongst known MLH1 mutation carrier female patients currently utilizing colorectal screening at various sites in Northern Cape.

This study was planned to coincide with the annual colorectal screening program at the four facilities in the Northern Cape, during a week-long screening period beginning September 2015. We visited Harrie Surtie hospital (Upington), Springbok hospital, Alexander Bay Community Health Centre and Garries hospital.

No power calculation was done as this study set out to determine gynaecological pathology in this patient population. We aimed to screen as many patients as possible that fitted our inclusion criteria. The sample size was determined by the time frame of the colorectal outreach and we anticipate that the results of our study would provide the basis upon which a larger, correctly powered study, be based on in the future.

### **Participants**

#### **Inclusion criteria:**

Proven hMLH1 mutation carrier or first degree relative of a carrier

Client able to give informed consent

Female patients above 18

Not pregnant/ pregnancy excluded

#### **Exclusion criteria**

Consent withheld

Clients not fulfilling inclusion criteria

By interviewing the female clients with Lynch syndrome who fulfilled the inclusion criteria, we aimed to gain insight into their reproductive history and current gynaecological profile. We assessed for certain risk factors predisposing to gynaecological malignancy, particularly endometrial and ovarian cancer.

Eligible patients not willing to participate in the study were assured that this would not jeopardise their current or future treatment. All patient queries were appropriately managed by the primary investigator. Participants were informed they could withdraw from the study at any time.

Their identity was coded on the data base and therefore not linked to the individual. They were guaranteed anonymity. Patients were not offered any remuneration to take part in the study.

Data including demographical information as well as a complete obstetrical and gynaecological history was collected from all consenting participants. This was followed by a clinical examination.

The patients were asked general and specific questions to assess for gynaecological cancer risk factors, information on smoking history, HIV status and previous personal and family history of gynaecological or other malignancies. (See Appendix 4)

The patients were all examined and had ultrasound imaging and endometrial sampling by either the primary investigator or supervisor. A cervical smear was taken to conclude a complete gynaecological screening. Thereafter, the uterus and adnexa were assessed by transvaginal ultrasonography (see Appendix 5); prior to endometrial sampling. Limitations in accurate assessment of the uterus and adnexa were documented.

The ultrasound machine ~~was used with~~ had the following specifications:

- Mindray® M7 portable unit
- Midrange TV probe at 4-10 MHz
- 7 MHz centre frequency

The following measurements were taken:

- Length of the uterus in mm (millimetre)
- Endometrial thickness (ET) in mm
- Left and right ovary, with the largest diameter measured in mm

An endometrial biopsy was taken where allowed, to screen for endometrial abnormalities.

The biopsy was fixed using a standardised technique with 10ml formaldehyde (10%). The specimens collected were safely transported back to the NHLS laboratory at GSH.

Measurement of serum CA-125 in all participants was considered but due to the low specificity of the test and the cost involved, serology was omitted.

### **Data Management and Statistical analysis**

The data were analysed using descriptive statistics. Numerical data were described using appropriate summary statistics, such as the mean, standard deviation, median and range. Results were graphically displayed using flow diagrams, bar graphs and proportional data as pie charts.

### **Data Collection and Management**

All data extracted was secured in an excel file and was password protected.

Each client was allocated a unique study number to protect client confidentiality.

### CHAPTER 3: RESULTS

#### Recruitment

Initially 48 women were identified to be screened gynaecologically.

