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HEREDITARY FACTORS IN ENDOMETRIAL CANCER

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HEREDITARY FACTORS IN ENDOMETRIAL CANCER

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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**“The more you know, the more you know you don't know.”
Aristotle**

**To my beloved mother Theoni, my father
Vasileios, my sister Polina
and Valia**

ABSTRACT

Endometrial carcinoma (EC) is the most common gynecological malignancy in Sweden and accounts for about 6 % of all female malignancies. The risk of EC increases with age and the majority of cases are diagnosed between age 50 and 60. Ninety percent of cases occur in women older than age 50. About 2% of EC may have a familial association related to Lynch syndrome (LS). About 80% of women with Cowden syndrome have the *PTEN* mutation, which increases their lifetime risk of developing EC. The benefit of EC surveillance among LS patients remains undetermined. Available studies are controversial concerning optimal age at which to initiate screening and screening modalities.

Our first study explored the prevalence of LS, Cowden syndrome (CS) and hereditary breast ovarian cancer syndrome in consecutively diagnosed women with EC. In addition, we explored the possibility of a familial association between uterine cancer and other specific malignancies. In all, we included 481 consecutively diagnosed cases of endometrial cancer. We used the Swedish Cancer Registry to confirm all diagnoses, as well as for a reference population for the years 1970 and 2010. We conducted mutation analyses on all families who met criteria for the syndromes referred to above to identify potential causal genes. Our study demonstrated familial clustering among relatives of our index EC cases; EC prevalence was twice as high in our study population as in the cancer population in Sweden at large (6% vs 4% and 3%). In addition, we identified LS in 1.5% of all women. No *BRCA1* and *BRCA2* mutations were identified. No families fulfilled the CS criteria. Among all the LS families in which mutation could be verified, only one had previously been diagnosed. Moreover, we found that onset of cancer at a young age in family members of EC patients and diagnosis of multiple malignancies in the same patient lend support to the concept of hereditary uterine cancer syndromes.

In study II, 54 Cowden syndrome-like families were identified from consecutively diagnosed EC patients. PCR and DNA sequencing analysis were carried out on genomic DNA to amplify all nine *PTEN* gene exons. Since we identified no germline mutations or polymorphisms, the implication is that these must be rare among CS-like families. Therefore strict Cowden syndrome criteria should be applied to identify CS patients.

Our third study involved a retrospective nationwide study of 170 women with Lynch syndrome. We gathered data on all diagnostic methodology employed for gynecological screening of LS and prophylactic surgery, including age at surgery. In all, 86 of the 117 women who were eligible for screening complied with the screening program. Gynecological surgery was carried out on 43 women prior to diagnosis with LS, for which reason they were inappropriate for screening. A lower incidence of cancer was found in the screened group than in the non-screened group. EC was diagnosed by endometrial biopsy in a large number of cases. In addition, the incidence of cancer was significantly reduced by prophylactic surgery.

In conclusion, the results from our studies will improve both characterization of EC and family screening while expanding genetic counseling, and thereby help to prevent endometrial cancer in high-risk patients by enrolling them in EC prevention programs before endometrial cancer develops. Our results will improve routine procedures used to investigate families and surveillance programs for patients at high risk. Such patients can then be offered a choice between participation in screening programs or surgery for prophylactic purposes. According to our results, LS patients should be screened for gynecological cancer with transvaginal ultrasound (TVUS) and endometrial biopsy (EB) by age 30-35. Once high-risk women have completed childbearing, prophylactic surgery should be made available.

POPULÄRVETENSKAPLIG SAMMANFATNING

Livmodercancer/ endometrie cancer (EC) är den fjärde vanligaste tumörformen hos kvinnor och utgör ca 6% av all kvinnlig cancer, vilket motsvarar ca 1400 fall/år i Sverige. Livstidsrisken för insjuknande är 2% i den totala befolkningen. Från 1960 har det skett en gradvis ökning av antalet nya fall: andelen gynekologiska cancerfall som utgörs av EC har stigit till 40 %. Risken för EC ökar med åldern, och de flesta fallen diagnostiseras mellan 50 och 60 års ålder. 90% av alla fall inträffar hos kvinnor äldre än 50 år. Mortaliteten i EC ligger på 7-10 per 100 000 kvinnor. Övervikt, högt blodtryck, fysisk inaktivitet, diabetes och ärftlighet är de mest välkända riskfaktorerna för utveckling av EC.

Ärftlig EC utgör 5 % av alla rapporterade EC-fall. I ca 1-2 % av alla EC är tumören en del av ett cancersyndrom med ökad risk för cancer i andra organ såsom Lynch syndrom (LS) och Cowden syndrom (CS).

LS, tidigare benämnd hereditary non-polyposis colorectal cancer, HNPCC, står för ca 1-2% av alla kolorektalcancerfall och EC-fall i Sverige, och för ca 9 % av all EC som uppkommer före 50 års ålder. Den ärvs autosomt dominant och har en frekvens mellan 1: 660 till 1: 2000. Kvinnor med LS har en 40 - 60% livstidsrisk att utveckla EC (lika stor risk som kolorektalcancer) och i ca hälften av fallen sker insjuknandet i EC före eventuellt kolorektalcancerinsjuknande. Medianåldern för insjuknande i EC hos personer med LS är mellan 46-62 års ålder. Man har även en ökad risk för andra tumörformer såsom ventrikel-, ovarial-, tunntarms- samt uretär cancer. LS orsakas av en nedärvd mutation från ena föräldern i någon av generna som kallas för DNA mismatch repairgenerna (MMR-genes); *MLH1*, *MSH2*, *MSH6* eller *PMS2*. Dessa gener kodar för proteiner som tillsammans bygger upp ett avancerat proteinkomplex kallat MMR-komplexet. Detta proteinkomplex' uppgift är att upptäcka och reparera baser som felaktigt satts in i cellens DNA-sträng vid kopiering av genomet, vilket är en grundförutsättning för att den genetiska informationen överförs på ett säkert sätt till de nya dottercellerna som bildas vid celledning. Om inte dessa gener kan bygga upp ett välfungerande MMR-komplex uppstår oundvikligen en pålagring av nya stavfel i DNA-koden för varje ny kopiering av genomet som sker. De uppkomna sekvenserna av stavfel kallas för microsatelliter, och syns i laboratoriet om man jämför de felkopierade kromosomerna med sina rättskrivna motsvarigheter. När man ser kromosomer med förekomst av microsatelliter säger man att genomet är utsatt för microsatellitinstabilitet (MSI). Som en logisk följd av detta samband mellan MMR-genernas funktion och uppkomst av MSI, så testar man för mutationer i MMR-generna på rutin om MSI upptäcks i tumörceller

för att eventuellt se om personen tillhör en LS-familj, vilket har stor betydelse både för den berörde individens egen risk att utveckla nya cancrar, men också för den personens släktingar. Dock har analys av MSI en låg känslighet och specificitet för att hitta LS-orsakade EC-fall, då även en stor del av de sporadiska EC-tumörerna också uppvisar MSI pga. nyuppkomna (somatiska) mutationer i samma MMR-gener, samt att de LS orsakade EC-tumörerna inte regelmässigt alltid behöver uppvisa MSI. Denna svåra ekvation gör att det idag inte finns biologiska markörer som med säkerhet kan säga vilka kvinnor med EC som kan ha LS. För närvarande används Amsterdam II eller Bethesda kriterierna, för att med hjälp av klinisk information identifiera misstänkta fall av LS, vilket skärper specificiteten men lämnar sensitiviteten oberörd. Det råder även oenighet om hur ett gynekologiskt kontrollprogram för kvinnor med LS ska utformas; när är det bäst tid att starta kontrollerna för kvinnor med LS, vilka diagnostiska metoder ska ingå i dem, och hur ska man behandla patienterna på längre sikt för att minska deras cancerrisk?

Cowden syndrom (CS) är en autosomt dominant sjukdom som kännetecknas av flera hamartom i bröstet, sköldkörteln, colon, njurarna och livmoderslemhinnan. Den globala incidensen är 1: 250,000. Nedärvda mutationer i tumör-suppressorgen *PTEN* bedöms vara ansvarig för mellan 35 till 80% av alla Cowden syndromfall. Förlust av *PTEN* aktivitet sker efter att man ärvt en muterad allel som åtföljs av en andra somatisk mutation av den normala allelen, vilket leder till förlust av proteinproduktens funktion med ökad fosforylering av viktiga signalproteiner i cellen som följd. Detta har en effekt på olika cellulära processer och signalvägar i cellen, såsom cellcykelprogression, metabolism, tillväxt, migration, invasion, angiogenes och apoptos.

Diagnosen av CS baseras på de National Comprehensive Cancer Network (NCCN) kriterierna. Livstidsrisken för att utveckla EC hos de med CS är 21-28%, med högsta incidensen av fall vid 35-45 års ålder.

Målsättningen med det aktuella projektet var att:

1. Kartlägga ärftligheten hos kvinnor med EC för att kunna erbjuda förbättrade riskberäkningar och omhändertagandet av kvinnor med familjär EC.
2. Utvärdera och optimera nuvarande kontrollprogram för EC för att kunna erbjuda kvinnor med risk för EC ett evidensbaserat preventionsprogram.

I den första studien undersökte vi frekvensen av Lynch syndrom, Cowden syndrom och ärftlig bröstcancer/ äggstockscancer syndrom bland kvinnor med livmodercancer. Vi undersökte också om det fanns en familjär koppling mellan livmodercancer och andra

cancrar. 481 kvinnor med diagnostiserad livmodercancer ingick i studien. Alla familjer som uppfyllde de kliniska kriterierna för ovannämnda cancersyndrom erbjöds mutationsanalys i syfte att identifiera möjliga orsaksframkallande gener.

Var sjätte kvinna som deltog i studien har haft åtminstone en nära släkting med livmodercancer som har insjuknat innan 50 års ålder. Prevalensen av LS i vår studiepopulation var 1,5%, och alla kvinnor utom en diagnosticerades med LS tack vara deltagande i studien. Då vi har kunnat påvisa en ökad förekomst av livmodercancer i vår studiepopulation jämfört med den svenska befolkningen (6% mot 3%) kan dessa resultat tyda på ett ärftligt livmodercancersyndrom skilt från LS.

I den andra studien identifierade vi 54 Cowden syndrom-liknande familjer bland kvinnor med livmodercancer. Med PCR och DNA-sekvenseringsanalys undersökte vi DNA från deras friska celler för att amplifiera alla nio exoner av *PTEN*-genen. Inga nedärvda mutationer eller polymorfismer identifierades, vilket tyder på att någon egentlig nedärvning från den tidigare generationen är sällsynt i CS-liknande familjer med livmodercancer.

Den tredje studien vände sig till alla kvinnor med konstaterat Lynch syndrom hemmahörandes i Syd- och Mellansverige. 170 kvinnor inkluderas i studien. Vi har samlat uppgifter som inkluderade alla diagnostiska metoder som används för gynekologisk kontroll för dessa kvinnor, och eventuell profylaktisk kirurgi. Resultaten visade på en betydligt lägre (15%) cancerförekomst hos de kvinnor som deltagit i kontrollerna jämförande med 81% för de som inte deltagit i ett kontrollprogram. 13 fall av livmodercancer upptäcktes hos kvinnor som deltog i gynekologiska kontroller varav användande av biopsi från livmodersslemhinna var avgörande för att upptäcka dem på kontrollbesöken innan symptom utvecklades. Två fall av äggstockscancer hittades med ultraljud. På lång sikt var profylaktiskt bortagande av livmoder och äggstockar den enda faktorn som tydligt sänkte cancerförekomsten hos kvinnor med LS.

Projektet har stor betydelse för hälso- och sjukvården. Våra resultat kommer innebära en förbättrad karakterisering av livmodercancer som diagnos, samt leda till en förbättrad släktutredning, där fler kommer att erbjudas genetisk rådgivning med möjlighet att inkludera alla kvinnor med ökad risk i preventionsprogram för EC och associerade tumörer redan innan de (eller någon annan släkting) utvecklar kliniska symtom av sitt syndrom. Projektet kommer att leda till förbättrade rutiner på kvinnokliniken vad gäller släktutredningar och kontrollprogram för riskindivider. De som har ärvt den sjukdomsorsakande mutationen kan då erbjudas profylaktisk kirurgi och/eller kontrollprogram.

LIST OF SCIENTIFIC PAPERS

- I. **Tzortzatos G**, Wersäll O, Danielsson KG, Lindblom A, Tham E, Mints M.
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- II. **Tzortzatos G***, Aravidis C*, Lindblom A, Mints M, Tham E.
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- III. **Tzortzatos G**, Andersson E, Soller M, Stenmark-Askmal M, Zagoras T, Goergii-Hemming P, Lindblom A, Tham E, Mints M.
The gynecological surveillance of women with Lynch syndrome in Sweden. *Manuscript* 2015.

***Implies equal contribution.**

ADDITIONAL PUBLICATIONS

- IV. Painter JN, O'Mara TA, Batra J, Cheng T, Lose FA, Dennis J, Michailidou K, Tyrer JP, Ahmed S, Ferguson K, Healey CS, Kaufmann S, Hillman KM, Walpole C, Moya L, Pollock P, Jones A, Howarth K, Martin L, Gorman M, Hodgson S; National Study of Endometrial Cancer Genetics Group (NSECG); CHIBCHA Consortium, de Polanco MM, Sans M, Carracedo A, Castellvi-Bel S, Rojas-Martinez A, Santos E, Teixeira MR, Carvajal-Carmona L, Shu XO, Long J, Zheng W, Xiang YB; The Australian National Endometrial Cancer Study Group (ANECS), Montgomery GW, Webb PM, Scott RJ, McEvoy M, Attia J, Holliday E, Martin NG, Nyholt DR, Henders AK, Fasching PA, Hein A, Beckmann MW, Renner SP, Dörk T, Hillemanns P, Dürst M, Runnebaum I, Lambrechts D, Coenegrachts L, Schrauwen S, Amant F, Winterhoff B, Dowdy SC, Goode EL, Teoman A, Salvesen HB, Trovik J, Njolstad TS, Werner HM, Ashton K, Proietto T, Otton G, **Tzortzatos G**, Mints M, Tham E; **RENDOCAS**, Hall P, Czene K, Liu J, Li J, Hopper JL, Southey MC; Australian Ovarian Cancer Study (AOCS), Ekici AB, Ruebner M, Johnson N, Peto J, Burwinkel B, Marme F, Brenner H, Dieffenbach AK, Meindl A, Brauch H; The GENICA Network, Lindblom A, Depreeuw J, Moisse M, Chang-Claude J, Rudolph A, Couch FJ, Olson JE, Giles GG, Bruinsma F, Cunningham JM, Fridley BL, Børresen-Dale AL, Kristensen VN, Cox A, Swerdlow AJ, Orr N, Bolla MK, Wang Q, Weber RP, Chen Z, Shah M, French JD, Pharoah PD, Dunning AM, Tomlinson I, Easton DF, Edwards SL, Thompson DJ, Spurdle AB. **Fine-mapping of the *HNF1B* multicancer locus identifies candidate variants that mediate endometrial cancer risk.** *Hum Mol Genet.* 2014 Nov 6. pii: ddu552. [Epub ahead of print]
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“Man is a being in search of meaning.”

