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HEREDITARY, MOLECULAR AND PROGNOSTIC FACTORS IN ENDOMETRIAL CANCER

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Hereditary, molecular and prognostic factors in Endometrial Cancer

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By

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“Live as if you were to die tomorrow.

Learn as if you were to live forever”

Mahatma Gandhi

To my amazing family

ABSTRACT

Aims: The overall aim of this thesis is to describe and evaluate hereditary uterine cancer syndrome both independent of and in relation to hereditary patterns of Lynch and/or Cowden syndrome for the purpose of recognizing and defining a unique pattern of familial uterine cancer.

The thesis also aims to determine whether the *TERT-CLPTM1L* region is a novel risk locus for endometrial cancer.

Finally, we aim to assess expression of two mitochondrial proteins, PGC1α and VDAC1, within the same population, as well as to consider the impact of different dietary and lifestyle factors on prognosis for endometrial cancer (EC).

Materials and methods: All studies are retrospective and based on a cohort population of women who were diagnosed with endometrial cancer and underwent surgery at Karolinska Hospital, Stockholm, Sweden, between January 1, 2008 and March 31, 2012.

All women who agreed to participate completed questionnaires, one concerning family history of cancer and the other regarding personal health history including comorbidity, parity, medications, lifestyle and dietary habits. Data on clinicopathological variables were obtained from the Take Care medical records system.

In study I we constructed pedigrees based on the questionnaires with verification by telephone interview, assessed the relative frequencies of various cancers among family members and compared those with the general Swedish population in 1970 and 2010.

Study II entails a collaborative effort, known as the Endometrial Cancer Association Consortium (ECAC), in which 5591 women of European ancestry with a history of histologically verified diagnosis of endometrial cancer were enrolled into 11 separate studies conducted in Western Europe, North America, and Australia. The Swedish contribution to this multicenter study derives from the Registry of Endometrial Cancer in Sweden (RENDOCAS), which is a hospital-based registry of consecutively occurring EC cases. The study included 262 cases of EC taken from the same group as Study I and enrollment continued until 2011. In addition to the above information, DNA samples for each patient were obtained from peripheral blood.

In **Study III,** both tumor and adjacent benign tissue were analyzed using immunohistochemistry and subsequently scored to assess PGC1α and VDAC1 expression in

both types of tissue and to correlate these findings with clinical data.

In **Study IV** a smart machine learning model, Random Survival Forest, was used to analyze the extensive data and correlate them with prognosis for endometrial cancer.

Results: In **Study I** we found an increased prevalence of EC among our study population compared with the general population in Sweden in 1970 and 2010. Lynch syndrome, as defined according to the Amsterdam II criteria, was found in 7 families. In all, 13% of index patients had at least one relative with EC and these families showed a tendency for more cases of early onset cancer among family members.

In **study II,** data concerning single nucleotide polymorphisms **(**SNPs) taken from the 5p15 region were available from 4401 cases and 28 758 controls. Using logistic regression, we found three imputed SNPs (rs7705526 (in SNP set 1), rs13174814 (SNP set 2) and rs62329728 (SNP set 3)) that each showed evidence of being independently associated with disease for which the respective ORs were 1.11, 0.87 and 1.27 by unconditional analysis. The linkage disequilibrium (LD) between these three SNPs is weak, which further suggests that they represent independent risk factors for endometrial cancer. When comparing with data taken from the cancer genome atlas (TCGA), we were able to identify higher expression of TERT-CLPTM1L RNA in EC tissue than found in normal tissue.

Study III found that both PGC1α and VDAC1 showed significantly lower expression in tumor tissue compared with benign tissue. We also observed a correlation trend indicating an association between low PGC1a expression and shorter time to death among patients in the FIGO I group.

The **study IV** analysis revealed that consumption of fried potatoes and carbonated soft drinks is higher among women with recurrent endometrial cancer and death.

Conclusions: Our study found an overrepresentation of EC among first- and second-degree relatives, as well as first cousins of endometrial cancer patients, when compared with the general population. Young age of onset and occurrence of multiple cancers in families with EC suggest the presence of additional factors relating to hereditary EC syndrome. We emphasize the importance of accurate diagnosis, with referral for genetic counseling, and improved surveillance of individuals at high risk for EC.

In **study II** we succeeded in uncovering a novel risk locus for EC and implicated three novel independent genetic variants within the 5p15 locus (already associated with several cancers) that increase risk of developing EC. Overexpression of TERT in cases of EC when compared with normal tissue suggests a potentially important role for this gene in tumorigenesis. Our findings may account for about 0.5% of the relative risk for familial EC.

Study III found downregulation of both PGC1α and VDAC1 in malignant tissue, as well as a correlation between low PGC1a expression and shorter time to death among patients in the FIGO I group. This correlation has important clinical implications since these patients are treated exclusively by surgery. Should lower expression of PGC1a correlate with increased risk of recurrence, new therapeutic strategies may be required.