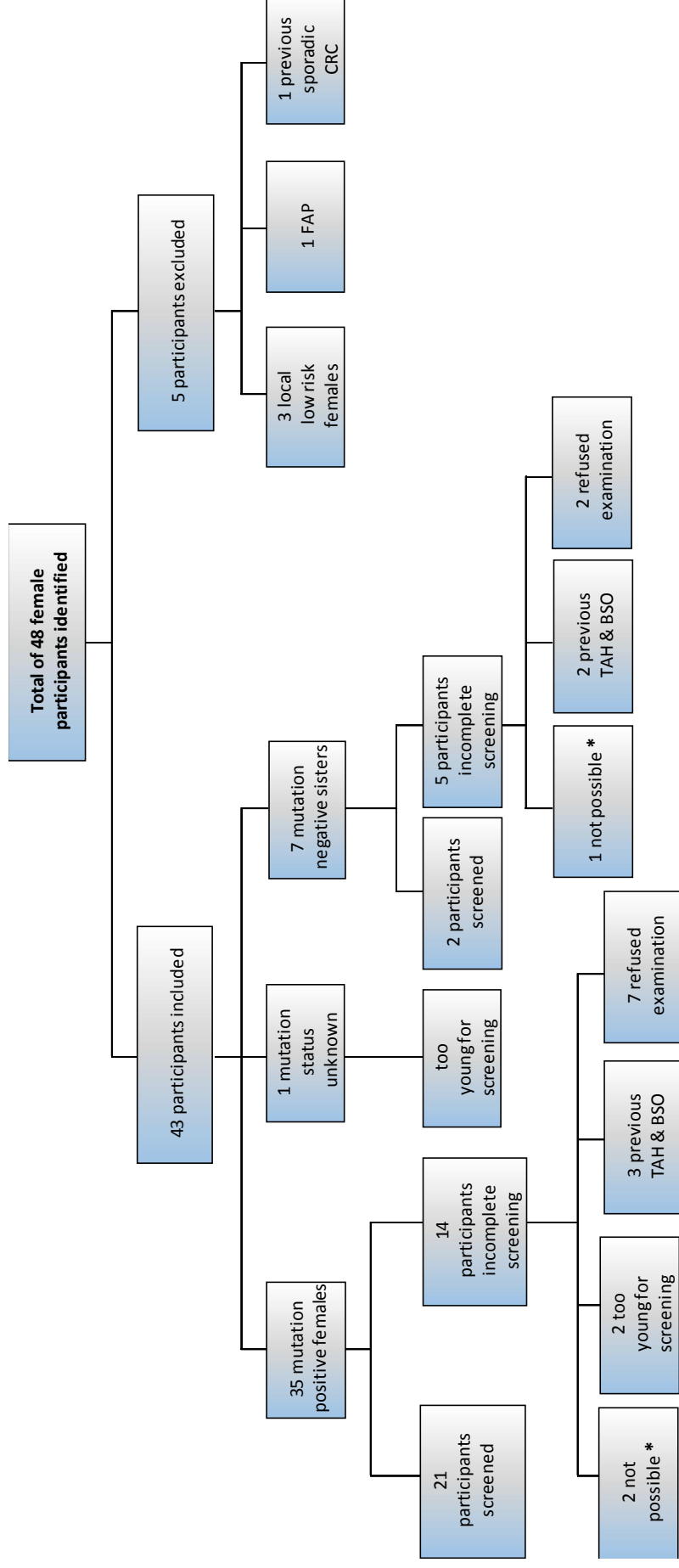
Five of these women were excluded from our study results as they have been previously assessed by the Genetics department of GSH to be of low risk (general population risk) for Lynch syndrome. These five women were still screened for gynaecological pathology as they were present at the screening facilities and requested to be seen by one of the gynaecologists. All of them were of no genetic risk to having Lynch syndrome. Two of the five women had surgical indications for annual colonoscopic screening.

In total, 43 women who met the entrance criteria were included in our study, of which 18 were postmenopausal and 25 premenopausal. Of these 43 participants, 35 had a proven hMLH1 mutation, 1 was awaiting her test result (blood sample genetic testing) and 7 participants were first line family members of an affected mutation carrier and tested negative for the mutation.

Thirty of the participants consented to examination and transvaginal ultrasonography. One patient was too ill to examine and was sent to the emergency unit of the screening facility. She had known chronic airway disease and had an acute episode of lower airway obstruction. The remaining twelve participants either refused examination or had a previous hysterectomy.

Twenty-one of the 35 hMLH1 mutation positive participants gave consent to full gynaecological screening which included a gynaecological examination (including a papsmear), transvaginal ultrasound and endometrial biopsy. Histology results were available for these twenty-one women. (See Figure 1)

Figure 1: Recruitment data



\* Note: included 1 sick patient, the other 2 had stenosed cervixes and a pipelle could not be passed

**Demographic and socio-economic status analysis of the 43 participants**

*Age distribution*

The age of the participants ranged from 19 to 70 years .The average age was 44 years.

