Tampere University

MARI KALAMO

Lynch Syndrome in Finnish Women

Endometrial cancer risk factors, surveillance, prophylactic surgery and family planning

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ACADEMIC DISSERTATION
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To Aarni and Valma

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ABSTRACT

The studies of this thesis were designed to gather further information on the gynecological health, risk factors and psychosocial aspects among carriers of Lynch Syndrome (LS).

LS is the most common hereditary cancer-predisposing genetic disorder in the world and it significantly increases the cancer risk in the gastrointestinal tract, urinary tract, endometrium and ovaries. LS carriers have pathogenic variants of mismatch-repairing (MMR) genes repairing oncogenic damages of DNA.
For the prevention of endometrial cancer (EC) and ovarian cancer (OC), risk-reducing hysterectomy and salpingo-oophorectomy are recommended for LS carriers after childbearing is complete. Gynecological surveillance has also been offered.

This thesis aimed to characterize the factors modifying the risk of endometrial cancer among LS carriers. Moreover, female LS carriers' reproductive health issues, attitudes towards risk-reducing surgery and psychological reactions associated with germline testing were assessed.

The study population consisted of 223 female verified LS pathogenic variant carriers identified from the Finnish Lynch syndrome research registry (LSRFi) and 290 non-carrier control EC patients from the Tampere University Hospital (TAUH) patient records. Data were collected by postal questionnaires which contained questions regarding EC risk factors and experiences on gynecological surveillance, risk-reducing surgery, reproductive health and psychosocial wellbeing. Patient record information was partially available for confirming the data.

In the first study we compared the lifestyle-related EC risk factors between 50 LS carriers and 110 non-carriers diagnosed with EC. The risk factors did not differ significantly between these groups, but the results showed a tendency for higher prevalence of endometriosis among the LS carriers.

The EC risk-modifying factors were further evaluated in a retrospective cohort study of 136 LS carriers. Type II diabetes, hypercholesterolemia and long-term use of HRT had significant associations with an elevated risk of EC among LS carriers in univariable and multivariable Cox regression analyses.

The aim of the third, descriptive study was to investigate the factors associated with the decision regarding the uptake of risk-reducing surgery. Seventy-six responders implicated no external factors affecting their decisions concerning the surgery. A majority of them considered the gynecological surveillance beneficial. Fifty-five percent had the prophylactic surgery performed at survey. The percentage of the responders who were satisfied with the counselling and information provided by medical experts was significantly higher among the prophylactically operated LS carriers. Pain experienced during endometrial sampling was mainly low or moderate.

In the fourth study we gathered information on the subjective experiences of genetic testing, and its' impacts among LS carriers. In this descriptive study, the majority of the 35 responders did not report LS as having any influence on their intimate relationships and only 20% reported an effect on their reproductive decisions. Most of the carriers implicated thankfulness and satisfaction with the gynecological surveillance provided.

In conclusion, the studies of this thesis provided data that could be applied to clinical counselling with female LS carriers. The results encourage the carriers to be recommended to maintain a healthy lifestyle and to avoid long-term HRT. The possible association of endometriosis with LS is an interesting target for future research. Surveillance seems to have positive psychosocial effects on the LS carriers. Our results emphasize the importance of adequate information and counselling with an emphatic attitude, provided by medical experts. The germline testing as such seems not to have a negative impact on the female LS carriers' self-image or to play a major role in their reproductive health decisions.

TIIVISTELMÄ

Tämän väitöskirjatyön tutkimukset suunniteltiin tiedon lisäämiseksi Lynchin syndrooman (LS) kantajien gynekologisesta terveydestä, riskitekijöistä ja psykososiaalisista näkökulmista.

LS on yleisin tunnettu syöpäriskiä lisäävä perinnöllinen häiriö ja se lisää merkittävästi riskiä ruoansulatauskanavan, virtsateiden sekä kohdun runko-osan ja munasarjojen syöpiin. Lynchin syndrooman kantajilla on jonkin DNA:n perimän vaurioita korjaavan mismatch repair (MMR) -geenin patogeeninen variantti. Kohtu- ja munasarjasyövän riskin poistamiseksi LS-kantajille suositellaan kohdun ja munasarjojen poistoa, kun lapsiluku on täynnä. Heille on tarjottu myös gynekologista seurantaa.

Väitöskirjan tavoitteena oli karakterisoida Lynchin syndroomaan (LS) liittyvän patogeenisen geenivariantin kantajien kohtusyövän riskiin vaikuttavia tekijöitä. Lisäksi haluttiin tutkia suhtautumista ennaltaehkäiseviin leikkauksiin sekä LS-diagnoosin vaikutuksia lisääntymisterveyteen ja psykososiaaliseen hyvinvointiin.

Tutkimusväestö koostui 223 geenitestauksella varmistetusta Finnish Lynch syndrome research registry (LSRFi) -rekisteriin kirjatusta naispuolisesta kantajasta sekä 290 Tampereen ylipistollisen sairaalan (TAUH) potilastietokannasta kerätystä kohtusyöpään sairastuneesta potilaasta ilman perinnöllistä syöpäalttiutta. Tiedot kerättiin postitse kyselylomakkeilla, joissa oli kysymyksiä kohtusyövän riskitekijöistä sekä kantajien kokemuksista liittyen seurantaan, ennaltaehkäiseviin leikkauksiin, perhesuunnitteluun ja psykososiaaliseen hyvinvointiin.

Ensimmäisessä osatyössä verrattiin 50 kohtusyöpään sairastuneen LS-kantajan ja 110 verrokki-kohtusyöpäpotilaan riskitekijöitä. Nämä tekijät eivät eronneet merkitsevästi ryhmien välillä, mutta endometrioosia vaikutti esiintyvän enemmän LS-kantajilla.

Kohtusyövän riskitekijöitä kartoitettiin lisää 136 LS-kantajan retrospektiivisellä kohorttitutkimuksella. Merkittävä yhteys kohonneeseen kohtusyövän riskiin todettiin muuttuja-analyyseissa tyypin II diabeteksella, hyperkolesterolemialla ja hormonikorvaushoidon pitkäaikaisella käytöllä.

Kolmas osatyö oli kuvaileva tutkimus, jossa selvitettiin 76 LS-kantajan osalta ennaltaehkäisevän leikkaushoidon päätöksentekoon vaikuttavia tekijöitä. Heistä 55% oli jo leikattu. Merkitsevästi päätökseen vaikuttavia tekijöitä ei havaittu. Suurin osa piti tarjottua seurantaa hyödyllisenä. Leikkaukseen jo päätyneiden joukossa tyytyväisyys terveydenhuollon ammattilaisten antamaan tietoon ja neuvontaan oli merkittävästi yleisempää. Kohdun limakalvonäytteiden aiheuttama kipu arvioitiin pääosin lieväksi tai kohtalaiseksi.

Neljännessä osatyössä kerättiin kuvailevaa tutkimusta varten LS -kantajien kokemuksia LS -diagnoosista ja sen vaikutuksista eri elämänalueisiin. Vastaajia oli 35. Heistä suurin osa ilmoitti, ettei LS ole vaikuttanut parisuhteeseen tai perhesuunnitteluun. Suurin osa oli tyytyväisiä seurantaan ja kiitollisia siitä.

Väitöskirjatutkimus tarjoaa tietoa, jota voidaan hyödyntää, kun neuvotaan naispuolisia LS -kantajia. Tulokset rohkaisevat ohjaamaan kantajia noudattamaan terveellisiä elintapoja ja välttämään pitkäaikaista hormonikorvaushoitoa. Endometrioosin mahdollinen yhteys Lynchin syndroomaan on kiinnostava löydös tulevia tutkimuksia ajatellen. Seurannalla vaikuttaa olevan positiivisia psykososiaalisia vaikutuksia. Tuloksemme korostavat asiantuntijoiden antaman riittävän tiedon ja neuvonnan sekä empaattisen asenteen tärkeyttä. Geenitestauksen tuloksella ei vaikuta olevan negatiivista vaikutusta LS-kantajien minäkuvaan tai merkittävää roolia heidän perhesuunnittelussaan.

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ABBREVIATIONS

ASA	Asetosalicylic acid
BMI	Body mass index
CRC	Colorectal cancer
CT	Computer tomography
EBRT	External beam radiation therapy
EC	Endometrial cancer
FIGO	International federation of gynecology and obstetrics
GUS	Gynecological ultrasound
HNPCC syndrome	Hereditary non-polyposis colon carcinoma syndrome
HRT	Hormone replacement therapy
IHC	Immunohistochemistry
INSiGHT	International Society for Gastrointestinal Hereditary Tumors
IUD	Intrauterine device
LS	Lynch syndrome
LSRFi	Finnish Lynch syndrome research registry
MMR	Mismatch repair
MRI	Magnetic resonance imaging
MSI	Microsatellite instability
NRS	Numeric pain rating scale
OC	Ovarian cancer
PCOS	Polycystic ovary syndrome
PET	Positron emission tomography
PLSD	Prospective Lynch syndrome database
POLE	(DNA) polymerase epsilon
ProMisE	Proactive molecular risk classifier for endometrial cancer
TCGA	The cancer genome atlas
VAS	Visual analogue scale
WHO	World health organisation

ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to by the Roman numerals as assigned below.

I Aaltonen MH*, Staff S, Mecklin JP, Pylvänäinen K, Mäenpää JU. Comparison of lifestyle, hormonal and medical factors in women with sporadic and Lynch syndrome-associated endometrial cancer: A retrospective case-case study. Mol Clin Oncol. 2017 May;6(5):758-764.

II Staff S, Aaltonen MH*, Huhtala H, Pylvänäinen K, Mecklin JP, Mäenpää JU. Endometrial cancer risk factors among Lynch syndrome women: a retrospective cohort study. Br J Cancer. 2016 Jul 26;115(3):375-81.

III Kalamo M, Mäenpää JU, Seppälä TT, Mecklin JP, Huhtala H, Sorvettula K, Pylvänäinen K, Staff S. Factors associated with decision-making on prophylactic hysterectomy and attitudes towards gynecological surveillance among women with Lynch syndrome (LS): a descriptive study. Familial Cancer 2020; 177:182.

IV Kalamo M, Mäenpää JU, Seppälä TT, Mecklin JP, Pylvänäinen K, Staff S. Descriptive study on subjective experience of genetic testing with respect to relationship, family planning and psychosocial wellbeing among women with Lynch syndrome. Hereditary Cancer in Clincal Practice 2021; 19:38.

* Publications I and II are published by the author's former last name, Aaltonen

AUTHOR'S CONTRIBUTIONS

The initial idea for this thesis came from the author together with prof. Jukka-Pekka Mecklin (JPM), doc. Synnöve Staff (SS) and prof. Johanna Mäenpää (JM). The author of this thesis collected the retrospective data in Studies I – IV via postal questionnaires and saved the data for the analyses. The data saving was performed together with MD Katariina Sorvettula in Study III. The data were confirmed and complemented by the author in collaboration with Kirsi Pylvänäinen (KP) from the LSRFi.

In study I, the author of this thesis designed the study and did the main work writing and revising the manuscript together with co-authors SS, JM and JPM.

Study II, a retrospective cohort study, was designed by the author together with SS. The manuscript was written and revised in equal contribution with the first author SS and in collaboration with the co-authors JM, JPM and M.sc. Heini Huhtala (HH).

The design of studies III and IV was built by the author of this thesis. The manuscripts were written and revised by the author with SS and in collaboration with JM and the co-authors. The statistical analyses in studies I-III were planned and carried out with HH and SS, and the results interpreted by the author together with them.

1 INTRODUCTION

Lynch syndrome (LS) is an autosomal dominantly inherited cancer syndrome deriving from the pathogenic germline variants (*path_MLH1*, *path_MSH2*, *path_MSH6* and *path_PMS2*) of the mismatch repairing (MMR) genes protecting the DNA from oncogenic mutations.

LS causes a genetic predisposition to cancers in the gastrointestinal system, gynecological organs, urinary tract and brain (glioblastoma). The risk of cancer in different organ systems among our study population; female carriers of LS pathogenic variants *path_MLH1*, *path_MSH2* and *path_MSH6* is presented in Figure 1. LS is the most common hereditary cancer syndrome in the world, affecting circa 1 in 300 individuals worldwide. (Aarnio et al., 1999), (Millar et al., 1999), (Møller et al., 2018)

The risk of endometrial and ovarian cancer is high among Lynch Syndrome pathogenic variant carriers. The lifetime risk of endometrial carcinoma (EC) varies between 31% and 57% and ovarian carcinoma (OC) between 4 and 13 % depending on a pathogenic variant type. The mean age at onset of LS-associated EC is 48 years and OC 40 to 45 years. (Møller et al., 2017)

In order to reduce the risk of gynecological cancer, hysterectomy, frequently together with bilateral salpingo-oophorectomy is recommended to the carriers of LS pathogenic variants after the childbearing is complete. Before the risk-reducing surgery, a gynecological surveillance has been widely implemented for individuals with LS pathogenic variants, including regular appointments with gynecological ultrasound (GUS) imaging and possibly endometrial biopsies. The benefits of surveillance in the prevention of EC are unclear and previous studies have showed no effect on the survival of the patients. (Auranen & Joutsiniemi, 2011), (Stuckless et al., 2013), (Ketabi et al., 2014)

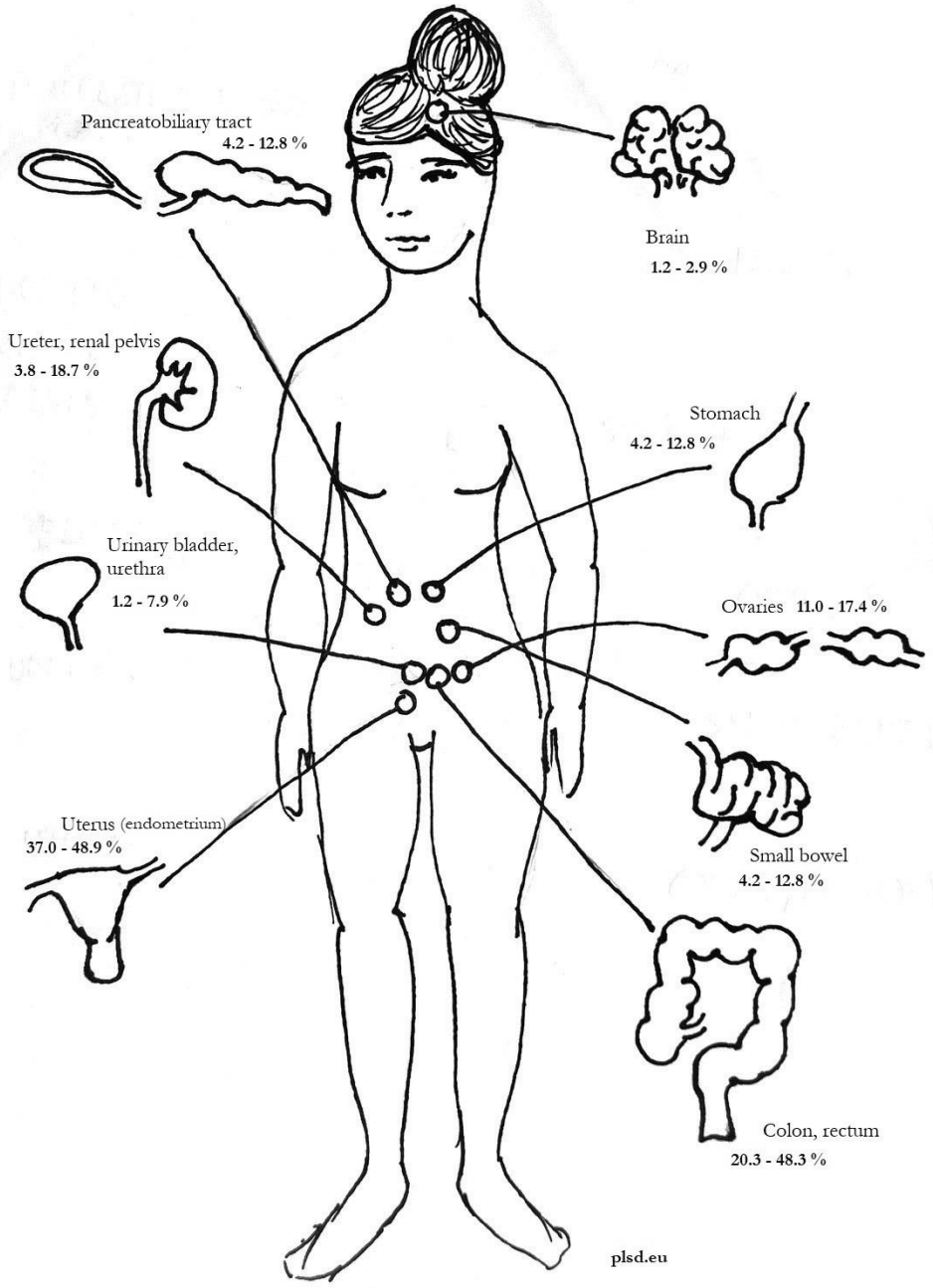

Figure 1. Risk of Lynch Syndrome -associated cancers by the age of 75 years in different organ systems among female carriers of *path_MLH1*, *path_MSH2* and *path_MSH6*.

In general population, the lifestyle-related risk factors for EC are well characterized. It is relatively unknown whether these factors, like obesity, metabolic syndrome, the use of estrogen without restriction from progestins, and nulliparity, increase the EC risk also among LS carriers. Moreover, it is not known whether the effect of EC protecting factors, for example hormonal contraception, is the same throughout the LS population.

The majority of LS carriers attend the gynecological surveillance if offered, and in previous reports, most of them consider it beneficial. In recent European guidelines for LS surveillance, regular annual or biannual GUS and endometrial biopsy are not encouraged due to the lack of evidence on the benefits over symptom-related interventions. (Crosbie et al., 2019) However, conducting gynecological examinations and informing the carriers and educating them on cancer symptoms after the LS diagnosis, is generally recommended. (Crosbie et al., 2019), (Gupta et al., 2019)
Risk-reducing surgery is usually offered to LS carriers according to recommendations. However, a part of them delay or refuse the surgery, and the reason for this is relatively unknown. When evaluating the decisions regarding prophylactic surgery, a small previous qualitative study implicated that some demographic and psychological factors can play a part in them. (Etchegary et al., 2015) According to another descriptive report, carriers felt their cancer worries relieved after prophylactic surgery but it also had a negative impact in form of deteriorated hormonal and sexual function. Pre-surgical information from experts seemed to diminish the negative outcomes. (Etchegary et al., 2018) Estrogen-only hormone replacement therapy (HRT) is considered safe to offer to LS carriers after surgery, if there are no pathological histology findings in the gynecological organs. (Crosbie et al., 2019)

Psychological effects of a genetic cancer predisposition are researched previously to some extent, but little is known about the psychosocial consequences of a LS diagnosis. In earlier studies, the psychological effects are suggested to depend significantly on the individual's psychological capacity to adjust. (Esplen et al., 2015) A temporary depressive reaction has been found among LS carriers soon after the germline testing results. (Aktan-Collan et al, 2013),(Galiatsatos et al., 2015) The influence of LS on reproductive decisions and family planning is also somewhat unknown.

This thesis aimed to investigate the factors which affect female *path_MLH1*, *path_MSH2* and *path_MSH6* carriers' risks for endometrial cancer and impact their decisions on risk-reducing surgery and family planning. We also wanted to study their attitudes towards testing and surveillance, and the psychological consequences of the LS diagnosis.

2 REVIEW OF THE LITERATURE

2.1 Endometrial carcinoma

2.1.1 Epidemiology

Endometrial carcinoma (EC), or endometrial cancer, or uterine corpus cancer, is the sixth most common cancer of women globally, and the fourth most common cancer among Finnish women. It represents half of all gynecological cancers diagnosed yearly in Finland.
Globally there are 320,000 new cases annually. The estimated age-standardized (ASRs, World standard) incidence is 8.3 per 100,000 women. (Ferlay et al., 2015) In Finland the incidence rate is 13.2 per 100,000 women, resulting in approximately 860 new cases diagnosed yearly and the trend has been rising. However, in last few years, the trend has moderated. (www.cancerregistry.fi)

Endometrial carcinoma is often diagnosed in the early stage and curative treatment can be offered. (Morice et al., 2016) The mean relative five-year survival rate in endometrial carcinoma is 82% (United States, 2005 to 2011). However, the survival rate depends on the extent of the cancer. In localized (stage I - II) endometrial carcinoma it is 95%, in regional extent 68% and in metastatic phase 17%. (Siegel et al., 2016) In the United States, endometrial cancer has been estimated to cause 4% (10,470) of all cancer deaths. (Siegel et al., 2016) Correspondingly, in Finland, endometrial cancer is responsible for 3.2% of all cancer deaths (www.cancerregistry.fi).
The International Federation of Gynecology and Obstetrics (FIGO) staging of endometrial cancer is presented in Table 1. Almost 72% of endometrial cancers are stage I, 12% are stage II, 13% are stage III, and 3% are stage IV. (Pecorelli, 2009)

Table 1. FIGO staging system for endometrial cancer (2009)

STAGE	DESCRIPTION
I A	Tumor confined to uterus, < 50 % myometrial invasion
IB	Tumor confined to uterus, ≥ 50 % myometrial invasion
II	Cervical stromal invasion
IIIA	Tumor invasion into serosa or adnexa
IIIB	Vaginal or parametrial involvement
IIIC1	Pelvic lymph node involvement
IIIC2	Para-aortic lymph node involvement
IVA	Tumor invasion into bladder or bowel mucosa
IVB	Distant metastases (including abdominal metastases) or inguinal lymph node involvement

(Pecorelli 2009)

2.1.2 Diagnosis

The average age at EC diagnosis is 63 years. The symptoms of EC usually include postmenopausal bleeding (90% of patients). (Siegel et al., 2016) A gynecological ultrasound (GUS) examination and endometrial sampling are used for the diagnosis. To design the treatment and assess the possible metastatic disease, additional imaging modalities (thoracic, abdominal and pelvic CT scan, MRI or PET scan) should be considered, depending on the clinical and pathologic risk. (Concin et al., 2021)

2.1.3 Classification

Histology

Endometrial cancer is divided into different histological subtypes. The WHO classification of endometrial carcinoma classifies the tumors based on histological subtype as follows; the prevalence marked with percents:

1.Endometrioid low grade (68%),

2. Endometrioid high grade (11%),

3. Serous (11%),

4. Carcinosarcoma (4%),

5. Mixed (3%),

6. Clear cell (1%),

7. Undifferentiated/dedifferentiated (1%),

8. Mucinous gastrointestinal type (<1%),

9. Mesonephric-like (<1%), and

10. Other types (< 1%).

(McCluggage et al., 2022)

Endometrioid endometrial carcinomas are graded with a 3-tier system developed by the FIGO: In general, FIGO1 (less than 5% nonsquamous solid component) and FIGO2 (6 to 50% nonsquamous solid component) are considered low grade and FIGO 3 (< 50% nonsquamous solid component)

high grade, accompanied by all serous and clear-cell endometrial carcinomas. (Soslow et al., 2019)

Types I and II

The very heterogenic group of endometrial carcinoma subtypes has traditionally been divided into two main groups based on histology, degree of differentiation and the occurrence of hormone receptors. (Bokhman, 1983), (Sorosky, 2012)

Type I, representing the majority of endometrial carcinomas, refers to low-grade, endometrioid, hormone-receptor positive endometrial adenocarcinoma with frequent microsatellite instability (40%) and a good prognosis (overall survival 85% at 5 years). Type I is usually estrogen-related and occurs in younger, obese, or perimenopausal women. Tumors commonly arise in the background of hyperplasia.
Type II endometrial carcinomas have been described as non-endometrioid (serous, clear-cell), high grade, TP53-mutated, hormone-receptor-negative tumors associated with a higher risk of metastasis and a poor prognosis (overall survival 55% at 5 years).
Type II represents 10 % of the endometrial carcinomas and typically occurs in an older cohort of women than type I. (Bokhman, 1983), (Sorosky, 2012), (DiSaia & Creasman, 2012)
Classification to type I and II is not clearly separable in practice as some tumors show intermediate characteristics. (Goebel et al., 2018)

Molecular classification

The traditional histological grading and classification system presented above has shown limited efficacy as a prognostic or predictive tool. With recently developed molecular classification, it is more probable to find EC cases at an increased risk of recurrence, enabling more accurate prognoses for the patients. (Cosgrove et al., 2018)

A collaborative project The Cancer Genome Atlas (TCGA) has discovered four individual prognostic EC subtypes based on genomic abnormalities that reflect EC tumor biology. (Alexa et al., 2021)

Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE) has been developed as a set of surrogate markers for TCGA A molecular classification for more practical clinical use, requiring less complicated samples and methods compared to TCGA. (Kommoss et al., 2018) ProMisE identifies four molecular subtypes that are related but not identical to the four genomic subtypes described in TCGA: mismatch repair deficient (MMR-D), showing loss of one or more mismatch repair protein(s) by immunohistochemistry (IHC), corresponding to the hypermutated subtype; DNA polymerase epsilon (POLE), with mutations in the exonuclease domain in exons 9–14, corresponding to the ultramutated subtype; p53 abnormal (p53abn) demonstrating aberrant p53 by IHC staining, corresponding to the copy number high subtype; and p53 wild-type (p53wt) corresponding to the copy number low subtype. (Kommoss et al., 2018)
TCGA and ProMisE classification systems are presented and compared in Table 2.