Plato

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LIST OF ABBREVIATIONS

AKT	Protein Kinase B
BRRS	Bannayan-Riley-Ruvalcaba syndrome
BSO	Bilateral salpingo-oophorectomy
CA 125	Tumor cancer antigen 125
CAH	Complex atypical hyperplasia
CDKN2A	Cyclin-dependent kinase inhibitor 2A
CS	Cowden syndrome
CT	Computed tomography
DTC	Differentiated non-medullary thyroid cancer
EB	Endometrial biopsy
EC/UC	Endometrial/Uterine cancer
EPCAM	Epithelial cell adhesion molecule
Exo1	Exonuclease 1
FAP	Familial adenomatous polyposis
HBOC	Hereditary breast and ovarian syndrome
HER2/ERBB2	Human epidermal growth factor receptor 2/ERBB2
HNPPC	Hereditary nonpolyposis colorectal cancer
IHC	Immunohistochemistry
LDD	Lhermitte –Duclos disease
LOH	Loss of heterozygosity
LS	Lynch syndrome

MLH1	Mutation L homologue 1
MMR	DNA mismatch repair
MRI	Magnetic resonance imaging
MSH2	MutS protein homolog 2
MSH6	MutS protein homolog 6
MSI	Microsatellite instability
PCNA	Proliferating cell nuclear antigen
PCOS	Polycystic ovarian syndrome
PET	Positron emission tomography
PGD	Preimplantation genetic diagnostics
PI3K	Phosphatidylinositol 3-OHkinase
PMS2	Postmeiotic segregation increased 2
PTEN	Phosphatase and tensin homolog
RASAL1	RAS Protein Activator Like 1
RPA	Replication protein A
SDH	Succinate dehydrogenase gene
SOE	Salpingo-oophorectomy
TP53	Tumor protein p53
TVUS	Transvaginal ultrasound

**“Everything you can imagine is real.”
Pablo Picasso**

1 INTRODUCTION

1.1 ENDOMETRIAL CANCER

Endometrial cancer (EC), which represents about 6% of all malignancies in women, is the most common gynecological malignancy in the developed world (National Board of Health and Welfare, 2012) (Burke et al., 2014). In addition, as the fourth most common form of cancer overall, about 3% of mortality from cancer is attributable to EC (Murali et al., 2014). There is an estimated 2-3% lifetime risk of developing EC (Salvesen et al., 2012), with a higher risk among Caucasian (2.88%) women than among African-American women (1.69%) (Burke et al., 2014). Endometrial cancer has steadily increased and annual incidence is currently estimated at 19-24 cases per 100,000 women (Murali et al., 2014). Mean age at diagnosis is about 60 years (Murali et al., 2014) (Sorosky et al., 2012) and fewer than 10-15% of cases are diagnosed prior to age 50 (Sorosky et al., 2012) (Burke et al., 2014).

Significant risk factors include obesity, hypertension, nulliparity, anovulation, diabetes mellitus and polycystic ovarian syndrome (PCOS) (Haidopoulos et al., 2010) (Bansal et al., 2009), also infertility and early age at menarche (Burke et al., 2014), as well as exposure to exogenous estrogens and tamoxifen (an estrogen receptor antagonist in breast tissue), when used for chemoprevention of breast cancer (Sorosky et al., 2012) (Burke et al., 2014). Factors that reduce risk of EC include high parity, oral contraceptives, progesterone-releasing IUDs and smoking (Murali et al., 2014) (Burke et al., 2014). Familial accumulation may occur in about 5% of cases (Olson et al., 2009).

1.1.1 Classification

Adenocarcinomas arising from epithelial cells account for up to 90% of endometrial carcinomas, including serous, endometrioid and clear cell tumors, which can be subdivided into two distinct types (Murali et al., 2014) (Sorosky et al., 2012) (Bansal et al., 2009). Type I tumors, which account for 70-80% of all endometrial cancers, mainly afflict younger, obese, pre- and perimenopausal women. Morphologically, type I is an endometrioid cancer. Unopposed estrogen stimulation contributes to estrogen-dependent hyperplasia, which precedes development of endometrial cancer in most type I tumors. These generally low-grade tumors are moderately to highly differentiated and carry a relatively good prognosis (Sorosky et al., 2012) (Murali et al., 2014) (Salvesen et al., 2012).

Type II tumors, which account for up to 10% of cases (Murali et al., 2014), typically show serous or clear cell morphology and are usually found in older, postmenopausal women. They arise directly from atrophic endometrium without hyperplasia (Sorosky et al., 2012) (Murali et al., 2014). These tumors, which are unrelated to estrogen stimulation, are believed to develop from a malignant lesion referred to as intraepithelial carcinoma. Type II tumors are characterized by higher grade and poor differentiation (Sorosky et al., 2012) (Murali et al., 2014) (Salvesen et al., 2012), and are therefore more aggressive (Sorosky et al., 2012) (Murali et al., 2014) (Salvesen et al., 2012).

1.1.2 Molecular Characterization

In addition to clinical and morphological differences, type I and type II tumors also differ at the molecular level, where they display different genetic alterations (Figure 1). The most commonly altered gene in type I EC is the *PTEN* tumor suppressor gene. Up to 80% of endometrial carcinomas and 55% of precancerous lesions (O’Hara et al., 2012) (Bansal et al., 2009) demonstrate *PTEN* inactivation due to either somatic mutations or loss of heterozygosity (LOH) (Doll et al., 2008).

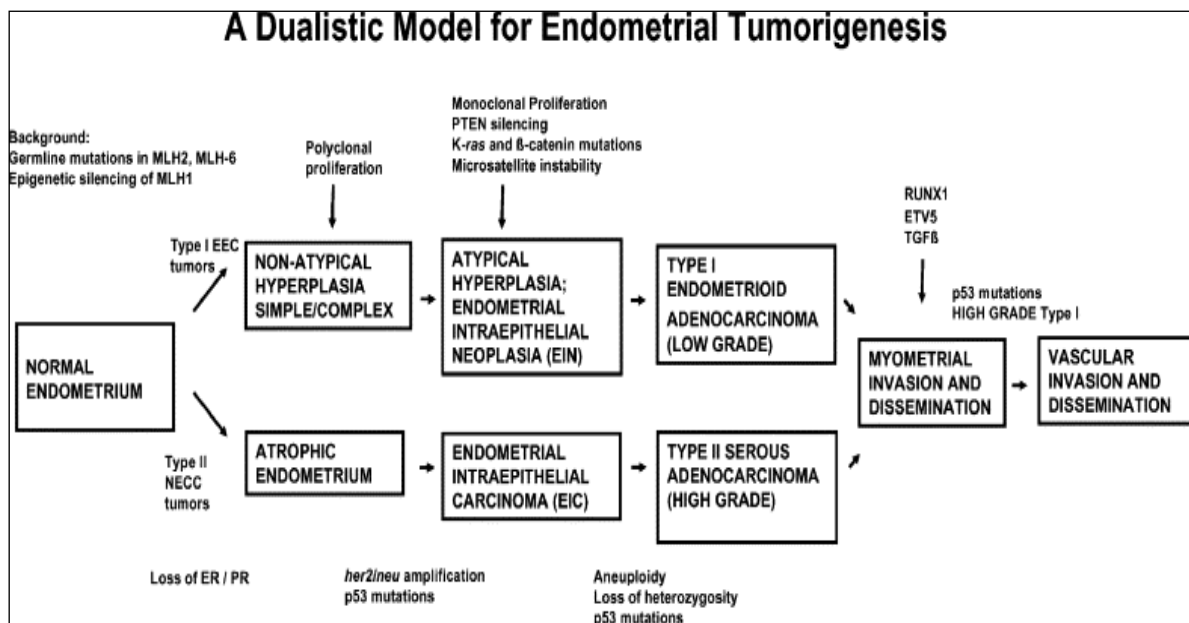


Figure 1: Dualistic model of endometrial carcinoma progression highlighting genetic abnormalities at the molecular level in both type 1 and type 2 endometrial cancers. *Reprinted from Doll et al., Journal of Steroid Biochemistry & Molecular Biology 2008, with permission from Elsevier (Doll et al., 2008).*

Up to 40% of endometrial carcinomas display microsatellite instability (MSI) (O'Hara et al., 2012) (Bansal et al., 2009) (Doll et al., 2008), which is caused by epigenetic silencing of the DNA mismatch repair gene (MMR) *MLH1* via promoter methylation (O'Hara et al., 2012).

Mutations have been reported for other genes in type I EC including *KRAS*, *PIK3CA* and *CTNNB1* (beta-catenin), occurring as often as 30%, 36% and 40% of the time, respectively (Salvensen et al., 2012) (O'Hara et al., 2012) (Bansal et al., 2009) (Sorosky et al., 2012).

Type I tumors are generally diploid and hormone-receptor positive (Salvensen et al., 2012). Dysregulation of the PI3K-PTEN-AKT signal transduction pathway in response to altered expression of *PTEN* and mutations in *PIK3CA* affects the molecular mechanisms of cell growth and proliferation, as well as survival and apoptosis (O'Hara et al., 2012) (Bansal et al., 2009).

Aneuploidy is common in type II tumors and as many as 90% of serous tumors exhibit *TP53* mutations, which cause accumulation of cells with damaged DNA (O'Hara et al., 2012) (Bansal et al., 2009). Other mutations that commonly occur in type II tumors include *CDKN2A/p16*, *HER2/ERBB2* amplification and inactivation of E-cadherin (O'Hara et al., 2012) (Bansal et al., 2009) (Doll et al., 2008) (Salvensen et al., 2012), which impact cell growth, cell signaling and cell motility, respectively (Bansal et al., 2009).

1.1.3 Diagnostics, surgical staging, treatment

Abnormal uterine bleeding, including postmenopausal bleeding, is the most common sign of EC. The diagnosis can be confirmed through transvaginal ultrasound (TVUS) with endometrial biopsy (EB) or by dilatation and curettage (D&C) (Sorosky et al., 2012, SGO Clinical Practice et al., 2014). When endometrial sampling yields negative results, hysteroscopy can be a particularly useful technique, especially when symptoms persist or for guided EB (Sorosky et al., 2012) (Burke et al., 2014).

Diagnosis is made through histopathological examination of endometrial biopsies. Magnetic resonance imaging (MRI) is superior to both computed tomography (CT) and ultrasound for determining tumor spread and elucidating the extent of myometrial and cervical invasion. Another promising technology is 18F- positron emission tomography (PET) –CT, which can

reveal metastatic lymph nodes, making it useful for post-therapy surveillance (Sorosky et al., 2012) (Salvesen et al., 2012).

The International Federation of Gynecologists and Obstetricians (FIGO) recommends surgical staging of EC in which surgical stage is based on tumor size and location, as outlined in table 1 (Pecorelli et al., 2009) and presented in figure 2.

FIGO stage	Extension of tumor
Stage I*	Tumor confined to the corpus uteri
IA*	No or less than half myometrial invasion
IB*	Invasion equal to or more than half of the myometrium
Stage II*	Tumor invades cervical stroma, but does not extend beyond the uterus
Stage III*	Local and/or regional spread of the tumor
IIIA*	Tumor invades the serosa of the corpus uteri and/or adnexae
IIIB*	Vaginal and/or parametrial involvement
IIIC*	Metastases to pelvic and/or para-aortic lymph nodes
IIIC1*	Positive pelvic lymph nodes
IIIC2*	Positive para-aortic lymph nodes with or without positive pelvic lymph nodes
Stage IV*	Tumor invades bladder and/or bowel mucosa, and/or distant metastases
IVA*	Tumor invasion of bladder and/or bowel mucosa
IVB*	Distant metastases, including intra-abdominal metastases and/or inguinal lymph nodes

***Either Grade 1, Grade 2 or Grade 3**

***Positive cytology requires separate reporting, with no change in stage**

Table 1: FIGO stages for endometrial cancer. *Reprinted from Pecorelli et al., International Journal of Gynecology and Obstetrics, with permission from Elsevier (Pecorelli et al., 2009).*

Surgery, including hysterectomy and bilateral salpingo-oophorectomy, is the currently accepted curative treatment for EC. Staging is carried out using peritoneal washing as well as pelvic and para-aortic lymphadenectomy (DeLeon et al., 2014). Advanced FIGO stage disease and certain rare high-risk histological subtypes require more extensive surgery (Sorosky et al., 2012). Stratification of patient risk depends on histological type, DNA ploidy status, myometrial invasion and lymph node involvement, as well as degree of metastatic involvement (Salvesen et al., 2012).