In **study IV** we observed that consumption of fried potatoes and sweetened carbonated beverages is associated with higher risk of recurrence and death from EC. Dietary modification may therefore be advisable for women with endometrial cancer.

LIST OF SCIENTIFIC PAPERS

I. Tzortzatos G, **Wersäll OC**, Gemzell DK, Lindblom A, Tham E, Mints M.
Familial cancer among consecutive uterine cancer patients in Sweden
Hereditary Cancer in Clinical Practice 2014, 12:14

II. Carvajal-Carmona LG, O'Mara TA, Painter JN, Lose FA, Dennis J, Michailidou K, Tyrer JP, Ahmed S, Ferguson K, Healey CS, Pooley K, Beesley J, Cheng T, Jones A, Howarth K, Martin L, Gorman M, Hodgson S; National Study of Endometrial Cancer Genetics Group (NSECG); Australian National Endometrial Cancer Study Group (ANECS), Wentzensen N, 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, Scott RJ, Ashton K, Proietto T, Otton G, **Wersäll O,** 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; 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, Pharoah PD, Dunning AM, Tomlinson I, Easton DF, Spurdle AB, Thompson DJ.
Candidate locus analysis of the TERT-CLPTM1L cancer risk region on chromosome 5p15 identifies multiple independent variants associated with endometrial cancer risk
Human genetics, 2015;134:231-245

III. **Wersäll OC**, Löfstedt L, Govorov I, Mints M, Gabrielson, M, Shoshan M.
PGC1 and VDAC1 expression in endometrial cancer
Molecular and clinical oncology, 2021; 14:42.

IV. **Wersäll OC**, Razumova Z, Govorov I, Mints M
Dietary habits and daily routines as the prognostic factors in endometrial cancer.
Submitted to Nutrition and Cancer

CONTENTS

LIST OF ABBREVIATIONS

AML	Admixture Maximum Likelihood
Bcl-2	B-cell lymphoma -2 family
BMI	Body Mass Index
CGRAN	Cancer Genome Atlas Research Network
CI	Confidence Interval
COGS	The Collaborative Oncological Gene–environment Study
CRC	Colorectal Cancer
CTNB1	Catenin Beta 1
DM	Diabetes Mellitus
EBRT	External Beam Radiotherapy
EC	Endometrial Cancer
ECAC	Endometrial Cancer Association Consortium
EEC	Endometrioid Carcinoma
ESGO	European Society of Gynecological Oncology
ESMO	European Society of Medical Oncology
ESTRO	European Society for Radiotherapy & Oncology
FDA	Food and Drug Administration
FDR	First-Degree Relatives
FIGO	International Federation of Gynecology and Obstetrics
GAPDH	Glyceraldehyde- 3-phosphate dehydrogenase
GI	Glycemic Index
GL	Glycemic Load
GWAS	Genome wide association study
HBOC	Hereditary Breast and Ovarian Cancer
HER2	Human Epidermal Growth Factor Receptor 2
HIR	High Risk
HK	Hexokinase
HNPCC	Hereditary Nonpolyposis Colorectal Cancer
HR	Hazard Ratio
iCOGS	Illumina iSelect High-Density Genotyping Array
IGF1	Insulin-like Growth Factor 1
IHC	Immunohistochemistry
KRAS	Kirstin Rat Sarcoma Viral Oncogene Homolog
LMH1	mutL Homolog 1
LS	Lynch syndrome
LVSI	Lymphovascular Space Invasion
MET	Metabolic Equivalent

MHT	Menopausal Hormone Therapy
MMR	Mismatch Repair Proteins.
MSI	Microsatellite Instability
mtDNA	mitochondrial DNA
N	Normal
NCCN	National Comprehensive Cancer Network
NEEC	Non-Endometroid Carcinoma
OR	Odds Ratio
OS	Overall Survival
OXOPHOS	Oxidative Phosphorylation
PCOS	Polycystic Ovarian Syndrome
PFS	Progression-Free Survival
PGC1α	Peroxisome proliferator-activated receptor γ coactivator 1
PH	Proportional Hazards
PPARγ	Peroxisome proliferator-activated receptor γ
ProMise	Proactive Molecular Risk Classifier for Endometrial Cancer
PRS	Polygenic Risk Score
PTEN	Phosphatase and Tensin Homolog
RCT	Randomized Controlled Trial
RENDOCAS	Registry of Endometrial Cancer in Sweden
RR	Relative Risk
RSF	Random Survival Forest
SCNA	Somatic Number Alteration
SHBG	Sex Hormone Binding Globulin
SNP	Single Nucleotide Polymorphism
SSB	Sugar-Sweetened Beverages
T	Tumour
TCGA	The Cancer Genome Atlas
TFAM	mitochondrial Transcription Factor A
VBT	Vaginal Brachytherapy
VDAC1	Voltage-Dependent Anion Channel Type 1
VIMP	Variable Importance

1. INTRODUCTION

1.1 INCIDENCE AND MORTALITY RATE

1.1.1 Incidence

Endometrial cancer (EC) is a predominant gynecological malignancy in the western world [1], which has gradually increased in incidence over recent decades while age of onset has decreased [2]. EC is driven by a variety of factors, including abnormal genetic and epigenetic alterations, as well as environment. Obesity, diabetes, hypertension and lack of physical activity have been well-studied as risk factors and continue to pose a challenge in western countries where their prevalence is increasing. The annual incidence in developed countries is between 19 and 25 cases per 100 000, while in developing countries, the incidence is much lower at 1 to 4 cases per 100 000 [3]. In 2020, 1467 women were diagnosed with EC in Sweden [3].