**Figure 2: Age distribution**

*Level of education*

Five (11.6%) women had some level of tertiary education, eight (18.6%), had matric level of education, 26 (60.4%) had high school level of education, 3 (6%) had a primary school level of education and 1 (2%) woman had received no formal education.

*Employment status*

21 (49 %) women were unemployed and 22 (51%) women had some formal employment.

*Smoking history*

At the time of interview 14(33%) smoked cigarettes while 9 (20%) had stopped smoking more than 6 months earlier.

*Access to health care***Table 1:** Distance travelled to nearest district hospital

| DISTANCE   | FREQUENCY | PERCENTAGE |
|------------|-----------|------------|
| 0-20 KM    | 12        | 28.0       |
| 21-50 KM   | 7         | 16.2       |
| 51-100 KM  | 11        | 25.6       |
| 101-200 KM | 10        | 23.2       |
| > 200 KM   | 3         | 7.0        |
| TOTAL      | 43        | 100.0      |

As illustrated in Table 1, more than half of the participants (55.8%) live more than 51 kilometre (km) from the nearest district hospital where the screening facilities were set up.

**Gynaecological and relevant medical history***Menarche*

The age range of menarche was 11 years to 19 years. Two participants could not remember the age of their menarche. The median age of menarche was 15 years old.

*Parity*

Five participants had never been pregnant and one had no live children with a previous miscarriage. 18.6% (n=8) gave birth to 1 child, 34.8% (n=15) gave birth to 2 children, 18.6% (n=8) gave birth to 3 children, 11.6% (n=5) gave birth to 4 children and 0.2% (n=1) gave birth to 5 children. There were 5 pregnancy losses, including 4 early miscarriages and 1 ectopic pregnancy.

**Table 2** Parity

| No of children | ≤ 1      | 2    | 3    | 4    | 5   |
|----------------|----------|------|------|------|-----|
| n              | 14 (6+8) | 15   | 8    | 5    | 1   |
| Percentage     | 32.6     | 34.9 | 18.6 | 11.7 | 0.2 |
| Total (n=43)   |          |      |      |      |     |