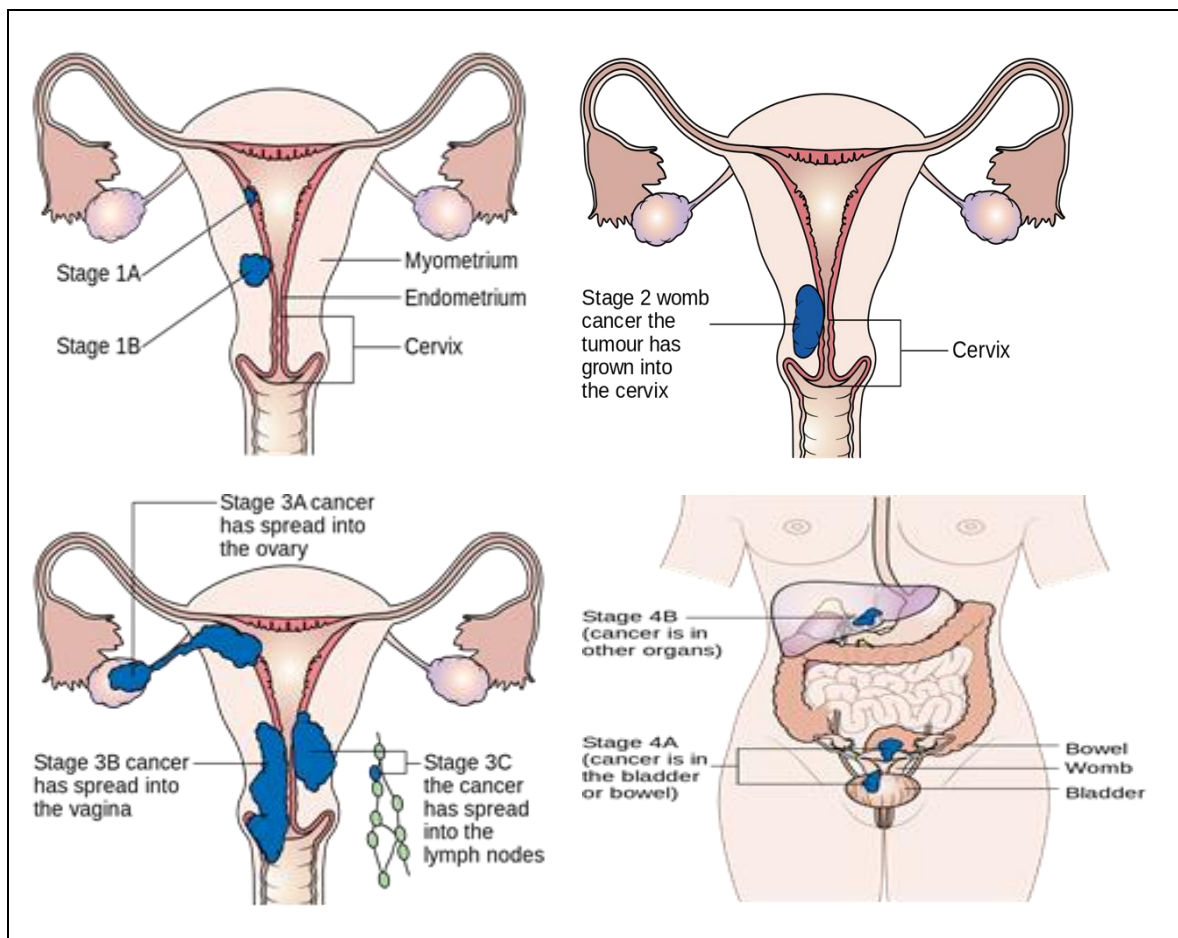


Figure 2: Diagram depicting the various stages of uterine cancer. *Image source: Cancer Research UK / Wikimedia Commons.*

Key signaling pathway changes with other potential biomarkers are currently being validated for their prognostic value (Salvesen et al., 2012).

Postoperative radiation therapy (vaginal brachytherapy or external beam radiation) and/or chemotherapy are limited to patients who are at high risk of local recurrence (Sorosky et al., 2012) (Burke et al., 2014) and/or advanced stage disease (Sorosky et al., 2012) (DeLeon et

al., 2014) (Salvesen et al., 2012) (Burke et al., 2014).

1.2 HEREDITARY FACTORS AND ENDOMETRIAL CANCER

1.2.1. Lynch Syndrome

1.2.1.1 History

The history of hereditary nonpolyposis colorectal cancer, also known as Lynch syndrome (LS/HNPCC), can be traced to 1895 when University of Michigan pathologist Dr. Aldred Warthin was inspired to investigate the family history of his seamstress, due to an abundance of various cancers (gastric, colonic or uterine cancer) in her family (Kastrinos et al., 2014) (Sehgal et al., 2014). His work, in which he referred to her family as “Family G,” was published in 1913 and after additional research, he hypothesized about the “influence of heredity on cancer” (Sehgal et al., 2014). In 1966 Dr. Henry Lynch described two families with colon, gastric and endometrial cancer and proposed a new familial cancer syndrome, ultimately called Lynch syndrome in 1984 (Kastrinos et al., 2014) (Martín-Lopez et al., 2013). This syndrome was termed HNPCC to distinguish it from familial adenomatous polyposis (FAP), since colorectal adenomatous polyps were fewer (Kastrinos et al., 2014) and extracolonic cancers were present (Martin-Lopez et al., 2013).

1.2.1.2 Etiology-Cancer risks

LS displays an autosomal dominant pattern of inheritance with an incidence ranging from 1:200 to 1:660 (de la Chapelle, 2005) and accounts for up to 4% of all colorectal cancers (Tutlewska et al., 2013) and 2% of all cases of uterine cancer; among women < age 50 prevalence may be as high as 9% (Garg et al., 2009). Not until 1993 were mutations in the DNA mismatch repair (MMR) genes *MLH1* (Mut L homologue), *MSH2*, *MSH6* (Mut S homologues) and *PMS2* (postmeiotic segregation, a Mut L homologue) found to be the cause of LS (Kastrinos et al., 2014) (de la Chapelle, 2005) (Walsh et al., 2010) (Lim et al 2010) (Sehgal et al., 2014). Meanwhile, although not a mismatch repair gene, the epithelial cell adhesion molecule gene (*EPCAM*) was recently identified as the gene that may cause LS (Tutlewska et al., 2013).

Mutations in *MLH1* and *MSH2* account for 90% of all mutations associated with LS (50% *MLH1* and 40% *MSH2*), while *MSH6* mutations account for 7-10%, *PMS2* <5% and *EPCAM*

1-3% (Kohlman et al., 2014) (Tutlewska et al., 2013) (Cohen et al., 2014). Moreover, lifetime risks linked to LS may vary depending on the specific gene mutations involved (Kastrinos et al., 2014) (Cohen et al., 2014) (Barrow et al., 2013).

The risk of developing colorectal cancer (up to age 70) lies between 40-80% in carriers of *MLH1* and *MSH2*, with a mean age at onset of 40-61 years; for *MSH6* the risk is 22% (mean age 54) and for *PMS2* up to 20% (mean age 61-66). Regarding endometrial cancer, lifetime risks vary from 40 to 60% for *MLH1*, *MSH2* and *MSH6* carriers (mean age of onset 47-62), while for *PMS2* the risk can be as high as 15% (mean age 50). (Kastrinos et al., 2014) (Cohen et al., 2014) (Barrow et al., 2013) (Kohlman et al., 2014) (Tafe et al., 2014). Endometrial cancer is often regarded as the sentinel cancer in women with Lynch syndrome (Cohen et al., 2014) (Lu et al., 2005).

Lifetime risk of developing ovarian cancer is highest in carriers of the *MSH2* mutation, followed by *MSH6* and *MLH1*, with estimated risks falling between 12 and 24% (Gerritzen et al., 2009) (Lu et al., 2005). Lifetime risks among the general population are 4.8% for colorectal cancer, 2.55% for endometrial and 1.4% for ovarian cancer (Kastrinos et al., 2014) (Cohen et al., 2014) (Barrow et al., 2013) (Kohlman et al., 2014). Concomitant ovarian and endometrial cancers are common among mutation carriers (Cohen et al., 2014), who are also at greater risk of gastric, ovarian, small intestine, urethral, hepatobiliary, skin (sebaceous gland tumors), brain and pancreas cancers (Cohen et al., 2014).

Researchers have also investigated the link between breast cancer and LS, but so far none has been found. Nevertheless, breast tumors have occurred in some LS patients with MMR deficiency, implying a possible link. In general, however, there is no significant difference in incidence of breast cancer among LS patients compared with the population at large (Cohen et al., 2014) (Win et al., 2013).

1.2.1.3 Mismatch repair mechanism; deletions in the *EPCAM* gene

The mismatch repair mechanism (MMR) is a proofreading system of the DNA replication process. The system identifies potential sites of DNA strand distortion around mismatched base pairs, as well as insertion-deletion loops (Kastrinos et al., 2014) (Guillotin et al., 2014). Two heterodimer proteins, known as MutL and MutS, form the MMR system. The two homologues of MutS, *MSH2* and *MSH6*, are able to recognize and bind to incorrectly matched base pairs. MutL, with its two homologues *MLH1* and *PMS2*, links up with MutS

and scans for single strand breaks in the preceding DNA sequence. When such breaks are found, MutL recruits exonuclease 1(Exo1) to catalyze removal of the daughter strand sequence up to and including the mutation where MutS resides.

Replication protein A (RPA) stabilizes the single-stranded DNA, thereby preventing Exo1 from undertaking further degradation. This process leaves behind a bare parental strand sequence with a large piece missing from the sequence of the daughter strand. Next the correct sequence is replaced in the missing daughter strand by DNA polymerase (Kastrinos et al., 2014) (Martín-Lopez et al., 2013), ligated by DNA ligase (Guillotín et al., 2014) (figure 3).

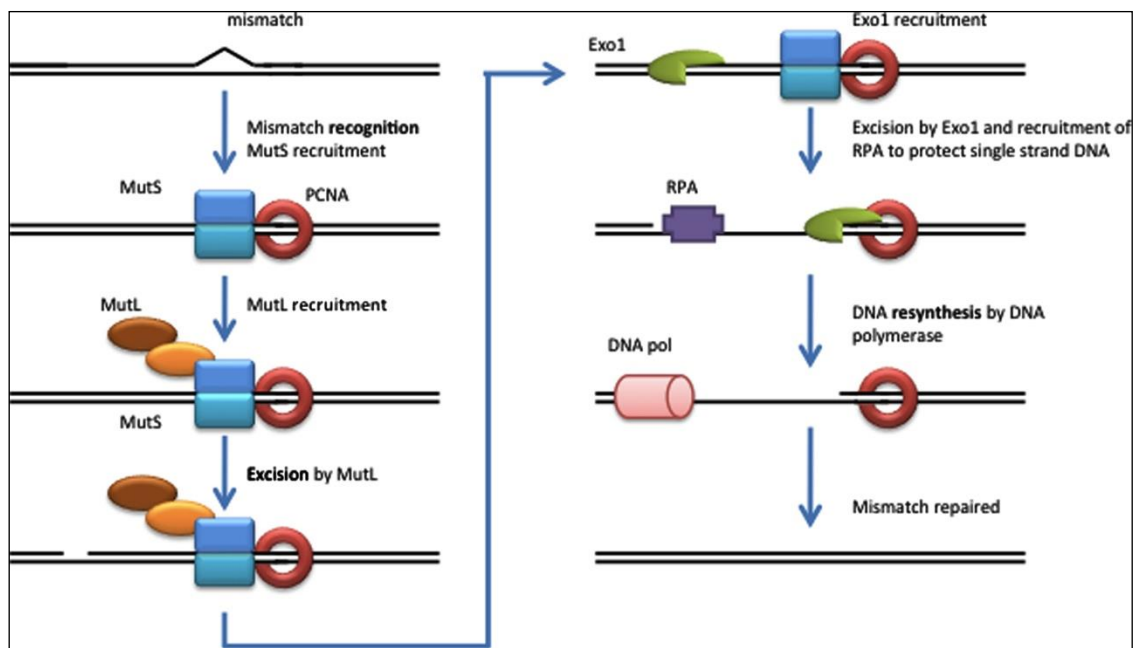


Figure 3: Mismatch repair mechanism including recognition, excision and resynthesis of the replicated DNA strand, in which base-base mismatches and insertion-deletion loops have occurred. Reprinted from Guillotin et al., *Experimental Cell research*, with permission from Elsevier (Guillotín et al., 2014).

Lynch syndrome is caused by loss of expression of one of the MMR proteins. Patients with LS inherit one germline mutation of one MMR gene. Dysfunction of one MMR gene results from somatic mutation or methylation (in *MLH1*), which culminates in tumor

development (Kastrinos et al., 2014) (Guillotin et al., 2014). Recent research has shown that one cause of LS in LS families that display deficient *MSH2* protein expression is germline deletions of the last two exons of the *EPCAM* gene. The location of *MSH2* is on chromosome 2 near the *EPCAM* gene. Deletions in the 3' end of *EPCAM* allow additional methylation of the *MSH2* gene promoter region, which reduces expression through a mechanism referred to as epigenetic silencing by promoter hypermethylation (Kastrinos et al., 2014) (Tutlewska et al., 2013).

1.2.1.4 Histology and molecular characteristics

When compared with sporadic carcinomas, colorectal cancer associated with Lynch syndrome demonstrates certain characteristics such as rapid progression from precancerous adenoma to cancer. Other characteristics include earlier onset of disease and more frequent involvement of the right colon. On the molecular level, these tumors display poor differentiation, tumor-infiltrating lymphocytes, mucinous cells and peritumoral lymphoid follicles (Cohen et al., 2013) (Tafe et al., 2014).

Most Lynch syndrome endometrial cancers are characterized by endometrioid histology, although both serous and clear cell carcinomas may also occur (Cohen et al., 2014) (Tafe et al., 2013) (Wang et al., 2013). Endometrial cancers exhibiting MMR deficiency share specific morphologic characteristics, including tumor-infiltrating and peritumoral lymphocytes (Cohen et al., 2014); in addition, they may demonstrate poor differentiation and other inflammatory infiltration of the tumor site (Wang et al 2013). In LS-related EC, involvement of the lower uterine segment is more common than in sporadic EC (Wang et al., 2013) (Tafe et al., 2014).

Lynch syndrome-associated ovarian cancer may include all histological types (e.g., endometrioid, clear cell, mucinous and serous cancers), although the incidence of serous cancers is low (Cohen et al., 2014) (Nakamura et al., 2014). At the time of diagnosis, most ovarian cancers are stage I or II and are moderately or well-differentiated (Nakamura et al., 2014).

1.1.2.5 Diagnosis of Lynch syndrome

When making a diagnosis of Lynch syndrome, a detailed family history concerning all cancer, regardless of site (including extra-colonic cancers), is of paramount importance, after which a pedigree can be constructed (figure 4). Lynch syndrome should be suspected when a clustering of LS-associated tumors is found, especially when onset is at an early age, and where an autosomal dominant pattern of inheritance can be established (Lynch et al., 2009).