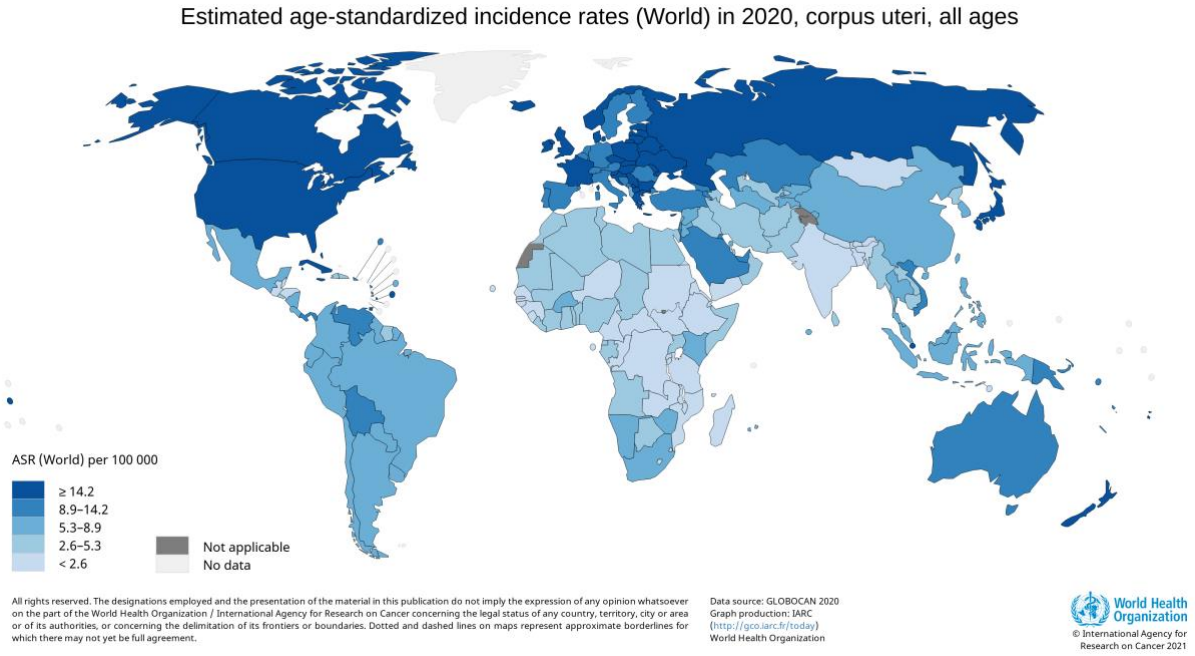

Figure 1. *Estimated age-standardized Incidence rates of endometrial cancer in 2020. [4]. Global Cancer Observatory: Cancer Today. Lyon, France: International Agency for Research on Cancer. Available from: https://gco.iarc.fr/today, accessed [30 Mars 2021]*

1.1.2 Mortality rate

The risk of developing EC increases with age; more than 90% of cases presenting in peri- and postmenopausal women, with peak incidence between the ages of 50 and 60[5]. EC is often diagnosed at an early stage, since symptoms such as abnormal vaginal bleeding present early, for which reason there is often a favorable prognosis with an overall 5-year survival rate greater than 80% [1]. In 2020, EC claimed the lives of 345 women in Sweden, making it the eighth leading cause of cancer deaths in the country and the second leading cause of death from gynecological cancer (after ovarian cancer)[3].