*Menopausal status*

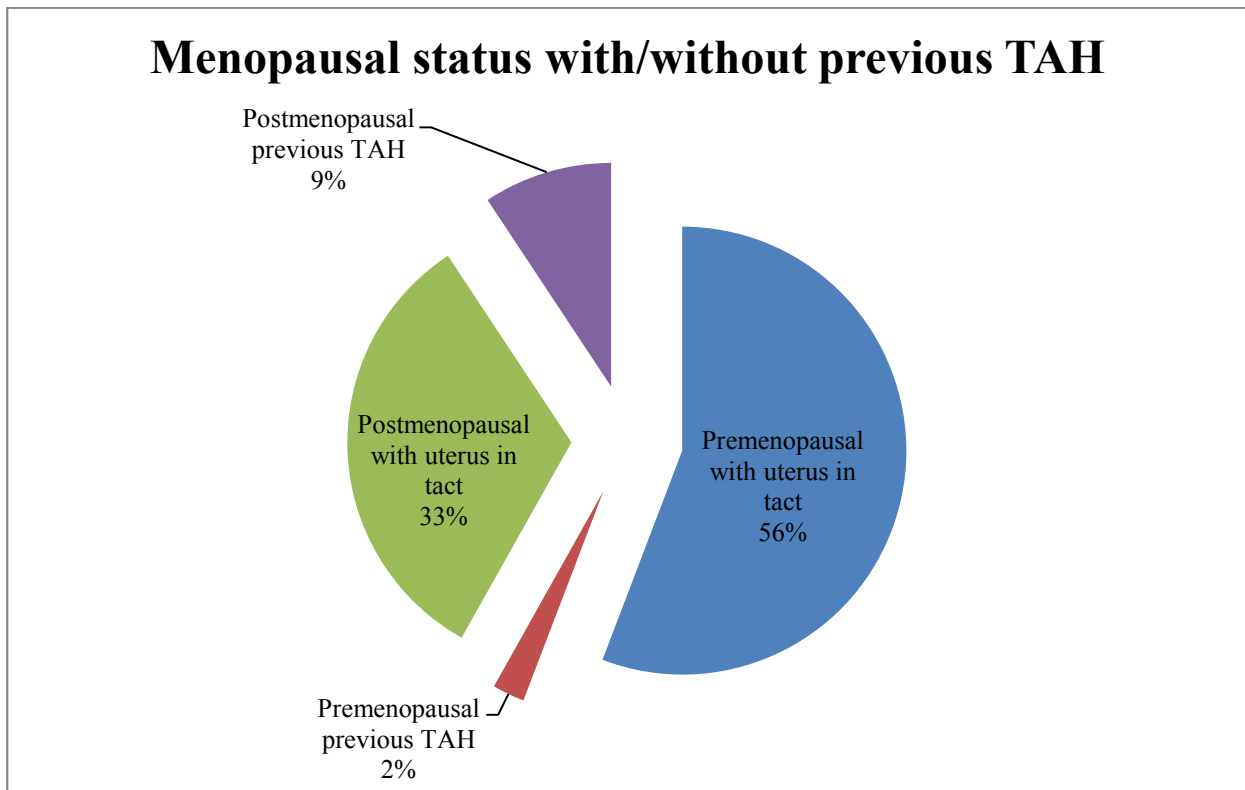
Of the 43 participants included, 18 were postmenopausal and 25 premenopausal. Five of the 18 postmenopausal clients had previous total abdominal hysterectomies. The indications for a hysterectomy were:

- 1 previous high grade cervical intra epithelial lesion
- 3 abnormal uterine bleeding

One premenopausal participant had a previous total hysterectomy. The indication was for a high grade cervical intra epithelial lesion.

None of these hysterectomies and/or salpingo-oophorectomies was done for endometrial or ovarian cancer. Histology results were regrettably not available.

Figure 4 Menopausal status



### *BMI*

BMI is usually categorised into four categories, underweight (BMI less than 20 kg/m<sup>2</sup>), normal (BMI between 20-25 kg/m<sup>2</sup>), overweight (BMI between 25 and 30 kg/m<sup>2</sup>) and obese (BMI over 30kg/m<sup>2</sup>). Of the 43 participants included, data for 7 were not available.

- One (2.7%) was underweight
- Seven (19.4%) were of normal weight
- Twelve (33.3%) were overweight
- Sixteen (44.4%) were obese

### *Contraceptive use*

Sixteen (37.2%) participants used hormonal contraception in their lifetime, four (9.3%) combined oral contraception and 12 (27.9%) DMPA (medroxyprogesterone acetate). The remaining 62.8% have never used hormonal contraception and 2 (4.6%) have had bilateral tubal ligation.

### *Previous cancer diagnosis*

Of the 43 participants included, 13 had previous colorectal surgery:

- 4 total colectomies for confirmed colon cancer
- 3 total colectomies for high grade dysplastic polyps
- 6 colectomies for low grade or multiple polyps (preventative surgery)

### *Other medical history*

Five participants were receiving treatment for hypertension. One of these five also had type2 diabetes.

Thirty-three participants had been tested for HIV previously. Four (12%) tested positive and of these 2 participants were receiving anti-retroviral treatment.

Twenty-one participants with proven hMLH1 mutation received full screening comprising of ultrasound, endometrial biopsy and cervical smear. (See table 2)