Table 2. Molecular TCGA and ProMisE -classifications of endometrial cancer

TCGA -classification	**Corresponding ProMisE -classification**
Copy-number high (serous-like)	p53abn
Copy-number low (endometrioid-like)	p53wt
POLE (ultramutated)	POLE
MSI (hypermutated)	MMR-D
(Kommoss et al 2018, Alexa et al 2021)	

2.1.4 Treatment

In apparent early-stage EC (stages I and II) the essential treatment is surgery with a minimal invasive approach via laparoscopy, when possible. Standard surgery is total hysterectomy with bilateral salpingo-oophorectomy without vaginal cuff resection. (Signorelli et al., 2009), (Concin et al., 2021) Infracolic omentectomy should be performed in clinical stage I serous endometrial carcinoma, carcinosarcoma, and undifferentiated carcinoma. (Concin et al., 2021), (Joo et al., 2015) Lymph node staging, mainly with sentinel lymph node detection and biopsy, is recommended in stage II EC and considered in stage IB disease with high-grade histology. It should be continued with pelvic and para-aortic lymphadenectomy, if lymph node involvement is present. (Tanaka et al., 2018), (Concin et al., 2021)
In stage III and IV EC, operative tumor debulking with enlarged lymph node removal can be considered, as well as debulking after neoadjuvant systemic therapy. (Barlin et al., 2010), (Bogani et al., 2018), (Concin et al., 2021)

The risk of recurrence in EC is evaluated using FIGO staging and the classifications explained in chapter 2.1.3. Risk level classification is presented as low risk, intermediate risk, high intermediate risk and high risk.
Adjuvant treatment is not recommended for patients with low-risk EC. In intermediate and high-risk disease without lymph node involvement, adjuvant brachytherapy is used to decrease vaginal recurrence. (Concin et al., 2021)
Adjuvant external beam radiation therapy (EBRT) is mainly used in high-risk EC and when lymph node status in high-intermediate risk is unknown.
In a high-risk disease, adjuvant therapy usually includes EBRT and chemotherapy, or sequential chemotherapy and radiotherapy. (Albeesh et al., 2019), (Concin et al., 2021)

2.1.5 Risk and protective factors

The most important risk factor for the development of endometrial carcinoma is the exposure to estrogen unrestricted by progestins. Estrogens stimulate the proliferation of cells in the endometrium and increase mitotic activity, which can induce malignant cell development. (DiSaia & Creasman, 2012), (Henderson & Feigelson, 2000), (Akhmedkhanov et al., 2001) It is suggested that progestins

decrease the risk of developing endometrial cancer by reducing cell proliferation and stimulating differentiation. (Akhmedkhanov et al., 2001) Early menarche and late menopause are known to be risk factors of EC. (Sorosky, 2012)

Obesity, especially with metabolic syndrome and diabetes mellitus type 2, is a significant EC risk factor, as the adipose tissue is the main source of estrogen in postmenopausal women, and the estrogen levels in the circulation of obese women in postmenopause are higher compared to women with a normal weight. (Fader et al., 2009), (Sheikh et al., 2014), (Raglan et al., 2019) Associated with obesity as well as physical inactivity, insulin resistance related to diabetes mellitus type 2 seems to be an independent risk factor. (Mu et al., 2012)

As for non-estrogen-dependent factors, age over 50 years, hypertension and thyroid disease are associated with an increased risk of EC. (Sorosky, 2012), (Braun et al., 2016)

Nulliparity is known to be associated with a twofold to threefold increase in the incidence of endometrial carcinoma. (Sorosky, 2012) Number of parity seems to protect dose-dependently from EC. Nulliparity is thought to be related to infertility rather than purposeful prevention of pregnancy. (Wu et al., 2015) Polycystic ovary syndrome (PCOS) and a history of infertility, probably interrelated to anovulation and progesterone deficiency, seem to increase the risk twofold to threefold as well, but in PCOS the risk is possibly at least partly associated with obesity. (Ignatov & Ortmann, 2020) Oral contraceptive use is known to protect from EC, and the benefit seems to persist for even decades after stopping the intake. (Gierisch et al., 2013)

Hormonal replacement treatment (HRT) increases the risk of EC, if estrogen intake is inadequately unopposed by progestins. It is suggested that sequential combined therapy increases the risk as well. However, continuous combined therapy seems safe or even tends to protect obese patients from EC. (D'Alonzo et al., 2019) The levonorgestrel-releasing intrauterine device seems to protect from endometrial cancer. (Soini et al., 2014), (Jareid et al., 2018)
Tamoxifen, a selective estrogen receptor modulator used for the adjuvant treatment and chemoprevention of breast cancer, creates stimulatory activity on the endometrium and increases the risk of endometrial cancer nearly threefold after three years of use. (Lee et al., 2020)

Hereditary cancer syndromes increase the risk of endometrial carcinoma markedly. (Sorosky, 2012) The most important one is Lynch syndrome, presented in Chapter 2.2, but mutations in the genes encoding PTEN, FOX01, PIK3CA, E-cadherin, β-Catenin, K-ras and P53 have also been linked with endometrial malignancies. Genetic disease can represent up to 10% of cases, of which 5% are Lynch syndrome. (Smith et al., 2001)

The risk factors and protective factors are collected in Table 3.

Table 3. Risk factors and protective factors of endometrial carcinoma

Risk factors	**Protective factors**
Estrogen-related:	Progestins
Obesity	Oral contraceptives
Insulin resistance and diabetes type II	IUD containing progestin (levonorgestrel)
Early age of menarche	
Late menopause	
Use of tamoxifen	
HRT without continuous progestin	
Other:	
Hereditary cancer syndromes	
Age over 50 years	
Hypertension	
Thyroid disease	

2.2 Lynch syndrome

2.2.1 Background and epidemiology

Lynch syndrome (LS), previously also called HNPCC (hereditary non-polyposis colon cancer) syndrome, is a dominantly inherited cancer syndrome deriving from pathogenic germ-line variants of the mismatch repairing (MMR) genes protecting the DNA from oncogenic mutations. These variants are manifested in all MMR genes including *path_MLH1*, *path_MSH2*, *path_MSH6* and *path_PMS2*. (Millar et al., 1999), (Peltomäki, 2016)

The name LS was first mentioned in the early publications of Henry Lynch, and the term HNPCC syndrome was taken in use in the 1980s. When one hundred years had passed since the first publication of a LS family by A. Warthin (1913), this syndrome was officially designated as Lynch syndrome. (Lynch & Lynch, 2013) As the most common hereditary cancer syndrome affecting approximately 1 in 300 individuals worldwide, Lynch syndrome is known to significantly increase the risk of cancers in the colorectum, endometrium, ovaries, stomach, small bowel, bile duct, pancreas, and urinary tract. (Millar et al., 1999), (Aarnio et al., 1999) (Møller et al., 2018)

2.2.2 Genetic testing

In addition to genetic testing of the high-risk family members (50% risk) in verified LS families, the provision of the pathogenic MMR variant testing for patients presenting with gastrointestinal and gynecological malignancies enables the prevention of cancer development among healthy carriers. (Crosbie et al., 2019) There are international guidelines for the screening of LS among patients with LS-associated cancers. (Seppälä et al., 2021) Screening should be started before the DNA testing by providing immunohistochemical (IHC) staining of the tumor by four MMR antigens to identify the lack of expression of the predisposing MMR gene. In endometrial cancer, IHC staining is recommended in following situations:

-

- Endometrial cancer diagnosed at 60 years of age or younger

- EC patient has one or more of following risk factors:

    - Personal history of metachronous or synchronous Lynch syndrome–associated cancer
    - First-degree relative with Lynch syndrome–associated cancer at 60 years of age or younger

    - Pathological features strongly suggestive of a Lynch syndrome–associated cancer

In ovarian cancer, LS testing is recommended if the patient is diagnosed at 50 years of age or younger or the tumor has non-serous and non-mucinous histology. (Crosbie et al., 2019)

2.2.3 Diagnostic criteria

Any individual genetically tested to be a carrier of *path_MLH1*, *path_MSH2*, *path_MSH6* or *path_PMS2* is designated to have Lynch syndrome, regardless of their clinical features.(Peltomäki, 2016) The criteria for the testing of gynecological tumors is presented in the previous chapter.

The Amsterdam II criteria (revised Amsterdam criteria) sets out clinical features used to recognize cancer-prone families to be offered testing for LS. (Lipton et al., 2004), (Samadder et al., 2017)
The Bethesda II guidelines for colorectal cancers suggest which gastrointestinal tumors should be tested for microsatellite instability (MSI; see chapter 2.2.5) and which patients should be offered genetic testing to identify pathologic variants for a Lynch syndrome diagnosis. (Lipton et al., 2004), (Umar et al., 2004) Both of these guidelines are presented in Table 4.

Table 4. Amsterdam II criteria and Bethesda II guidelines for detection of LS

Amsterdam II criteria	Bethesda II guidelines
- At least three relatives with one of the following LS-associated cancers: large bowel, small bowel, endometrium, ureter or renal pelvis	- Colorectal cancer diagnosed in a patient under 50 years of age
- One affected person is a first-degree relative of the other two	- Presence of synchronous, metachronous LS -associated tumors, regardless of age
- At least two successive generations are affected	- Colorectal cancer with the MSI-high genotype diagnosed in a patient under 60 years of age
- At least one person was diagnosed before the age of 50 years	- Colorectal cancer diagnosed in one or more first-degree relatives with a Lynch syndrome -related tumor, with one of the cancers diagnosed under 50 years of age
- Familial adenomatous polyposis has been excluded	- Colorectal cancer diagnosed in two or more first- or second-degree relatives with Lynch syndrome -related tumors, regardless of age
- Tumors have been verified by pathologic examination	
(Lipton, Johnson et al, 2004)	(Umar et al, 2004)

2.2.4 Databases

In 1994, the International Society for Gastrointestinal Hereditary Tumors INSiGHT established an international database of pathogenic MMR variants identified in families with Lynch syndrome. The data deposited in the database is mostly collected by individual investigators and gathered from published literature reports. (Plazzer et al., 2013)

PLSD, prospective Lynch syndrome database, has been developed by the LS researchers and established in 2012. It is an international, multicentre, prospective observational project aiming to make available the age and organ-specific information on cancer risk and survival according to MMR gene pathogenic variant and gender. (www.plsd.eu)

2.2.5 MMR gene function

During cellular division, errors in replicated DNA are normally identified and adjusted by the MMR protein complexes to maintain genomic stability. (Li, 2007)

In Lynch Syndrome, the mutations affecting the functions in the MMR proteins MLH1, MSH2, MSH6 or PMS2 may produce errors in the DNA replication. These proteins identify and correct DNA nucleotide excision repair damages caused by the DNA polymerase during replication, which occurs especially in microsatellites. This can take place in tumor suppressor genes or proto-oncogenes leading to carcinogenesis. (Li, 2007)

MMR pathogenic variants *path_MLH1*, *path_MSH2*, *path_MSH6* and *path_PMS2* tend to fail this DNA mismatch correction. DNA replication errors are transmitted to daughter cells, leading to repetitive DNA sequences to become unstable (microsatellite instability, MSI). In tumors associated with Lynch Syndrome, usually more than one mutated microsatellite sequences can be found, and this type of tumor is titled as MSI-high (MSI-H). (Li, 2007), (Peltomäki, 2016)

Most MMR gene pathogenic variants are inherited from a parent and the mutations are rarely new. The majority of all MMR gene pathogenic variants are specific to a particular family. Some prevalent recurrent mutations are also known: these mutations may appear de novo or instead represent founder mutations. (Peltomäki, 2016)

2.2.6 Gynecological cancers in Lynch Syndrome

The cancer spectrum in Lynch syndrome includes colorectal cancer (approximately 47 % of all Lynch-related tumors) and extracolonic cancers (53 %) consisting of other epithelial malignancies: endometrial, ovarian, gastric, small intestine, hepatobiliary tract, kidney and urinary tract cancers. (Quehenberger et al., 2005), (Watson & Riley, 2005), (Samadder et al., 2017)

Endometrial or ovarian cancer can be the first diagnosed malignancy, and endometrial cancer is as or even more prevalent as colorectal cancer among LS carriers with an uterus and ovaries. Their risk of endometrial cancer is estimated in recent studies to be 31 - 49 % during a lifetime with the risk being the highest for individuals carrying *path_MLH2*. (E. Stoffel et al., 2009), (Bonadona et al., 2011),(Dominguez-Valentin et al 2020)

Endometrial cancer associated with Lynch syndrome is diagnosed roughly 10 years earlier than in the general population. The mean age at diagnosis is 48 years. (Vasen et al., 1999) The majority of endometrial cancers is endometrioid in type. Certain histopathologic features such as mucinous differentiation, solid-cribriform growth pattern, high grade and possible necrosis might suggest that a tumor is a result of a mismatch repair defect. (Vasen et al., 1999) Loss of hMLH1 protein expression occurs in endometrial cancer associated with the Lynch syndrome but may also occur in 15–30% of unselected cancers due to abnormal promoter methylation. (McCarthy et al., 2019) MLH1 methylation analysis has been used to exclude sporadic EC cases from MLH1-deficient tumors and select non-methylated cases for LS screening. This has been suggested to be more cost-effective when performed age-selectively, since LS-related EC is rare among the elderly. (Pasanen et al., 2022) Abrogation of MSH2 and/or MSH6 protein expression seems to be a relatively specific indicator for Lynch syndrome. (Peltomäki, 2016)

In a study evaluating the endometrial samples of a cohort of LS carriers, early molecular changes in tumor development, such as MMR deficiency and tumor suppressor gene promoter methylation, were detected in almost all samples with carcinoma precursors (complex hyperplasia with or without atypia), in 40% of biopsies with simple hyperplasia and even in 7% of samples with a histologically normal endometrium as early as 12 years before EC gynecological cancer was diagnosed, which suggests a potential additional tool for cancer prevention. (Niskakoski, Pasanen, Lassus, et al., 2018) (Nieminen et al., 2009)

Endometrial carcinomas of the lower uterine segment are relatively rare in the general population – less than 5 % of endometrial cancers – but occur more frequently in individuals with Lynch syndrome. The reason for this is unknown. (Westin et al., 2008)

Endometrial cancer survival rates in Lynch syndrome population are excellent, with 10-year survival of 98%. (Watson & Riley, 2005)

Ovarian cancer represents approximately 13,4 % of all Lynch syndrome -related cancers. Previous studies estimate the lifetime risk of ovarian cancer among LS mutation carriers to be 4-17 % with the risk highest for individuals with *path_MSH2* and *path_MSH6*. (Watson et al., 2008) (Grindedal et al., 2010) Mean age at diagnosis of LS-related ovarian cancer is significantly lower than in the general population: from 43 to 45 years compared to 59 years, respectively. The survival rate of LS-associated OC is significantly higher compared to sporadic OC: in a large cohort study, five-year survival rates for stage III-IV diseases were 59% vs. 28%. (Grindedal et al., 2010)
The LS OC tumors seem to demonstrate a variety of histopathological subtypes, mostly epithelial and invasive, with 22 % occurring with synchronous primary endometrial cancer. (Watson et al., 2008) Invasive epithelial ovarian cancers among LS carriers are predominantly endometrioid (35%), serous or clear cell subtype according to a study of 63 cases in Sweden and Denmark. (Ketabi et al., 2011)
In comparisons of molecular features of LS-associated and sporadic OC tumors, the tumor suppressor gene methylation has been shown to be more frequent and the expression of tumor suppressor genes normal significantly more in OC of LS pathogenic variant carriers. This can partially explain the different function of LS-related ovarian malignancies. In these studies, the LS OC tumors were mainly of the non-serous type (endometrioid and clear-cell carcinomas). (Niskakoski et al., 2013) (Niskakoski et al., 2014)

When evaluating the molecular concordance of synchronous EC and OC tumors of LS carriers, the results have suggested that these carcinomas arise from shared origins, representing metastatic tumors from one site to another. The same finding applies to the EC precursor, complex hyperplasia, when compared to EC and OC in LS patients. (Niskakoski, Pasanen, Porkka, et al., 2018)

The average risks of different cancers in female Lynch Syndrome pathogenic variant carriers are presented in Table 5. (Dominguez-Valentin et al., 2020)

Table 5. Risk (%) of LS-associated cancers to age 75 years among female carriers

Cancer type:	MLH1	MSH2	MSH6	PMS2*
Any cancer	81 [74-88]	84 [77-91]	62 [47-78]	34 [19-60]
Colorectal	48 [41-57]	47 [39-55]	20 [12-41]	10 [3-41]
Endometrial	37 [30-47]	49 [40-61]	41 [29-62]	6 [3 - 11]
Ovarian	11[7-20]	17 [12-31]	11 [4-39]	2 [<1 - 5]

Data from PLSD database, Dominguez-Valentin M, 2020 (www.plsd.eu) **to age 80 years*

Lifestyle EC risk factors and protective factors among LS carriers

In addition to genetic risk factors, certain lifestyle factors, which are known to generally increase the risk of endometrial carcinoma, should be taken into account also in LS carriers. (Chapter 2.1.5)

The Manchester International Consensus Group was congregated in 2017 to develop distinct and integrated clinical guidance for the management of Lynch syndrome, based on existing data and opinions from medical professionals and patients. These guidelines were published in 2019. (Crosbie et al., 2019)

Asetylsalicylic acid (ASA, Aspirin) has been shown to decrease the risk of LS-related CRC and is therefore recommended in moderate doses, unless there are contraindications for its use. (Rothwell et al., 2011) However, the effect of ASA in prevention of endometrial cancer is unclear. (Burn et al, 2020)
In general, based on the knowledge on EC risk factors, LS carriers are advised to avoid obesity, take regular exercise and have a healthy diet, avoid smoking, avoid excess use of alcohol, and to avoid known carcinogens as a part of maintaining healthy lifestyles. (Crosbie et al., 2019)
The use of peroral contraception is suggested to prevent EC among LS carriers. (Lu & Daniels, 2013)

The recommendations of the consensus group regarding risk-reduction are concentrated in Table 6.

Table 6. Recommendations for EC risk reduction for female LS carriers

- Opportunity to discuss fertility and contraceptive needs with a specialist
- Combined oral contraceptive pills preferred for carriers wishing contraception
- Aspirin for chemoprevention, when not contraindicated
- Healthy body mass index maintained
- Healthy diet, regular exercise, no smoking, moderate alcohol use or no alcohol

(Crosbie et al., 2019)

2.2.7 Prophylactic (risk-reducing) surgery

Because of the high risk of EC among Lynch syndrome MMR pathogenic variant carriers, prophylactic total hysterectomy and bilateral salpingo-oophorectomy are generally recommended after possible childbearing at age of approximately 40 years. (Schmeler et al., 2006)
If surgery is avoided because of the risk of complications or some other reason, surveillance could offer an alternative.