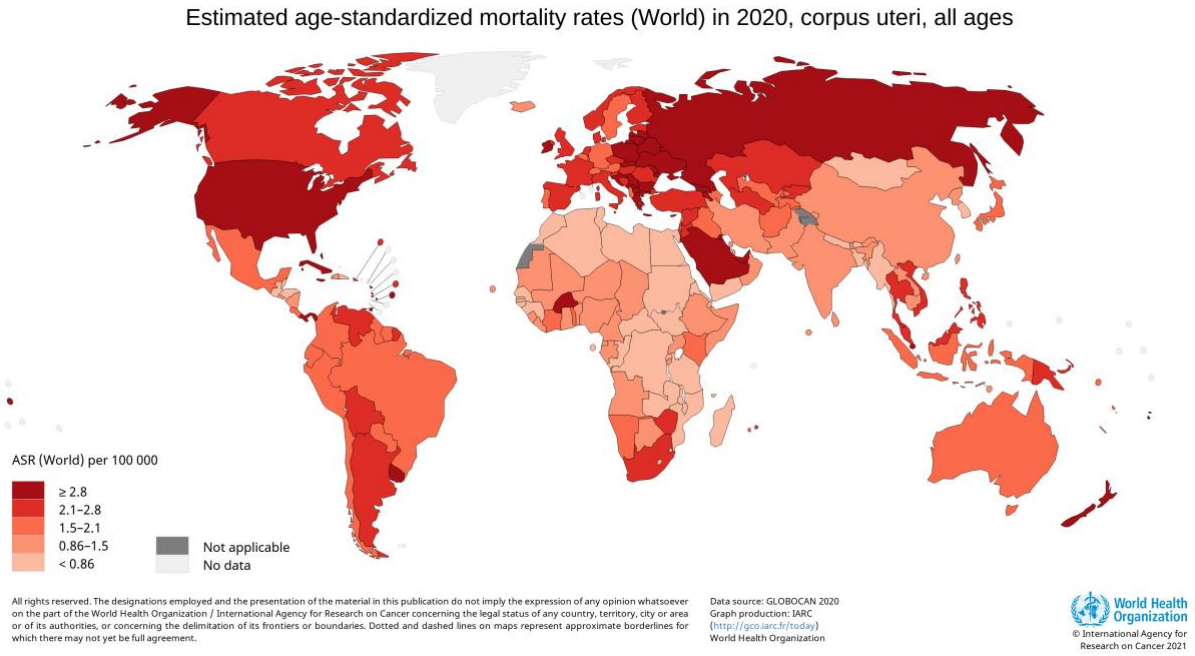

Figure 2. *Estimated age-standardized Mortality rates of endometrial cancer in 2020[4].*
Global Cancer Observatory: Cancer Today. Lyon, France: International Agency for Research on Cancer. Available from: https://gco.iarc.fr/today, accessed [30 Mars 2021]

1.2 ETIOLOGY

EC arises from the endometrial layer that lines the uterine cavity and is traditionally classified into two subtypes based on clinical, pathological, and molecular criteria: Type I, endometrioid, and Type II, non-endometrioid. EC must be distinguished from uterine sarcoma, which most commonly arises from either connective tissue or from the muscle layer [6]. Type I accounts for about 80% of all EC cases.

1.2.1 Classification

Type I is hormone-sensitive, correlates with high levels of unopposed estrogen, and most commonly affects postmenopausal women. Type II, in distinction to Type I, is not hormone sensitive and includes diagnoses such as serous carcinoma, carcinosarcoma (extremely poorly differentiated carcinomas), clear cell cancers, and others [1]. Type I tumors are endometrioid carcinomas (EECs) that bear a morphological resemblance to normal endometrial tissue when well differentiated and are often associated with or follow endometrial hyperplasia. Mucinous adenocarcinoma, classified as a Type I endometrial lesion, is quite rare (<5%). Tumor growth is usually confined to the uterus, the histological grade is generally low, and cure is usually achieved through hysterectomy. In contrast, Type II endometrial carcinomas tend to occur among postmenopausal women with atrophic non-neoplastic endometrium. These generally high-grade serous or clear cell carcinomas are referred to as non-endometrioid carcinomas (NEECs). They are non-estrogen dependent, and etiologically are believed to originate from lesions referred to as intraepithelial carcinoma. Clinically, NEECs often demonstrate aggressive behavior.

Molecular data also help to distinguish between the subtypes. Typically, the predominant genetic mutations associated with type I etiology include *PTEN* (phosphatase and tensin homolog), *PIK3CA KRAS2, CTNB1* (b-catenin-gene), and *LMH1* genes, as well as microsatellite instability. In contrast, Type II EC is characterized by p53 mutations and HER2/neu [7, 8].

Furthermore, the genetic alterations that distinguish EECs from NEECs differ from one another [5]. The Cancer Genome Atlas (TCGA) Research Network used array and sequencing-based technologies to characterize the genomic changes typifying EC by exploring the integrated genomic, transcriptomic, and proteomic characteristics in 373 cases of endometrial carcinoma. EC can be grouped into four categories based on combinations of somatic nucleotide substitutions, microsatellite instability (MSI), and somatic copy number alterations (SCNAs), figure 3: 1) *POLE* ultra-mutated, 2) microsatellite instability, hypermutated 3) copy-number low, and 4) copy-number high. Certain genomic features have been identified that are common to serous EC, ovarian serous carcinoma and basal-like breast carcinoma[9].

Most low-grade (FIGO grades 1 and 2) EECs can be mapped to the copy number low and MSI-H categories, while Grade 3 EEC can be found in any category. EEC is generally associated with the endometrioid genomic profile, with presence or lack thereof of the *TP53* mutation or high copy number alterations. In contrast, serous carcinomas are characterized by *TP53* mutations as well as high copy number alterations [10].