**Table 3:** Twenty-one mutation positive females screened

| #  | Age | BMI   | Parity | Hyst Y/N | Post-Meno pausal | Menstrual irregularity  | Ultrasound |      |         |              | Endometrial biopsy             | Papsmear                 |
|----|-----|-------|--------|----------|------------------|-------------------------|------------|------|---------|--------------|--------------------------------|--------------------------|
|    |     |       |        |          |                  |                         | Uterus     | ET   | Left    | Right        |                                |                          |
| 1  | 40  | 34.05 | 0      | N        | N                | 0                       | 72         | 10.6 | 33 x 25 | 23 x 26      | Proliferative endometrium      | Normal                   |
| 3  | 66  | 43.06 | 3      | N        | Y                | 0                       | 14         | 4.5  | *NS     | <b>67x21</b> | Specimen not optimal           | Normal                   |
| 6  | 27  | 33.5  | 1      | N        | N                | Amenorrhoea             | 78         | 6.3  | 34x26   | 32x23        | Inactive endometrium           | Normal                   |
| 8  | 52  | 28.23 | 2      | N        | Y                | 0                       | 43         | 3    | NS      | NS           | Suboptimal                     | Normal                   |
| 10 | 43  | 33.53 | 1      | N        | N                | 0                       | 76         | 6.4  | 34x29   | NS           | Suboptimal                     | Normal                   |
| 12 | 44  | 33.33 | 2      | N        | N                | Intermenstrual spotting | NS         | 7.6  | NS      | NS           | Proliferative endometrium      | Normal                   |
| 15 | 56  | 38.54 | 3      | N        | Y                | 0                       | 71         | 11.4 | NS      | NS           | Weakly secretory endometrium   | Normal                   |
| 16 | 59  | 26.67 | 4      | N        | Y                | PMB                     | 84         | 21.4 | 20x16   | NS           | <b>Endometroid cancer Grd2</b> | Normal                   |
| 17 | 43  | 33.35 | 2      | N        | N                | 0                       | 45         | 3.3  | 28x17   | 23x22        | Menstrual endometrium          | Normal                   |
| 18 | 37  | 29.75 | 2      | N        | N                | 0                       | 84         | 3    | 24x23   | 24x20        | Secretory endometrium          | Cyto normal, Trichomonas |

**GROOTE SCHUUR OBSTETRICS AND GYNAECOLOGY DEPARTMENT**

| #  | Age | BMI   | Parity | Hyst Y/N | Post-Meno pausal | Menstrual irregularity | Ultrasound |       |         | Endometrial biopsy    | Papsmear                      |
|----|-----|-------|--------|----------|------------------|------------------------|------------|-------|---------|-----------------------|-------------------------------|
|    |     |       |        |          |                  |                        | Uterus     | ET    | Ovaries |                       |                               |
|    |     |       |        |          |                  |                        | Left       | Right |         |                       |                               |
| 19 | 46  | 22.43 | 4      | N        | N                | Oligomenorrhoea        |            |       |         | Breakdown endometrium | Normal                        |
| 24 | 32  | 19.23 | 1      | N        | N                | Amenorrhoea            | 66         | 3.6   | 23x16   | 24x23                 | Cyto normal                   |
| 27 | 53  | 31.11 | 5      | N        | Y                | 0                      | 53         | 2.1   | 16x11   | 24x10                 | Normal                        |
| 30 | 38  | 38.05 | 2      | N        | N                | Amenorrhoea            | 79         | 2.4   | 21x15   | 19x11                 | No endocervical cells Rpt3/12 |
| 31 | 64  | 34.21 | 4      | N        | Y                | 0                      | 59         | 4.4   | 22x12   | 20x12                 | Normal                        |
| 34 | 42  | 29.38 | 4      | N        | N                | Amenorrhoea            | 63         | 1.7   | 16x8    | 23x11                 | No endocervical cells Rpt3/12 |
| 36 | 45  | 28.88 | 2      | N        | N                | 0                      | 79         | 6.3   | 24x10   | NS                    | Normal                        |
| 38 | 52  | 35.38 | 2      | N        | N                | Oligomenorrhoea        | 56         | 2.7   | 12x16   | 19x19                 | Normal                        |
| 41 | 45  | 0     | 2      | N        | N                | 0                      | 69         | 5     | NS      | NS                    | Normal                        |
| 42 | 55  | 27.78 | 3      | Y        | Y                | 0                      | 56         | thin  | NS      | 17x11                 | Normal                        |
| 43 | 44  | 25.39 | 3      | N        | N                | Amenorrhoea            | 63         | 3.4   | 32x15   | NS                    | Cyto normal, Gardnerella      |

\*N/S = Not seen

ET = Endometrial Thickness

### *Gynaecological examination*

Pelvic examination of the patients screened revealed no abnormalities.