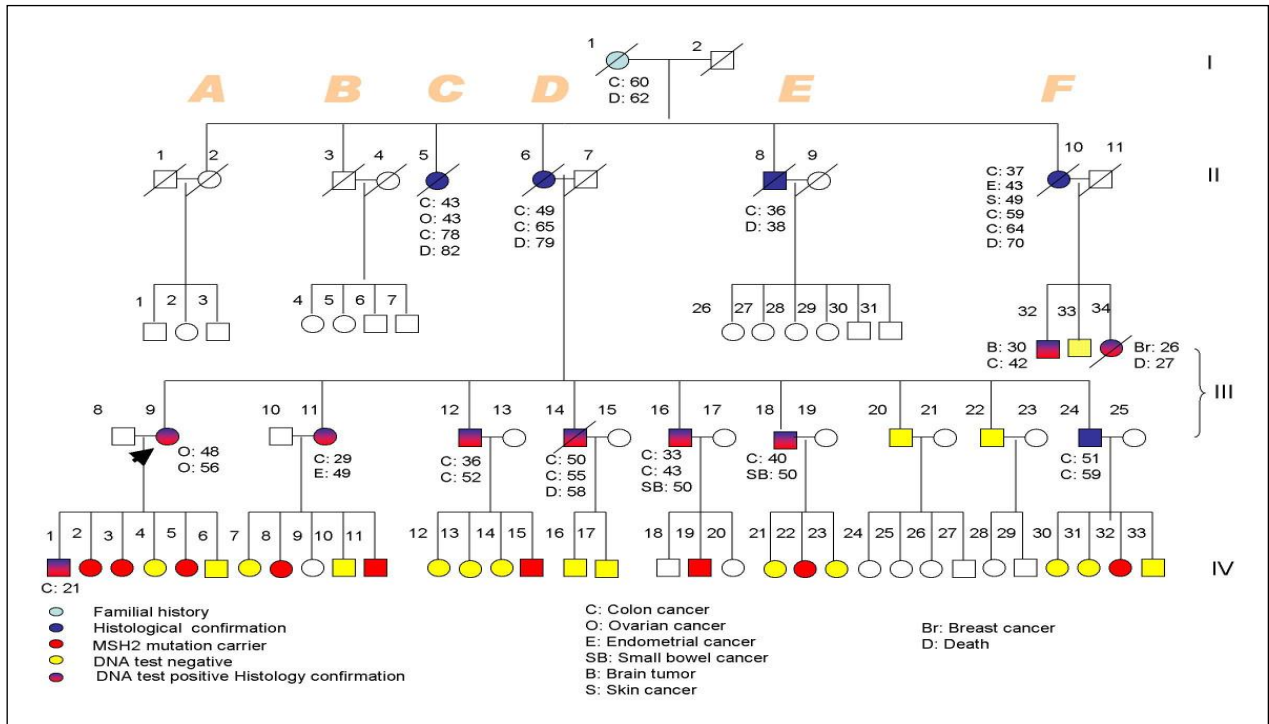


Figure 4: Typical pedigree found in a Lynch syndrome family with an *MSH2* mutation, with incidence of both colorectal and extracolonic cancers. Reprinted from Akoum et al., *Hereditary Cancer in Clinical Practice* 2009, 7:10, under the Creative Commons Attribution (CC-BY) license.

The causative genes in Lynch syndrome may be identified by applying the Amsterdam Criteria I/II (Vasen et al., 1999) and the revised Bethesda guidelines (Seghal et al., 2014) (Lynch et al., 2009) (Kohlman et al., 2014) (table 2). Since the sensitivity of the Amsterdam II criteria ranges from 50 to 87% (Sjursen et al., 2010) for colorectal cancer but only 20-30 % for endometrial cancer (Lu et al., 2005) (Leenen et al., 2012), some Lynch syndrome families may be missed, especially regarding colorectal cancer (Manchada et al., 2009). Specificity is as high as 70% (Cohen et al., 2014). Patients with the MMR gene mutation may be missed up to 25% of the time when the Bethesda guidelines are applied (Seghal et al., 2014).

Amsterdam Criteria I

At least three relatives with histologically verified colorectal cancer:

- 1. One is a first-degree relative of the other two;**
- 2. At least two successive generations affected;**
- 3. At least one of the relatives with colorectal cancer diagnosed at <50 years of age;**
- 4. Familial adenomatous polyposis (FAP) has been excluded.**

Amsterdam Criteria II

At least three relatives with an hereditary nonpolyposis colorectal cancer (HNPCC)-associated cancer [colorectal cancer, endometrial, stomach, ovary, ureter/renal pelvis, brain, small bowel, hepatobiliary tract, and skin (sebaceous tumors)]:

- 1. One is a first-degree relative of the other two;**
- 2. At least two successive generations affected;**
- 3. At least one of the syndrome-associated cancers should be diagnosed at <50 years of age;**
- 4. FAP should be excluded in any colorectal cancer cases;**
- 5. Tumors should be verified whenever possible.**

Revised Bethesda Guidelines

Colorectal tumors from individuals should be tested for MSI in the following situations:

- 1. Colorectal cancer diagnosed in a patient who is <50 years of age.**
- 2. Presence of synchronous or metachronous colorectal, or other HNPCC-associated tumors regardless of age.**
- 3. Colorectal cancer with microsatellite instability-high (MSI-H) histology diagnosed in a patient who is <60 years of age.**
- 4. Colorectal cancer diagnosed in one or more first-degree relatives with an HNPCC-related tumor, with one of the cancers being diagnosed under age 50 years.**
- 5. Colorectal cancer diagnosed in two or more first- or second-degree relatives with HNPCC-related tumors, regardless of age.**

Table 2: Amsterdam Criteria I/II and revised Bethesda guidelines as used to identify patients with Lynch syndrome. *Adapted from Sehgal et al., 2014, Genes under the Creative Commons Attribution License for open access from MDPI AG (Sehgal et al., 2014).*

Microsatellites are small repetitive DNA nucleotide sequences (e.g. AAAAA or CGCGCGCG) that usually acquire errors in the presence of MMR dysfunction. Therefore, when tumors arise due to MMR gene dysfunction, a deviant number of microsatellite nucleotide repeats is found (compared with normal tissue). This is known as microsatellite instability (MSI), which can be identified by subjecting tumors from suspected Lynch syndrome patients to molecular tissue analysis to help make the diagnosis (Kohlmann et al., 2014) (Ma et al., 2013). Both tumor and normal tissues are subjected to analysis to determine the extent of microsatellite instability; tumors may be characterized as being MSH-high (instability shown in two or more markers), MSI-low (instability shown in only one marker) or MS-stable (no instability). Up to 80% of colon adenomas associated with Lynch syndrome may be MSI-high.

Certain limitations apply to MSI testing for EC. *MSH6* mutations may be MSI-low or MS-stable (Hampel et al., 2006), which can create problems when diagnosing Lynch syndrome in EC patients. Moreover, *MLH1* promoter hypermethylation in sporadic cases of EC may result in MSI-high endometrial tumors (up to 75% of MSI-high cases in EC) (Tafe et al., 2014) (Ma et al., 2013). Other advantages to using MSI testing to aid in diagnosis of Lynch syndrome include: 1) effective in identifying tumors caused by MMR dysfunction, 2) little tissue required and 3) reproducible results (Kohlmann et al., 2014).

In addition to the limitations mentioned above, MSI testing has other disadvantages. Testing is not universally available since it requires a lab equipped for molecular analysis and microdissection. In addition, this technique may not be cost-effective since MSI testing must be followed by molecular testing to identify the mutated genes (Kohlmann et al., 2014).

Immunohistochemistry (IHC), with sensitivity of up to 92% (Ma et al., 2013) (Kohlmann et al., 2014), may also be used to evaluate the presence or absence of MMR gene protein expression in tumor tissue. Most hospital pathology labs are equipped for this test, which costs less than MSI testing and may allow identification of the specific gene mutation through targeted mutational analysis (Kohlmann et al., 2014). As with MSI testing, it must be determined whether *MLH1* promoter hypermethylation or germline mutations are responsible for loss of *MLH1* expression in EC. One disadvantage of IHC is the weak staining pattern often encountered in tissue sample preparation. Use of small tissue samples also makes this technique less reliable (Kohlmann et al., 2014) (Tafe et al., 2014) (Ma et al., 2013).

Various organizations have established guidelines for risk assessment, screening, genetic testing, treatment and surveillance of patients and families with LS, the largest of which is the National Comprehensive Cancer Network guidelines (Seghal et al., 2014) (Lynch et al., 2009) (NCCN guidelines version 2.2013).

Under current recommendations, MSI testing or immunohistochemistry should be carried out on all endometrial cancers (<70 years) (excluding *MLH1* promoter hypermethylation in EC) and on all colorectal tumors (<70 years) to help identify LS (Vasen et al., 2013) (Seghal et al., 2014).

1.2.2 Cowden Syndrome

A young woman named Rachel Cowden presented to the hospital in 1962 with cystic and ulcerative breast disease. In addition, she exhibited thyroid disease, papillomatous growths of the oral cavity and central nervous system lesions, as well as other unusual findings. Other members of her family were found to have similar disorders. Suspecting a new syndrome, her doctors named it after the patient (Mester et al., 2014).

Cowden syndrome, with an incidence of 1:250,000, is a rare autosomal dominant disorder with incomplete penetrance and variable expressivity (Farooq et al., 2010). Typical findings include multiple hamartomas (especially cutaneous), macrocephaly and an increased risk for developing various cancers including breast, thyroid and endometrial carcinoma (Farooq et al., 2010), as well as renal cancer, colorectal cancer and melanoma (Mester et al., 2014) (Pilarsky et al., 2013). Facial trichilemmomas, papillomatous papules and acral keratosis are the most common mucocutaneous lesions. Lhermitte-Duclos disease (cerebellar hamartomas), is a component of Cowden syndrome and can manifest as headaches, cerebellar ataxia and visual disturbances, as well as increased intracranial pressure (Farooq et al., 2010). The diagnosis is made according to the National Comprehensive Network (NCCN) clinical criteria (NCCN guidelines, v1 2012) (table 3). Patients who demonstrate either some or a combination of typical characteristics, and who display the CS phenotype, while failing to strictly meet NCCN criteria, are referred to as Cowden syndrome-like patients.

Different authors vary as to what criteria are required for the Cowden syndrome-like classification (Marsh et al., 1998) (Rustad et al., 2006) (Bennett et al., 2006) (Ni et al., 2008) (Orloff et al., 2013) (Tzortzatos, Aravidis et al., In press January 2015).

Pathognomonic criteria	Major criteria	Minor criteria
<ul style="list-style-type: none"> • Adult Lhermitte-Duclos disease • Mucocutaneous lesions: • Facial trichilemmomas • Acral keratosis • Mucosal lesions 	<ul style="list-style-type: none"> • Breast cancer • Non-medullary thyroid cancer • Macrocephaly (>97th percentile) • Endometrial carcinoma 	<ul style="list-style-type: none"> • Benign thyroid lesions (goiter/nodules) • Mental retardation (IQ < 75) • Hamartomas in the gastrointestinal canal • Lipomas • Fibrocystic breast disease • Fibromas • Genitourinary tumors (renal cancer) and malformations
<p>A diagnosis of CS can be made in an individual who meets one of the following criteria:</p> <ol style="list-style-type: none"> 1) Pathognomonic lesions alone given the presence of: <ul style="list-style-type: none"> • Six or more facial papules, three or more of which must be trichilemmoma or • Facial cutaneous papules and oral mucosal papillomatosis or • Oral mucosal papillomatosis and acral keratoses or • Six or more palmoplantar keratoses 2) Presence of two major criteria, one of which must be macrocephaly 3) Presence of one major plus three minor criteria 4) Presence of four minor criteria 		

Table 3: Clinical diagnostic criteria for Cowden syndrome (*Adapted from NCCN guidelines v1, 2012*).

Cowden syndrome belongs to a group of diseases linked to germline mutations in the *PTEN* gene, which along with Bannayan-Riley-Ruvalcaba syndrome (BRRS), Lhermitte-Duclos disease and autism disorders associated with macrocephaly constitute the *PTEN* hamartoma tumor syndrome (Mester et al., 2014). BRRS, a childhood disorder, is also characterized by

macrocephaly, hamartomatous intestinal polyps, lipomas and pigmented macules of the penis, intellectual disability/developmental delay (Farooq et al., 2010) (Mester et al., 2014) (Pilarski et al., 2013).

Earlier reports indicated that as many as 80% of patients who met the clinical criteria for Cowden syndrome had *PTEN* germline mutations (Farooq et al., 2010); however, more recent research (Pilarski et al., 2013) indicates that *PTEN* mutations occur in up to 35% of CS patients and in 23-42% of patients who fulfill the BRRS criteria. In classical CS, most *PTEN* germline mutations affect exon 5 (up to 40%) as well as exons 7 and 8 (Orloff et al., 2008). Large deletions in the *PTEN* gene have only been found in 1% of cases, while about 10% of all patients with classic CS demonstrate mutations in the promoter region (Zhou et al., 2003).

Among CS and CS-like patients without *PTEN* involvement, mutations in succinate dehydrogenase genes (*SDHB/C/D*), which exert an effect on mitochondrial function, may be responsible for activation of pathways that are similar to those affected by *PTEN* mutations (Ni et al., 2008). Other mutations have been noted in the *RASAL1* (RasGTPase activating protein gene) tumor suppressor gene, which affects thyroid tumorigenesis (Ngeow et al., 2014), the *PIK3CA* and *AKT1* genes (Orloff et al., 2013), as well as hypermethylation of the *KILLIN* tumor suppressor gene that occupies the same location on the chromosome as the *PTEN* gene. The *KILLIN* gene, which is transcribed in the opposite direction from *PTEN*, is regulated by *p53* and plays a role in cell cycle arrest (Bennet et al., 2010) (Mester et al., 2014).

In women, the *PTEN* germline mutation is associated with an 85% lifetime risk for developing breast cancer, as well as a 34% risk for renal cancer and a 28% risk of developing endometrial cancer, 35% thyroid cancer (Tan et al., 2012) (Mester et al., 2014). The elevated risk of developing EC begins when women reach their late 30s to early 40s (Tan et al., 2012) (Nieuwenhuis et al., 2014) (Bubien et al., 2013).

1.2.3 *PTEN* gene

The *PTEN* gene (phosphatase and tensin homologue) is localized to chromosome 10q23.3. This 9-exon tumor suppressor gene encodes for a protein consisting of 403 amino acids (Farooq et al., 2010) (Nakanishi et al., 2014) (Black et al., 2005). It exerts a negative regulatory effect on the phospho-inositide 3-kinase (PI3K)/AKT/mTOR pathway (figure 3) by decreasing the activity of kinases (PDK-1, AKT, mTOR, S6K1) found downstream from PI3K, through conversion of phosphatidylinositol 3,4,5-triphosphate (PIP3) into phosphatidylinositol 4,5-bisphosphate (PIP2) (Nakanishi et al., 2014) (Hollander et al., 2011). When *PTEN* activity is reduced or lost, the resultant increase in phosphorylation of several crucial cellular proteins (figure 3) (Hollander et al 2011) may impact various processes including cell cycle progression, metabolism, translation, growth, migration, invasion, angiogenesis, apoptosis and cell survival (Farooq et al., 2010) (Hollander et al 2011) (Nakanishi et al., 2014).