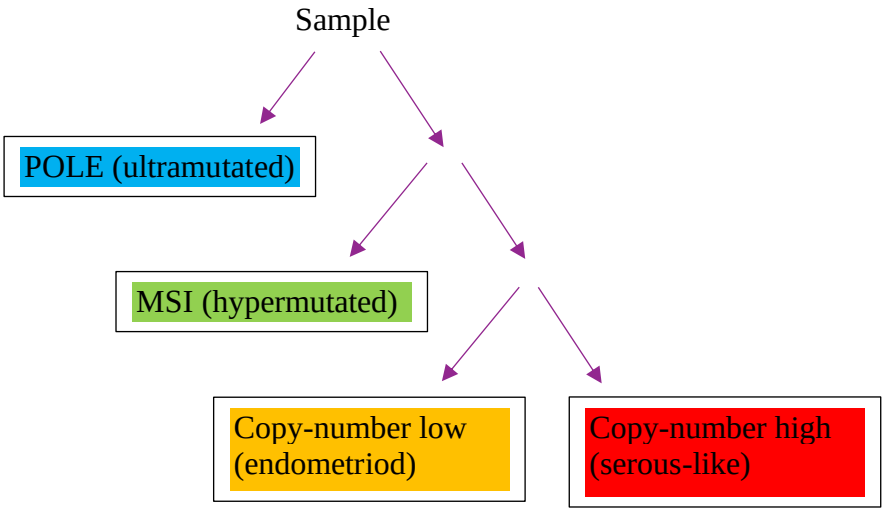

Figure 3. *The genomic classification according to TCGA. Figure reproduced with permission*

Moreover, the proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE) further developed the TCGA classification system to facilitate its application. Under this system, EC cases are divided into four subgroups based on a combination of protein expression and mutations [11-13].

As follows. First, immunohistochemistry (IHC) analysis is applied to investigate occurrence of the mismatch repair proteins PMS2 and MSH6. The next step is to evaluate potential POLE mutations, and finally assessment for aberrant expression of p53 using IHC to yield p53abnormal and p53wild type.

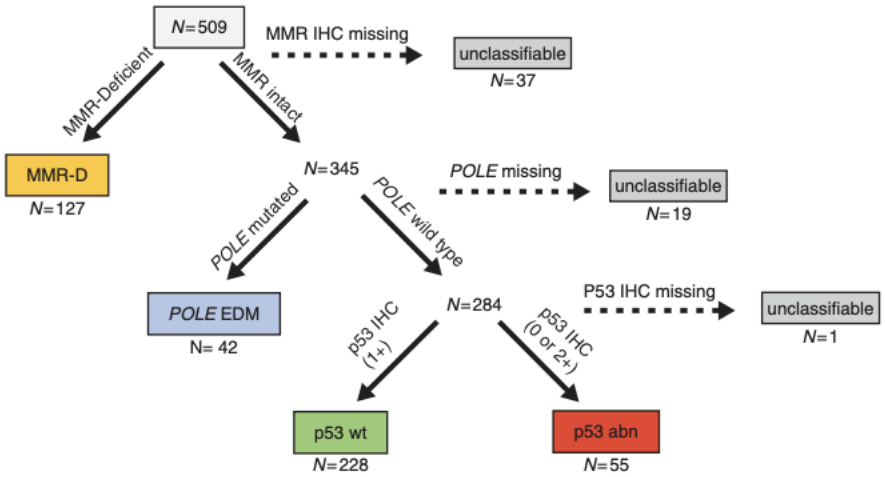

Figure 4. *ProMisE classification [13]. The ProMisE algorithm. Figure reproduced with permission*

1.3 RISK FACTORS

Risk factors associated with EC, especially of the endometrioid type, have been well defined and involve identifiable endogenous or exogenous sources of unopposed excess estrogen.

The endometrial tissue undergoes a number of dynamic changes over the course of a woman's lifetime. Because estrogen is a steroid hormone, it stimulates differentiation and decidualization, thereby promoting endometrial cell growth through both genomic and non-genomic mechanisms. The process entails binding to the estrogen receptor, which modulates transcription of a variety of proteins. In addition, estrogen may indirectly activate various pathways through non-genomic processes [14]. Moreover, the ovaries are the primary estrogen producers in premenopausal women, while later in life, peripheral tissues convert androgens to estrone and estradiol under the influence of the aromatase enzyme [15].

1.3.1 Overweight and obesity

Endometrial cancer patients typically have a clinical profile that includes high body mass index (BMI), generally defined as overweight (BMI 25–30) or obese (BMI 30), and often have signs of metabolic syndrome, including hypertension and diabetes. Globally, overweight and obesity continue to increase, both in developed and undeveloped countries. In the US, for example, by 2010 no state had a prevalence under 20%, with most hovering around 30% [16]. High BMI is usually associated with favorable prognostic indicators of EC, including low histological grade, endometrioid histology, and a tendency for identification at an early stage. Women with metabolic syndrome are at a 1.89 relative risk (RR) for developing endometrial cancer [1]. One population-based case-control study of 668 patients examined the association between longtime overweight and endometrial cancer and found that women with chronic OW/obesity were at almost five times greater risk of developing EC.