According to the Manchester International Consensus group, it is strongly recommended that risk-reducing surgery is offered to *path_MLH1*, *path_MSH2* and *path_MSH6* carriers at 35–40 years of age when childbearing is complete. (Crosbie et al., 2019)
Individualized counselling regarding the optimal timing of the procedure, considering the patient's personal risk assessment and state of health, is recommended. Risk-reducing surgery is not strongly recommended to PMS2

pathogenic variant carriers due to the lack of adequate evidence on its benefits. (Crosbie et al., 2019)

Thorough preoperative counselling with an expert, regarding the risks and benefits, is recommended before the surgery, supplemented with patient-friendly written information. A preoperative pelvic ultrasound examination and endometrial biopsy are recommended for the detection of occult gynecological cancer.
If the patient has not attended colorectal surveillance as planned, a preoperative colonoscopy is recommended to detect possible colorectal malignancies. This enables simultaneous colorectal surgery, which is recommended if necessary. (Crosbie et al., 2019)

In accordance with the recommendations, if no histopathology is found, patients should be offered estrogen-only hormone replacement treatment after the prophylactic oophorectomy at least until the time of normal menopause at 51 years of age, with regard to their own views and gender identity. (Crosbie et al., 2019)

2.2.8 Surveillance

Widely, the EC surveillance before prophylactic surgery is performed on MMR pathogenic variant carriers and individuals from families that fulfil the clinical criteria for Lynch Syndrome. The surveillance has generally been performed by means of a gynecological examination, gynecological ultrasound (GUS) and blind or hysteroscopy-guided endometrial biopsy; either routinely or optionally in cases with endometrial thickness exceeding the designated criteria. (Renkonen-Sinisalo et al., 2007), (Auranen & Joutsiniemi, 2011) The interval of the examinations has varied from one to three years and the initiation of surveillance from 25 to 35 years of age. The surveillance for EC seems more effective with the use of endometrial biopsies in addition to GUS alone. (Renkonen-Sinisalo et al., 2007)

The purpose of the surveillance is to detect premalignant endometrial changes or early-stage carcinoma, with the aim of reducing cancer-related mortality among female LS pathogenic variant carriers. The benefit of EC surveillance has remained unclear and the data relating to its results is contradictory. Both atypical hyperplasia and stage 1 EC have been diagnosed in asymptomatic patients undergoing surveillance. Moreover, there is no evidence that the surveillance leads to an

improved chance of survival. (Auranen & Joutsiniemi, 2011), (Stuckless et al., 2013), (Ketabi et al., 2014)

The Manchester International Consensus Group has given updated recommendations for the gynecological surveillance of non-operated LS carriers. Routine gynecological surveillance is not recommended in MMR pathogenic variant carriers, due to the lack of evidence that this improves outcome over symptom-related investigations. (Crosbie et al., 2019) Many countries, including Finland, have followed the recommendations and discontinued the regular surveillance.

Earlier recommendations for surveillance have been given in Europe by the European Society of Medical Oncology (ESMO) 2016 and in United States by the American Society of Clinical Oncology (ASCO) 2015. Latest recommendations have been stated in the US by the National Comprehensive Cancer Network (NCCN) 2021. A comparison of all these guidelines is presented in Table 7.

Table 7. Surveillance recommendations for asymptomatic female LS carriers

Type of intervention	ASCO2015[1]	ESMO2016[2]	Manchester 2019[3]	NCCN 2021[4]
Informing carriers, educating on cancer symptoms	Yes	Yes	Yes	Yes
Gynecological examination	Yes	Yes	Yes	Yes
Transvaginal pelvic US examination	Yes	Yes	No	Yes
Endometrial biopsy	From age 30-35, annually	From age 30-35, annually	No	From age 30-35, annually/biannually

1) (E. M. Stoffel et al., 2015) 2) (Paluch-Shimon et al., 2016) 3) (Crosbie et al., 2019) 4)(Gupta et al., 2019)

2.2.9 Psychological aspects of genetic testing and gynecological screening

It has been shown that the psychological impact of genetic testing in hereditary cancer syndromes, including Lynch Syndrome, is highly dependent on an individual's pre-testing psychological adjustment capacity rather than the results themselves. Studies suggest that the most vulnerable group are the members of cancer families who have a high level of cancer anxiety, a history of depression, or who decline genetic testing. Testing itself can have a beneficial effect if the test results - even when revealing carrier status - are provided with sufficient information and opportunity for adequate planning. (Meiser et al., 2000), (Aktan-Collan et al., 2013), (Esplen et al., 2015)

It is also suggested that the family test results interacting with personal results, or in some cases, family test results alone seem to predict psychological outcomes more precisely than personal results alone. (Eliezer et al., 2014)

An earlier seven-year follow-up study did not show adverse psychosocial consequences among the LS carriers after the genetic testing. (Aktan-Collan et al, 2013) A review of 18 articles on the psychosocial impact of LS implies a transient increase in depression after testing among the pathogenic variant carriers, tending to normalize in six to twelve months. (Galiatsatos et al., 2015)

The psychological effects of gynecological screening in LS families have been evaluated in a study which did not demonstrate any adverse psychological effect in the screened population, even in those with false positive screening results. In this population, the screening included GUS, an office hysteroscopy, endometrial biopsy and ovarian tumor marker assessment (CA12-5). (NJ Wood et al., 2008)
Pain experienced in the endometrial sampling during the surveillance has shown to have a partial impact on prophylactic surgery decisions. In a relatively small study of LS carriers, 7 out of 52 (13%) mentioned the pain during endometrial biopsy as an important reason to ask for the surgery, along with a fear of cancer. The median VAS score of the pain experienced was 5. (Helder-Woolderink et al., 2017)
A positive experience regarding the relationship and communication with the screening physicians appears to increase trust in the efficacy of the surveillance and the ability to manage the Lynch Syndrome. However, it does not seem to engage patients to the screening more efficiently. (McGarragle et al., 2019)

Family and fertility

The impact of the discovery of a genetic cancer syndrome on the carriers' family planning and relationships has been studied to some extent among BRCA1/2 carriers. It has been shown that among BRCA mutation carriers, one of the main reasons for attending genetic testing was to know whether their children were at risk of carrying the mutation. (Fortuny et al., 2009)
Couples tend to separate more frequently among BRCA1/2 carriers compared to non-carriers, especially before childbearing, but the BRCA mutation does not tend to influence the number of pregnancies. (Mancini et al., 2015)

Positive Lynch Syndrome genetic testing results have been shown to emphasize underlaying conflicts and increase tension in families with previously poor relations. However, the impact appears to be relationship-strengthening in families with good relations. (Carlsson & Nilbert, 2007) It is not known, whether Lynch Syndrome affects fertility, the planned number of pregnancies, or intimate relationships. In young female Lynch syndrome patients with colorectal carcinoma, fertility has been shown to decrease after the diagnosis and treatment. (Stupart et al., 2015)

2.3 Present study

This review of the literature implicates that despite of the widespread research, the knowledge on LS carriers' EC risk factors is limited and incoherent and the psychosocial effects and influence of the syndrome on the family and reproductive decisions are relatively unknown. The factors impacting the carriers' decisions on the prophylactic surgery are remotely investigated. We aimed to collect more information on these topics and design a series of studies, of which the detailed objectives are presented in the next chapter.

3 AIMS OF THE STUDY

The objectives of this thesis were to investigate the possible similarities and differences in the risk factors of endometrial carcinoma among Lynch Syndrome pathogenic variant carriers and non-carriers, and to collect information about the impact of the known EC risk factors on LS carriers with pathogenic variants *path_MLH1*, *path_MSH2* and *path_MSH6*. Furthermore, we wanted to study the aspects of the pathogenic variant carriers themselves: the impact of positive germline testing results on their lives, the attitudes towards the surveillance and the factors that have an influence on their decision to undergo risk-reducing surgery. The initial aim was to learn more about the most beneficial ways to inform, guide and treat the female pathogenic variant carriers to maintain their physical and mental health and minimize the risk of gynecological cancer.
Specific aims of the study were:

1. To compare the lifestyle-related risk factors of endometrial carcinoma between Lynch syndrome pathogenic variant carriers with EC and sporadic EC patients.
2. To investigate the associations of lifestyle, reproductive, hormonal and medical factors with the risk of endometrial carcinoma in a cohort of Lynch syndrome pathogenic variant carriers.
3. To evaluate the process of decision-making for risk-reducing surgery, the attitudes towards surveillance and the pain experienced during endometrial biopsies among the LS pathogenic variant carriers.
4. To gather information about the impacts of pathogenic variant germline testing on relationships, family planning and self-image of the female LS carriers.

4 MATERIALS AND METHODS

4.1 Study subjects

4.1.1 LS and control subjects

The studies of this thesis were conducted in Tampere University Hospital (TAUH), Finland. The study population consisted of pathogenic MMR gene variant (*path_MLH1*, *path_MSH2*, *path_MSH6*) carriers identified from the Finnish Lynch Syndrome Research registry (LSRFi; Studies I – IV). Control patients were identified from the TAUH patient records including patients diagnosed and treated for sporadic EC between January 2002 and December 2009. Only patients with no familial history of cancer were included (Study I). The study protocol was approved by the TAUH Science Centre (January 2011). Informed consent was obtained from all the study subjects and controls.

In Study I, a total of 78 female Lynch syndrome carriers with diagnosis of endometrial cancer and 290 non-carrier controls with diagnosis of endometrial cancer were included.
The Study II population included 223 female LS pathogenic variant carriers.
In Study III, the population consisted of 112 female LS carriers with age of 30 years or older and no diagnosis of EC.
The Study IV subjects were 79 female LS carriers, tested before the age of 45 years.

4.1.2 The Finnish Lynch Syndrome Research registry

In March 2023, the Finnish Lynch Synrome Research registry (LSRFi) included over 420 LS families and approximately 1800 verified germline pathogenic variant carriers.

All the LSRFi subjects of this thesis have given a general informed consent to participate in any LSRFi initiated clinical studies and permitted LSRFi researchers to use their medical information in addition to the specific informed consent associated to Studies I – IV.
In March 2023, there were 933 female pathogenic variant carriers in the LSRFi registry. At survey, 377 female carriers had given their informed consent to participate in LSRFi's clinical studies. Carriers younger than 25 years of age or older than 90 years of age were excluded from Studies I – IV. Contact information (mailing address) of 223 women fulfilling the inclusion criteria was available.

4.2 Study design

Study I was a retrospective case-case study comparing lifestyle-related risk factors of endometrial carcinoma between the LS pathogenic variant carriers and non-carriers diagnosed with EC. In Study II, the lifestyle risk factors were evaluated in a cohort study of the LS pathogenic variant carriers only. Studies III and IV were descriptive studies evaluating the attitudes and insights of healthy pathogenic variant carriers. In Study III, the factors affecting the decision-making on risk-reducing surgery and the experiences of the surveillance and counselling by the experts were investigated, and the experience of pain during endometrial biopsies was evaluated with NRS scaling. Study IV focused on the impacts of positive germline testing to reproductive health decisions, relationships and psychological wellbeing among carriers tested for LS before the age of 45.
A summary of Studies I – IV is presented in Table 8.

Table 8. Summary of studies I - IV

	STUDY I	STUDY II	STUDY III	STUDY IV
DESIGN OF THE STUDY	Retrospective case – case - study	Retrospecive cohort study	Retrospecitve descriptive study	Retrospective descriptive study
METHOD OF DATA COLLECTION	LSRFi and TAUH patient records: questionnaire 1	LSRFi: questionnaire 1	LSRFi: questionnaires 1 and 2	LSRFi: questionnaires 1, 2 and 3
NUMBER OF PATIENTS	LS EC Cases: 78 Sporadic EC cases: 290	223	112	79
NUMBER OF RESPONDERS	LS EC Cases: 50 Sporadic EC cases: 110	136	76	35
INCLUSION CRITERIA	LS carrier with diagnosis of EC/ Non-carrier with diagnosis of EC	LS carrier	LS carrier Age ≥ 30 years No diagnosis of EC or OC	LS carrier Age at LS testing < 45 years No hysterectomy/ oophorectomy before testing
EXPOSURE DATA	Lifestyle factors, medical and reproductive history	Lifestyle factors, medical and reproductive history	Factors potentially affecting decision of risk-reducing surgery; Satisfaction with surveillance and counselling; Pain level (NRS) at endometrial biopsy	Reproductive history, family planning and relationship status; psychological wellbeing aspects
MAIN OUTCOME MEASURES	-	Diagnosis of EC	, -	-
OBJECTIVE OF THE STUDY	Comparison of EC risk factors among sporadic and LS EC patients	Detection of lifestyle-related EC risk factors among LS pathogenic variant carriers	Evaluation of the risk-reducing surgery decision-making process and attitudes towards surveillance	Clarification of the impacts of positive germline testing on reproductive decisions and psychological wellbeing

4.3 Methods

The data were collected via questionnaires sent to the study subjects (Studies I – IV) and controls (Study I). One re-sending was performed if no answer was received within three months.
In all of the studies, the LSRFi data was used to determine the types of pathogenic variants carried by the study subjects. The LSRFi data was also used to verify medical record data (EC diagnosis, prophylactic operations, time of germline testing) obtained from the questionnaires. The EC diagnoses of the non-carriers in Study I were confirmed from TAUH medical records.

4.3.1 The questionnaires

The questionnaires were sent by mail with a return envelope and filled in manually by the study subjects.
The contents of the questionnaires are summarized in Tables 9, 10 and 11.

Questionnaire 1

The aim of the Questionnaire 1 was to collect data on the health condition, lifestyle factors, parity, use of hormonal preparations and possible other medication of the LS carriers. This questionnaire was sent to all study subjects, including the controls with sporadic endometrial carcinoma diagnosis, for comparison of these factors with the LS carriers in Study I. The questions were mainly simplified to be answered with "yes" or "no" (Y/N) or with numeric measurements.

Questionnaire 2

In addition to the information from Questionnaire 1, the subjects of Studies III and IV were asked to fill in Questionnaire 2, aiming to collect data on their views on germline testing, surveillance and risk-reducing surgery and their possible impacts. This questionnaire was sent to the LS pathogenic variant carriers with no diagnosis of EC. The questionnaire was designed to be simple to answer, mainly with numeric

measurements and Y/N answers. The pain level at endometrial biopsies was estimated by the responders via Numeric Pain Rating Scale (NRS) from 0 (no pain) to 10 (worst imaginable pain).

Questionnaire 3

The subjects participating in Study IV were asked to fill in all three questionnaires. Questionnaire 3 was designed only for this study population consisting of LS pathogenic variant carriers with no diagnosis of EC and tested before the age of 45 years. This questionnaire collected descriptive data on the possible impact of LS to the personal relationships and family planning and self and body image of the carriers. The design of Questionnaire 3 was similar to the previous questionnaires with simple response measurements but it provided more possibilities for open comments.

Table 9. Summary of Questionnaire 1

All study subjects		
Question topic	**Further information**	**Measurement/ response**
Age at menarche		years
Age at menopause	If achieved	years
Height		cm
Weight	At present time	kg
	At age of 18, estimate	kg
	At age of 40, estimate	kg
Number of pregnancies		number
HRT use, ever		Y/N
If yes:	Duration	years
Ovulation failure		Y/N
PCO		Y/N
Endometriosis		Y/N
If yes:	Any medication	Y/N
	Oral contraceptives	Y/N
	Oral progesterone	Y/N
	Progesterone IUD	Y/N
Cancer other than EC		Y/N
If yes:	Which cancer/cancers?	list
LS surveillance duration, if any	Asked from LS carriers	years
Regular smoking, ever		Y/N
If yes:	Duration	years
	Cigarettes per day	number
Alcohol consumption		Y/N
If yes:	Duration	years
	Servings per week	number
Atherosclerosis		Y/N
Diabetes		Y/N
Hypertension		Y/N
Coronary disease		Y/N
Hypothyreosis		Y/N
Hypercholesterolemia		Y/N
Other chronic condition		Y/N
If yes:	which condition/conditions	list
Hormonal contraception, ever		Y/N
If yes:	Duration	years
Medication	Currently or previously used	list

Table 10. Summary of Questionnaire 2

Subjects of studies III and IV		
Question topic	**Further information**	**Measurement/ response**
Date of germline testing		Date/year
Age at germline testing		years
Relationship status before testing	In a relationship?	Y/N
Present relationship status	In a relationship?	Y/N
Risk-reducing surgery performed?		Y/N
Has attended follow-up?		Y/N
Considers follow-up beneficial?	If "yes" to previous question	Y/N
Parity	Number of deliveries	number
Pain level at endometrial biopsy (Numeric pain rating scale, NRS)	NRS 0-10	number
Satisfied with the advice provided by professionals?	During testing and possible surveillance/ surgery	Y/N
Sufficient information provided on possible adverse effects of risk-reducing surgery?	In general	Y/N
	On gynecological prolapses	Y/N
	On urinating complaints	Y/N
	On G-I tract complaints	Y/N
Has felt pressure for risk-reducing surgery?		Y/N
Satisfied with decision to have surgery?	If performed	Y/N
Planning to have surgery?	If not performed	Y/N
Cancer other than gynecological cancer in family?		Y/N
Which cancer?	If "yes" to previous question	Description
Family member died of gynecological cancer?		Y/N
Experience of personal state of health	Poor/intermediate/good	0/1/2
Poor tolerance of insecurity?	Own experience	Y/N
Strong fear of cancer?		Y/N
Strong fear of surgery/operations?		Y/N
Experience of surgery as responsibility?		Y/N

Table 11. Summary of Questionnaire 3

Subjects of study IV		
Question topic	**Further information**	**Measurement/ response**
Germline testing influenced on responder's relationship?	Own experience	Y/N
Pregnancy/pregnancies before testing?		Y/N
Pregnancy/pregnancies after testing?		Y/N
Induced abortion(s) before testing?		Y/N
Induced abortion(s) after testing?		Y/N
Plans for pregnancy before testing?		Y/N
Plans for pregnancy after testing?		Y/N
Sterilization performed before testing?		Y/N
Sterilization performed after testing?		Y/N
Germline testing influenced on responder's family planning?	Own experience	Y/N
Further description for previous question	Voluntary	Description
Did germline testing have negative influence on responder's self and body image?		Y/N
Open comments on responder's subjective experiences of germline testing	Voluntary	Open comments

4.3.2 Statistical analysis

In Studies I-III, statistical analyses were performed with IBM SPSS statistics software, version 22 (IBM SPSS, Inc., Armonk, NY, USA).

Two-tailed p values less than 0.05 were considered to indicate statistically significant differences.

In Study I, data on lifestyle-related risk factors of EC were statistically compared between patients with LS and sporadic EC. Student's t-test was used for comparison of mean values, and the chi-squared test for the comparison of categorical variables. The comparison of prevalence of HRT or hormonal contraception use, smoking, endometriosis, ovulation failure, diabetes, atherosclerosis, hypercholesterolemia, hypertension and hypothyreosis was performed using a chi-square test, as well as the comparison of prevalence of the cancers of the gastrointestinal tract, urinary tract, breasts and ovaries.

In Study II, the associations between lifestyle-related factors and the risk of EC in female LS carriers was estimated with the Cox regression model. Age was used as a timescale for EC risk estimation. Starting from birth, the time at risk was considered to end at the time of the survey, diagnosis of EC or prophylactic hysterectomy. The age at menarche and menopause, BMI, annual weight change and the duration of hormonal contraception and HRT use were divided into two categories by the median values for the univariable analyses. These variables were also analysed as continuous variables in the Cox regression model. Two categories were also used for BMI, using cut-off point values 25 (overweight) and 30 (obese). Nonparametric testing was used for the comparison of BMI as a continuous variable between the study subjects with diabetes and the non-diabetic subjects.

In Study III, the chi-squared test was used to evaluate the association of categorized variables with decisions of risk-reducing surgery. The association of continuous variables with performed prophylactic surgery was analysed using the t-test or a nonparametric test when appropriate. The categorization was used for NRS scores (0 to 5 versus 6 or more) and the number of deliveries (0 versus 1 or more) in the statistical analyses.

Study IV was a descriptive study, in which no statistical analyses were performed.

4.3.3 Ethical aspects

All studies were registry-based and questionnaire-based and required contact (by mail) with the study participants. All of the LS pathogenic variant carriers recruited had given their informed consent to be contacted regarding scientific studies by the LRSFi. Ethics approval was obtained from the Ethics Committee of Pirkanmaa Hospital District: (4th January 2011, decision code ETL R10079). All the subjects recruited gave their informed consent to participate in these four studies.

5 RESULTS

5.1 Distribution of pathogenic variant types (I, II, III, IV)

The LS carriers in Studies I, II, III and IV presented pathogenic variant types *path_MLH1* (significant majority of the responders), *path_MSH2* and *path_MSH6*.

Table 12 presents the distribution of different pathogenic variants in the responders of each study. The total amount of the subjects is not counted, as some of the women have participated in more than one of the studies.

Table 12. Distribution of LS pathogenic variant types among responders of Studies I, II, III and IV

Study population description	*path_MLH1* n (%)	*path_MSH2* n (%)	*path_MSH6* n (%)	Total n
Study I LS carriers with endometrial cancer	39 (78.0%)	8 (16.0%)	3 (6.0%)	50
Study II LS carriers	112 (82.4%)	15 (11.0%)	9 (6.6%)	136
Study III LS carriers with no EC	47 (62.0%)	22 (29.0%)	7 (9.0%)	76
Study IV LS carriers with no EC, tested before age of 45 years	28 (80.0%)	4 (11.4%)	3 (8.6%)	35

5.2 Comparison of EC risk factors between LS carriers and sporadic controls with EC (I)

A total of 50 EC patients with Lynch syndrome and 110 patients with sporadic EC returned the questionnaire. The response rates were 67% and 38% among the study subjects respectively.

The age of Lynch syndrome carriers was significantly lower at the time of the survey (mean age 65.0 years vs. 72.4 years) as well as at the time of their EC diagnosis (mean age 49.2 years vs. 55.6 years) compared to the sporadic EC patients.

There was no difference in the reported mean BMI values (27,2 among LS EC patients vs. 27.5 among the sporadic EC patients at survey) or the proportion of overweight persons (BMI > 25; 62.0% vs. 66.0% at survey, respectively) between the study groups.

The self-reported lifestyle habits, for example smoking and alcohol consumption measures, as well as the prevalence of chronic illnesses were also similar between the LS carrier group and sporadic EC group.

Among the reproductive factors, a difference was found in the percentage of subjects with one or more spontaneous abortions (10.0% of LS carriers vs. 24.0% of sporadic EC patients, p=0.043) and the ever use of hormonal contraception (56.0% vs. 32.7% respectively, p=0.004).

The prevalence of endometriosis seemed higher (16.0% vs 8.0%) among the LS carriers, but the differences did not reach statistical significance (p=0,137).

The prevalence of ovarian cancer, gastrointestinal tract cancer and urinary tract cancer was higher among the LS carriers.

The main results of Study I are summarized in Table 13.

Table 13. Study I: Summary of main characteristics and results.

Parameter	LS carriers with EC (n=50)	Subjects with sporadic EC (n=110)	p value of difference	All subjects of study I (n=160)
BMI (mean) at survey	27.2	27.5	0.697	27.4
Smoking (Percentage of subjects who smoke/have smoked)	30%	20%	0.137	23%
Mean alcohol use (servings per week)	1.7	2.1	0.354	1.9
Mean number of pregnancies	2.25	2.04	0.431	2.15
Spontaneous abortions (percentage of subjects with 1 or more)	10.0%	23.6%	**0.043**	19.4%
Induced abortions (percentage of subjects with 1 or more)	6.0%	15.5%	0.094	12.5%
HRT use (percentage of users)	50%	55%	0.521	54%
Mean duration of HRT use, years	11.3	9.7	0.407	-
Hormonal contraception use (percentage of users)	58%	33%	**0.004**	41%
Endometriosis (percentage of subjects with diagnosis)	16%	8%	0.137	11%
Diabetes (percentage of subjects with diagnosis)	12%	15%	0.665	14%
Hypertension (percentage of subjects with diagnosis)	36%	47%	0.183	44%

5.3 Endometrial cancer risk factors among Lynch syndrome carriers (II)

In Study II, 136 subjects answered the questionnaire, resulting in a response rate of 61%.