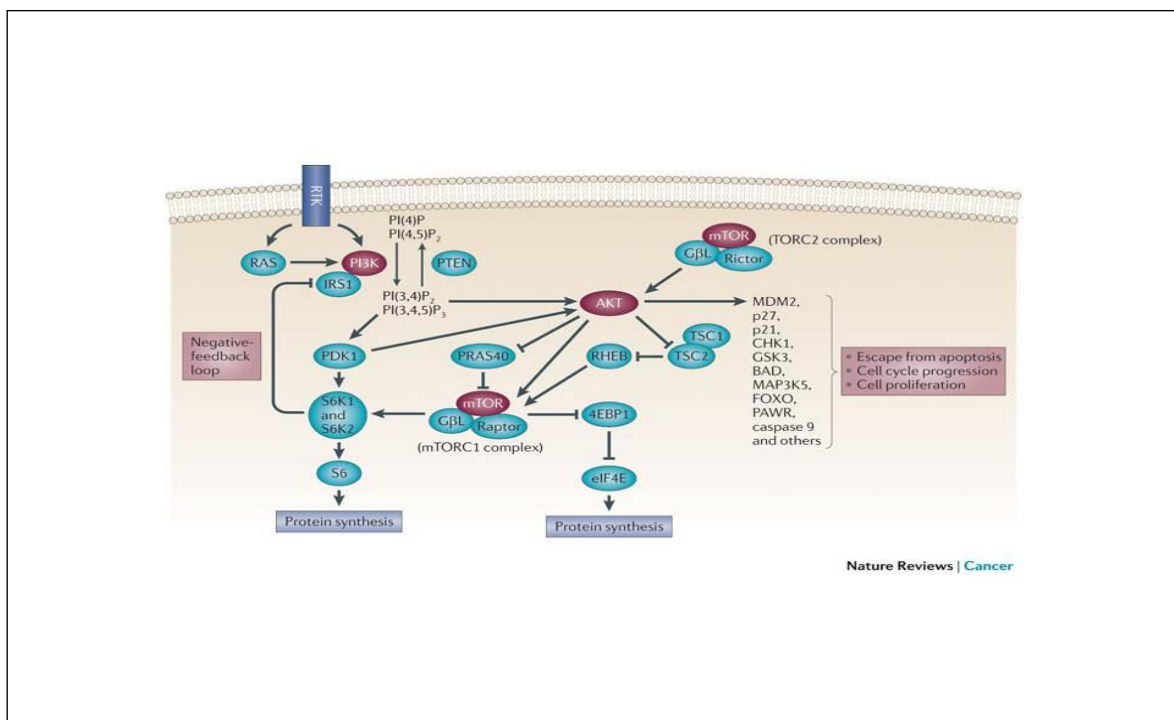


Figure 5: *PTEN* phosphatase activity and its interaction with various signaling pathways through the PI3K-AKT/mTOR pathway. Reprinted from Hollander et al., *Nature reviews/Cancer*, with permission from Macmillan Publishers Ltd (Hollander et al., 2011).

Somatic *PTEN* alterations are common in a variety of sporadic tumors. Such mutations can be found in breast, endometrial (up to 35-55% of sporadic endometrial carcinomas) (Peterson et al., 2012), thyroid, prostate and renal cancers, as well as in melanomas (Tan et al., 2012) (Hollander et al., 2011) (Farooq et al., 2010). *PTEN* inactivation may be caused by gene deletions, small insertions and mutations or alterations that may occur throughout the entire coding region, most commonly in exon 5 (Hollander et al., 2011).

1.2.4 Hereditary breast and ovarian syndrome

The cause of hereditary breast ovarian cancer syndrome (HBOC) is through mutation of the *BRCA1* and *BRCA2* genes (Lynch et al., 2013). Up to 10% of all breast cancer (Kobayashi et al., 2013) and up to 15% of ovarian cancers (Meaney-Delman et al., 2013) can be attributed to this syndrome. Lifetime risk among mutation carriers of developing breast cancer is 45-80%. Among *BRCA1* carriers, there is a 45-60% lifetime risk of developing ovarian cancer, while the corresponding figures for *BRCA2* carriers is 11-35% (Paul et al., 2014).

When HBOC is suspected in Sweden, the following criteria are used in counseling situations to test for *BRCA1* and *BRCA2* mutations:

- 1) At least three cases of breast cancer in first-degree relatives, one of whom was under the age of 50 at the time of diagnosis
- 2) Two first-degree relatives with breast cancer, one before the age of 40 years
- 3) One case of breast cancer before 35
- 4) Any combination of breast cancer and ovarian cancer in a family regardless of age
- 5) One case of ovarian cancer before age 45

(The Swedish Society of Medical Genetics, (SFMG), Guidelines 2014) (von Wachenfeldt et al., 2007).

BRCA1 and *BRCA2* mutation carriers play no role in the development of endometrial cancer, except among patients who have used tamoxifen (Shai et al., 2014). However, a recent association has been implied between serous a endometrial cancer and *BRCA1* gene mutation, which suggests a possible association with HBOC syndrome (Pennington et al., 2013).

1.2.5 Gynecologic surveillance in Lynch syndrome

Controversy persists regarding the benefit of screening Lynch syndrome patients for gynecological cancer and what diagnostic modalities to employ (Auranen et al., 2011) (Vasen et al., 2013). Surveillance is recommended for early detection of gynecological cancer because of the elevated risk for endometrial and ovarian cancer (Barrow et al., 2013).

Current recommendations suggest beginning surveillance at age 30-35 years with an annual/biannual gynecologic examination, to include transvaginal ultrasound, endometrial biopsy (Barrow et al., 2013) (Vasen et al., 2013) and possibly CA-125 and hysteroscopy (Barrow et al., 2013). Inclusion of endometrial sampling in the recommended screening program may entail some discomfort (Elmarsy et al., 2009) that could discourage patients from being compliant with rechecks and gynecological screening (Crispens et al., 2012).

One regimen that has recently been tested combines colonoscopy with endometrial sampling under sedation, thereby reducing pain, discomfort and anxiety. Results are promising, but have not yet been adopted as standard clinical practice (Huang et al., 2011).

Prophylactic hysterectomy and bilateral salpingo-oophorectomy (BSO) should be offered to LS mutation carriers (Vasen et al., 2013), for whom this strategy has proven effective to minimize the risk of developing gynecological cancer (Schmeler et al., 2006). According to current international recommendations the procedure should be timed with completion of childbearing (> 40years) after informing the patient about the risks and benefits of surgery (Vasen et al., 2013). Both prophylactic gynecological surgery and planned colorectal surgery may be carried out at the same time (Vasen et al., 2013).

Both reproductive and clinical genetic counseling should be offered to young patients with Lynch syndrome who have not yet completed childbearing in order to discuss options related to prenatal and/or preimplantation diagnosis (Cohen et al., 2014).

1.2.6 Family history

A variety of studies have addressed the possibility of a familial association with respect to EC. Increased risk of developing EC at a younger age (<55 years) is found among women whose first-degree relatives have been diagnosed with this disease (Parazzini et al., 1994) (Lucentaforte et al., 2009). A family history of colorectal and ovarian cancer in first-degree relatives has been linked to EC (Lucentaforte et al., 2009) (Hemminki et al., 2004). In addition, increased risk of endometrial cancer (Kazerouni et al., 2002) has been found to be associated (von Wachenfeldt et al., 2007) with a history of personal or familial breast cancer.

“The only true wisdom is in knowing you know nothing.”

Socrates

2 AIMS OF THE THESIS

This thesis aims to expand our current understanding of EC and to analyze hereditary factors among women with EC to ultimately improve risk assessment and surveillance of women at risk for familial EC. Furthermore, we aimed to identify possible disease-causing genes in families with suspected high-risk genes. Another aim was to evaluate and optimize current EC screening programs in order to create an evidence-based EC prevention program for women at risk.

Specific aims for each paper:

- I. To investigate the frequency of hereditary uterine cancer syndromes, including LS, Cowden syndrome and hereditary breast and ovarian cancer, among uterine cancer patients in Sweden. To study what familial association might exist between uterine cancer and other selected cancers.
- II. To examine the prevalence of germline *PTEN* mutations in a significant proportion of Cowden and Cowden-like families of endometrial cancer patients.
- III. The aim of this study is to examine how and with what kind of diagnostic modalities the gynaecological surveillance of LS patients is performed in Sweden.

3 MATERIAL AND METHODS

3.1 PAPER I

3.1.1 Study design

The Department of Obstetrics and Gynecology, Karolinska University Hospital, Stockholm, Sweden serves as the referral center for all cases of uterine cancer in Stockholm County.

Of the 890 patients operated for uterine cancer between January 2008 and March 2012 who were invited to participate in the current study, 481 accepted (index patients). We obtained information regarding diagnosis and age at onset for the various cancers (i.e., colorectal, breast, ovarian and other cancers in the index patient, as well as in her first and second-degree relatives, including first cousins), height, weight, parity, history of diabetes mellitus, hormone replacement therapy, lipid-lowering drugs, and prior cancer diagnoses. All information was updated at the end of the study period using the patient's medical records and the Swedish Cancer Registry.

A blood sample for DNA extraction was taken from all patients in accordance with Registry of Endometrial Cancer biobank procedures in Stockholm, Sweden. Telephone interviews were undertaken to obtain relevant information concerning first- and second-degree relatives and first cousins of index patients. We recorded relevant information regarding current age or age at death, type of cancer and age when cancer was diagnosed. All diagnoses (for both index patients and their relatives) were histopathologically verified. We also examined data from the Swedish Cancer Registry, medical records and/or death certificates.

Pedigrees were constructed for each patient using the information collected, after which all pedigrees were examined for the presence of Lynch syndrome, Cowden syndrome and hereditary breast and ovarian cancer in accordance with the Amsterdam II criteria (Seghal et al., 2014), the National Comprehensive Cancer Network guidelines (NCCN guidelines, v1 2012) and HBOC criteria (von Wachenfeldt et al., 2007), respectively.

We followed current standard procedures to screen mutations for causative genes, including *MLH1*, *MSH2*, *MSH6*, *BRCA1* and *BRCA2*. Pedigrees were also assessed for occurrence of cancer among close relatives, especially focusing on putative hereditary endometrial cancer, as well as colorectal, breast and ovarian cancer.

When examining family history of cancer among participating patients we only linked either the maternal or paternal family, depending on which side had the most cancers, to each index patient. Among family members who had > one cancer, each type of cancer was counted individually.

3.1.2 Reference population

All physicians and pathologists report all new cancer cases to the Swedish Cancer Registry, which was founded in 1958. The registry was used to verify the various cancer diagnoses among both index patients and their family members, and served as a reference population for this study. The population of Sweden was 8.08 million (2.621.732 > 50years) in 1970 and 9.4 million (3.492.146 >50 years) in 2010, at which points in time the total numbers of cases of cancer reported annually to the Cancer Registry were 28,594 and 54,342, respectively.

3.2 PAPER II

3.2.1 Study design

We selected participants from the cohort of patients with endometrial cancer who had surgery between January 2008 and March 2012 and who also participated in study no. I. The pedigrees created by the process outlined in study no. I, were all assessed for possible Cowden syndrome and Cowden syndrome-like families.

Cowden syndrome-like families can be defined by the presence of at least one case of endometrial cancer and one case of breast cancer, in addition to at least one additional tumor associated with Cowden syndrome (endometrial, breast, thyroid, colon, or renal cancer) in a given individual, or among first-degree relatives. We applied NCCN guidelines (NCCN guidelines, v1 2012) and the definition for Cowden syndrome-like families (as presented above and in paper II) (Tzortzatos, Aravidis et al., In press 2015) to assess whether any patients met the criteria for Cowden syndrome and/or CS-like families.

3.2.2 Touchdown PCR/ DNA sequencing

Genomic DNA was extracted from peripheral blood leucocytes following standard procedure at the Department of Clinical Genetics, Karolinska University Hospital (MagneSil Genomic, large volume system, Promega, Madison, WI, USA in a Tecan robot serial no. 904004850, Männedorf, Switzerland). All nine *PTEN* gene exons, including adjacent introns, were then amplified by subjecting the extracted genomic DNA to polymerase chain reaction. We used the Primer3Plus platform online tool (<http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi/>) to design the PCR primers and produce amplicons ranging in size from 230 to 399 bp, which also contained the area of interest. For verification that each primer pair would generate its own unique amplicon from the entire genomic DNA sequence the UCSC In-Silico PCR tool was used (<http://genome.ucsc.edu/cgi-bin/hgPcr?command=start>). We used AmpliTaq Gold DNA PolymeraseTM (Roche Molecular Diagnostics, Pleasanton, CA, USA) to amplify each fragment in a 25 µl final PCR volume that contained both the pair of primers at a concentration of 10 µM and 50 ng of DNA template from each patient. PCR reactions were carried out in 96-well PCR plates (Bio-Rad, Hercules, CA, USA) using a 2720 thermal cycler (Applied Biosystems Foster City, CA, USA) or a DNA Engine Tetrad^R 2 Peltier Thermal Cycler (Bio-Rad, Hercules, CA, USA). Using the temperature independently designated based on the melting temperatures (T_m) for each primer pair, we applied a “touchdown” PCR protocol. Specifically, we carried out two stages of PCR cycles following initial DNA denaturation. Stage one involved 7 cycles of gradient temperature decrease covering a range including melting temperatures (T_m) of both the forward and reverse primer. Stage two entailed 30 cycles at a stable annealing temperature, identical to that of the last cycle in stage one. After stages one and two, a final extension phase was allowed to continue for 10 minutes, followed by a rapid thermal ramp to 4°C, which was held until purification occurs. The resultant PCR products were subjected to analysis using 1.5% agarose gel electrophoresis and then examined under ultraviolet lighting. Clear bands occurring at the appropriate size range were interpreted as confirming positive amplification. ExoSAP-IT[®] (USB Affymetrix, Santa Clara, CA) was used for clean-up of the PCR products, which were subsequently sequenced overnight using a 48-capillary 3730xl DNA Analyzer (Applied Biosystems). The resulting sequences were analyzed using Seqscape software version 2.7 (Life Technologies, Carlsbad, CA, USA) with reference sequence NM_000314.4. Table 1 from paper II presents the primer pairs for each amplicon (forward and reverse), including their corresponding annealing temperatures, GC percentage content and size of PCR product.