The presence of such risk factors may also correlate with earlier onset of disease [17].

1.3.2. Diabetes mellitus

Type II diabetes mellitus (DM) has long been considered to be an independent risk factor, with an approximate RR of 2.0. Because obesity is common with type II DM, it may represent a confounding risk factor, raising the question of the independent role of DM [1].

Because of the high correlation between obesity and insulin resistance, many studies have focused on investigating the role of DM as an independent risk factor. One comprehensive meta-analysis of 29 studies consistently found a nearly twofold increase in risk of endometrial cancer in women with DM. The potential benefits of metformin have also been well studied

and while one meta-analysis indicated a reversible effect on hyperplasia, others showed no such benefits, though all demonstrated improved overall survival (OS) and a reduction in risk of recurrence [18, 19].

1.3.3 Unopposed estrogen

Hyperestrogenism is also related to other identified risk factors, such as unopposed estrogen therapy (especially with duration of treatment longer than five years), as well as to estrogen-producing tumors, such as ovarian granulosa cell tumors and theca cell tumors, each of which may increase risk of developing EC by as much as 20%. Early onset menarche (age <12 years) and late menopause (age >55 years) may double the risk for occurrence of EC [1].

Nulliparity is also a recognized risk factor. In contrast, among women who have given birth, data suggest a 40% reduction in risk for EC [20]. Polycystic ovarian syndrome (PCOS) is often associated with chronic anovulation and possible infertility, once again raising the issue of unopposed estrogen, since it is associated with a nearly threefold increased risk of EC (OR 2.79–2.89)[21]. However, as in the case of diabetes, obesity may once again be a confounding factor [1].

Hormone replacement therapy (HRT) to treat menopause is one source of exogenous estrogen among postmenopausal women. Unopposed estrogen has shown a correlation with increased risk of EC even when taken for less than five years [22]. Estradiol stimulation of endometrial proliferation is both time- and dose-related, as shown by many studies [23], whereas use of continuous-combined (cc) HRT with synthetic progestins reduces this risk, as shown in a systematic review of 31 studies [24]. However, long-term use (>5 years) of estrogen combined with micronized progesterone increases the absolute risk of developing EC [25, 26]. Nevertheless, a more recent systematic review demonstrates a significant protective effect from combined therapy when a more appropriate dose regimen is used [27] In summary, all studies show a correlation between EC and HRT, where type of progesterone (synthetic or micronized), dosage and regimen each may play a pivotal role.

1.3.4 Other risk factors

Adult-attained height: this has been implicated by several studies as a risk factor for EC. A direct biological explanation is unlikely, for which reason the genetic and environmental factors that affect female growth are likely linked to the risk of developing EC [20].

Ethnicity: black women of non-Hispanic are at significantly higher risk for developing and dying from EC than non-Hispanic white women, RR 1.55 (95% CI 1.5-1.61) [28].

Genetics: A familial association with EC has been found in about 5% of cases, though the remaining 95% are essentially sporadic. Among younger women, genetic predisposition is the most significant risk factor. Lynch syndrome as a monogenic cause of EC has been extensively studied [17]. However, other genetic risk factors related to inheritance, including obesity and high BMI, are common issues that impact risk for developing EC [29].

Smoking: A variety of studies have shown that smoking reduces risk of EC, likely due to antiestrogenic properties. The risk of developing EC is lower among both current and former smokers than among never smokers [20].

Physical activity: Physical activity has demonstrated an inverse association with risk of developing EC [20, 30], while also improving survival among those who develop this diagnosis, thereby underscoring both a preventive and therapeutic role [31, 32].

Hypertension – Even after adjusting for confounders such as BMI, smoking, oral contraception and parity, hypertension appears to be associated with a risk of EC. Although the exact pathological mechanism has not been defined, the suggestion is that chronic hypertension inhibits apoptosis and promotes cellular aging [20].

1.4 TUMOUR CHARACTERISTICS

EC generally presents early with abnormal uterine bleeding, facilitating diagnosis at an early stage, at which time surgical treatment alone is usually curative [33]. Meticulous preoperative staging of early EEC, however, is necessary to avoid over or undertreatment with surgery.

1.4.1 Histopathology and grade

According to the WHO/International Society of Gynecological Pathology, EC may be histologically classified into one of two categories: adenocarcinoma or carcinosarcoma. Adenocarcinoma can be further subdivided into serous, mucinous, endometrioid, clear cell, and mixed tumors. Under the FIGO system, endometrioid carcinomas may be histologically classified using a three-tiered grading system based on degree of differentiation: well differentiated, moderately differentiated and poorly differentiated. On pathology, EC may manifest as a polypoid, plaque-like, or extensively infiltrating mass. EC may spread to involve the adnexa, parametrium, distal uterus, and the cervix. Histopathological grading of adenocarcinoma is important from a prognostic standpoint. The proportion of solid to glandular components can be used to describe three architectural grades [5].