The median age of the subjects was 58.0 years at the time of survey (range 29-85). 50 subjects (37.8 %) had a diagnosis of EC at survey, and the median age at diagnosis was 49.5 years.

Of the 86 carriers not affected with EC, risk-reducing hysterectomy had been performed to 52 (60.5 %) at the median age of 45 years.

In Cox regression analyses of the study, non-insulin-dependent diabetes (type II diabetes) and hypercholesterolemia were associated with a higher risk of EC (HR 3.21 (95% CI 1.34–7.78), p=0.009; HR 2.08 (95% CI 1.11–3.90), p=0.02; respectively). A history of endometriosis (HR 1.96 (95% CI 0.90-4.28), P=0.09) and the use of HRT continuing for more than 9 years (HR 2.03 (95% CI 0.89-4.62), p=0.09) also showed a trend to a higher EC risk.
In the multivariable Cox regression analysis, diabetes and duration of HRT use significantly associated with the risk of EC (HR 4.18 (CI 1.52-11.52), p=0.006; HR 1.07 (CI 1.02-1.13), p=0.010; respectively).

BMI was not associated with the risk of EC at any age among the study subjects.

A summary of the characteristics of the subjects in Study II is presented in Table 14, and the main results of univariable and multivariable Cox regression analyses are summarized in Tables 15 and 16.

Table 14. Study II: Characteristics of study subjects.

Parameter	LS carriers with EC (n=50)	LS carriers with no EC (n=86)	LS carriers total (n=136)
Age, years (median)			
At survey	49.5	45.0	47.0
At menarche	13.0	13.0	13.0
At menopause	50.0	50.0	50.0
Number of live births (%)			
None 1-2 3 or more	9 (18.0%) 26 (52%) 15 (30%)	9 (10.5%) 51 (59.3%) 26 (30.2%)	18 (13.2%) 77 (56.6%) 41 (30.2%)
BMI and weight			
BMI at age of 18 (mean)	21.5	20.9	21.1
BMI at survey (mean)	27.2	25.9	26.4
Change of weight per year, mean (kg)	+0.3	+0.4	+0.4
Hormonal contraception use			
Percentage of ever users	56.0%	76,7%	69.1%
Mean duration of use, years	6.6	9.2	8.4
HRT use			
Percentage of ever users	50.0%	41.9%	44.9%
Mean duration of use, years	11.3	9.1	10.0
Chronic illness: percentage of subjects with diagnosis			
Diabetes	12.0%	1.2%	5.1%
Hypertension	19.8%	36.0%	25.7%
Hypercholesterolemia	9.3%	28.0%	16.2%
Endometriosis	11.6%	16.0%	13.2%
Smoking and alcohol consumption			
Percentage of ever smokers	30.0%	46.5%	40.4%
Alcohol, servings per week (mean)	1.2	2.0	1.7

Table 15. Study II: Results from univariable Cox regression analysis

Statistically significant or nearly significant results: Associations between endometrial cancer risk and medical factors for women with Lynch Syndrome				
Univariable analysis	**Number of women with EC**	**Total number of women**	**HR (95% CI)**	**p value**
History of diabetes	6 (85.7%)	7	3.21 (1.34-7.68)	0.009
History of hypercholesterolemia	14 (63.6%)	22	2.08 (1.11-3.90)	0.02
History of endometriosis	8 (44.4%)	18	1.96 (0.90-4.28)	0.09
Use of HRT for 9 years or more	16 (51.6%)	31	2.03 (0.89-4.62)	0.09

Table 16. Study II: Results from multivariable Cox regression analysis

Associations between endometrial cancer risk and medical factors for Lynch Syndrome carriers		
Multivariable analysis[1]		
Total number of carriers n=136	**HR (95% CI)**	**p value**
History of diabetes	4.18 (1.52 – 11.52)	0.006
History of hypercholesterolemia	1.47 (0.70 – 3.09)	0.308
Duration of HRT use (years)[2]	1.07 (1.02 – 1.13)	0.010
History of endometriosis	0.97 (0.39 – 2.42)	0.943

CI = confidence interval, HR = hazard ratio
1: Adjusted for age at survey (as continuous variable, parity (nulliparous vs. parous), duration of hormonal contraceptive use (as continuous variable), age at menarche (as continuous variable) and ascertainment (as categorised variable).
2: Continuous variable

5.4 Factors associated with the decision on prophylactic surgery among LS carriers (III)

In Study III, the response rate was 68% with 76 answered and returned questionnaires.

Hysterectomy for non-prophylactic medical reasons, such as myomas, excessive menstrual bleeding (without endometrial hyperplasia) or pelvic floor prolapses was performed to ten subjects. They were excluded from the analyses regarding risk-reducing surgery.

Risk-reducing surgery was performed on 42 subjects (55% of the responders) at the median age of 42 years. 24 subjects had not had hysterectomy. Eight (33.3%) of them reported that they are not planning to have surgery at all, and 16 (67.0%) had not made a decision about the surgery at the time of the survey or did not state it if they had.

Relationship status, a history of cancer in the family, the experiences regarding the state of one's own health, experiences of poor tolerance of insecurity, a fear of cancer, a fear of surgical operations, the experience of risk-reducing surgery as a responsibility, pressure felt towards risk-reducing surgery, or pain experienced during endometrial biopsy, seemed not to have an influence on the decision regarding prophylactic surgery.

The percentage of carriers satisfied with the LS-related information provided by medical professionals was significantly higher among the subjects who had undergone risk-reducing surgery (73.2%) compared to the non-operated subjects (31.8%). Additionally, the operated carriers tended to report having more information from professionals on certain postoperative conditions including gastrointestinal tract complaints and pelvic floor prolapses.

5.5 Gynecological surveillance (III)

A history of regular attendance at the gynecological surveillance provided was similar (87.5% vs. 88.1%) between non-operated subjects and subjects with risk-reducing

surgery performed. The majority of the responders (84.2%) considered the surveillance beneficial.

At survey, the LS carriers who had not undergone risk-reducing surgery had been under surveillance for a median time of 11 years (range from 6 to 29 years). This was the time interval between the LS pathogenic variant testing and the survey. The subjects with prophylactic surgery performed had a median time interval of 6 years (range from 0 to 14 years) between testing and surgery, representing the surveillance time.

5.6 Pain experienced during endometrial biopsy (III)

A total of 54 study subjects (71.1% of the responders) answered the question about pain experienced during endometrial biopsies, estimated with NRS scale in Questionnaire 2. The median NRS result was 3.5 on the scale from 0 to 10. The pain was estimated mild or intermediate or no pain at all (NRS 0-5) by 72.2% of the responders. 22 subjects did not respond to this question.

As mentioned in Chapter 5.3, the NRS scale result was not associated with the decision on risk-reducing surgery.

The results and the characteristics of the responders are presented in Table 17.

Table 17. Study III: Summary of responses from female LS carriers with no EC

Parameter description	**Risk-reducing surgery performed n=42**[1] n (%)	**No risk-reducing surgery n=24** n (%)	**p value of difference**	**Total responders n=76**[1] n (%)
One of more live births	37 (88.1%)	21 (87.5%)	1.000	66 (86.8%)
Health status intermediate/good	24 (58.5%)	18 (75.0%)	0.282	50 (65.8%)
Attended gynecological appointments regularly	37 (88.1%)	21 (87.5%)	1.000	68 (89.5%)
Other than gynecological cancer in family	39 (92.9%)	24 (100.0%)	0.295	73 (96.0%)
Family member died of gynecological cancer	12 (29.3%)	4 (16.7%)	0.373	17 (22.3%)
Poor tolerance of insecurity	5 (11.9%)	5 (20.8%)	0.477	13 (17.1%)
Strong fear of cancer	19 (45.2%)	10 (41.7%)	0.803	32 (42.1%)
Strong fear of surgical operations	6 (14.3%)	4 (16.7%)	1.000	12 (15.7%)
Experience of surgery as responsibility	12 (29.3%)	3 (12.5%)	0.142	16 (21.0%)
Has felt pressure to have surgery	14 (35.9%)	5 (20.8%)	0.391	20 (26.3%)
Satisfied with information and advice	30 (73.2%)	7 (31.8%)	**0.003**	43 (56.6%)
Endometrial biopsy pain NRS score (0-10)	n=28[2]	n=19[2]		n=54[2]
0 - 5	21 (75.0%)	11 (57.9%)	0.339	39 (72.2%)
6 - 10	7 (25.0%)	8 (42.1%)		15 (27.8%)

1: 10 subjects with non-prophylactic hysterectomy were excluded from comparison
2: Total of 54 subjects answered this question (22 subjects did not respond)

5.7 Female LS carriers' subjective experience of genetic testing and its impacts (IV)

After two mailings, 35 subjects returned the questionnaires of study IV, resulting in a response rate of 44.3%.

The median age at genetic testing was 31 years and median age at survey was 44 years among the responders.

97% of the responding subjects reported that the LS genetic testing results have not had any influence on their relationships.

Seven individuals (20% of the subjects) stated that positive germline testing had an impact on their family planning. Six of them offered more detailed information in the open comments section. Some of them told they wanted to have children as quickly and early as possible. Some stated that finding out about pathogenic variant limited the number of pregnancies they would have otherwise planned.

14% (five individuals) reported a negative impact on their self and body image by the positive test results.

The majority (86%) of the responders had been pregnant before the germline testing and 49% after receiving the test results. 6 subjects (17.1%) had undergone risk-reducing surgery after the testing by the time of survey. There were no significant differences in the numbers of induced abortions and sterilization procedures before and after the germline testing results.

Half of the 12 open comments given by the subjects indicated satisfaction and thankfulness over the testing, information and surveillance. Several responders told they will encourage their children to have genetic testing. A portion of the answers described worry and fear of health problems. One responder was disappointed in the negative manner in which she was informed about the pathogenic variant.

The measurable results of Study IV are summarized in Table 18.

Table 18. Study IV: Information derived from questionnaire for female LS carriers

Carriers with no EC, tested before age of 45 years (responders, n=35)	
Was in a relationship at the time of genetic testing	32 (91.0%)
Was in a relationship at survey	28 (80.0%)
Testing did have influence on relationship	1 (3.0%)
Pregnancy/pregnancies before testing	24 (69.0%)
Pregnancy/pregnancies after testing	17 (49.0%)
Induced abortion(s) performed before testing	2 (5.7%) (8.7% of women who had been pregnant before testing)
Induced abortion(s) performed after testing	2 (5.7%) (11.8% of women who had been pregnant after testing)
Has planned pregnancy before testing	30 (86%)
Has planned pregnancy after testing	15 (42.9%)
Sterilization performed before testing	5 (14.3%)
Sterilization performed after testing	5 (14.3%)
Testing did have influence on family planning	7 (20.0 %)
Testing did have negative influence on self-image and/or body image	5 (14.3%)

6 DISCUSSION

While discovering a hereditary cancer syndrome is a health risk, it is presumably also a stress-inducing factor in an LS carrier's life and has an influence on the family members. The syndrome does not only affect the carriers: their children have a 50% risk of inheriting the cancer predisposing pathogenic variant. Medical evaluation and thorough information given by a professional constitute a potential way to reduce the psychological burden associated with the genetic disorder. The common objective of this thesis was to gather information to help in the counselling of the female carriers of Lynch Syndrome to promote their health and wellbeing.

6.1 Endometrial cancer risk and protective factors in LS (I & II)

In Study I, the risk factors of endometrial cancer were compared between the LS carriers with EC and non-carriers with EC. In Study II, the EC risk factors were investigated among a cohort of LS carriers only. In both studies, the LS carriers were all tested and verified to have the MMR gene pathogenic variant. In some previous studies on EC risk factors, the comparison was performed between women with and without a CRC family history, without results of germline testing. (Fornasarig et al., 1998)(Wang et al., 2009)

The response rate among the LS carriers in Studies I (64%) and II (61%) was satisfying. In Study I, the response rate of sporadic controls was markedly lower (38%).

6.1.1 Obesity and metabolic syndrome

Obesity has previously been shown to be associated with the risk of EC among the general population. (Jenabi & Poorolajal, 2015) In some previous studies, EC patients with a CRC family history appeared to be less obese than patients with no familial history of CRC. (Fornasarig et al., 1998)(Wang et al., 2009) (Yoo et al., 2012) In our studies, this finding was not repeated as the BMI of the LS carriers with EC

and sporadic EC patients did not differ in Study I. However, this finding was in line with another previous study that reported no significant difference in BMI between these groups of women. (Blokhuis et al., 2010)
In contrast to the general population, BMI or annual increase in weight among LS carriers in Study II did not correlate with the risk of EC. This result is in line with the previous studies conducted among MMR pathogenic variant carriers. (Win et al., 2011)(Dashti et al., 2015)

Type II diabetes is strongly associated with obesity. It has also been shown to independently increase the risk of EC in the general population. (Liao et al., 2014) Coherently with this, the correlation of elevated EC risk and type II diabetes and hypercholesterolemia was found among LS carriers in Study II. In Study I, no difference was found in the prevalence of diabetes and hypercholesterolemia between the LS carriers with EC and the sporadic EC patients, which is in line with previous suggestions that these groups could have similar risk factors. (Win et al., 2011)(Dashti et al., 2015) It has been shown that endometrial carcinomas found in LS carriers, similarly as in the general population, are mainly of the endometrioid type and seem to develop via precancerous stages (hyperplasia, complex hyperplasia, atypical hyperplasia) which indicates the involvement of DM type II as a risk factor. (Nieminen et al., 2009) However, obesity is very strongly linked to DM II and insulin resistance and some reports suggest that DMII as an independent risk factor should be researched more, as obesity may account for some of the results. (Luo et al., 2014)

Although it is unclear whether obesity increases the EC risk among the LS pathogenic variant carriers, the association of elevated EC risk and diabetes type II would suggest that it could be beneficial to guide the LS carriers to maintain a normal BMI and use a healthy diet to avoid the metabolic comorbidities of overweight. This is also implicated by the authors of a relatively recent review study on LS carriers. (Coletta et al., 2019)

6.1.2 Hormonal risk and protective factors

In previous studies, hormone-related risk factors have been shown to have a similar impact on the EC risk among LS pathogenic variant carriers and the general population. (Lu et al., 2013)(Ali, 2014)(Dashti et al., 2015)

In Study II, hormonal contraception did not appear as a protective factor against EC in the LS carrier population. The results were the same concerning late menarche and parity. However, the protective impact of these factors has been shown in a previous large retrospective cohort study. (Dashti et al., 2015) The difference could be explained with a smaller population causing a lack of statistical power or the different ethnic background of the subjects in Study II. However, the lack of the protective impact of hormonal contraception also appeared in previous study among a relatively small population of *path_MLH1* carriers. (Blokhuis et al., 2010)

In Study I, the ever use of hormonal contraception was higher among LS carriers than sporadic EC patients. This could be explained with information and advice given to the pathogenic variant carriers to prevent gynecological cancers, and possibly with careful family planning.

In Study II, the use of hormonal replacement therapy for menopausal symptoms seemed to increase the risk of EC among the LS carriers. The duration of HRT correlated with a significantly higher risk in a multivariable analysis. The same impact is suggested in the previous studies on hormonal factors mentioned above. These results imply that unoperated LS carriers should presumably be recommended to avoid the long-term use of HRT.

Hormonal replacement therapy use seemed more frequent among LS carriers compared to the sporadic EC patients in Study I. However, the younger age of the LS women at survey presumably explains this and was the only significant co-variant in the logistic regression analysis.

6.1.3 Endometriosis

In Study I, a trend for higher reported prevalence of endometriosis was found among the LS carriers compared to sporadic EC patients. This trend has not been reported in earlier LS studies. LS women reported a diagnosis of endometriosis twice as often as the controls. The result was not statistically significant in this study population, but it possibly intimates a hypothesis that germline factors predisposing to EC could to some extent be involved in the pathogenesis of endometriosis. In previous studies,

LS carriers have been shown to have an increased risk for clear-cell OC and endometrioid-type OC compared to the general population. (Aarnio et al., 1999) These malignancies are suggested to possibly arise via a malignant transformation of the endometriotic tissue in the abdominal cavity and ovaries. (Prowse et al., 2006) (Fadare & Parkash, 2019) In gynecological organs removed in risk-reducing surgical operations, endometriosis has been found in up to 20% of the specimens. (Karamurzin et al., 2013) These findings could suggest a connection between LS and endometriosis.

6.2 Risk-reducing surgery and surveillance (III)

Attitudes towards gynecological surveillance and risk-reducing surgery decisions among LS pathogenic variant carriers were investigated in Study III.

No factors, in addition to the cancer risk, seemed to impact the women's decision for prophylactic hysterectomy and salpingo-oophorectomy. In a small previous study, age, parity and psychosocial factors, such as cancer worries, had an influence on the decision-making. (Etchegary et al., 2015) These results were not repeated in Study III.

In our study, having the prophylactic surgery performed was significantly associated with positive attitudes towards gynecological surveillance and the satisfaction with the information and advice obtained from medical professionals. However, the compliance with the surveillance was similar between the operated and non-operated women, and the majority of the responders considered the surveillance beneficial. The same findings on the experienced benefits of the surveillance have been revealed in previous studies. (Etchegary et al., 2015), (Helder-Woolderink et al., 2017) These results emphasize the importance of adequate consulting and discussions with professionals soon after the positive germline testing results, and at the follow-up appointments before the prophylactic surgery.

A few unoperated study subjects reported not having any information about the surveillance and not being provided gynecological appointments. It is possible that

these women have refused the risk-reducing surgery due to a lack of information, thus being continuously exposed to the risk of EC.

Results of Study III imply the importance of profound and detailed information about risk-reducing surgery and the possible side effects of the operations. Some of the LS carriers in this study reported information about the risks and possible adverse effects of surgery being either inadequate or entirely unavailable.
This is in line with previous qualitative studies on this subject, showing that LS carriers are mainly satisfied with their decisions to have surgery performed, but women, who are still considering surgery, are not perfectly content with the information they receive. (Schmeler et al., 2006), (Etchegary et al., 2018) However, more detailed information about the potential long-term side effects and risks is possibly not always provided until the decision to undergo risk-reducing surgery has been made. These results could suggest that female LS carriers should be informed in a detailed and structured way about the risk-reducing surgery readily after the germline testing and repeatedly at appointments with professionals. The study also emphasizes the importance of clear national guidelines for informing and treating female LS pathogenic variant carriers.

The experience of pain during endometrial samples among the LS carriers did not have an influence on prophylactic surgery decisions in Study III. However, the study provided beneficial information about the level of pain experienced. In an earlier study, severe pain during endometrial biopsy has been showed to be a reason to stop surveillance and possibly precipitate the risk-reducing surgery. (Helder-Woolderink et al., 2017) The pain associated with endometrial sampling by the subjects of Study III was rarely severe. This can be considered a positive and relieving result as GUS alone without endometrial sampling has been shown to be an inefficient technique for surveillance in terms of EC prevention for LS carriers. (Auranen & Joutsiniemi, 2011)

6.3 Effects of LS diagnosis and surveillance on female carriers at reproductive age (IV)

It is logical to assume that inherited MMR pathogenic variants could have an influence on the carriers' decisions regarding family planning and, possibly, their intimate relationships. Knowledge on this subject could be beneficial for medical professionals when guiding and informing the LS pathogenic variant carriers. In Study IV, we wanted to collect the subjective views and experiences of female LS carriers tested during their reproductive age and collect information on the impacts of germline testing on their family relationships and reproductive decisions.
Previous studies have shown that inherited cancer predisposing genes do not physically affect an individual's fertility. (Stupart et al., 2015) (Duffour et al., 2016) However, some reports suggest that cancer syndromes can have an influence on reproductive decisions among the carriers and their families, (Smith et al., 2004) (Dewanwala et al., 2011) and some MMR gene pathogenic variant carriers are reported to even consider prenatal testing. (Dewanwala et al., 2011) A similar trend was not found in Study IV: the majority of responders in this study did not report significant impacts on reproductive decisions or a negative effect on their intimate relationships. Their mean parity was even fractionally higher than in the general Finnish population and the number of sterilizations or induced abortions before and after genetic testing did not differ at all. This could imply that among the Finnish LS carrier population, the fear and worry of one's children inheriting the pathogenic variant does not limit family planning. One hypothetical explanation could be that counselling has not highlighted the risks for the offspring. However, some women reported worry and insecurity in their open comments. A small minority of them also reported the germline testing results had, to some extent, harmed their self and body image. This emphasizes the importance of a delicate and encouraging attitude on the part of the medical professionals when counselling LS carriers.

In line with Study III, the responders of Study IV had a positive and thankful attitude towards gynecological surveillance and considered it beneficial. As mentioned before, the fear of passing the pathogenic variant on seems not to restrict family planning among them. One reason for this could be regular surveillance and consulting with experts. All the women in Study IV were undergoing the surveillance, which was performed widely in Finland at the time of the survey. This implies that the surveillance may have a positive psychological impact on the LS

carriers, and therefore raises the question of whether the provision of some regular surveillance should be considered, even though it has not been shown to be efficient in the prevention of the life-threatening endpoint, gynecological cancer. (Auranen & Joutsiniemi, 2011)

6.4 Strengths and weaknesses of the study

The subjects in Studies II-IV and the LS subjects in Study I were all LSRFi verified LS pathogenic variant carriers with germline testing performed and the type of pathogenic variant confirmed, which can be considered to increase the reliability of the results. We were able to verify a part of the data from the medical records of the LSRFi subjects. The data in Studies I-IV were mainly collected via self-report questionnaires and a portion of the subjects were elderly women who had to recall measurements from a long time ago. This could be considered a weakness in the data reliability. However, in earlier reports it has been shown that recalled measures correlate satisfyingly, and self-reported information appears to be valid. (Perry et al., 1995) (Baier et al., 2000)

We considered the questionnaires a suitable method to collect data on the subjective experiences and opinions of the pathogenic variant carriers themselves. The LSRFi registry-related informed consent given by the subjects in advance implies that they had at least some interest in the research and could be assumed to give careful and thought-out responses. This positive attitude towards questionnaire studies is possibly one reason to the significantly higher response rate of LS pathogenic variant carriers compared to the sporadic EC controls in Study I, which has potentially produced some bias into the analyses. Furthermore, the significant age difference between the LS carriers and the controls and the fact that information was only collected from cancer survivors, presenting subjects with a more favourable outcome, can be hypothesized to be bias-introducing weaknesses in Studies I and II. A similar limitation occurs in Study III, as the mean age of prophylactically operated responders was significantly older than the mean age of unoperated women. The responders in Study III also had a fractionally higher rate of performed risk-reducing surgery than the non-responders, which must be noted when reviewing the results.