3.3 PAPER III

3.3.1 Study design

Our group undertook a study of all Swedish women with known LS nationwide. To identify patients for recruitment we contacted the regional departments of clinical genetics in Lund, Stockholm, Linköping, Uppsala and Gothenburg, thereby covering all of Sweden except the extreme north.

After searching the registries to identify women with Lynch syndrome, we contacted 260 candidates. In all, 170 agreed to participate, while 160 of them had clinical data sufficient for inclusion in the study.

We reviewed the medical records of study participants to collect information regarding history of gynecological surveillance of LS patients, biopsy results (if any), and any genetic records. Additional information was obtained concerning the details of surveillance, with special focus on endometrial biopsy (EB), transvaginal ultrasound (TVUS), CA-125 testing, hysteroscopy, number of visits, prophylactic surgeries with age at time of procedure, current age, age at LS diagnosis and screening/surveillance location, where relevant

3.4 Statistical analysis

Population data for paper I were obtained from official Swedish statistics for two separate years (1970 and 2010) to compensate for any differences in the incidence of cancer over time. Regarding different cancers among relatives of index patients, the corresponding proportions and confidence intervals (CIs) were calculated for each site. We compared the CIs obtained concerning the proportions of cancers at each site with the proportions of various cancers in the general population in 1970 and 2010. Any CI interval that failed to match the proportion from 1970 and 2010 revealed a difference for that year. Only when a significant difference in the proportion of malignancies was demonstrated compared with the population at large was under-representation or overrepresentation considered to be present.

For categorical data we used the Pearson's chi-square test, while any statistically significant differences in unpaired groups was evaluated using the Wilcoxon rank sum test. Statistical analysis was carried out using R software (www.r-project.org, R Core team, 2012).

For paper III we used the Statistica[®] software (Statsoft.se) package to analyze data. Differences in groups were calculated using the Pearson's chi-square test and Fisher's exact test, Kaplan-Meier estimator, while testing of multiple groups was carried out using the Kruskal-Wallis test. P values <0.05 was considered significant.

3.5 Ethical considerations

The Regional Ethical Review Board at Karolinska Institutet, Stockholm, Sweden, approved the studies in this paper (papers I and II: 2010/1536-31/2, paper III: 2012/885-31/1). Written informed consent was provided by all participants. For the purpose of verifying histology results concerning diagnosis of cancers in relatives of female participants for papers I and II, written informed consent was obtained directly from relatives or their nearest surviving relative. The biobank number for the project is Bbk443.

4 RESULTS

4.1 PAPER I

We followed index patients for a median period of 24 months. Table 4 presents details regarding the characteristics of the patients.

Median age at time of diagnosis for index patients was 67 years (range 34-95 years). Endometrioid carcinoma was found by histological examination in 82% of index patients, with most tumors (86%) confined to the uterus (FIGO stage 1) (Table 1). At follow-up rechecks, 17 index patients (3.5%) presented with recurrent disease (median age 70.5 years); 12% were originally diagnosed with sarcomas, 6% with clear cell carcinoma, and 7% with endometrioid carcinoma. Among index patients with recurrent disease, 12% were originally diagnosed with stage 3 or 4 disease (compared with 7% of the cohort as a whole), while 47% demonstrated low-grade differentiation (compared with 22% in the cohort as a whole).

4.1.1 Proportion of different cancer types among relatives

A total of 1316 cancers were reported among relatives of index patients. Uterine cancer (6%) showed up in a higher proportion than in the cancer population at large in both 1970 (4%) and 2010 (3%) (Table 3 in paper I). When we examined first-degree relatives alone, and first- and second-degree relatives combined, we found a similar overrepresentation. While cancers such as stomach/unspecified abdomen, larynx and bone were also overrepresented among relatives, other cancers including breast (16%), colon (8%), rectal (3%) and ovarian (2%) were not.

In fact, certain cancers were underrepresented, including cancers of the rectum, pancreas, urinary tract, non-Hodgkin lymphoma, lip/tongue/mouth, endocrine glands (excluding thyroid), pharynx, small intestine, peritoneum, nose, mediastinum, eye and myelofibrosis (table 3 in paper I).

Characteristics	Number/Total	(%)*	Median	Range [Min, Max]
Age at diagnosis, years			67	[34, 95]
Body mass index at diagnosis			26.3	[17.6, 55.1]
Hormone replacement therapy	239/452	(52.9)		
Parity			2	[0, 8]
Diabetes mellitus	51/462	(11)		
Lipid-lowering drugs	102/455	(22.4)		
Histology				
Endometrioid	394/481	(81.9)		
Serous or mixed	56/481	(11.6)		
Clear cell	9/481	(1.9)		
Sarcoma	20/481	(4.2)		
Hyperplasia with atypia	2/481	(0.4)		
FIGO stage				
1A	316/480	(65.8)		
1B	95/480	(19.8)		
2	34/480	(7.1)		
3A	16/480	(3.3)		
3B	7/480	(1.5)		
3C	2/480	(0.4)		
4	3/480	(0.6)		
4B	7/480	(1.5)		
Grade				
1	193/480	(40.2)		
2	181/480	(37.7)		
3	106/480	(22.1)		
Depth of myometrial invasion				
None	64/481	(13.3)		
<50%	282/481	(58.6)		
≥50%	128/481	(26.6)		
Spread through serosa	7/481	(1.5)		
Relapse	17/481	(3.5)		

Table 4: Characteristics: 481 index patients

4.1.2 Hereditary cancer syndromes

After assessing the pedigrees nine of 481 index patients (2%) met the Amsterdam II criteria for diagnosis of LS. Endometrioid cancer was present in all nine patients and seven mutations were identified: three in *MLH1* (c.546-2A>G; c.790+1G>C and deletion of exon 1-3) and four in *MSH2* (c.1147C>T; c.1786_1788del; deletion of exon 7-10 and deletion from exon 3 of the *EPCAM* gene to exon 6 of *MSH2*). While one patient was previously known to belong to an LS family, six new LS families were now subsequently diagnosed. Interestingly, two LS index patients had no known colorectal cancer in their family history (figure 6).

Since none of the index patients met the National Comprehensive Cancer Network guidelines for Cowden syndrome, they were not screened for *PTEN* gene mutations.

Six of the nine patients who met the HBOC criteria were screened for *BRCA1* and *BRCA2* mutations, but none were found. The remaining three had refused further genetic counseling and/or investigation.

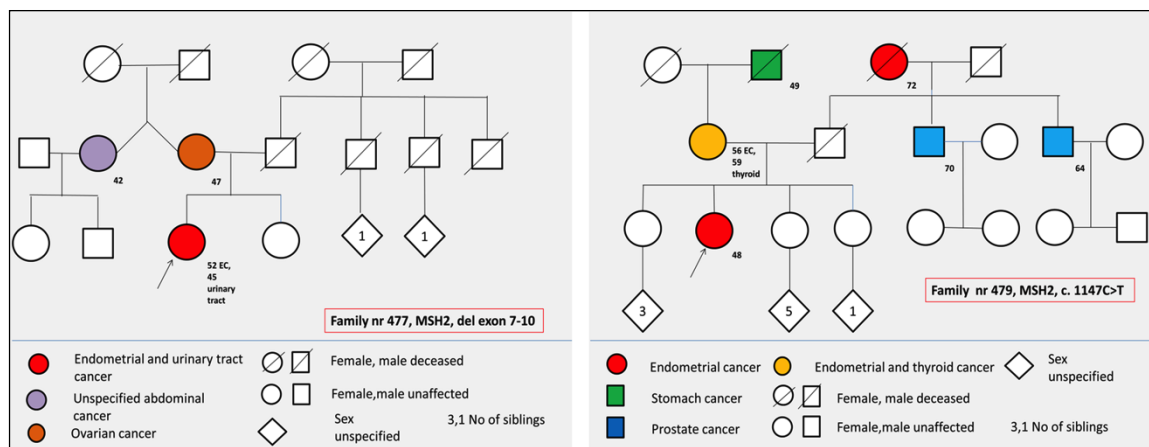


Figure 6: Pedigrees of two families diagnosed with LS. *MSH 2*, del exon 7–10 and *MSH2*, c1147C > T mutations were diagnosed during the study. Notably, none of the families had any history of known colorectal cancer. Adapted from *Tzortzatos et al. Hereditary Cancer in Clinical Practice 2014 12:14*, under the Creative Commons Attribution (CC-BY) license.

4.1.3 Family history of cancer

Table 1 in paper I shows the family history of cancer for index patients, among index whom 17% had a family history of breast cancer, 12% colorectal cancer and 6% ovarian cancer.

We compared the families of the 64 index patients who had at least one relative with uterine cancer (13%) with the families of the 417 index patients who had no relatives with uterine cancer (Table 4 in paper I). We found a significant difference in the number of family members diagnosed with cancer ($p < 0.001$) between the two groups, but no differences were found in histology, age at diagnosis, stage, relapse, ploidy and presence of multiple cancers. Lynch syndrome could be identified in four of the thirty families that had relatives with uterine cancer and in two families in which uterine cancer occurred before age 50 in at least one relative or in the index case (12 of 64 families).

We found six families, three in which the index patient was diagnosed before age 50, with at least two cases of uterine cancer but with no other cancers, possibly indicating site-specific heredity for uterine cancer.

4.1.4 Multiple cancers in index patients

We also searched for the presence of other cancers in index patients and found that 16% had at least one additional cancer (table 5, paper I). Of these, breast cancer occurred together with uterine cancer in 45% of cases. Uterine cancers predominantly demonstrated endometrioid histopathology (80%), although other types were also found, including 6% each of serous carcinoma, clear cell carcinoma and sarcoma, as well as 3% mixed type.

In addition, colorectal cancer was present in 19% of index patients and within this group, 86% of uterine cancers were endometrioid type, while 7% were serous carcinoma and 7% clear cell carcinoma.

In four index cases (5%), the diagnosis of ovarian cancer accompanied that of uterine cancer, with histopathology demonstrating three endometrioid carcinomas and one serous carcinoma.

As shown by table 5, paper I, nine index patients were diagnosed with at least three cancers. Four of the 75 patients with multiple cancers were identified as belonging to LS families.

4.2 PAPER II

No patients met the NCCN diagnostic criteria for Cowden syndrome, but 54 patients were identified as having CS-like families. No germline mutations or polymorphisms were found to involve any of the nine exons of the *PTEN* gene.

4.3 PAPER III

Prior to being diagnosed with LS, 43 patients had undergone hysterectomy with or without bilateral salpingo-oophorectomy; these women made up the *non-screened group* as their prior surgery precluded meaningful screening/surveillance. This left 117 women eligible for screening (screened group), of whom 26 women aged 20-30 had not yet reached the age to participate in the screening process, while three other women had not been informed about screening and two more chose not to attend surveillance, leaving a total of 86 patients who did attend screening.

4.3.1 Mutation spectrum and cancer incidence

Mutations found among the 160 LS patients were as follows: 79 with *MLH1*, 51 with *MSH2*, 25 with *MSH6*, and 5 with *PMS2* mutations. The corresponding figures for the preceding mutational spectrum among the 117 eligible patients were as follows: 62, 31, 19, and 5, respectively. The corresponding figures for the 86 patients in the *screened group* were 40, 26, 17, and 3. Finally, the figures for the *non-screened group* were 17 with *MLH1*, 20 with *MSH2*, 6 with *MSH6*, and none with *PMS2* mutations.

Across the board, EC/complex atypical hyperplasia (CAH) developed in 20% of *MLH1* carriers, 11.5% of *MSH2*, 11.7% of *MSH6*, and 0% of *PMS2* of the 86 patients. Moreover, OC afflicted 4.38% of *MSH2* carriers.

4.3.2 Cancer incidence

Among the 86 patients in the *screened group* the total incidence of gynecological cancer was 15% (13% EC/CAH, 2% OC).

Meanwhile, among women in the *non-screened group* 35 were diagnosed with EC (81.4%), three with OC (7 %) and one with colorectal cancer. When comparing mean age at time of cancer diagnosis there was no significant difference between the screened group, 48.5 years

(range 40-80 years), and the *non-screened group* who had undergone hysterectomy before LS was diagnosed, 52 years (range 35-68 years).

Prophylactic surgery in the group of screened women was carried out on 41 patients at a median age of 53 (range 40-77 years) with the following breakdown of surgical procedures: 32 women with hysterectomy and bilateral salpingo-oophorectomy (BSO), seven with hysterectomy alone and two with BSO alone. Postoperative histopathological findings showed EC/CAH in four patients (9.8%) at a median age of 47.5 (range 42-58 years).

The screened group included 45 women, median age 41 (range 24-84 years), who presented for annual gynecological screening. Eleven (24%) of these women subsequently developed gynecological cancer; nine were diagnosed with EC/CAH (20%) and two with OC (4%). Gynecological screening detected five cases of EC/CAH (median age 48, range 42-80 years), while symptoms of intermittent bleeding led to the discovery of four more cases (median age 46.5, range 40-59 years) (table 1). The two cases of ovarian cancer, involving women aged 38 and 45, were found by TVUS during recheck visits (table 1, paper III).

4.3.3 Diagnostic screening modalities

Transvaginal ultrasound examination (TVUS)

All women who presented for gynecological screening underwent TVUS. Endometrial thickening, as noted by TVUS, was found in two of four patients with EC and intermittent bleeding symptoms. TVUS also revealed the two cases of OC.

Endometrial biopsy (EB)

In all, 28 women (33%) underwent endometrial biopsy as part of their gynecological screening. A significant proportion of the women who presented for screening were found to have cancer, as diagnosed through EB.

EB contributed to the diagnosis of all cancers and premalignant lesions among both symptomatic and asymptomatic patients who presented for screening. However, one patient who previously had a negative EB was later found to have EC/CAH following prophylactic hysterectomy.

Tumor marker cancer antigen (CA) 125

The Ca-125 marker for ovarian cancer was assessed in 27 patients (29%). Of the two patients who were found to develop ovarian cancer, a borderline elevation of Ca-125 (36kU/L compared with the reference limit of 35kU/L) was found in the single patient who was screened.