- Grade 1 (G1): well-differentiated, less than 5% of nonsquamous, solid growth pattern.
- Grade 2 (G2): Moderately with 6-50% nonsquamou, solid growth pattern.
- Grade 3 (G3): Poorly differentiated, greater than 50% nonsquamous, solid growth pattern.
- Serous and clear cell tumours are always classified as grade 3, though this assessment does not address squamous epithelial differentiation.
-

1.4.2. FIGO staging and classification

In 2009, a surgical staging system for endometrial cancer was revised based on the earlier International Federation of Gynecology and Obstetrics (FIGO) system (table 1) [34]. Surgical staging is the most important factor for prognosis and serves as a guide for treatment. Stage I tumors are confined to the uterine corpus. The stage I A, B, and C subgroups are based on depth of myometrial invasion. Stage II tumors are defined by presence of endocervical gland infiltration, while stage III entails regional spread. Stage IV is defined by the presence of distant metastases such as bladder or rectal involvement. The combination of surgical staging and histological grading provides a comprehensive assessment, divided into low, moderate, and high-risk categories [35].

Table 1. FIGO staging system for EC, 2009 [35]

Stage I	Tumor confined to the corpus uteri
IA	No or less than half of the myometrium
IB	Invasion 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 and/or adnexa
IIIB	Vaginal and/or parametrial involvement
IIIC	Metastases to the pelvic and/or para-aortic lymph nodes
IIIC1	Positive pelvic 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

1.5 HEREDITARY SYNDROMES AND GENTICS

1.5.1 *Lynch syndrome*

Lynch syndrome (LS), previously known as Hereditary Nonpolyposis Colon Cancer (HNPCC), is a hereditary autosomal dominant disorder with a mendelian inheritance pattern; the disorder is also associated with a high risk of endometrial cancer. LS is one of the more common hereditary disorders that predisposes carriers to cancer [36], for which the underlying mechanism involves germline mutations in DNA mismatch repair genes (MMR). As far back as 1895, Warthin observed a familial cluster of cancer cases that were subsequently described by Henry Lynch in 1966 [37]. Two families had presented with many cases of similar carcinomas, with onset in early age, arousing suspicion of autosomal dominant inheritance. The prevalence of LS in the general population is about 0.35% [36].

From a genetic standpoint, four MMR genes (*MLH1, MSH2, MSH6*, or *PMS2*) are integrated into a system that is responsible for recognizing and repairing bases that have been incorrectly inserted, deleted, or misincorporated, while also serving to repair DNA damage, situations that may arise during DNA replication and recombination [38]. DNA repair is essential for genome stability, and mutations in MMR genes lead to increased somatic mutations and microsatellite instability. Microsatellites refer to areas of specific sequentially repeating non-coding DNA motifs within the genome. Multiple repeats may occur, and microsatellite replication errors may arise. Defective MMR genes may induce accumulation of microsatellite replication errors in tumors, referred to as microsatellite instability (MSI) [39].

LS is associated with many types of cancer that occur early in life (<50 years) and carries with it a 46% lifetime risk for developing colorectal cancer, while a 51% risk of endometrial cancer has been found. Concerning ovarian cancer, lifetime risk is also high at 15% [40, 41]. The first malignancy to present in patients with LS is often EC [42]. Other cancers for which LS carriers are at greater risk include brain, gastric, urethral, renal (pelvis), small intestine, and biliary tract cancers. About 2% of all EC cases are associated with LS, but unlike sporadically occurring EC, LS carriers are generally diagnosed at an earlier stage. The risk among the general population of developing early onset EC (<50 years) is 4%-18% [40], while 9% of all women diagnosed with EC before age 50 have LS [43], which creates a challenge when considering cancer prevention and surveillance programs for women of childbearing age.

1.5.1.1 Diagnosis and screening methods for LS

A diagnosis of LS is made according to the revised Amsterdam II clinical criteria and revised Bethesda Criteria [39].

Amsterdam II Criteria [44]:

- At least three relatives with any Lynch syndrome-associated cancer – colorectal (CRC), EC, intestinal, ureter or renal pelvis, etc.
- One should be a first-degree relative of the other two
- At least two successive generations should be affected
- At least one should be diagnosed before age 50
- Familial adenomatous polyposis should be excluded in CRC(s) cases
- Tumours should be verified by pathological examination

Revised Bethesda criteria [45]

- Individual with CRC diagnosed by age 50
- Individual with synchronous or metachronous CRC or other LS-associated tumours regardless of age.
- Individual with CRC and MSI-H histology diagnosed by age 60
- Individual with CRC and more than 1 FDR with an LS-associated tumour, with one cancer diagnosed by age 50
- Individual with CRC and more than 2 FDRs or SDRs with an LS-associated tumour, regardless of age