In all the studies, the study populations were relatively small, which is a limitation to many LS-related studies. The response rate was the lowest in study IV, only 44,3%.

The women in this study received three different questionnaires, which may have been tiring. A small portion of the subjects returned blank questionnaires with a note stating that they do not want to be constantly reminded of the LS and forced to think about passing it on to their offspring. It can also be assumed that the women selected for Study IV have been in a busy phase of their lives with family and work commitments.
Because the population in our study did not include any *path_PMS2* carriers, the results cannot be applied to them.

6.5 Clinical implications and future views

An implication for a higher prevalence of endometriosis among the LS endometrial carcinoma patients compared to controls, derived from Study I, is an interesting finding for future research. This trend was detected despite the more frequent hormonal contraception use among the LS women. It suggests that endometriosis among female LS carriers should be actively observed and treated. Although in Study II we could not detect a protective effect with hormonal contraception, it is a known EC protective factor in the general population, has been shown to be effective in the treatment of endometriosis (Grandi et al., 2019) and hence could be recommended to female LS carriers.
The results from Studies I and II suggest that the risk factors of EC do not markedly differ between the LS carriers and the general population. Encouraging the carriers to maintain a normal BMI and healthy lifestyle and avoid the long-term use of HRT could be beneficial.
Results of Study III suggest that it is beneficial to offer the female LS pathogenic variant carriers profound information about the aim of the surveillance and risk-reducing surgery - including the possible adverse effects - throughout their adulthood, regardless of the timing of the surgery.
The LS carriers in our study considered gynecological surveillance beneficial and relieving. The pain experienced in endometrial sampling was mainly estimated to be low or moderate. The women in reproductive age undergoing the surveillance mainly reported that the positive germline testing did not have negative psychological effects or a restricting influence on their reproductive decisions. Although the surveillance has not been shown to be effective in the prevention of EC, it could be

conductive to re-evaluate the recommendations and take the psychological benefits of surveillance into account. This consideration would probably warrant some further study on the psychological aspects and outcomes.

7 SUMMARY AND CONCLUSIONS

The main findings and conclusions in this study were:

1. The risk factors of EC did not differ significantly between the LS carriers with EC and sporadic EC patients. The prevalence of endometriosis showed a higher trend among the LS carriers. (Study I)

2. Diabetes type II and use of HRT may increase the risk of EC among LS carriers. (Study II) This emphasizes the benefits of lifestyle guidance for the carriers.

3. The gynecological surveillance has been well accepted by the LS carriers (Study III). The results indicate that systematic and profound information on risk-reducing surgery and its possible adverse effects should be provided to the carriers regardless of the phase in their lives.

4. Among Finnish women with LS, detecting the pathogenic variant in fertile age does not seem to have significant negative effect on their reproductive decisions, intimate relationships or self and body image. Surveillance, empathetic counselling and care provided by the medical experts seems to decrease the concerns, fear and anxiety of the LS carriers.

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PUBLICATION I

Comparison of lifestyle, hormonal and medical factors in women with sporadic and Lynch syndrome-associated endometrial cancer: A retrospective case-case study

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Comparison of lifestyle, hormonal and medical factors in women with sporadic and Lynch syndrome-associated endometrial cancer: A retrospective case-case study

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Abstract. Data available on lifestyle-associated hormonal and medical factors among endometrial cancer (EC)-affected women who carry the Lynch syndrome (LS) mutation is limited. The aim of the present retrospective case study was to compare the reproductive and medical history, as well as lifestyle-associated factors, among patients with LS and sporadic EC. The study population consisted of 50 verified germline mismatch repair (MMR) gene mutation carriers diagnosed with EC, and 110 sporadic EC patients. Data were collected using postal questionnaires. Apart from the mean age at the time of the EC diagnosis (LS, 48.7 years compared with sporadic patients, 55.2 years; P<0.0001), the characteristics of sporadic and LS EC patients were similar with regard to body mass index (BMI) at age 18, 40 or at the time of the survey, and smoking and alcohol consumption. LS women reported a significantly lower rate of spontaneous abortion (P=0.043) and also more frequent use of contraceptives (P=0.004). The prevalence of co-morbidities, including diabetes, atherosclerosis, hypercholesterolemia and hypertension, was similar between the LS and the sporadic groups. A trend for a higher prevalence of endometriosis among mutation carriers was detected (16.0 vs. 8.1%, P=0.137). As anticipated, the prevalence of gastrointestinal tract, urinary tract and ovarian cancer was higher among the LS women (P<0.0001, P=0.006 and P=0.056, respectively). Co-morbidity and lifestyle-associated factors appeared to be comparable among patients with LS and sporadic EC. The reported difference in the use of contraceptives warrants further investigation. Future studies are also required to address the possible association between LS and endometriosis.

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Introduction

Lynch syndrome (LS), also called hereditary non-polyposis colorectal cancer (HNPCC), is a dominantly inherited cancer predisposition syndrome caused by germline mutations in the DNA mismatch repair (MMR) genes, MLH1, MSH2, MSH6, and PMS2. In addition to the early occurrence of colorectal cancer (CRC), LS is also characterized by certain extracolonic cancers (ECCs), of which EC is the most common (1). The lifetime risk of EC varies between 32 and 60%, according to previous studies (2-4). Inactivation of the LS genes leads to loss of MMR proteins and results in microsatellite instability (MSI), which is typical for LS-associated EC. MSI is present in 64% of LS-associated EC tumors, and only in 15% of sporadic EC tumors (5-7).

The common risk factors for EC in the general population, i.e. in non-carriers of MMR mutations, have been well-characterized in several studies: The EC risk rises by nulliparity, obesity, hypertension, high blood glucose levels, ovulation failure, non-use of hormonal contraceptives, estrogen use, estrogen-producing tumors and use of tamoxifen (8,9). Few studies have correlated MMR expression with body mass index (BMI), lifestyle habits and medical history in unselected EC, suggesting an association between lower BMI and a loss of MMR expression (10-12). However, data are limited on lifestyle, hormonal and medical factors in mutation-verified LS-associated EC. Previous studies reporting BMI in EC-affected LS women have elicited contradictory results (13-16).

The aim of the present study was to characterize lifestyle factors and the medical and reproductive history, in EC-affected verified LS mutation carriers and in sporadic EC patients having no familial history of cancer.

Materials and methods

Study subjects. The present retrospective case study was performed at Tampere University Hospital (TAUH), Tampere, Finland. The study protocol was approved by the TAUH Ethical Committee. The study population consisted of Finnish female LS mutation carriers diagnosed with EC in eight Central and five University Hospitals across Finland between January 1992 and December 2010 (Table I). All LS patients with EC tested positive for germ-line mutations associated with LS between January 1996 and December 2009, and this Finnish LS registry has been previously characterized (9). The distribution of different germ-line mutations among LS EC patients (n=50) was as follows: MSH2, 8 patients (16%); MLH1, 39 patients (78%); and MSH6, 3 patients (6%).

The control population consisted of Finnish women with sporadic EC treated at Tampere University Hospital between January 2002 and December 2009 showing no familial history of cancer. Questionnaires addressing reproductive and medical history were mailed to 78 LS and 290 sporadic EC patients. Questionnaires were re-sent to patients who did not return questionnaires in 6 months from the first mailing. Finally, data from the returned questionnaires were collected from a total of 50 LS and 110 sporadic EC patients. All participants in the present study provided their informed consent.

Questionnaires. Participants in the study were recruited to complete a questionnaire collecting data on height, present weight and weight at the ages of 18 and 40 years, parity, number of abortions and miscarriages, age at menarche and menopause, history of ovulation failure, polycystic ovaries and endometriosis, use of hormone replacement therapy (HRT) and hormonal contraception, smoking habits, alcohol consumption, history of other types of cancer and chronic illnesses. A detailed description of the questionnaire content is presented in Table II.

Statistical analysis. IBM SPSS statistics software, version 22 (IBM SPSS, Inc., Armonk, NY, USA) was used for the statistical analyses. BMI, alcohol consumption, the cumulative number of smoked cigarettes as pack-years, number of deliveries, number of induced and spontaneous abortions, age at menarche and menopause, and the duration of HRT or contraceptive use were statistically compared among patients with LS and sporadic EC. The comparison of means was performed using Student's *t*-test, and comparison of categorical variables (induced and spontaneous abortions) was performed using a Chi-square test. The prevalence of ever use of HRT or hormonal contraception, ever smoking, endometriosis, ovulation failure, diabetes mellitus (DM), atherosclerosis, hypercholesterolemia, hypertension and hypothyreosis was compared among patients with LS and sporadic EC using a Chi-square test. The prevalence of gastrointestinal tract, urinary tract, breast and ovarian cancer (OC) types was also compared among patients with LS and sporadic EC using a Chi-square test. Two-tailed P<0.05 values were considered to indicate statistically significant differences.

Table I. Hospitals of LS EC patients featured in the present study.

Name of hospital	Location
Tampere University Hospital	Tampere, Finland
Helsinki University Hospital	Helsinki, Finland
Oulu University Hospital	Oulu, Finland
Kuopio University Hospital	Kuopio, Finland
Turku University Hospital	Turku, Finland
Jyväskylä Central Hospital	Jyväskylä, Finland
Päijät-Häme Central Hospital	Lahti, Finland
Kanta-Häme Central Hospital	Hämeenlinna, Finland
Seinäjoki Central Hospital	Seinäjoki, Finland
Rovaniemi Central Hospital	Rovaniemi, Finland
Pohjois-Karjala Central Hospital,	Joensuu, Finland
Kotka Central Hospital	Kotka, Finland
Satakunta Central Hospital	Pori, Finland

LS, Lynch syndrome; EC, endometrial carcinoma.

Results

Questionnaire response rates were 67 and 38% among patients with LS and sporadic EC, respectively. The mean age of the patients at the time of EC diagnosis was 49.2 (range, 36-66) and 55.6 (range, 42-72) years among patients with LS and sporadic EC, respectively (P<0.0001). Patients with LS and sporadic EC at the time of the survey were also significantly younger compared with their sporadic EC counterparts (mean age, 65.0 vs. 72.4 years; P=0.0001). The mean BMI values reported in the survey at the ages of 18 or 40 years were similar, as was the proportion of overweight patients (reporting a BMI >25) among mutation carriers in comparison with sporadic EC patients.

Self-reported lifestyle habits, including smoking and alcohol consumption, did not differ among patients with LS and sporadic EC.

Reproductive factors appeared to be similar among the study and control patients, with the exception of the number of spontaneous abortions and the use of hormonal contraception. Only 10% of the patients with LS and sporadic EC reported one or more spontaneous abortions compared with 24% of sporadic EC patients (P=0.043), and the ever use of hormonal contraception was more frequent among mutation carriers compared with sporadic EC women (56.0 vs. 32.7%; P=0.004).

LS women seemed to use HRT more frequently in the survey responses compared with sporadic EC patients (16.0 vs. 6.3%; P=0.05), although in the logistic regression analysis, only a younger age in the survey among LS women remained as a significant co-variant.

A total of 16% of the patients with LS and sporadic EC, and 8.1% of the sporadic EC patients had been diagnosed with endometriosis (P=0.137). Self-reported prevalence of chronic illnesses, including hypertension, atherosclerosis, DM, hypothyreosis and hypercholesterolemia, was similar among patients with LS and sporadic EC. The prevalence of

Table II. Details of the questionnaire sent on to patients with LS and sporadic EC.

Feature	Further information	Measurement/response
Height		cm
Weight	At an age of 18	kg
	At an age of 40	kg
	At present	kg
Age at menarche		Years
Age at menopause if achieved		Years
Number of pregnancies		Number
	Deliveries	Number
	Miscarriages	Number
	Induced abortions	Number
Vaginal HRT use?		Y/N
Systemic HRT use?		Y/N
If yes:	Systemic HRT duration	Years
	Systemic HRT at present	Y/N
Ovulation failure		Y/N
PCO		Y/N
Endometriosis		Y/N
If yes, any treatment?		Y/N
	Contraceptive tablets	Y/N
	Progesterone po	Y/N
	Progesterone-IUD	Y/N
Cancer other than EC?		Y/N
If yes:	GI tract cancer	Y/N
	Urinary tract cancer	Y/N
	Breast cancer	Y/N
	Ovarian cancer	Y/N
Operated for cancer?		Y/N
LS gynecological follow-up duration		Years
Regular smoker?		Y/N
If yes:	Cigarettes per day	Number
	Duration of smoking	Years
Alcohol consumption?		Y/N
If yes:	Servings/week	Number
	Duration of consumption	Years
Diabetes?	Insulin treatment	Y/N
	Tablet treatment	Y/N
Hypertension?		Y/N
MCC?		Y/N
Hypothyreosis?		Y/N
Hypercholesterolemia?		Y/N
Atherosclerosis?		Y/N
Any other serious condition? If so, which?		List
Hormonal contraception?		Y/N
If yes:	Duration of use	Years
Medication		List

LS, Lynch syndrome; EC, endometrial carcinoma; HRT, hormone replacement therapy; PO, peroral; PCO, polycystic ovaries; IUD, intrauterine device; MCC, coronary heart disease; GI, gastrointestinal.

gastrointestinal tract cancer (48 vs. 0%; P<0.0001), urinary tract cancer (12 vs. 2%; P=0.006) and OC (6 vs. 0.01%; P=0.056) was higher among LS patients in comparison with sporadic EC patients.

Table III. BMI and lifestyle habits among LS women with EC compared with patients with sporadic EC.

Parameter	Total EC, (n=160)	LS-associated EC (n=50)	Sporadic EC (n=110)	P-value
BMI, mean (SD)				
At age of 18	21.6 (2.7)	21.4 (2.1)	21.7 (3.0)	0.525[a]
At age of 40	24.4 (4.0)	24.5 (4.5)	24.3 (3.7)	0.828[a]
At survey	27.4 (5.4)	27.2 (5.3)	27.5 (5.4)	0.697[a]
BMI >25, n (%)				
At age of 18	18 (11.3)	3 (6.0)	15 (14.0)	0.157[b]
At age of 40	68 (42.5)	28 (56.0)	40 (36.0)	0.334[b]
At the time of the survey	104 (65.0)	31 (62.0)	73 (66.0)	0.592[b]
Tobacco use, n (%)				
Yes	37 (23.0)	15 (30.0)	22 (20.0)	0.164[a]
No	123 (77.0)	35 (70.0)	88 (80.0)	
Smoking, pack years[c]	7.55	5.53	9.95	0.137[b]
Alcohol consumption[d]	1.9	1.7	2.1	0.354[a]

[a]According to Student's *t*-test. [b]According to Pearson's Chi-square test. [c]A 'pack year' is defined as 20 cigarettes a day for 1 year. [d]Mean number of servings of alcohol per week. BMI, body mass index; EC, endometrial cancer; LS, Lynch syndrome; SD, standard deviation.

Table IV. Prevalence of factors associated with reproduction among LS women with EC compared with patients with sporadic EC.

Factor	Total EC (n=160)	LS-associated EC (n=50)	Sporadic EC (n=110)	P-value
No. of pregnancies[a]	2.15	2.25	2.04	0.431[b]
No. of deliveries[a]	1.79	1.84	1.73	0.594[b]
Spontaneous abortions, n (%)				
0	129 (80.6)	45 (90.0)	84 (76.4)	0.043[c]
≥1	31 (19.4)	5 (10.0)	26 (23.6)	
Induced abortions, n (%)				
0	140 (87.5)	47 (94.0)	93 (84.5)	0.094[c]
≥1	20 (12.5)	3 (6.0)	17 (15.5)	
Age at menarche[a]	13.6	13.4	13.7	0.375[b]
Age at menopause[a]	50.4	50.3[d]	50.5[e]	0.878[b]
Duration of HRT use, years[a,f]	-	11.3	9.7	0.407[b]
Duration of hormonal contraception use, years[a]	-	6.6	6.9	0.83

[a]The mean values are indicated. [b]According to the Student's *t*-test. [c]According to the Pearson Chi-square test. [d]n=21; [e]n=69. [f]Regarding ever users of HRT, n=25 LS patients and n=61 sporadic EC patients. EC, endometrial cancer; LS, Lynch syndrome; HRT, hormone replacement therapy.

Comparisons of BMI, lifestyle habits, reproductive history, prevalence of chronic illnesses, hormonal therapy use and history of cancer among the patients with LS and sporadic EC are summarized in Tables III-VI.

Discussion

In the present case study, data on self-reported reproductive and medical histories in verified LS mutation carriers in comparison with sporadic EC patients are reported. According to these results, co-morbidity and prevalence of lifestyle-associated factors appeared to be comparable among patients with LS and sporadic EC. However, ever use of hormonal contraceptives was more common among mutation carriers, who also reported having fewer spontaneous and induced abortions compared with their sporadic counterparts. Furthermore, a trend of higher prevalence of endometriosis among mutation carriers was detected. As expected, GI-tract, urogenital tract and ovarian malignancies were more frequent among LS mutation carriers.

Previously published studies describing BMI or environmental factors in suspected LS populations have presented contradictory results. These studies have suggested that EC women with a family history of CRC or suspected LS appear

Table V. Self-reported prevalence of chronic medical conditions and use of hormonal therapy among LS women with EC compared with patients with sporadic EC.

Therapy or condition, n (%)	Total EC (n=160)	LS-associated EC (n=50)	Sporadic EC (n=110)	P-value[a]
Ever use of HRT				
Yes	86 (54)	25 (50)	61 (55)	0.521
No	74 (46)	25 (50)	49 (45)	
HRT use at present				
Yes,	15 (9)	8 (16)	7 (6)	0.05[b]
No	145 (91)	42 (84)	103 (94)	
Ever-use of hormonal contraception				
Yes	65 (41)	29 (58)	36 (33)	0.004
No	95 (59)	21 (42)	74 (67)	
Ovulation failure				
Yes	16 (10)	4 (8)	12 (11)	0.570
No	144 (90)	46 (92)	98 (89)	
Endometriosis				
Yes	17 (11)	8 (16)	9 (8)	0.137
No	143 (89)	42 (84)	101 (92)	
Diabetes mellitus				
Yes	23 (14)	6 (12)	17 (15)	0.665
No	137 (86)	44 (88)	93 (85)	
Atherosclerosis				
Yes	10 (6)	1 (2)	9 (8)	0.134
No	150 (94)	49 (98)	101 (92)	
Hypercholesterolemia				
Yes	51 (32)	14 (28)	37 (34)	0.478
No	109 (68)	36 (72)	73 (66)	
Hypertension				
Yes	70 (44)	18 (36)	52 (47)	0.183
No	90 (56)	32 (64)	58 (53)	
Hypothyreosis				
Yes	21 (13)	6 (12)	15 (14)	0.776
No	139 (87)	44 (88)	95 (86)	

[a]According to the Pearson Chi-square test. [b]In the logistic regression analysis, only the age at the time of the survey remained a statistically significant covariate. EC, endometrial cancer; LS, Lynch syndrome; HRT, hormone replacement therapy.

to be obese less often compared with EC women with no CRC family history (14-18). However, Lynch mutation carriers with EC have also been reported to be more obese compared with women with sporadic EC (16), whereas others (13) have reported no significant difference in BMI between these two patient groups, which is in line with the results presented in the current study. Even though our LS cohort included only 50 EC cases, all of them were verified germline MMR gene mutation carriers, and in comparison with previous descriptive studies, the cohort was relatively large.

Several studies of unselected EC cohorts have revealed a positive association between MSI or MMR protein expression positivity and a higher BMI and older age at the time of EC diagnosis (10-12). However, these previous studies have used indirect measurements of LS (i.e., absent MMR protein expression or MSI) instead of germline mutation testing. Consistently with previous studies (14,16), no differences in the prevalence of DM or other chronic illnesses between the study groups were identified in the current study. With regard to BMI and other co-morbidities, the similarities between LS and sporadic EC patient cohorts may imply that sporadic and hereditary EC patients share a common risk factor profile. However, such conclusions cannot be drawn from the type of data presented in the current study.

To date, only two comprehensive retrospective cohort studies of verified LS populations have been published with regard to EC risk (19,20). According to these studies, risk factors are partially shared in sporadic and hereditary EC, since the BMI appeared not to have an effect on EC risk, although parity, longer use of hormonal contraceptives and a later age at menarche reduced the risk of EC in LS women (19,20). It has been suggested that LS women have more non-endometrioid

Table VI. Cancer prevalence among women with EC with or without an inherited predisposition.

Type of cancer, n(%)	Total EC (n=160)	LS-associated EC (n=50)	Sporadic EC (n=110)	P-value[a]
GI-tract cancer				
Yes	24 (15)	24 (48)	0 (0)	<0.0001
No	136 (85)	26 (52)	110 (100)	
Urinary tract cancer				
Yes	8 (5)	6 (12)	2 (2)	0.006
No	152 (95)	44 (88)	108 (98)	
Breast cancer				
Yes	5 (3)	3 (6)	2 (2)	0.159
No	155 (97)	47 (94)	108 (98)	
Ovarian cancer				
Yes	4 (3)	3 (6)	1 (1)	0.056
No	156 (97)	47 (94)	109 (99)	

[a]According to the Pearson Chi-square test. EC, endometrial cancer; GI, gastrointestinal; LS, Lynch syndrome.

tumors compared with sporadic patients, which could at least partly explain certain of the differences in the reported risk factor profiles (19-21).

LS mutation carriers in the present study used hormonal contraceptives more frequently than non-carriers. This may reflect EC risk-reducing strategies recommended for mutation carriers, or improved standards of advisory family planning. Only a few studies have previously addressed the influence of contraceptive use on ECC risk among LS women (13,19). No significant effect of contraceptive use on the ECC risk was detected among MLH1 mutation carriers in the study of Blokhuis *et al* (13), although that study included only 12 cases of EC in 87 mutation-positive females, in comparison with 121 mutation-negative female relatives. However, the previously described large retrospective cohort study revealed a marked EC risk reduction among LS mutation carriers with a history of contraceptive use extending to 1 year (19). The results of the present study, demonstrating fewer spontaneous and induced abortions among mutation carriers, may also be interpreted as more premeditated family planning being carried out for the LS mutation carriers tested at a fertile age, and this warrants further investigation.

LS women reported endometriosis two times more frequently than sporadic EC patients. However, the present study was not able to detect statistically significant associations between more frequent diagnosis of endometriosis and LS-associated EC. It is intriguing to speculate that genetic factors conferring EC predisposition may also be partly involved in the pathogenesis of endometriosis. LS women also have an increased risk for OC, as also demonstrated in the present case study and, more specifically, for endometrioid OC and the clear-cell type of OC (2). Endometrioid and clear-cell OC are speculated to possibly originate from endometriotic foci undergoing a malignant transformation (22). Notably, a small series of LS prophylactic surgery specimens revealed endometriosis in up to 20% of samples (23). Taken together, this interesting finding of possible association of LS with endometriosis warrants future studies at a larger scale.