### *Papanicolaou tests*

Twenty nine cervical smears were done. Cytology testing revealed one ASCUS (atypical squamous cells of unknown significance). Three smears had absent endocervical cells and had to be repeated. Two smears showed Trichomonas and one Gardnerella infection.

### *Ultrasound*

In total, thirty patients agreed to transvaginal ultrasound scans. One adnexal mass was seen in a 69 year old female. She was referred to the local district hospital for repeat pelvic ultrasound. The endometrium thickness, size and morphology of the ovaries were measured where possible.

### *Endometrial sampling*

Twenty-three endometrial biopsies were taken. One (4%) patient was diagnosed with grade-2 endometroid adenocarcinoma. No endometrial hyperplasia was diagnosed on biopsy. Two biopsies were not possible due to cervical stenosis. Ten biopsies were histologically suboptimal for diagnosis.

### CHAPTER 4: DISCUSSION AND RECOMMENDATIONS

The literature states that women with Lynch syndrome have an increased risk for developing endometrial cancer and ~~in~~ to a lesser degree, ovarian cancer. Screening recommendations have therefore been advised to diagnose early stage disease or premalignant lesions. Less invasive treatment options would benefit the patient.

Annual colonoscopy has proven to be of value to decrease colorectal cancer. Although the international community recommends screening from the age of thirty, the efficacy of gynaecological screening is unclear in detecting cancer and also premalignant pathology.

We screened a small group of women with a proven hMLH1 mutation or first degree family members of a known mutation carrier. These women have never been screened for gynaecological cancer. In our study of 43 participants, 21 had a proven hMLH1 mutation and fulfilled our inclusion criteria to be screened. The screening tests involved a transvaginal ultrasound and endometrial sampling. Only one endometrioid adenocarcinoma (1/21) has been detected. One participant, with an adnexal mass was seen on ultrasound and this was consistent with a hydrosalpinx. One cytology test was abnormal and showed ASCUS (atypical squamous cells of unknown significance).

We assessed these patients demographic and gynaecological risk profile. The majority of the participants (77%) had an increased BMI. This is a well-known risk factor for developing endometrial cancer. More than half of the participants (53%) were previous or current smokers. Smoking cessation should be advised. There were no other modifiable risk factors to gynaecological disease seen in this patient population.

These patients have to travel long distances to their closest health care facilities. Counselling aimed at informing the patients with Lynch syndrome of their increased risk of uterine and ovarian cancer should be offered at these annual screening opportunities. Risk reducing strategies could possibly involve lifestyle modification

The one participant diagnosed with grade 2 endometrioid endometrial cancer received surgery. It is important to note that this patient was symptomatic and had postmenopausal bleeding for longer than 8 months. She however, did not seek medical treatment despite adequate counselling by the genetic counsellor and research sister.

There has also been reluctance from the women undergoing annual colorectal screening to be seen by the gynaecologists. The main reason the women gave for not wanting gynaecological screening was that they already had a colonoscopy and were reluctant for more invasive testing.

Although endometrial sampling improves the sensitivity of the screening offered, it is invasive and not completely risk or pain free. The quality of the specimen obtained is also limited and was reflected in the histology results. Eleven (11/23) endometrial biopsies were assessed by the pathologist to be suboptimal. These 11 correlated with an endometrial thickness of less than 6mm. Endometrial sampling using a pipelle is a blind technique and ideally hysteroscopic directed biopsies should be performed. Transvaginal ultrasonography was well tolerated and might be a more acceptable approach for gynaecological screening in future.

The low prevalence of gynaecological pathology in this group of patients could possibly be linked to their genetic mismatch repair system mutation. There have been numerous studies to suggest females with the hMLH1 and hMSH2 gene mutation have a different phenotype to hMSH6 carriers. hMSH6 carriers are much more prone to develop endometrial cancers while hMLH1 carriers seem to have a higher propensity toward proximal colorectal cancer [29]. The number of subjects in our study is however, small.