Hysteroscopy

Hysteroscopy, in three cases motivated by the finding of a suspicious polyp on TVUS, was carried out on four patients who presented for screening. In two of these three cases, a benign polyp was confirmed by hysteroscopy, while the third case showed normal endometrium.

Intermittent bleeding was the motivation for hysteroscopy in the fourth patient, for whom findings were also normal.

Prophylactic surgery vs. no surgery

When comparing the group of women who underwent prophylactic surgery with those who had no surgery, the incidence of cancer/premalignant lesions in the latter group was 24% (11 cases=9 EC/CAH, 2 OC, 20% EC/CAH, 4% OC), while the incidence in the former group was 9.75% (2 cases each of EC and CAH based on postoperative histopathological findings), which represents a significantly lower incidence of cancer in the operated group ($p=0.036$).

Screening setting

We wanted to find out whether incidence of cancer differed based on screening methodology or screening setting (private, county, university).

We found no difference in incidence of cancer based on screening setting. Patients who attended screening in the private setting and those who underwent prophylactic surgery tended to have a higher age and a broader age interval, although the difference in comparison with the other two settings was not significant. Moreover, compared with university and county clinics, the private setting was associated with more screening visits and fewer prophylactic procedures. CA-125-testing was done more frequently in private clinics, while endometrial biopsy was equally common in all settings.

Educational level

The educational level of patients in the screening group was not associated with any differences in the number or type of recheck visits.

5 DISCUSSION

5.1 PAPER I

To determine whether cancer in the family history is a risk factor for uterine cancer we studied a Swedish patient population with uterine cancer and confirmed an association between family history and occurrence of uterine cancer. Our research showed that among index patients at least 13% had a family member with uterine cancer (7% with at least one FDR with diagnosed uterine cancer) and that families in our cohort experienced an increased relative proportion of uterine cancer, compared with incidence of cancer in the population at large for the years 1970 and 2010.

The combination of multiple cancers present in any one individual and early age (<50years) at diagnosis of cancer is suspicious for hereditary cancer syndrome. We found that among patients in our cohort who had at least two cases of uterine cancer in the same family, 47% had family members diagnosed with cancer at an early age (<50 years). LS, a known hereditary syndrome, was identified in only 13% of that cohort. Moreover, at least one extra cancer was found in 17% of index patients (Tzortzatos et al. 2014).

As many other studies on uterine cancer have shown, we found an increased risk of this disease among first-degree relatives of uterine cancer patients, with even greater odds for developing uterine cancer among relatives of patients who were diagnosed before age 50 (Parazzini et al., 1994) (Lucentaforte et al., 2009) (Hemminki et al., 2004) (von Wachenfeldt et al., 2007) (Hemminki et al., 1999) (Gruber et al., 1996) (Parslov et al., 2000).

The risk for first-degree relatives of uterine cancer patients to develop endometrial cancer is increased when environmental factors, including obesity, may interact with genetic susceptibility (Seger et al., 2011). No difference in BMI (median 26.6) was seen in our study when comparing index patients with or without additional cases of uterine cancer.

The relative proportion of laryngeal, stomach/abdominal and skeletal cancer was increased in our study, which we suggest may be due to misclassification regarding metastasis (skeletal cancer), or possible problems with recall bias concerning information and classification of cases (particularly various abdominal cancers). To date uterine cancer has not been shown to have an association with laryngeal cancer.

An overrepresentation of endometrial cancer among non-*BRCA1/2* breast cancer families was demonstrated in a comparison with the cancer population at large, which suggests a new breast cancer syndrome (von Wachenfeldt et al., 2007). However, our study of endometrial cancer patients did not find that breast cancer was overrepresented. Nevertheless, there may be an association between endometrial cancer and breast cancer, as implied by the finding in our study that 45% of our index patients suffering from multiple primary cancers had both EC and BC, a higher proportion than found by Delin et al. (31%) (Delin et al., 2004) and Uccella et al. (10%) (Uccella et al., 2011). In addition, the risk of endometrial cancer regardless of family history (Kazerouni et al., 2002) and endometrial serous carcinoma in younger women (<55 years) (Liang et al., 2011) is elevated in patients with a history of breast cancer. A recent study found that among seven (5%) women with uterine serous carcinoma who had mutations in breast cancer genes, only two had a family history of breast cancer (Pennington et al., 2013). Although 6% of our index patients had both breast cancer and serous carcinoma, we found no *BRCA1/2* mutations.

Research has found that the risk of developing endometrial carcinoma increases with tamoxifen use (RR 2.2-4), especially among postmenopausal women (Fisher et al, 1998) (Braithwaite et al., 2003). Our study identified 12 women with a history of tamoxifen treatment for breast cancer who subsequently developed uterine cancer. Although there is a higher cumulative incidence of endometrial cancer after five years of tamoxifen treatment, 13/1000 compared with 5.4/1000 among women who never used tamoxifen (Braithwaite et al 2003), we are unable to attribute cases in which uterine cancer developed in our study to tamoxifen.

Ovarian cancer was not found to be overrepresented in our study. Although our study population was not large enough to establish an association between EC and OC, 5% of our index patients did have OC. A similar figure (4%) was found by Uccella et al. (Uccella et al., 2011), while a much higher figure (29%) was reported by Delin et al. (Delin et al., 2004). Both an increased risk of synchronous or consecutive OC following EC (especially of the endometrioid type) and an increased risk of EC following primary OC were reported by Hemminki et al. (Hemminki et al., 2003).

Colorectal cancer was not overrepresented in our study, either; it was found in 17% of our index patients who had more than one cancer. The corresponding figure reported by Uccella et al. was lower (3%) (Uccella et al., 2011), while Delin et al. reported a figure similar to ours (11%) (Delin et al., 2004). LS was identified in two of 14 index patients with metachronous colorectal cancer.

The Amsterdam II criteria were met by nine families (1.9%) in our study; of these, seven (1.5%) were found to have LS following verification of mutation. The *MLH1* gene was mutated in three families and the *MSH2* in the other four. Similar percentages of LS (1.8-4%) have been reported by other studies in unselected cases of uterine cancer (Hampel et al., 2006) (Leenen et al., 2012) (Ollikainen et al., 2005) (Egoavil et al., 2013). However, our finding of 1.5% LS among our cases may represent an underestimate because of the low sensitivity for identifying endometrial cancer (20-30%) when using the Amsterdam II criteria (Hampel et al., 2006) (Leenen et al., 2012). Moreover, carriers of the *MSH6* mutation are less likely to fulfill the Amsterdam II criteria (Sjursen et al., 2010). They are at lower risk of both colorectal cancer (10-22% cumulative risk by age 70) and of other LS-related cancers (Baglieto et al., 2010) (Bonadona et al., 2011).

We find it interesting to postulate that the small number of families we identified with two or more cases of uterine cancer alone may represent site-specific uterine cancer, which is distinct from LS.

5.2 PAPER II

Although some small studies have searched for *PTEN* mutations in families with CS or CS-like phenotype, ours is the first involving CS-like families with uterine cancer.

Marsch et al. (Marsch et al., 1998) searched for germline *PTEN* mutations in a study of 64 CS-like families with a family history of breast and thyroid cancer, but not EC. One cryptic germline mutation c.209T>C, was found in exon 3 in one family that did not strictly fulfill the criteria for CS, suggesting that while the international CS diagnostic criteria remain robust, other genes may be involved in the CS-like phenotype.

Rustad et al. (Rustad et al., 2006) found that only the six families with CS had germline *PTEN* mutations, while the two families suspected of having CS and the eight families in which both breast and thyroid cancers were present did not.

Black et al. examined *PTEN* for germline mutations in a series of 240 consecutive ECs (both type I and type II). They were only able to identify an intronic deletion, a rare polymorphism in one patient, but no disease-causing mutations were found. This patient had a family history of sarcoma, as well as breast, lung and colon cancers. The researchers concluded that *PTEN* germline mutations do not increase the risk of EC in an unselected population outside the context of CS (Black et al., 2005).

Since differentiated non-medullary thyroid cancer (DTC) may affect 3-10% of individuals with germline *PTEN* mutations, Nagy et al. studied the frequency of these mutations in an unselected population of 259 cases of DTC, 17 of which fulfilled CS criteria. The authors found a very low mutation rate (0.8%), but were able to identify two deleterious mutations in two individuals who did meet CS criteria. They suggested combining germline *PTEN* mutational screening with histology and clinical evaluation of thyroid cancer patients (Nagy et al., 2011).

Laugé et al. studied a series of 20 women with breast cancer who also had a personal history and/or family history of breast/brain tumors. They excluded patients with a personal or family history of Cowden disease as well as patients with a family history of breast cancer in which germline *BRCA1* and *p53* mutations were present. They performed point mutation analysis of the *PTEN* gene and found two previously described polymorphisms (insertion of a T in intron 4, IVS4-29insT, and a T to G transition in intron 8, IVS8+32T/G), but no disease-associated mutations (Laugé et al., 1999).

Lynch et al. selected a series of 25 families to sequence for germline *PTEN* mutations in order to investigate whether *PTEN* mutations predispose to breast cancer.

Of these families, three had CS and five had CS plus breast cancer, while four had breast and thyroid cancer without a definite diagnosis of CS. The remaining 13 families were at high risk of breast, ovarian and/or prostate cancer, with wild-type *BRCA1* and *BRCA2* sequences. Mutational analysis and DNA sequencing of the *PTEN* gene identified seven (five nonsense and two missense) mutations in 6 CS families and one CS-like family. Consequently, all seven of these mutations were identified in patients from CS and CS-suspected families (by clinical characteristics) and none were found among the remaining 13 families referred to above. (Lynch ED et al.1997).

A recently published study (Castéra et al., 2014) of a large series of 708 consecutive patients who fulfilled HBOC criteria did not identify any germline *PTEN* mutations through next-generation sequencing. Kurian et al. carried out multiple gene-sequencing analysis in 198 women suspected of having HBOC. *PTEN* was one of the tested genes and no germline mutations were found (Kurian et al., 2014). Neither study showed any association between germline *PTEN* mutation and HBOC.

One study carried out germline mutation analysis of the succinate dehydrogenase (*SDH*) gene on 375 CS and CS-like individuals in whom *PTEN* mutations were not found. CS-like individuals were defined as those who fell one or two criteria short of meeting all existing CS guidelines. Ten germline mutations/variants in the *SDHB* and *SDHD* genes were identified in these patients that were not found in healthy controls. *SDHx* gene mutations affect mitochondrial function related to the Krebs cycle and may be associated with activation of pathways similar to those that *PTEN* mutations affect. Significantly higher frequencies of breast cancer, as well as thyroid and renal cell carcinomas were found among carriers of the *SDHx* mutation compared with carriers of germline *PTEN* mutations. They postulate that this gene may be an indicator of susceptibility among CS and CS-like individuals when germline *PTEN* mutations are not present (Ni et al., 2008).

Bennet et al. showed hypermethylation of *KILLIN* in 30% of all cases among 123 CS and CS-like individuals who tested negative for germline *PTEN* mutations. Disruption of *p53*-activation was also seen and these changes were associated with increased risk of breast and renal cancer among *PTEN* mutation-positive patients. CS-like individuals shared some features of CS without meeting diagnostic criteria (Bennet et al., 2010).

Another study of 103 patients with primary breast cancer and 25 patients with familial breast cancer identified no germline *PTEN* mutations, leading the authors to conclude that *PTEN* gene alterations are rare in relation to breast cancers. (Freihoff et al., 1999).

A recent study examined 91 CS and CS-like individuals, who did not exhibit any *PTEN/SDHx/KILLIN* mutations, for the presence of mutations in other genes along the AKT/PIK3CA/mTOR pathway. A total of 8.8% were found to have germline *PIK3CA* mutations and 2.2% *AKT1* mutations. The authors showed that this resulted in increased cellular PIP3 and phosphorylation of AKT1, suggesting that *PIK3CA* and *AKT1* are CS susceptibility genes (Orloff et al., 2013).

Other studies have shown that about 10% of *PTEN* mutation-negative CS patients have nucleotide variants within the full length of the promoter region that can cause either a decrease in *PTEN* protein expression or loss of function. Since 89% of these patients had breast cancer, the authors suggested that these mutations had very high penetrance for breast cancer (Zhou et al., 2003). Teresi et al. examined miscellaneous *PTEN* promoter nucleotide variations of unknown significance in CS patients and found that some of these variations led to decreased *PTEN* expression through dysfunctional translation, rather than by affecting transcription (Teresi et al., 2007). Liu et al. identified a novel *PTEN* mutation located in 1.312 (G<T) within the promoter region in a patient whose pedigree suggested CS. Since this is the p53-binding sequence region, it may affect p53-induced *PTEN* expression. No mutations were identified in the nine exons of *PTEN*. However, the authors were unable to determine whether oncogenesis in this patient could be attributed to a *KILLIN* mutation or *PTEN* hypermethylation (Liu et al., 2013).

Our study has two major flaws: the small number of patients and the lack of a detailed phenotypic evaluation of patients to obtain information on head-circumference (associated with increased risk of cancer in CS patients) or on non-cancer phenotype. On the other hand, this project was carried out in the real clinical world on CS-like families referred for germline mutation screening based on their family history alone.

To conclude, we detected no germline *PTEN* mutations in our cohort of CS-like patients, suggesting that screening for *PTEN* mutations in such patients has no clinical relevance unless patients meet strict CS diagnostic criteria. We did not search for large genomic deletions/duplication of one or more exons. Large deletions are common in somatic alterations, but are not found as constitutional *PTEN* mutations (Zhou et al., 2003). We did

not check for mutations in the promoter region because germline mutations in that area are uncommon (Zhou et al., 2003).

5.3 PAPER III

We showed that screening Lynch syndrome patients for gynecological malignancy reduces the incidence of cancer. Endometrial biopsy is an effective method for diagnosing endometrial cancer and precancerous lesions. Prophylactic hysterectomy with or without bilateral salpingo-oophorectomy significantly reduces the incidence of cancer.

In the non-screened group we found an 81.4% incidence of EC, compared with 13% in the screened group. Most cases of EC are detected at an early stage and patients are cured by surgery. Therefore it remains unclear whether EC screening reduces morbidity and mortality.

One study (de Jong et al., 2006) showed a decrease in mortality among patients who attended an annual screening program that included TVUS and CA-125, but further studies are needed to evaluate the efficacy of screening programs regarding morbidity and mortality.