There were limitations to our study. First, the study was descriptive and did not provide data on actual environmental EC risk factors for genetically predisposed LS women, but nevertheless produced qualitative data on features of sporadic and hereditary EC cohorts. As anticipated, the response rate was markedly higher among LS mutation carriers, and this may have introduced bias into the analysis. The positive family history and verified mutation status may be associated with a higher participation rate and a more positive attitude towards questionnaire studies. EC patients with LS were younger than sporadic patients at the time of diagnosis and at the time of the survey, which could have had an influence on the distribution of time-dependent factors, and this should be taken into account when interpreting the results. The data were only collected from EC survivors, and self-reported retrospective data were based on patients' memory. This may have led to bias and under-reporting. However, it has been demonstrated that, for example, weight measures based on patients' memory actually correlate well (24).

In conclusion, the present case study has reported on self-reported reproductive and medical histories in verified LS mutation carriers compared with sporadic EC patients. The BMI, co-morbidity and lifestyle-associated factors appeared to be comparable between LS and sporadic EC patient cohorts. Ever use of hormonal contraceptives was more common among mutation carriers, and they appeared to have undergone fewer spontaneous and induced abortions. These findings may reflect more premeditated family planning in LS mutation carriers tested for mutations at a fertile age, providing an interesting target for future research. A trend of higher prevalence of endometriosis among mutation carriers was also detected, similarly warranting further investigation at a larger scale.

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PUBLICATION II

Endometrial cancer risk factors among Lynch syndrome women: a retrospective cohort study

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Endometrial cancer risk factors among Lynch syndrome women: a retrospective cohort study

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Background: Lynch syndrome (LS) is associated with a significant lifetime risk of endometrial cancer (EC). There are limited data on factors modifying the EC risk in LS patients.

Methods: The study cohort included 136 LS mutation-positive women. Exposure data were collected by postal questionnaires. Cox regression model was used to estimate the associations between lifestyle, hormonal, reproductive and medical factors and the risk of EC.

Results: Increased EC risk was associated with type II diabetes and hypercholesterolaemia in univariable (HR 3.21, (95% CI 1.34–7.78), $P=0.009$ and HR 2.08, (95% CI 1.11–3.90), $P=0.02$; respectively) and with diabetes and duration of hormone replacement therapy (HRT) in multivariable analysis (HR 4.18 (95% CI 1.52–11.52), $P=0.006$ and HR 1.07 (95% CI 1.02–1.13), $P=0.010$; respectively).

Conclusions: Prevention of diabetes and avoiding long-duration HRT are potential targets for reduction of EC risk in women with LS.

Lynch syndrome (LS) is a cancer predisposition syndrome with autosomal-dominant inheritance pattern caused by germ-line mutations in DNA mismatch repair (MMR) genes *MLH1*, *MSH2*, *MSH6* and *PMS2* (Vasen *et al*, 1999). LS is associated with significantly increased lifetime risks of both colorectal and endometrial cancer (EC), ranging from 20% to 51% depending on the type of the mutation (Møller *et al*, 2015).

Factors increasing EC risk in general population all relate to conditions of oestrogen dominance over progesterone. EC risk has been shown to increase with nulliparity, early age at menarche, late age at menopause, obesity, metabolic syndrome, ovulation failure, non-use of hormonal contraceptives, and oestrogen or sequential hormone replacement therapy (HRT) (Ali, 2014; Barry *et al*, 2014; Trabert *et al*, 2015). Data on the influence of these risk factors on EC risk of genetically predisposed LS women are, however, limited. An intervention study of oral contraceptive and medroxyprogesterone acetate in LS women suggested a protective effect on endometrial proliferation similar to the general population (Lu *et al*, 2013). This was further supported by a recent large retrospective study, where EC risk in LS women decreased with parity, use of hormonal contraceptives and later age at menarche (Dashti *et al*, 2015).

The association of high body mass index (BMI) and other metabolic syndrome-related factors with EC risk of LS women is

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not clear. Studies addressing the association of BMI with MMR protein expression or microsatellite instability in unselected EC have been contradictory (McCourt *et al*, 2007; Cohn *et al*, 2008; Gonzalez *et al*, 2012; Joehlin-Price *et al*, 2014). Only few comprehensive studies have been conducted in well-characterised study populations with germ-line mutation testing. According to these studies, BMI may not be associated with EC risk among LS women (Win *et al*, 2011; Dashti *et al*, 2015).

To date, hysterectomy provides the only means for EC risk reduction or prevention in high-risk women. Therefore, research on the impact of environmental factors on EC risk in LS women is needed. Here we have estimated the associations between lifestyle, hormonal, reproductive and medical factors and the risk of EC in a cohort of MMR germ-line mutation carrier women.

MATERIALS AND METHODS

Study patients. This retrospective cohort study was carried out in Tampere University Hospital (TAUH), Finland. Study cohort included Finnish women with inherited pathogenic MMR gene mutation identified from the nationwide Finnish LS Registry (Jarvinen *et al*, 2009). The Finnish LS Registry consists data of original research cohort including 81 kindreds ascertained through family history strongly suggestive of LS and clinic-based cohort including patients referred to clinical genetic units of five University hospitals in Finland for suspected LS (Mecklin *et al*, 1987; Gylling *et al*, 2009). The index patients belonging to the research cohort have been directly tested for germ-line MMR mutations without prescreening for MMR protein loss in the tumours. Patients of clinic-based cohort have been screened for MMR deficiency in tumour tissue prior to germ-line testing from blood samples. Counselling and possible germ-line mutation testing have been systematically offered for family members of index patients up to second- or even to third-degree relatives. Mutation analyses have been performed by direct exon sequencing or by multiplex ligation-dependent probe amplification (Gylling *et al*, 2009). The pathogenicity of MMR gene sequence variants has been evaluated by InSiGHT criteria (Thompson *et al*, 2014). At present, the Finnish LS Registry includes 260 families and approximately 1400 verified germ-line MMR mutation carriers (http://www.hnpcc.fi/).

Questionnaires addressing lifestyle factors, medical and reproductive history were mailed to 223 MMR germ-line mutation carrier women living across Finland and having previously consented for LS Registry inquiries. Content of postal questionnaires is summarised in Table 1. Questionnaires were re-sent to non-responding patients in 6 months after first mailing. EC diagnoses were confirmed from the pathology reports and medical records obtained from district hospitals. Informed consent was obtained from all study participants and the study protocol was approved by TAUH Ethical Committee.

Statistical analysis. SPSS statistics software (version 22, IBM, Armonk, NY, USA) was used for the statistical analyses. Cox regression model was used to estimate the associations between parity, age at menarche and menopause, duration of HRT or hormonal contraception, BMI, annual weight change, alcohol consumption and the risk of EC in LS women. Age was used as a timescale for EC risk estimation. The time at risk was considered to start from birth and end at the diagnosis of EC, prophylactic hysterectomy or the time of the survey, whichever occurred first. For the univariable analyses, age at menarche and menopause, BMI, annual weight change, duration of hormonal contraception and HRT were divided into two categories by the median values of the variables. These variables were also analysed as continuous variables in the regression model. In addition, BMI was also categorised using cutoff points 25 (=overweight) and 30 (=obese). The comparison of BMI as a continuous variable between diabetic and non-diabetic patients was performed using nonparametric testing.

As the LS women in the study were ascertained from multiple case cancer families or because of EC diagnosis, the selection of women may not have been random with respect to disease status. Therefore, ascertainment was adjusted for in the multivariable analyses by taking into account the time of germ-line testing with respect to the end of time at EC risk (i.e., germ-line testing performed before EC diagnosis, prophylactic hysterectomy or survey in healthy non-hysterectomised women compared with germ-line mutation testing after EC diagnosis or prophylactic hysterectomy). Parity, age at menarche and duration of hormonal contraceptive use as continuous variables were also adjusted for in the multivariable analysis as they have been previously reported to associate with EC risk in LS women (Dashti *et al*, 2015).

Two-tailed P values of <0.05 were considered as statistically significant.

RESULTS

One hundred and thirty-six women returned the questionnaire resulting in a 61% response rate. Median age at survey was 58 years (range 29–85). Distribution of the different germ-line mutations was as follows: 82.4% of *MLH1*, 11% of *MSH2*, and 6.6% of *MSH6* mutations. Fifty women (36.8%) had been diagnosed with EC at median age of 49.5 years. Prophylactic surgery had been performed in 52 out of 86 (60.5%) of EC unaffected women at median age of 45 years. Characteristics of the study patients and exposure data are summarised in Table 2.

In univariable Cox regression analysis, non-insulin-dependent diabetes and hypercholesterolaemia were associated with an elevated risk of EC (HR 3.21 (95% CI 1.34–7.78), $P=0.009$; HR 2.08 (95% CI 1.11–3.90), $P=0.02$; respectively). Diabetic LS women were more overweight than non-diabetic LS women at survey (median BMI 29.7 *vs* 25.0, $P=0.012$, Mann–Whitney *U*-test), but BMI at the age of 18 or 40 years or at survey did not associate with the risk of EC (HR 1.03, (95% CI 0.91–1.17), $P=0.6$; HR 1.04, (95% CI 0.98–1.11), $P=0.19$; HR 1.02 (95% CI 0.97–1.08), $P=0.42$; respectively). Among ever users of HRT (n=61), the duration of use (>9 years) showed a trend for association with EC risk (HR 2.03 (95% CI 0.89–4.62), $P=0.09$). History of endometriosis showed also a trend for association with EC risk (HR 1.96 (95% CI 0.90–4.28), $P=0.09$).

In multivariable Cox regression model, diabetes and duration of HRT use were associated with a statistically significant increase in the risk of EC (HR 4.18 (95% CI 1.52–11.52), $P=0.006$; HR 1.07 (95% CI 1.02–1.13), $P=0.010$; respectively).

Summary of univariable and multivariable Cox regression analyses is presented in Table 3.

DISCUSSION

We report here the associations between EC risk and lifestyle, medical and hormonal factors in a retrospective cohort of verified MMR mutation carriers. These findings suggest that type II diabetes and postmenopausal hormone therapy may associate with an elevated risk of EC in LS. Even though diabetic LS women were more overweight than non-diabetic women at survey, BMI at any time point or annual weight change did not associate with the risk of EC. Our results are in contrast to the previous observations of BMI as an EC risk factor in general population (Jenabi and Poorolajal, 2015) but are in line with studies reporting no

Table 1. List of exposure data collected by postal questionnaires sent to MMR mutation carriers

	Description		
Height			cm
Weight		At age of 18 At age of 40 At present	Kg Kg Kg
Age at menarche	Age when you had your first periods		Years
Age at menopause if achieved	Age when you had your last periods		Years
Number of pregnancies			Number
Deliveries			Number
Spontaneous abortions			Number
Induced abortions			Number
Vaginal HRT use	Local/vaginal oestrogen therapy		Y/N
Systemic HRT use ever	Reply yes, if you have received any oestrogen therapy (pill, patch, gel) for postmenopausal symptoms (e.g., hot flushes, sweating)		Y/N
If yes:	Try to estimate the duration of use in years Describe here the type of oestrogen you use at present (pill, patch, gel).	Systemic HRT duration Systemic HRT at present	Years Y/N
Ovulation failure	Have you ever been diagnosed with irregular menstrual bleeding, which was caused by ovulation failure (i.e., the egg not being released from the ovary)?		Y/N
PCOS	Have you been diagnosed with polycystic ovary syndrome?		Y/N
Endometriosis	Have you been diagnosed with endometriosis, which can cause dysmenorrhea and/or pelvic pain? In endometriosis, tissue that normally lines the inside of your uterus (endometrium) can grow outside your uterus		Y/N
If yes, any treatment	Describe here the modalities of treatments that you have received for endometriosis? Estimate here the duration of use for each treatment modality	 Contraceptive tablets Progesterone po Progesterone-IUD	 Y/N Y/N Y/N
Cancer other than endometrial cancer	Have you been diagnosed with other cancers besides endometrial cancer?		Y/N
If yes:	Describe here which cancers and the time of diagnosis	GI[a] tract cancer Urinary tract cancer Breast cancer Ovarian cancer	Y/N Y/N Y/N Y/N
Operated for cancer	List here the type of cancer and the time of surgery		Y/N
Gynaecological follow-up duration	For how long have you participated in regular gynaecological follow-up (i.e., clinical examination, ultrasound and possibly endometrial sampling)? Describe here the time interval		Years
Regular smoking ever	Have you ever smoked regularly (at least one cigarette per day)?		Y/N
If yes:	Try to estimate for how long you have been smoking (years) and approximately how many cigarettes per day	Cigarettes per day Duration of smoking	Number Years
Alcohol consumption	Do you currently use or have you used alcohol?		Y/N
If yes:	Try to estimate how many servings per week you use or have used in average. 1 serving = 12 cl wine or 4 cl hard alcohol or 0.33 l bottle of beer/cider Try to estimate for how long you have used alcohol as you described above	Servings/week Duration of consumption	Number Years
Diabetes	Have you been diagnosed with diabetes, which means that you have too high level of blood glucose? Describe the year of diagnosis		
If yes:	Describe here the different treatments you have received for diabetes	Insulin treatment Tablet treatment	Y/N Y/N
Hypertension	Have you been diagnosed with hypertension, which means that your blood pressure is too high? Describe here the year of diagnosis		Y/N
Hypothyreosis	Have you been diagnosed with impaired thyroid function (low levels of thyroxin hormone and high levels of thyroid-stimulating hormone)? Year of diagnosis?		Y/N
Hypercholesterolaemia	Have you been diagnosed with high blood levels of total cholesterol?		Y/N
Any other serious condition, which	Describe here	List	
Hormonal contraception	Have you used hormonal contraception?		Y/N
If yes	Describe here the duration of use in years.	Duration of use	Years
Medication	List here other regular medication you use or have previously used		List

Abbreviations: GI = gastrointestinal; HRT = hormone replacement therapy; IUD = intrauterine device; MMR = mismatch repair; N = no; PCOS = polycystic ovary syndrome; Y = Yes.
[a]Data are available only from 100 study participants, and no patient reported to have been diagnosed with the condition.

Table 2. Characteristics of study women with Lynch syndrome			
	No endometrial cancer, *N* = 86 (63%)	**Endometrial cancer, *N* = 50 (37%)**	**Total *N* = 136**
Age (years)[a]			
Mean (s.d.)	46.6 (8.7)	48.4 (6.9)	47.2 (8.1)
Median (range)	45 (29–72)	49.5 (28–62)	47 (28–72)
Mismatch repair gene mutated, *n* (%)			
MLH1	72 (83.8)	40 (80.0)	112 (82.4)
MSH2	7 (8.1)	8 (16.0)	15 (11.0)
MSH6	7 (8.1)	2 (4.0)	9 (6.6)
GI-tract cancer			
Yes	26 (30.2)	24 (48.0)	50 (36.8)
No	60 (69.8)	26 (52.0)	86 (63.2)
Urinary tract cancer			
Yes	3 (3.5)	6 (12.0)	9 (6.6)
No	83 (96.5)	44 (88.0)	127 (93.4)
Age at menarche			
Mean (s.d.)	13.2 (1.5)	13.4 (1.5)	13.3 (1.5)
Median (range)	13.0 (10–17)	13.0 (11–16)	13.0 (10–17)
Age at menopause			
Mean (s.d.)	50.4 (3.0)	50.7 (3.3)	50.5 (3.1)
Median (range)	50.0 (46–55)	50.0 (43–58)	50.0 (43–58)
Number of live births, *n* (%)			
No	9 (10.5)	9 (18.0)	18 (13.2)
1–2	51 (59.3)	26 (52.0)	77 (56.6)
⩾3	26 (30.2)	15 (30.0)	41 (30.2)
Ever use of hormonal contraception,*n* (%)[b]			
Yes	66 (76.7)	28 (56.0)	94 (69.1)
No	20 (23.3)	21 (42.0)	41 (40.1)
Missing	0 (0)	1 (2.0)	1 (0.8)
Duration of hormonal contraception use (years)[c]			
Mean (s.d.)	9.2 (6.9)	6.6 (5.7)	8.4 (6.7)
Median (range)	7.00 (1–30)	4.5 (1–24)	6.0 (1–30)
Ever use of hormone replacement therapy, *n* (%)			
Yes	36 (41.9)	25 (50.0)	61 (44.9)
No	50 (58.1)	25 (50.0)	75 (55.1)
Duration of hormone replacement therapy (years)[c]			
Mean (s.d.)	9.1 (6.8)	11.3 (8.0)	10.0 (7.4)
Median (range)	7.5 (1–35)	10.0 (2–36)	9.0 (1–36)
Ever use of vaginally administered hormone replacement therapy, *n* (%)			
Yes	23 (26.7)	24 (48.0)	47 (34.6)
No	63 (73.3)	26 (52.0)	89 (65.4)
Ovulation failure, *n* (%)			
Yes	11 (12.8)	4 (8.0)	15 (11.0)
No	75 (87.2)	46 (92.0)	121 (89.0)
Body mass index at age 18 years			
Mean (s.d.)	20.9 (2.6)	21.5 (2.1)	21.1 (2.4)
Median (range)	20.3 (16.0–28.3)	21.6 (16.9–26.9)	20.8 (16.0–28.3)
Body mass index at age 40 years[d]			
Mean (s.d.)	24.0 (4.9)	24.3 (4.5)	24.2 (4.7)
Median (range)	23.2 (17.4–45.0)	23.4 (18.0–41.2)	23.2 (17.4–45.0)
Body mass index at survey			
Mean (s.d.)	25.9 (4.8)	27.2 (5.3)	26.4 (5.0)
Median (range)	24.6 (17.8–43.1)	26.3 (15.2–43.7)	25.4 (15.2–43.7)
Change in weight per year (kg)[e]			
Mean (s.d.)	0.4 (0.4)	0.3 (0.3)	0.4 (0.4)
Median (range)	0.3 (−0.2–1.96)	0.3 (−0.4–1.4)	0.3 (−0.4–1.96)
Endometriosis[f], *n* (%)			
Yes	10 (11.6)	8 (16.0)	18 (13.2)
No	76 (88.4)	42 (84.0)	118 (86.8)
Diabetes[f,g], *n* (%)			
Yes	1 (1.2)	6 (12.0)	7 (5.1)
No	85 (98.8)	44 (88.0)	129 (94.9)

Table 2. (Continued)

	No endometrial cancer, *N* = 86 (63%)	Endometrial cancer, *N* = 50 (37%)	Total *N* = 136
Hypertension[f], *n* (%)			
Yes	17 (19.8)	18 (36.0)	35 (25.7)
No	69 (80.2)	32 (64.0)	101 (74.3)
Hypercholesterolaemia[f], *n* (%)			
Yes	8 (9.3)	14 (28.0)	22 (16.2)
No	78 (90.7)	36 (72.0)	114 (83.8)
Hypothyreosis[f], *n* (%)			
Yes	10 (11.6)	6 (12.0)	16 (11.8)
No	76 (88.4)	44 (88.0)	120 (88.2)
Smoking[h], *n* (%)			
Yes	40 (46.5)	15 (30.0)	55 (40.4)
No	46 (53.5)	35 (70.0)	81 (59.6)
Smoking as pack years[i]			
Mean (s.d.)	8.5 (7.8)	5.5 (4.5)	7.7 (7.2)
Median (range)	5.0 (1.0–30.0)	3.0 (1.0–16.0)	5.0 (1.0–30.0)
Number of alcoholic servings consumed per week			
Mean (s.d.)	2.0 (2.5)	1.2 (1.7)	1.7 (2.3)
Median (range)	1.0 (0–12)	0.5 (0–7)	0.5 (0–12)

Abbreviation: GI = gastrointestinal.
[a]Age of diagnosis of endometrial cancer for affected women; age of prophylactic hysterectomy or survey for endometrial cancer-unaffected women (whichever occurred first).
[b]Ever use was defined as regular use lasting for at least 1 year.
[c]Data presented only from women reported to have regularly used hormonal contraception ($n = 94$) or postmenopausal hormone therapy ($n = 61$).
[d]BMI at 40 years is available from 127 women aged ⩾40 years at survey.
[e]Change in weight per year was calculated as kilograms starting from age 18 years until the date of survey.
[f]Medical conditions (endometriosis, hypertension, diabetes, hypercholesterolaemia and hypothyreosis) were reported only if diagnosed by a medical doctor and/or having required regular medication.
[g]All reported cases of diabetes were non-insulin dependent.
[h]Smoking was defined as current or ever smoking (regularly minimum of 1 cigarette per day for at least 1 year) as compared with never smoking.
[i]Pack year is defined as smoking 20 cigarettes a day for 1 year. Pack years were calculated only for current and ever smokers ($n = 55$).