We found that the apparent prevalence of gynaecological disease in this study population was lower than expected (4.76%).

We conclude however, that this high risk group with limited resources available to them warrants regular gynaecological screening. This should include a detailed history taking and clinical examination. Early symptom reporting should be advised to these women in order to detect possible early malignant changes.

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**APPENDICES:**

***Appendix 1: Information leaflet***

**PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM**

Title of the research project:

**The prevalence of gynaecological disease in patients with Lynch Syndrome**

**REFERENCE NUMBER:**

**PRINCIPAL INVESTIGATOR: Dr M Lerm**

**CO-INVESTIGATORS: Dr N Mbatani**

**ADDRESS: University of Cape Town, WESTERN CAPE**

**CONTACT NUMBER: 021- 404 4488**

You are being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved.

Your participation is entirely voluntary and you are free to decline to participate. If you say no, this will not affect your care in any way whatsoever. You can at any time withdraw from the study, even if you did agree to take part.

This study has been approved by the Health Research Ethics Committee at the University of Cape Town and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

**What is this research study all about?**

We want to screen women with a known predisposition to colon cancer for any gynaecological abnormalities. We will do this research study when you come for your annual colonoscopy check-up at your clinic. We are hoping to get more than 50 women to participate in the screening for gynaecological disease.

**Why have you been invited to participate?**

We are keen to talk to females with your condition as we hope to establish scientific information to improve knowledge of gynaecological cancers related to this genetic condition. We would also like to test for any other gynaecological conditions. Our aim is to establish whether females with this genetic disorder are indeed at much higher risk than females without the disorder.

If you would like to participate in this study, the researchers will ask you a series of questions pertaining to your gynaecological health to ensure you are eligible to participate.

### **Study Procedures**

Your involvement in the study will require one interview with the researcher during your yearly examination to look for colon cancer. We will ask some questions regarding your monthly period, contraception use, previous pregnancies and births. We will also perform a pregnancy test to ensure you are not pregnant when we do further examinations. We will then do an ultrasound examination to look at the size and condition of your ovaries.

When you are put to sleep (sedated) we will do a Pap smear to look for any early changes at the mouth of the womb that can result in cancer of the womb. Then we will take a small biopsy of the lining of your womb. This might result in some cramping afterwards which should disappear within minutes.

### **Potential Benefits**

You will be screened for gynaecological abnormalities. In the case that we pick up any abnormalities the results will be discussed with your local doctor for further treatment. By participating in this study you will be making an important contribution to research that may benefit others in the future, as little is known about gynaecological problems in females with your condition.

### **Potential Risks**

The ultrasound examination may cause you some discomfort but most women tolerate the procedure without any problems. As you will be asleep with the Pap smear and biopsy of the lining of your womb you will not feel any pain or discomfort. There might be some abdominal cramping and vaginal bleeding afterwards which should settle within minutes.

### **Compensation**

No compensation will be offered for participation, but there will also be no costs involved for your gynaecological consultation.

### **Confidentiality**

Your participation is regarded as strictly confidential and not shared with other parties. You will be given a number so that your name will not be connected to your questionnaire. Any data reported from this study would not identify individuals. All information will be kept in locked file cabinets accessible only to the principal investigator and the research team. Any publications/lectures/reports resulting from this study will not identify you by name or any other means.

**The right to ask questions/withdraw from this study**

Your participation in this study is entirely voluntary. You have the right to withdraw at any time. If you decide not to partake / to withdraw from this study, this will not jeopardize you or the treatment you may require in any way. If you have any queries, you can contact Dr Marlize Lerm at 021 404 4488.

You can contact the Health Research Ethics Committee at 021 406 6626 for any concerns or complaints that have not been adequately addressed by your study doctor. You will receive a copy of this information and consent form for your own records.