We found that the use of EB in screening settings was of benefit to LS women, since more cases of EC were found when EB was used.

The findings in our study are consistent with those of Renkonen-Sinisalo et al. (Renkonen-Sinisalo et al., 2007). These authors demonstrated clear differences in screening accuracy when comparing the various diagnostic tools used to detect cancer. They also showed that the ability to detect EC at the screening visit largely depended on whether or not EB was used. Another important result from that study was the finding of 14 additional premalignant cases using EB that were missed on TVUS, thereby leading to fewer cases of cancer and ultimately to decreased mortality and morbidity within the screened group.

Nowadays, since TVUS is routinely used for screening in every gynecological practice we were unable to compare results between TVUS and non-TVUS groups in our study. However, no EC was found by TVUS in any of our reported cases, which contradicts a study by Helder-Woolderink et al. (Helder-Woolderink et al., 2013), in which annual TVUS detected all premalignant cases, with no added value from EB. Meanwhile, a screening program study that followed 292 LS women for 13 years using only TVUS screening did not detect any cases of EC (Dove-Edwin et al., 2002). This was a large study and therefore

clearly shows that TVUS alone is insufficient to detect EC, which is well in line with our results.

The drawbacks of annual EB are the small risk of complications (infection and possible tissue damage, such as perforation of the uterus) and discomfort from the procedure (Elmarsy et al., 2009). Therefore the risk is that women may choose not to attend screening, thereby lowering compliance (Crispens et al., 2012). They may also opt for prophylactic surgery because of the screening procedure (Helder-Woolderink et al., 2013). However, other authors suggest that both hysteroscopy and EB are well-tolerated outpatient procedures (Manchada et al., 2012) (Järvinen et al., 2009). A newly proposed strategy is to perform EB simultaneously with colonoscopy rechecks in an effort to reduce pain, discomfort and anxiety. This strategy has been tested at some centers and has been shown to improve screening accuracy and compliance (Huang et al., 2011).

Hysterectomy with/without bilateral SOE as a method of preventing cancer is almost 100% effective regarding both EC and OC (Schmeler et al., 2006). Owing to the small number of patients in prophylactic surgery groups, it has not been possible to ascertain the reduction in mortality in any of these studies (Renkonen-Sinisalo et al., 2007) (Crispens et al., 2012) (Schmeler et al., 2006) (Boilesen et al., 2008). The disadvantages of prophylactic hysterectomy include general surgical complications and premature menopause associated with bilateral SOE (Nakamura et al., 2014) (Schmeler et al., 2006). Patients should be informed not only about the probable reduction in risk of cancer from prophylactic surgery, but also about negative effects on childbearing, as well as potential secondary surgical complications and complications associated with premature menopause. The literature is nearly unanimous in recommending total prophylactic hysterectomy with/without bilateral SOE once childbearing is completed (NCCN guidelines, 2013) (Vasen et al., 2013) (Järvinen et al., 2009) ((Schmeler et al., 2006) (Lachiewicz et al., 2014) and after all the pros and cons of prophylactic surgery have been discussed (Vasen et al., 2013) (Nakamura et al., 2014), or the procedure can be done in conjunction with colorectal cancer surgery (Nakamura et al., 2014). Many studies underscore how important it is for gynecologists to be aware of the possibility that malignancy may already be present when they undertake prophylactic surgery (Schmeler et al., 2006) (Lachiewicz et al., 2014) (Lu et al., 2013) (Backes et al., 2011).

There is no consensus about the age at which a patient should be included in a gynecological surveillance program. Since the youngest cancer patient in our material was 35 years old and the oldest 80 years old we suggest that gynecological surveillance should begin at least five

years prior to the earliest case, i.e. from 30 years of age and continue into old age. To date no upper age limit for surveillance has been defined (Vasen et al., 2013) (Auranen et al., 2011) (Ketabi et al., 2014).

In our material, two women in the screened group developed ovarian cancer (1.7%) which is in consistent with other studies (Auranen et al., 2011) (Renkonen-Sinisalo et al., 2007) (Järvinen et al., 2009) (Boilesen et al., 2008). TVUS detected both cases without any diagnostic contribution from Ca-125. The cases of OC reported by Renkonen-Sinisalo et al. (Renkonen-Sinisalo et al., 2007) were not discovered through surveillance, but only diagnosed as a result of symptoms or incidentally at surgery. Similarly, no cases of OC were discovered through surveillance in the studies reported by Auranen et al. (Auranen et al., 2011). Screening results for OC among LS patients are very few and usually not significant due to the low number of cases. Gynecologists use both TVUS and the Ca-125 tumor marker to screen for OC, but so far neither test has proven to be significantly effective for preventing mortality from OC (Gaarenström et al., 2006). The prognosis for ovarian cancer in LS patients may be better than in patients with sporadic ovarian cancer (Nakamura et al., 2014) (Backes et al., 2011) (Grindedal et al., 2010), although not all studies agree (Crijnen et al., 2005).

No differences were found in the number or types of screening visits in relation to educational level in our cohort, but we were unable to measure the impact on compliance. Increased compliance may correlate with higher educational levels, as suggested by Ketabi et al. (Ketabi et al., 2012), perhaps due to a better understanding among highly educated women regarding the risks of EC and OC.

We found no significant differences in the type of screening or incidence of cancer among the various medical settings where surveillance was conducted. The only difference between county and university hospitals was that the former used Ca-125 more frequently than the latter. These two types of gynecological clinics were similar in all other respects, perhaps due to the small number of patients overall, or this result may reflect a trend among gynecologists to comply with national guidelines for LS patients.

One limitation of our study is its retrospective design. Selection bias may be present. Women who are aware of their increased risk of cancer may be more likely to participate in the study. No information is available about the women who chose not to participate. However, all

cancer diagnoses were obtained from medical records, thereby eliminating any recall or ascertainment bias.

6 GENERAL CONCLUSIONS

- In an unselected uterine cancer population we identified an overrepresentation of uterine cancer among first-degree and second-degree relatives and first cousins of patients. We suspect that a common genetic factor, and/or common environmental and lifestyle factors may account for this.
- Our data raise suspicion of a possible hereditary uterine cancer syndrome because we observed an increased incidence of cancer occurring before age 50 in relatives of patients with uterine cancer, as well as an increased incidence of multiple cancers among index patients.
- We also determined that the prevalence of Lynch syndrome is about 2% and of the seven families diagnosed with LS in our study, only one was previously known. The family history of all these families should have alerted physicians to suspect LS.
- All gynecologists should be aware that the prevalence of LS among endometrial cancer patients is at least 2% and a careful family history should be obtained from these patients. In addition, relatives of uterine cancer patients are at increased risk of uterine cancer. Gynecologists should not hesitate to refer suspected cases for genetic counseling and investigation. Both gynecologists and clinical geneticists should work toward improving strategies for identification, follow-up and surveillance of individuals at increased risk for uterine cancer.
- We showed that germline *PTEN* mutations are rare in CS-like families with endometrial cancer.
- Screening for *PTEN* mutations among endometrial cancer patients with CS-like phenotype is expensive and has been routine procedure at oncogenetic clinics. We suggest that testing should be aimed only at patients who meet strict CS criteria.
- Gynecologists should also be aware of CS criteria and should apply them in daily clinical practice in order to identify and refer women with possible CS who present with endometrial cancer.
- Gynecologists should inform female LS patients about the advantages and disadvantages of prophylactic surgery and the importance of gynecological surveillance, as well as about early symptoms of gynecological cancer. Emphasis should be placed on the risk of cancer and average age at onset.

- We suggest that screening should begin at age 30-35 and include TVUS and probably EB to improve diagnostic accuracy. Prophylactic surgery should be recommended after childbearing at a suitable age.
- All gynecologists should be regularly updated about current national recommendations for screening, treatment and follow-up of female LS patients.

7 FUTURE PERSPECTIVES

- Our center may also conduct a large LS prevalence study in the future using both IHC and MSI to examine all endometrial cancer tumors. Such a study may also include tumors from other cancer centers in Sweden. Possibly it could reveal that the prevalence of LS in consecutive endometrial cancer patients may be higher than expected, as we postulated in our first study. A future study could also assess whether screening for LS by IHC or clinical criteria among endometrial cancer patients is cost-effective.
- A future study might appropriately examine family history of cancer and the prevalence of hereditary cancer syndromes such as LS and HBOC in an unselected group of ovarian cancer patients using the same approach and methodology as described in paper I.
- In the future, larger population-based studies covering a longer period and with more patients enrolled could be used to evaluate the possible impact of environmental and lifestyle factors (other than obesity) on the development of endometrial cancer.
- One focus for current and future studies is the identification of possible low-risk genes that may explain familial predisposition for uterine cancer. Genome-wide association studies are underway looking for possible disease-causing loci/genes that may play a role in endometrial carcinogenesis. Such studies could shed sufficient light on the biological pathways leading to EC to improve future screening for at-risk patients and enable formulation of targeted treatments. Our research group RENDOCAS is participating in such research through collaboration with other international groups.
- Future studies involving greater numbers of CS and CS-like patients may focus on the analysis of other genes, such as *SDHx*, *PIK3CA*, *AKT1* and *KILLIN*, as well as the promoter region of the *PTEN* gene, when no germline *PTEN* mutations are present in order to investigate the prevalence of other mutations in such patients.

- Large population-based studies covering a long time span and having access to cancer registries may help evaluate the effect of surveillance and prophylactic surgery, especially regarding morbidity and mortality over time for women with LS.
- Further studies are needed to assess anxiety in female patients who have been informed that they have LS, especially since attending a surveillance program may cause significant distress. Other studies might address various psychological issues, including anxiety and distress due to awareness of the increased lifetime risk for developing cancer among LS patients. Furthermore, it would be important to ascertain whether LS affects the decision to have children and whether women with LS would plan to complete childbearing earlier than other women in order to have prophylactic surgery.
- Future studies should also address other issues such as clinical application of tests for LS and Cowden syndrome in a gynecological and oncogenetic setting, as well as cost-effectiveness and usefulness to gynecologists and/or clinical geneticists.
- Long-term follow-up studies could also determine whether to offer preimplantation genetic diagnostics (PGD) and/or prenatal testing to female LS patients and determine what proportion of patients would avail themselves of this option and the outcomes. It would also be valuable to determine how much anxiety and concern female carriers experience regarding the well-being of their offspring, especially if neither PGD nor prenatal screening have been conducted.

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**“IT IS NEVER TOO LATE TO BE WHAT YOU MIGHT HAVE BEEN.”
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“Ithaka

As you set out for Ithaka
hope the voyage is a long one,
full of adventure, full of discovery.
Laistrygonians and Cyclops,
angry Poseidon—don’t be afraid of them:
you’ll never find things like that on your
way
as long as you keep your thoughts raised
high,
as long as a rare excitement
stirs your spirit and your body.
Laistrygonians and Cyclops,
wild Poseidon—you won’t encounter them
unless you bring them along inside your
soul,
unless your soul sets them up in front of
you.

Hope the voyage is a long one.
May there be many a summer morning
when,
with what pleasure, what joy,
you come into harbors seen for the first
time;
may you stop at Phoenician trading stations
to buy fine things,
mother of pearl and coral, amber and
ebony,
sensual perfume of every kind—
as many sensual perfumes as you can;
and may you visit many Egyptian cities
to gather stores of knowledge from their
scholars.

Keep Ithaka always in your mind.
Arriving there is what you are destined for.
But do not hurry the journey at all.
Better if it lasts for years,
so you are old by the time you reach the

Ιθάκη

“Σα βγεις στον πηγαιμό για την Ιθάκη,
να εύχεσαι νάναι μακρύς ο δρόμος,
γεμάτος περιπέτειες, γεμάτος γνώσεις.
Τους Λαιστρυγόνες και τους Κύκλωπας,
τον θυμωμένο Ποσειδώνα μη φοβάσαι,
τέτοια στον δρόμο σου ποτέ σου δεν θα
βρεις,
αν μόν’ η σκέψις σου υψηλή, αν εκλεκτή
συγκίνησις το πνεύμα και το σώμα σου
αγγίζει.
Τους Λαιστρυγόνες και τους Κύκλωπας,
τον άγριο Ποσειδώνα δεν θα συναντήσεις,
αν δεν τους κουβανείς μες στην ψυχή σου,
αν η ψυχή σου δεν τους στήνει εμπρός
σου.

Να εύχεσαι νάναι μακρύς ο δρόμος.
Πολλά τα καλοκαιρινά πρωιά να είναι
που με τι ευχαρίστησι, με τι χαρά
θα μπαίνεις σε λιμένας πρωτοειδωμένους·
να σταματήσεις σ’ εμπορεία Φοινικικά,
και τες καλές πραγμάτειες ν’ αποκτήσεις,
σεντέφια και κοράλλια, κεχρμπάρια κ’
έβενους,
και ηδονικά μυρωδικά κάθε λογής,
όσο μπορείς πιο άφθονα ηδονικά
μυρωδικά·
σε πόλεις Αιγυπτιακές πολλές να πας,
να μάθεις και να μάθεις απ’ τους
σπουδασμένους.

Πάντα στον νου σου νάχεις την Ιθάκη.
Το φθάσιμον εκεί είν’ ο προορισμός σου.
Αλλά μη βιάζεις το ταξίδι διόλου.
Καλλίτερα χρόνια πολλά να διαρκέσει·
και γέρος πια ν’ αράξεις στο νησί,

island,
wealthy with all you have gained on the
way,
not expecting Ithaka to make you rich.

Ithaka gave you the marvelous journey.
Without her you would not have set out.
She has nothing left to give you now.

And if you find her poor, Ithaka won't
have fooled you.
Wise as you will have become, so full of
experience,
you will have understood by then what
these Ithakas mean.”
— C.P. Cavafy, *Collected Poems*, 1911

πλούσιος με όσα κέρδισες στον δρόμο,
μη προσδοκώντας πλούτη να σε δώσει η
Ιθάκη.

Η Ιθάκη σ' έδωσε τ' ωραίο ταξίδι.
Χωρίς αυτήν δεν θάβγαινες στον δρόμο.
Άλλα δεν έχει να σε δώσει πια.

Κι αν πτωχική την βρεις, η Ιθάκη δεν σε
γέλασε.

Έτσι σοφός που έγινες, με τόση πείρα,
ήδη θα το κατάλαβες η Ιθάκες τι
σημαίνουν.”

— C.P. Cavafy, 1911