Table 3. Univariable and multivariable Cox regression analyses for associations between the risk of endometrial cancer and reproductive, medical and lifestyle-related factors for women with Lynch syndrome

Univariable analysis	Number of women with endometrial cancer (%)	Total number of women	HR (95% CI)	*P* value
Age at menarche, years				
<13 years	16 (35.5)	45	1.00	
⩾13 years	34 (37.4)	91	1.08 (0.59–1.96)	0.81
Live births				
Nulliparous	9 (50.0)	18	1.00	
Parous	41 (34.7)	118	0.74 (0.36–1.52)	0.42
Ever use of hormonal contraceptive				
No	21 (51.2)	41	1.00	
Yes	28 (29.8)	94	1.06 (0.59–1.9)	0.85
Use of hormonal contraceptive[a]				
<6 years	38 (44.7)	85	1.00	
⩾6 years	11 (22.0)	50	0.66 (0.34–1.30)	0.23
Ever use of systemic hormone replacement therapy				
No	25 (33.3)	75	1.00	
Yes	25 (41.0)	61	0.93 (0.53–1.63)	0.80
Use of hormone replacement therapy[b]				
<9 years	9 (30.0)	30	1.00	
⩾9 years	16 (51.6)	31	2.03 (0.89–4.62)	0.09
Ever use of vaginally administered hormone therapy				
No	26 (52.0)	63	1.00	
Yes	24 (48.0)	23	1.48 (0.84–2.58)	0.18
Endometriosis				
No	42 (35.6)	118	1.00	
Yes	8 (44.4)	18	1.96 (0.90–4.28)	0.09
Ovulation failure				
No	46 (92.0)	121	1.00	
Yes	4 (8.0)	15	0.52 (0.19–1.44)	0.21

Table 3. (Continued)

Univariable analysis	Number of women with endometrial cancer (%)	Total number of women	HR (95% CI)	*P* value
Diabetes				
No	44 (34.1)	129	1.00	
Yes	6 (85.7)	7	3.21 (1.34–7.68)	0.009
Hypertension				
No	32 (31.6)	101	1.00	
Yes	18 (51.4)	35	1.63 (0.91–2.92)	0.10
Hypercholesterolaemia				
No	36 (72.0)	114	1.00	
Yes	14 (28.0)	22	2.08 (1.11–3.90)	0.02
Hypothyreosis				
No	44 (88.0)	120	1.00	
Yes	6 (12.0)	16	0.81 (0.34–1.91)	0.63
Body mass index at age 18 years[c]				
<20.8	17 (25.8)	66	1.00	
⩾20.8	33 (47.1)	70	1.55 (0.86–2.79)	0.14
Body mass index at age 40 years[c]				
<23.2	24 (38.1)	63	1.00	
⩾23.2	26 (40.6)	64	1.18 (0.64–1.95)	0.69
Body mass index at survey[c]				
<25.4	20 (29.9)	67	1.00	
⩾25.4	30 (43.5)	69	1.20 (0.68–2.11)	0.53
Gain in weight per year (kg)[d]				
<0.3	26 (40.6)	64	1.00	
⩾0.3	24 (33.3)	72	0.81 (0.47–1.42)	0.47
Smoking				
No	35 (43.2)	81	1.00	
Yes	25 (45.5)	55	0.74 (0.40–1.35)	0.33
Alcohol consumption[e]				
No	19 (57.6)	33	1.00	
Yes	31 (30.1)	103	0.83 (0.47–1.48)	0.53

	Total number of women $n = 136$	HR (95% CI)	*P* value
Multivariable analysis[f]			
History of diabetes		4.18 (1.52–11.52)	0.006
History of hypercholesterolaemia		1.47 (0.70–3.09)	0.308
Duration of hormone replacement therapy (years)[g]		1.07 (1.02–1.13)	0.010
History of endometriosis		0.97 (0.39–2.42)	0.943

Abbreviations: CI = confidence interval; HR, hazard ratio.
[a]The duration of hormonal contraceptive use was categorised using the median duration (6 years) as the cutoff point.
[b]The duration of hormonal replacement therapy use was categorised using the median duration (9 years) as the cutoff point. Data are presented only from ever users of hormone replacement therapy ($n = 61$).
[c]Body mass index variables at ages 18 and 40 years and at survey were categorised using median value as the cutoff point.
[d]Gain in weight per year (kg) variable was categorised using median value as the cutoff point.
[e]Alcohol intake was categorised either as full abstinence or any consumption.
[f]Adjusted for age at survey (as continuous variable), parity (nulliparous *vs* parous), duration of hormonal contraceptive use (as continuous variable), age at menarche (as continuous variable) and ascertainment (as categorised variable).
[g]Continuous variable.

association among MMR mutation carriers (Win *et al*, 2011; Dashti *et al*, 2015). Our data regarding BMI therefore partially supports the view that pathogenesis of EC in LS could be independent of oestrogenic pathway (Win *et al*, 2011). However, hormonal risk factors have been shown to act similarly on EC risk in both general and LS population (Lu *et al*, 2013; Ali, 2014; Dashti *et al*, 2015). Recently, a large retrospective cohort study showed a reduction of EC risk in LS women with longer use of hormonal contraceptives, later age at menarche and parity (Dashti *et al*, 2015). These findings were not repeated in our cohort possibly owing to different ethnic background or smaller sample size and therefore lack of statistical power. An association between postmenopausal HRT and EC risk was detected in multivariable analysis, which can be interpreted as in-line with previous findings concerning the influence of hormonal factors. However, it should be noted that neither the type of hormonal contraceptives nor the type of HRT (i.e., unopposed oestrogen or oestrogen opposed by sequential or continuous progestin) was specified in our study.

The reported positive associations between diabetes and HRT use and increased EC risk are novel in verified MMR germ-line mutation carriers and are in line with studies regarding EC risk in general population (Trabert *et al*, 2013; Liao *et al*, 2014). In the present study, five out of six women had been diagnosed with diabetes prior to EC diagnosis (the mean time interval between diabetes and EC diagnoses was 5 years). All reported cases of diabetes in the present study were non-insulin dependent, which generally are strongly linked to obesity (Nathan, 2015). Even if BMI itself may not affect the EC risk in MMR mutation carriers,

the positive association between diabetes and EC risk suggests weight control to be beneficial for LS women in prevention of diabetes and therefore also EC.

There are several limitations to the study. The sample size of the cohort was relatively small but, on the other hand, included only verified MMR mutation carriers. Exposure data were collected by self-reported questionnaires possibly causing bias. For instance patients older at the time of survey had to recall their weight and duration of hormonal contraception back a long time. Nevertheless, it has been shown that recalled weight measures actually correlate well (Perry *et al*, 1995). Finally, the cohort was subjected to potential immortal bias and may have been overrepresented with EC cases of a more favourable outcome, as they represent survivors who may have been fit enough to complete the questionnaires.

In conclusion, our data suggest that diabetes and use of postmenopausal HRT may increase the risk of EC in LS women. If these results are replicated, lifestyle modifications aiming at prevention of diabetes may be beneficial for MMR mutation carrier women in terms of reduction of EC risk. As regards to postmenopausal HRT, the present results imply that long-term HRT should not be encouraged.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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PUBLICATION III

Factors associated with decision-making on prophylactic hysterectomy and attitudes towards gynecological surveillance among women with Lynch syndrome (LS): a descriptive study

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ORIGINAL ARTICLE

Factors associated with decision-making on prophylactic hysterectomy and attitudes towards gynecological surveillance among women with Lynch syndrome (LS): a descriptive study

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Abstract
To prevent endometrial carcinoma in Lynch syndrome (LS), regular gynecological surveillance visits and prophylactic surgery are recommended. Previous data have shown that prophylactic hysterectomy is an effective means of cancer prevention, while the advantages and disadvantages of surveillance are somewhat unclear. We aimed to evaluate female LS carriers' attitudes towards regular gynecological surveillance and factors influencing their decision-making on prophylactic surgery that have not been well documented. Pain experienced during endometrial biopsies was also evaluated. Postal questionnaires were sent to LS carriers undergoing regular gynecological surveillance. Questionnaires were sent to 112 women with LS, of whom 76 responded (68%). Forty-two (55%) had undergone prophylactic hysterectomy by the time of the study. The majority of responders (64/76; 84.2%) considered surveillance appointments beneficial. Pain level during endometrial biopsy was not associated with the decision to undergo prophylactic surgery. The level of satisfaction the women had with the information and advice provided during surveillance was significantly associated with the history of prophylactic hysterectomy (satisfaction rate of 73.2% versus 31.8% of nonoperated women, $p = 0.003$). The women who had undergone prophylactic surgery were older than the nonoperated women both at mutation testing (median of 42.3 years versus 31.6 years, $p < 0.001$) and at the time of the study (median of 56.9 years versus 46.0 years, respectively, $p < 0.001$). Women with LS pathogenic variants have positive experiences with gynecological surveillance visits, and their perception of the quality of the information and advice obtained plays an important role in their decision-making concerning prophylactic surgery.

Key words Lynch syndrome · HNPCC · Surveillance · Prophylactic surgery

Introduction

Lynch syndrome (LS), previously called hereditary nonpolyposis colorectal cancer (HNPCC), is a cancer predisposition syndrome with a dominant inheritance caused by pathogenic (path_) germline variants in the DNA mismatch repair (MMR) genes *MLH1*, *MSH2*, *MSH6*, and *PMS2* [1]. In addition to the early occurrence of colorectal cancer (CRC), LS is also characterized by certain extracolonic cancers (ECCs), of which endometrial carcinoma (EC) is the most common [1]. Carriers of different *path_MMR* variants exhibit distinct patterns of cancer risk and survival. The cumulative incidence of EC for *path_MLH1*, *path_MSH2*, *path_MSH6* and *path_PMS2* is 42.7%, 56.7%, 46.2% and 26.4% at the age of 75 years, respectively [2].

ECs associated with *path_MMR* variants usually occur at younger ages than in the general population. The average age

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at EC diagnosis in women with LS in a recent retrospective series was reported to be 47–49 years (range 26–87) [1, 3]. The steepest increase in the cumulative incidence of EC was between 50 and 60 years of age in the Prospective Lynch Syndrome Database (PLSD) [2].

The clinical practice and guidelines for gynecological surveillance and prophylactic surgery for female LS variant carriers vary in different countries [4]. Common practice in countries performing surveillance in Europe, Australia, North America and South America is either annual on biannual gynecological examination [5]. Based on current published studies, there are no adequate data for evidence-based clinical decisions based on findings during surveillance [6]. In Finland, after predictive genetic testing was nationally introduced in 1995, annual gynecological examinations have become common clinical practice, including pelvic ultrasound examination and endometrial biopsy, starting at approximately 35 years of age [7]. Prophylactic surgery, or hysterectomy with or without bilateral salpingo-oophorectomy or salpingectomy, has usually been performed after the age of 40 years, when having children is complete, or at the age of menopause, depending on the mutation carrier's preference [3, 4]. However, some pathogenic variant carriers disagree with the surgery recommendation and refuse to undergo prophylactic hysterectomy. The cancer-preventing effects of prophylactic surgery have been proven by clinical trials [6]. A few previous studies have evaluated the process of decision-making on prophylactic surgery, the effects of gynecological surveillance and prophylactic hysterectomy on the quality of life, and the pain associated with endometrial sampling of the mutation carriers [4, 8–10].

Since data on the attitudes of LS mutation carriers towards prophylactic surgery and gynecological surveillance are limited and even absent in Finland, we wanted to evaluate the decision-making process, satisfaction with surveillance, and pain associated with endometrial biopsies in this questionnaire-based study.

Materials and methods

Study subjects

The present retrospective cohort study was performed at Tampere University Hospital (TAUH), Tampere, Finland. Informed consent was obtained from all study participants, and the study protocol was approved by the TAUH Ethical Committee (decision code ETL R10079, dated 4.1.2011).

The study cohort included Finnish women with inherited pathogenic MMR gene variants identified from the nationwide Finnish LS Registry (LSRFi), [11] which has been described in more detail previously [12, 13, 14, 15]. Briefly, the LSRFi includes 300 families and approximately 1400 verified germline MMR variant carriers (http://www.hnpcc.fi/). Healthy women belonging to a Finnish LS family receive counseling from clinical geneticists and gynecologists. After counseling, the decision to undergo mutation testing and its timing are based on the woman's individual choice. Regular follow-up of the mutation-positive women starts after the mutation testing. Prophylactic hysterectomy is generally recommended for all female mutation carriers after 35–40 years of age, when the mutation carrier is no longer wishing for a pregnancy. Surgery is recommended by the age of menopause at the latest. If prophylactic surgery has not been performed by the age of 40, annual follow-up visits are recommended. The removal of the ovaries is discussed with mutation carriers and is usually performed if the woman is peri- or postmenopausal or if she, after receiving information, decides to have them removed before menopause. Finally, salpingo-oophorectomy is recommended at the time of menopause at the latest.

One hundred and twelve female LS carriers at least 30 years of age, with no history of endometrial or ovarian cancer and having previously consented regarding registry inquiries, were identified from the LSRFi. The study cohort is described in Table 1. A postal questionnaire was sent to these 112 women and was re-sent to those who did not return questionnaires within 6 months of the first mailing.

Questionnaires

Study participants completed a retrospective questionnaire collecting data on their history of other types of cancer,

Table 1 Characteristics of the LS cohort (112 Finnish females with a *path_MMR* variant)

	Whole LS cohort (N = 112)	Study population (responders) (N = 76)
Age		
Median (range)	49 (30–89)	52 (30–82)
Age at mutation testing		
Median	38 (20–72)	36 (22–65)
Distribution of MMR genes		
MLH1	72 (64%)	47 (62%)
MSH2	32 (29%)	22 (29%)
MSH6	8 (7%)	7 (9%)
History of other cancer (Y/N)		
Y	42 (38%)	24 (33%)
N	70 (62%)	49 (67%)
Prophylactic surgery performed		
Y	63 (56%)	42 (68%)
N	49 (44%)	24 (32%)

LS Lynch syndrome, *path_MMR* pathogenic variant of DNA mismatch repair gene

family history, parity, and age at mutation testing and prophylactic gynecological surgery, if performed. Data on the subjects' attitudes towards gynecological surveillance and prophylactic surgery and their experiences with these procedures were also collected. Pain associated with endometrial biopsies was evaluated with a numeric rating scale (NRS; 0–10, 0 = no pain and 10 = worst imaginable pain). Subjects who stated they could not recall or evaluate the pain level did not answer this question. A detailed description of the questionnaire content is presented in Table 2.

Statistical analysis

IBM SPSS statistics software, version 22 (IBM SPSS, Inc., Armonk, NY, USA), was used for the statistical analyses. The association of categorized variables with prophylactic surgery decisions was performed using the chi-squared test. Two-tailed $P < 0.05$ values were considered to indicate statistically significant differences. The association of continuous variables (e.g., NRS and number of deliveries) with the history of prophylactic surgery was carried out using the t test or a nonparametric test when appropriate. NRS scores and the number of deliveries were also categorized (NRS 0 to 5 versus 6 or more and parity of 0 versus 1 or more deliveries) in the statistical analyses. When assessing factors possibly influencing prophylactic surgery decision-making, patients who had a hysterectomy for nonprophylactic reasons were excluded from the analyses.

Results

Seventy-six women returned the questionnaire, resulting in a 68% response rate. The distribution of the affected genes was as follows: 62% *MLH1*, 29% *MSH2* and 9% *MSH6* mutations. A prophylactic hysterectomy was performed on 42 subjects of this population (55%) at the median age of 42.0 years (range 32.0–67.0). Twenty-four subjects had not had a hysterectomy at the time of the survey, and 10 subjects had a nonprophylactic hysterectomy performed for benign medical reasons, such as uterine myomas, menorrhagia without endometrial hyperplasia, and pelvic floor prolapses, for which they were excluded from the analyses concerning prophylactic hysterectomy. The characteristics of the study cohort (both responders and nonresponders) are summarized in Table 1.

Among subjects not having had a hysterectomy performed at the time of the study, eight (33.3%) reported they

Table 2 Details of the questionnaire used

Feature	Further information	Measurement/response
Time of predictive testing		Date/year
Age at predictive testing		Number
Relationship status before testing	In a relationship?	Y/N
Relationship status on study	In a relationship?	Y/N
Prophylactic surgery performed		Y/N
Has attended follow-up appointments		Y/N
Considers follow-up beneficial	If "yes" to previous	Y/N
Parity	Number of deliveries	Number
Experienced pain in endometrial biopsy	NRS 0–10	Number
Satisfied with the advice provided by the professionals	In general	Y/N
Enough information provided on possible adverse effects of prophylactic surgery	Gynecological prolapses	Y/N
	Urinating complaints	Y/N
	G-I tract complaints	Y/N
Has felt pressure for prophylactic surgery		Y/N
Satisfied with decision to have surgery	If performed	Y/N
Planning to have prophylactic surgery	If not performed	Y/N
Cancer other than gynecological cancer in family	Personal history or family member	Y/N
	Which cancer	Description
Family member died of gynecological cancer		Y/N
Experience of personal state of health	Poor/intermediate/good	0/1/2
Poor tolerance of insecurity	Own experience	Y/N
Strong fear of cancer		Y/N
Strong fear of surgery/operations		Y/N
Experience of surgery as responsibility		Y/N

were not planning to have a prophylactic hysterectomy at all, and 16 (66.7%) reported not having decided yet about the surgery or did not respond.

The median age at mutation testing among subjects with a prophylactic hysterectomy performed was 42.3 years (range 25–65), compared to 31.6 years (range 22–48) for subjects with no hysterectomy performed ($p < 0.001$). At the time of the study, the median age of subjects with a prophylactic hysterectomy performed was 56.9 years (range 43–72), compared to 43.2 years (range 30–76) for subjects with no hysterectomy performed ($p < 0.001$).

The median time interval between mutation testing and the study survey was 11 years (range 6–29 years) among the study subjects still in surveillance (not having undergone prophylactic surgery). The median duration of surveillance (median time interval between mutation testing and prophylactic surgery) was 6 years (0–14 years), and the median time interval between surgery and the study questionnaire was 9 years (1–38 years) among the prophylactically operated subjects.

Sixty-eight (89.5%) of the responders reported attending regular surveillance appointments that were provided. Six subjects reported not having been offered appointments at all, and two subjects did not respond to this question. Sixty-four (84.2%) of the subjects considered appointments to be beneficial, 10 subjects did not respond to this question and only two patients considered appointments unbeneficial.

Pain associated with endometrial sampling measured by NRS, overall satisfaction with the given information and all the background factors possibly having an influence on women's attitudes and decisions on prophylactic surgery obtained from the questionnaires are summarized in Table 3. Fifty-four subjects evaluated pain associated with endometrial biopsy, while 22 (29%) of the subjects did not respond

Table 3 Background characteristics and factors collected from questionnaires obtained from prophylactically operated vs. nonoperated mutation carriers

Reported variables	Study population (N = 76)[a]	Prophylactic hysterectomy performed (N = 42)[b]	Nonoperated (N = 24)	p value[c]
	n (%)	n (%)	n(%)	
1. Parity: 1 or more deliveries	66 (86.8)	37 (88.1)	21 (87.5)	1.000
2. Own health considered intermediate or good	50 (65.8)	24 (58.5)	18 (75.0)	0.282
3. In a relationship at mutation diagnosis	67 (88.2)	40 (95.2)	19 (79.2)	0.089
4. Attended gynecological appointments regularly	68 (89.5)	37 (88.1)	21 (87.5)	1.000
5. Cancer other than gynecological cancer in family	73 (96.0)	39 (92.9)	24 (100.0)	0.295
6. Family member died of gynecological cancer	17 (22.3)	12 (29.3)	4 (16.7)	0.373
7. Poor tolerance of feeling of insecurity	13 (17.1)	5 (11.9)	5 (20.8)	0.477
8. Strong fear of cancer	32 (42.1)	19 (45.2)	10 (41.7)	0.803
9. Strong fear of surgery/operations	12 (15.7)	6 (14.3)	4 (16.7)	1.000
10. Experience of surgery as responsibility	16 (21.0)	12 (29.3)	3 (12.5)	0.142
11. Feels/has felt pressure for surgery	20 (26.3)	14 (35.9)	5 (22.7)	0.391
12. Satisfied with information and advice in general	43 (56.6)	30 (73.2)	7 (31.8)	0.003
13. Satisfied with information on possible postoperative disadvantages				
a. Urinary complaints	18 (23.6)	11 (28.9)	2 (9.1)	0.106
b. Chronic pelvic pain	20 (26.3)	12 (31.6)	3 (13.6)	0.215
c. GI-tract complaints	19 (25.0)	12 (31.6)	2 (9.1)	0.061
d. Pelvic prolapses	19 (25.0)	12 (31.6)	2 (9.1)	0.061
14. Endometrial biopsy pain (NRS score)	(N = 54)[d]	(N = 28)[d]	(N = 19)[d]	
a. 0–5	39 (72.2)	21 (75.0)	11 (57.9)	
b. 6–10	15 (27.8)	7 (25.0)	8 (42.1)	0.339
15. Median age, years	Year (range)	Year (range)	Year (range)	
a. At mutation testing	35.2 (22–65)	42.3 (25–65)	31.6 (22–48)	< 0.001
b. At survey	48.8 (30–76)	56.9 (43–72)	43.2 (30–76)	< 0.001

[a]Defined by study questionnaire responders

[b]10 subjects with nonprophylactic hysterectomy excluded from comparison

[c]Comparison between nonoperated and prophylactically operated subjects

[d]Total of 54 subjects answered this question (22 subjects did not respond)

to this question. The median NRS among Women with LS was 3.5. Most women (72.2%) reported mild or intermediate pain associated with endometrial biopsy measured by NRS (NRS 0–5), and strong pain (NRS 6–10) was reported by 27.8% of women. Approximately 40% of participants reported pain to be very mild or there was no pain at all (NRS 0 to 2). Pain levels during endometrial biopsy did not influence the rate of prophylactic surgeries when analyzed either as a continuous variable or when categorized. Regardless of the history of prophylactic hysterectomy, a majority of women (43/76; 59.7%) reported satisfaction with the information and advice regarding LS in general and prophylactic surgery provided by gynecologists. Only four subjects did not answer this question. The self-reported satisfaction with general LS-associated information and advice by experts was dependent on the history of prophylactic hysterectomy: 73.2% of the operated patients were satisfied versus only 31.8% of the nonoperated patients ($p = 0.003$; Table 3). The compliance rate with gynecological surveillance was similar among operated and nonoperated women (88.1% versus 87.5%, respectively, $p = 1.00$; Table 3).

In addition, there was a trend for women who chose prophylactic hysterectomy to have received more information on certain postoperative complications than women who had not chosen surgery yet ($p = 0.061$ for information on GI-tract postoperative complications and pelvic prolapses; Table 3).

Discussion

In this study, LS pathogenic variant carriers' attitudes towards gynecological surveillance and satisfaction with the advice and information provided by experts were significantly associated with having had prophylactic surgery. To our knowledge, this finding highlights the importance of general information in this context and emphasizes the role of attending medical staff. On the other hand, compliance with surveillance was similar between prophylactically operated and nonoperated women, suggesting that the quality of information may play a significant role in decision-making.

Parity and experienced pain during endometrial sampling did not correlate with the decision to undergo prophylactic surgery. A previous study indicated that parity influences decision-making, but the data were derived from a very small study population of ten women with LS [8]. Severe pain experienced during endometrial sampling has been previously shown to be the main reason to quit screening, thus possibly lowering the threshold for surgery [10]. Different populations may explain differences in the results concerning the experience of pain during endometrial sampling. Since ultrasound examination is not sufficient as a single surveillance method in terms of EC prevention, [16] it is a relief that pain associated with endometrial sampling was not a significant factor for decision-making, at least in our study population. The association of older age at mutation testing and at survey was expected since all recommendations for the initiation of surveillance and the timing of prophylactic surgery are age-dependent.

The majority of subjects considered gynecological surveillance to be beneficial in general. There have been some previous qualitative studies on the topic showing experienced benefit [8, 10]. We show that some of the study subjects reported being either inadequately or not at all informed about the risks and possible long-term side effects of prophylactic surgery. Earlier qualitative studies evaluating surgery decisions have reported similar results: mutation carriers are mainly satisfied with prophylactic surgery decisions, but nonoperated women are not completely satisfied with the information they receive [6, 8]. One possible explanation for this is that more detailed surgery-related information is provided only when the decision to undergo surgery has been made. From this retrospective analysis, it is not possible to draw straightforward conclusions, but it is probable that women with LS may warrant more detailed and structured information on surgery during surveillance.