***Appendix 2: Consent form***

**Declaration by participant**

By signing below, I ..... agree to take part in a research study entitled (The prevalence of gynaecological disease in patients with Lynch syndrome)

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is voluntary and I have not been pressurized to take part.
- I may choose to leave the study at any time and will not be penalized or prejudiced in any way.
- I may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

**Signed at (*place*) ..... on (*date*) ..... 2015.**

**Signature of participant      Signature of witness**

**Declaration by investigator**

I (*name*) ..... declare that:

- I explained the information in this document to .....
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above
- I did/did not use a interpreter. (*If a interpreter is used then the interpreter must sign the declaration below.*)

**Signed at (*place*) ..... on (*date*) ..... 2015.**

**Signature of investigator      Signature of witness**

**Declaration by interpreter**

I (*name*) ..... declare that:

- I assisted the investigator (*name*) ..... to explain the information in this document to (*name of participant*) ..... using the language medium of Afrikaans/Xhosa.
- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.
- I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her question satisfactorily answered.

**Signed at (*place*) ..... on (*date*) ..... 2015.**

**Signature of interpreter**

**Signature of witness**

***Appendix 3: Research approval letter from HREC***

**Appendix 4: Data sheet demographic and clinical history**

Study nr:

Age:

Address/ Local clinic:

Level of education:

Employment status:

G\_\_ P \_\_ M \_\_ E\_\_ T\_\_

Menarche:

Last menstrual period:

Menstrual irregularities:

Amenorrhoea / Oligomenorrhoea / intermenstrual spotting/ menorrhagia

Comment: \_\_\_\_\_

Contraception:

|               |       |     |      |                |
|---------------|-------|-----|------|----------------|
| Nil/<br>Other | Depot | COC | IUCD | Tubal ligation |
|---------------|-------|-----|------|----------------|

Last used / comment: \_\_\_\_\_

Pregnancy status/ NA/ Prognostix POS / NEG

Menopausal : Y / N            If Y, Duration (in years):

Menopausal symptoms: \_\_\_\_\_

Any family history of a gynaecological cancer? \_\_\_\_\_

Is affected member gene mutation pos/unknown

Smoking history: Y / N / Previous

HIV status: Pos / neg / unknown

Previous Pap Smear result if any:

|                       |            |           |
|-----------------------|------------|-----------|
| Less than 3 years ago | 3-10 years | >10 years |
|-----------------------|------------|-----------|

*Appendix 5: Data sheet examination and ultrasound*

**EXAMINATION**

**Body Mass Index (BMI):**

Weight:      Height:

Examination findings:

    General:

    Breast/thyroid:

    Abdomen:

PV and speculum:

**Cervix normal Y / N**

**Endometrial biopsy done Y / N**

**Comment:** \_\_\_\_\_

**Transabdominal and/or transvaginal ultrasound:**

**Uterus**

|       |             |                        |
|-------|-------------|------------------------|
| Size: | Morphology: | Endometrial thickness: |
|       |             |                        |

COMMENT:

**Assess ovaries**

|  |  |          |
|--|--|----------|
| Size:<br>Left: _____<br>Right: _____<br><br>Ascites? Y / N | Morphology:<br>Normal / Cystic /<br>Septated / Solid | COMMENT: |
|  |  |          |

*Appendix 6: ICG-HNPPC Amsterdam criteria I and II*

**Chart 1.** Amsterdam criteria I and II

| Amsterdam criteria I   |
|--|
| At least three relatives need to have histologically verified colorectal cancer (CRC)<br>One needs to be a first-degree relative of the other two<br>At least two successive generations need to be affected<br>At least one of the relatives with CRC needs to have received the diagnosis before age 50<br>Familial adenomatous polyposis needs to have been ruled out   |
| Amsterdam criteria II  |
| At least three relatives need to have a cancer associated with hereditary nonpolyposis colorectal cancer (colorectal, endometrial, stomach, ovary, ureter or renal-pelvis, brain, small bowel, hepatobiliary tract, or skin [sebaceous tumors])<br>One needs to be a first-degree relative of the other two<br>At least two successive generations need to be affected<br>At least one of the relatives with CRC needs to have received the diagnosis before age 50<br>Familial adenomatous polyposis should have been ruled out in any relative with colorectal cancer<br>Tumors should be verified whenever possible |