Some of our study subjects were not satisfied with the surveillance protocol. A few LS carriers reported not being informed at all about gynecological surveillance appointments. This probably influenced their decision-making on prophylactic surgery and may have led them to refuse it, thus keeping them susceptible to EC. This finding emphasizes the importance of structured national guidelines for the management of LS.

The strengths of our study include a well-defined population of women with LS who were all verified as germline pathogenic variant carriers and were not just women who had a strong family history of EC or CRC. The study cohort identified from the LSRFi included 112 women, and the response rate was quite high (68%), which is in line with previous questionnaire-based studies among subjects with a hereditary cancer predisposition [14, 15].

There are some limitations to our study. The setting is retrospective, and a questionnaire survey is subject to the risk of misremembering background factors. This misremembering may therefore cause recall bias. However, we consider that a questionnaire-based survey is also a valuable method to collect the points of view and experiences of women with LS. Prophylactically operated subjects were expectedly significantly older at the time of mutation diagnosis and at study than nonoperated women, which can also cause some bias. A comparison of responders to nonresponders did not reveal any major concerns other than the slightly more frequent rate of prophylactic hysterectomy among the responders, which may cause potential bias and must be taken into account when interpreting the present results. Some of the study subjects had a hysterectomy for nonprophylactic

reasons, and they had to be excluded from the analyses when estimating the factors influencing the decision-making about prophylactic surgery.

In conclusion, we show here new descriptive data on the attitudes towards surveillance and factors associated with the history of prophylactic surgery in a Finnish cohort of women with LS. Based on our results, surveillance is well accepted. Considering the results of our study, we suggest that the mutation carriers should be systematically informed about surveillance and its aims and about prophylactic surgery. We suggest that information should be offered regardless of the timing of the prophylactic surgery.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interest.

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PUBLICATION IV

Descriptive study on subjective experience of genetic testing with respect to relationship, family planning and psychosocial wellbeing among women with lynch syndrome

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Hereditary Cancer in Clinical Practice

RESEARCH Open Access

Descriptive study on subjective experience of genetic testing with respect to relationship, family planning and psychosocial wellbeing among women with lynch syndrome

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Abstract

Background: Due to increased risk of endometrial and ovarian cancer, women belonging to known Lynch Syndrome (LS) families are recommended to undergo germline testing. Current practice in Finland is to offer counselling to women with pathogenic variant and advocate risk-reducing surgery (RRS) after completion of childbirth. The present study aimed to clarify the impacts of positive germline testing on family planning and reproductive decisions of these women, which are relatively unknown.

Methods: Seventy-nine carriers of germline MMR gene pathogenic variant (*path_MMR)* were identified from the Finnish LS Registry as having genetic testing performed before the age of 45 years and not having undergone hysterectomy or oophorectomy. These women were sent a questionnaire concerning family planning, intimate relationships and psychosocial wellbeing.

Results: Thirty-five women (44.3%) responded. Parity of *path_MMR* carriers (2.1) was slightly higher than parity among Finnish women in general (1.8). No significant differences were found between parity, number of induced abortions or sterilizations before and after genetic testing. Only minority of subjects reported any influence on family planning (20%) or negative impact on feminine self and body image (14%).

Conclusions: The positive germline testing does not seem to have a major negative impact on family planning, intimate relationships or feminine self and body image. According to the open comments, counselling, supportive and empathic attitude of the professionals seem to have a significant impact on this. These results are a valuable addition to the counselling of LS women at reproductive age.

Keywords: Lynch syndrome, Hereditary cancer, Testing, Relationships, Psychosocial wellbeing

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Background

Women with a pathogenic MMR gene variant (*path_MMR*) associated with Lynch syndrome (LS) carry a 40–70% lifetime risk for endometrial cancer and a 7–15% lifetime risk for ovarian cancer [1–3]. For female carriers of *path_MMR*, international guidelines recommend an annual review with an clinician after age of 25 and, if certain symptoms e.g. abnormal bleeding occur, also gynecological referral [4]. At the time of the present study, the procedure in Finland was a gynecological surveillance including pelvic ultrasound examination and endometrial biopsy with one or 2 years interval, beginning at age of 35 [5]. However, with no clear evidence of survival benefit supporting the gynecological screening procedure [5], Finland has recently revised the national guidelines concerning the gynecological screening (FinGOG guidelines, accessed December 2019: https://gynekologiyhdistys.fi/pienryhmat/onkologia).

Soon after positive germline testing and counselling by a clinical geneticist, Finnish women with LS are offered a visit at a tertiary hospital with an expert gynecologist providing additional counselling and clinical gynecological and ultrasound examination. Thereafter, routine screening visits are not any more recommended to symptomless individuals. Visits, including gynecological ultrasound examination and endometrial biopsy, are suggested if dysfunctional bleeding occurs. However, risk-reducing hysterectomy, possibly with oophorectomy, is recommended when childbearing is complete or at the age of 50 years at the latest and carriers of *path_MMR* are usually invited to discuss the timing of RRS at the age of 40 years [5].

Finnish LS Registry (LSRFi) comprises all known families with LS-associated inherited MMR variant in Finland [6]. The germline testing of members of these families is mainly performed in early adulthood depending on the individual's preference. Germline testing as such may be associated with psychological distress and anxiety [7]. Having inherited *path_MMR* predisposing to gynecological cancers may also have an impact on intimate relationship, family planning and psychosocial wellbeing. There is a relative lack of data available concerning these aspects with respect to any hereditary cancer syndrome [8, 9]. A few studies have been performed on patient-physician -relationship and effects of surveillance, the *path_MMR* carrier's knowledge about the surveillance and decision-making concerning the prophylactic surgery in LS [10–14]. However, little is known about influence of positive germline testing on parity, age and timing of childbearing, induced abortions, sterilizations, intimate relationships, feminine self and body image. This information would be useful and valuable to professionals when counselling and communicating with young women diagnosed with LS-associated germline variant.

In the present study, we aimed to collect information and aspects from female carriers of *path_MMR* considering their subjective experience of positive germline testing with respect to relationship, family planning and psychosocial wellbeing.

Methods

Study subjects

The present study was performed at Tampere University Hospital (TAUH), Tampere, Finland. The study protocol was approved by TAUH Ethical Committee (January 2011) and an informed consent was obtained from all the study participants.

The present study is a part of a large retrospective cohort study among Finnish women with LS aiming at characterization of factors associated with gynecological health and morbidity in general [14, 15, 16]. The entire female LS study population, the present study and the previously published sub-studies are presented in a schematic flow chart (Fig. 1). The Finnish LS Registry (LSRFi) consists data of original research cohort including 81 kindreds ascertained through family history of LS and finally includes data of 1700 carriers of verified germline variant [6]. The women in the present study have given their informed consent to participate in LSRFi initiated clinical studies and permitted LSRFi researchers to use their address and medical information. They have all been voluntarily tested positive for MMR-gene pathogenic variant associated with Lynch syndrome, thus receiving appropriate information and counselling by the professionals.

The study population included women with pathogenic MMR gene variants identified from LSRFi and no history of endometrial cancer. Inclusion criteria for this study was germline testing before the age of 45 years (i.e. women considered at fertile age) and no hysterectomy or oophorectomy performed before germline testing (possibility to conceive after testing). Sterilization was not an exclusion criteria, as we consider it possible to wish for pregnancy and conceive through fertility treatments even after sterilization. Finally, 79 women were identified and a postal questionnaire concerning family planning and psychosocial effects of germline testing was sent to them. The questionnaire was re-sent within 6 months after first mailing to non-responding subjects. Demographics of the carriers included in the present study are presented in Table 1.

Questionnaire

The study questions included in the questionnaire are presented in Table 2. The questionnaire also included an opportunity to give open comments considering the effects of germline testing on family planning and further personal information about the topic.

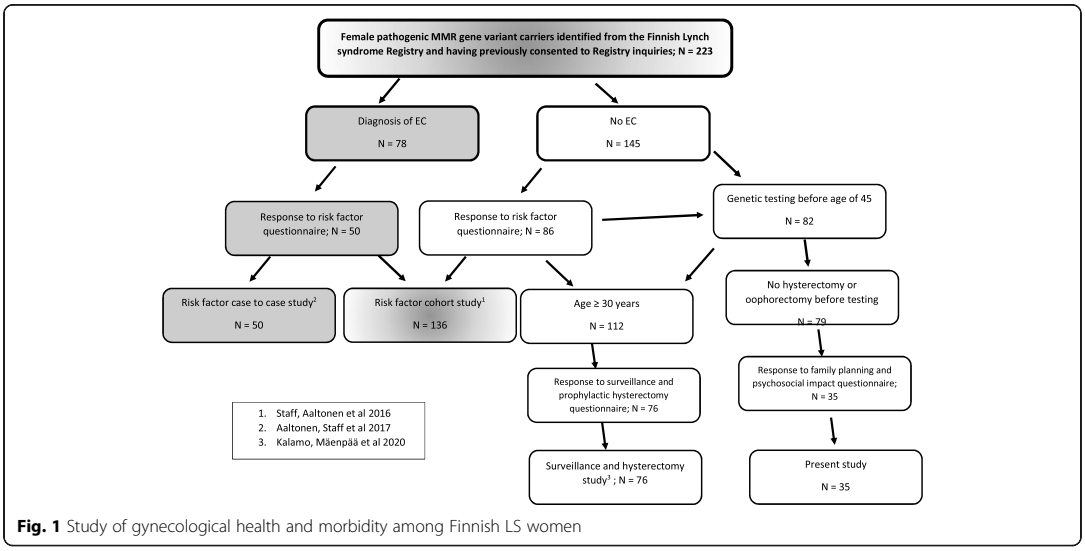

Fig. 1 Study of gynecological health and morbidity among Finnish LS women

Results

Finally, 35 women returned the questionnaire after two mailings, resulting in a 44.3% response rate. All of them reported attending the gynecological surveillance regularly.

Median age of the responders at study was 44 (31–59) years and their median age at germline testing was 31 (21–42) years. Median time interval between testing and the study was 13 (10–17) years. Mean parity of the responders was 2.1 (0–4). Mean parity of the non-responders was 2.3 (0–5) and mean parity of the whole study population was 2.2 (0–5).

The most common gene with MMR variant among the responders was *MLH1* (80.0%), reflecting the high percentage of *MLH1* carriers in LSRFi. The overall characteristics between the responders and non-responders were quite similar with no statistically significant differences. Details on responders as well as non-responders are summarized in Table 1.

All the responders had at least secondary vocational education. Twenty percent had a university degree. Most women reported being in a relationship at the time of genetic testing at a median age of 31 (91%), and practically all of them stated that testing had no influence on their relationships (97%). Before genetic testing, 86% of women had been pregnant and approximately half of the responders had also been pregnant after the testing. The number of reported induced abortions and sterilization procedures was similar before and after testing (5.7 vs 5.7% and 14.3 vs 14.3%, respectively). Only seven women experienced that positive germline testing influenced their family planning (20%). Only a small proportion of women reported negative impact on feminine self or body image (14%). Educational details and responses to questionnaire of the study responders are presented in Table 2.

Six out of 7 women who reported any impact of germline testing on family planning gave detailed information

Table 1 Pathogenic MMR germline variant carriers included in the study

	Responded to questionnaire = study population (*N* = 35)	Non-responders (*N* = 44)	All (*N* = 79)
Median age at study	44 (31–59)	40 (24–56)	41 (24–59)
Median age at genetic testing	31 (21–42)	28 (19–44)	30 (19–44)
Hysterectomy performed after testing	6 (17.1%)	9 (20.4%)	15 (19.0%)
Parity (mean)	2.1 (0–4)	2.3 (0–5)	2.2 (0–5)
Gene:			
MLH1	28 (80,0%)	36 (81.8%)	64 (81.0%)
MSH2	4 (11.4%)	5 (11.3%)	9 (11.3%)
MSH6	3 (8.6%)	3 (6.8%)	6 (7.6%)

($p > 0,1$ on all variables compared to responders)

Table 2 Questionnaire with given responses ($N = 35$)

Highest educational degree	High school	2 (5.7%)
	Vocational school	15 (42.9%)
	Uni. applied sciences	11 (31.4%)
	University	7 (20.0%)
Questions with answer options "yes" and "no":		
1.Were you in a relationship at the time of testing?	Answer "yes"	32 (91.0%)
2. Were you in a relationship at the time of study?	Answer "yes"	28 (80.0%)
3. Did genetic testing have influence on your relationship?	Answer "yes"	1 (3.0%)
4. Have you been pregnant before testing?	Answer "yes"	24 (69.0%)
5. Have you been pregnant after testing?	Answer "yes"	17 (49.0%)
6. Have you had induced abortion(s) before testing?	Answer "yes"	2 (5.7%) (8.7% of women who answered "yes" to question 4.)
7. Have you had induced abortion(s) after testing?	Answer "yes"	2 (5.7%) (11.8% of women who answered "yes" to question 5.)*
8. Have you planned pregnancy before testing?	Answer "yes"	30 (86.0%)
9. Have you planned pregnancy after testing?	Answer "yes"	15 (42.9%)
10. Have you been sterilized before testing?	Answer "yes"	5 (14.3%)
11. Have you been sterilized after testing?	Answer "yes"	5 (14.3%)
12. Did genetic testing have influence on your family planning?	Answer "yes"	7 (20.0%)
13. Did genetic testing have negative influence on your feminine self and body image?	Answer "yes"	5 (14.3%)

*difference between the amount of abortions not statistically significant: $p > 0,5$

on this topic, and are presented in Table 3. Some women gave spontaneous, open descriptions of reactions related to germline testing and they are summarized in Table 4. Half of these comments included feelings of gratefulness and appreciation towards the testing procedure and health care professionals.

Discussion

This descriptive study reveals the subjective views of carriers of *path_MMR* on the influence of germline testing to their important life decisions. Inherited cancer syndromes such as LS do not affect only the individual carrying the germline variant, but also the *path_MMR* carriers' children have a 50% chance of inheriting the cancer predisposing gene. It is therefore likely that inherited MMR gene variants may affect individual's decision-making regarding family planning, or relationships in general. Since there were no PMS2 pathogenic variants (PV) in the study population, we were unable to comment on women's perceptions of being a PMS2 PV carrier.

Table 3 Effects of genetic testing on family planning (Question 12 on Table 2: Reported by 7 women and 6 described the effects in more detail as abstracted here)

Age 34, tested at 27, 1 child before testing and 1 after testing, gene *MLH1*	Wanted to attend colonoscopy as planned and this had influence on pregnancy timing
Age 41, tested at 33, 1 child before testing and 3 children after testing, gene *MLH1*	Genetic finding limited the number of children, wanted to have them quickly after testing. Considered that pregnancies and breastfeeding have positive effects on health.
Age 39, tested at 21, 2 children after testing, gene *MLH1*	After genetic finding decided to have children as early as possible
Age 51, tested at 39, 2 children before testing, gene *MSH2*	Decided not to have more children after genetic finding.
Age 32, tested at 22, 1 child after testing, gene *MLH1*	Does not plan pregnancy after 35, thinks endometrial sampling affects fertility
Age 46, tested at 29, 1 child before testing, gene *MLH1*	After genetic finding did not want more children because of risking to pass the pathogenic variant on to offspring

Table 4 Abstracted open comments on subjective experiences of genetic testing in general (Opportunity to this given at the end of the questionnaire)

Age 46, tested at 36, 4 children before testing, gene *MLH1*	Very afraid of cancer and death, demanded for hysterectomy straight after testing, but was not operated until at age of 45.
Age 37, tested at 27, 1 child before testing and 3 children after testing, gene *MLH1*	No influence on family planning. Genetic finding has caused other difficulties in life. Encourages her children to have genetic testing.
Age 32, tested at 22, 1 child after testing, gene *MLH1*	Considers the uterus and ovaries a risk. Plans to have surgery after menopause.
Age 42, tested at 26, 1 child before testing and 2 children after testing, gene *MLH6*	Genetic finding has caused uncertainty and anxiety. Grateful for surveillance.
Age 52, tested at 35, 2 children before testing and 1 after testing, gene *MLH1*	First reaction was fear and disgust towards upcoming surveillance procedures. Later grateful for information and her children's possibility for genetic testing.
Age 58, tested at 42, 1 child before testing, gene *MLH1*	Grateful and positive thoughts. Considers herself safe and privileged for surveillance.
Age 42, tested at 27, 2 children after testing, gene *MLH1*	Grateful for supportive professionals. Tells that surveillance appointments were nor provided automatically at regional hospital, had to insist them.
Age 34, tested at 27, 1 child before testing and 1 after testing, gene *MLH1*	Had depression for 6 months after genetic finding. Other reasons influenced as well. Considers results reported to her in an unfriendly and negative manner. Felt that prophylactic removal of gynecological organs deteriorates self-esteem.
Age 41, tested at 33, 1 child before testing and 3 children after testing, gene *MLH1*	Grateful for testing and surveillance. Worried for her children.
Age 39, tested at 21, 2 children after testing, gene *MLH1*	Genetic finding had negative influence on feminine self-image.
Age 30, tested at 23, 2 children after testing, gene *MSH2*	Was missing peer support, then got it from her own sister after her testing. Grateful for surveillance. Worried for her children.
Age 36, tested at 27, 1 child after testing, gene *MLH1*	Feels safe and does not have worries. Grateful for surveillance.

There is a paucity of data concerning family planning among individuals with inherited cancer syndromes. Even though inherited gene variants conferring gynecological cancer risks do not have impact on fertility as such [17, 18], some reports have implicated that germline testing results have impact on reproductive decisions [8, 9]. Some individuals with pathogenic MMR variants have even been reported to consider prenatal genetic testing and consider it ethical [8, 19]. Therefore, it is very important to collect LS carriers' subjective views and experiences of genetic testing on their reproductive decisions in order to guide and help clinicians in counselling.According to our results, testing positive for a LS-causing variant appears not to have significant impact on family planning or negative influence on relationships among Finnish women. Only a minority of responders reported any influence or negative impact. Parity of the carriers of *path_MMR* in this study was 2.1, which, to our surprise, was even higher than that of Finnish women in general. The mean parity among Finnish women was 1,8 in 2012 (data from Statistics Finland, stat.fi). The educational background may not explain this, since the education level of the study population does not differ significantly from the general Finnish population.

The number of sterilizations and induced abortions was exactly the same before and after germline testing possibly implying that the fear of passing the pathogenic variant forward is not a major determinant of reproductive decisions. However, in open comments some reported worry, deteriorating of feminine self and body image and fear of having more children. Due to the inclusion criteria, study subjects were relatively young, both at the time of germline testing and at the time of study. However, the mean time interval between testing and present study was 13 years, thus we can assume that these women have been adapted to being carriers of *path_MMR* and not in the initial phase of accepting it. Study subjects were all in surveillance phase and contemporarily considered to have increased risk of endometrial cancer compared to general population.

As in our earlier study [14], women with LS consider the surveillance and the information given by medical professionals of high-quality and very beneficial. According to these women's subjective opinion, the role of adequate information can be considered very significant in avoiding possible negative psychological impact associated with carrying a *path_MMR*. These variant carriers' views highlight the experienced impact of regular surveillance on managing the psychological side-effects associated with positive germline testing. This aspect can be underestimated in gynecological surveillance trials, where survival benefit is usually considered as the primary endpoint. Moreover, according to our results, there

is a subjective variation how an individual carrier of *path_MMR* experiences the impact of surveillance on her psychosocial wellbeing and it probably should be taken into account in an effort of tailoring the carriers' counselling and management.

In Finland, women in LS families mainly seem to have adequate knowledge of gynecological cancer screening and they are aware of their entitlement to participate in it. In addition to the clinical specialists, LSRFi offers support and information for the carriers of *path_MMR*. In the present study, all responding women reported attending the gynecological surveillance regularly. None of the subjects in our study implicated not having known about the surveillance. However, concerning the present national guidelines, information of practice, benefits and impact of the gynecological counselling and RRS could probably be improved. Even some false perceptions of the screening were present among the answers. One woman also reported she had to ask for surveillance as it was not provided automatically.

The present study had some limitations. The study population was relatively small and the response rate was low (44.3%). This is possibly due to several study questionnaires that were sent to these women as a part of the larger LS study entity. Some women returned empty questionnaires, implicating in a note that they do not want to be reminded of their cancer predisposition and give additional thought to their genetic risk as they already have to attend the surveillance. Majority of the subjects in the present study were middle-aged or younger at the time of study. Therefore, they were probably in a relatively busy phase of their life and this could partly explain the somewhat low response rate. Data in the present study was self-reported, including the surveillance behavior, but the main goal of the present study was to highlight the true subjective, personal experiences of the carriers of *path_MMR*. Moreover, earlier studies have supported the validity of self-reported information [20]. The strength of the present study is the inclusion of study subjects that are verified carriers of germline MMR gene variant identified from the LSRFi and access to their medical data was used for verification of parity data, time of germline testing etc. It can be also considered a strength that the study subjects were not from a single center but represented Finnish women with LS from various parts of Finland.

Conclusions

In conclusion, testing positive for a germline variant in their fertile age does not seem to have a significant negative impact on women's reproductive decisions among the Finnish women with *path_MMR MLH1, MSH2 or MSH6*. The positive germline testing does not seem to confer a negative impact on intimate relationships or on feminine self and body image. Almost all women responding in this study experienced regular surveillance beneficial. The results of the present study can be considered of valuable addition to the counselling of women with LS after germline testing and enables clinicians to share reassuring peer-derived data of reproductive issues to women carrying the *path_MMR*. In addition to preventing gynecological cancer, counselling and caring by specialists after germline testing seems to decrease concerns about variant carriers' future life. Supportive and empathic attitude of the professionals seems to be a significant factor in avoiding anxiety and fears of the carriers of *path_MMR*. Similar conclusions have been presented in earlier studies on carriers of cancer-related genetic variants [12, 13].

Authors' contributions

M.Kalamo collected and analyzed the patient data and designed the research questionnaire forms with S.Staff. M. Kalamo, S. Staff, J. Mäenpää and T. Seppälä were major contributors in writing the manuscript. J-P Mecklin, M. Kalamo and S. Staff were initial designers of the study. J-P Mecklin and K. Pylvänäinen allowed the access to LSRFi patient database. K Pylvänäinen provided the contact information and additional statistical details of the study subjects. All authors have read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study protocol was approved by TAUH Ethical Committee (4.1.2011/ Ref. ETL R10079) and an informed consent was obtained from all the study participants.

Consent for publication

All the study participants gave their informant consent to publish their personal data and comments collected by the questionnaire.

Competing interests

Toni Seppälä is the CEO and co-owner of Healthfund Finland Oy (outside the submitted work), and reports a fee from Boehringer-Ingelheim (outside the submitted work). The other authors declare that they have no competing interests.

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