Patients must meet all revised Amsterdam II criteria to be eligible for germline genetic testing of MMR, but meeting just one Bethesda criterion entitles the patient to laboratory analysis for confirmation of MSI which, if positive, qualifies the patient for further DNA germline testing. Sensitivity for the Bethesda criteria can reach as high as 90%, but with a positive predictive value of only 3%-5%. The initial step in diagnosing LS is to confirm a characteristic mutation through tumor immunohistochemistry for MMR and then use PCR to test for MSI. The benefit of screening individuals for LS is realized through a reduction in the risk of any attendant cancer. For example, colonoscopy screening reduces CRC incidence and improves overall survival. Moreover, prophylactic hysterectomy and bilateral salpingo-oophorectomy may be indicated to prevent Lynch-associated EC and ovarian cancer [36].

More recently, immunotherapy has been used for treatment of MSI tumors, underscoring the importance of an accurate diagnosis [46].

1.5.1.2 Lynch syndrome and endometrial cancer

The risk of LS-associated EC is highly mutation-dependent. The highest lifetime risk of developing EC is thought to be associated with the MSH2 hand MSH6 with an incidence of about 50%, while MLH1 34% and PMS2 24% carry with them a 34% and 24% risk respectively. The age of onset is also reduced compared to the general population and is median on 49y [47].

As in sporadic EC, most LS-associated cases will be diagnosed at an early stage with well-differentiated histology. Nevertheless, some variation in types of EEC and NEEC have been noted among women with LS.

1.5.2 Screening of EC among LS patients

In 2020, the National Comprehensive Cancer Network (NCCN) published the following guidelines to screen for LS among women in the US presenting with EC [48]:

- EC is often diagnosed at an early stage thanks to early onset of symptoms, for which reason women should be encouraged to report abnormal vaginal bleeding or postmenopausal bleeding. Endometrial biopsy should be included in the workup.

- Hysterectomy may come into consideration as a risk-reducing option, but it has not been shown to decrease mortality though it does reduce incidence. Hysterectomy should be timed according to individual risk factors, including comorbidity, family history and specific mutations.

- Annual screening with endometrial biopsy is both a highly sensitive and highly specific diagnostic procedure, which should begin at age 30-35.

- Transvaginal ultrasound screening for endometrial cancer is not recommended for premenopausal women, but can be considered in postmenopausal women, while bearing in mind that the procedures is associated with low sensitivity and specificity in postmenopausal women.

1.5.3 Genetics and endometrial cancer

Endometrial cancer has multifactorial etiology, with an array of genetic and environmental factors that play a role in risk of developing the disease. Although Lynch Syndrome, as described above, is associated with a high risk for developing EC, it accounts for only about 2% of cases. Regardless of presence of LS, family history of EC significantly increases the risk of developing the disease [49, 50].

Regarding family history, a first degree relative with EC doubles the risk of developing this disease and risk is even higher if the relative was diagnosed at a young age [51].

Data regarding the importance of genetic factors in the etiology of this disease can be obtained by studying families, twins, and adopted individuals. In the case of monogenic disorders, linkage analysis can be used to test for various genetic markers across the entire genome; affected individuals in some families always inherit certain risk loci, whereas healthy individuals do not. Linkage analysis is reliant on ascertaining specific family structures and identifying high-risk genes, which imposes certain limitations. Association studies that search for population associations were designed to reveal disease susceptibility genes and are superior to the linkage analysis technique for detection of common or weak susceptibility alleles.

Genome-wide association studies (GWAS) are used to reveal associations between various single-nucleotide polymorphisms (SNPs) and specific diseases. Genetic variation in common SNPs occurs within the general population and GWAS can be used to compare them and associate them with various diseases [52]. Once an association is identified through such testing, detailed studies concerning the locus in question can be carried out to ascertain whether the SNPs in question are causally related to an increased risk of disease, or whether genetic variations in adjacent exons or genes in linkage disequilibrium may be responsible. Additional studies are usually carried out on other populations to confirm findings.

The GWAS technique has been successful in identifying genetic variants that are associated with a modest increase in risk for developing different common types of cancer, which suggests that some common variants may at least in part be responsible for an elevated risk of familial cancer [53].

In 2011, Spurdle et al. conducted a genome-wide association study that yielded convincing evidence for an association between EC and SNP rs4430796 near the *HNF1B* gene on chromosome 17q [53], later confirmed through a follow-up study focused on how altered *HNF1B* gene expression affects risk of developing EC [54].
Subsequently, additional GWAS studies have discovered an additional 16 genetic risk regions associated with EC. Additional post-GWAS analyses were able to confirm involvement of these genes and pathways in EC carcinogenesis [55-57].
Figure 5, which summarizes the current state of knowledge regarding hereditary risk, shows a model that illustrates the risk associated with various mutations, how common they are, and their influence on predisposing for disease.

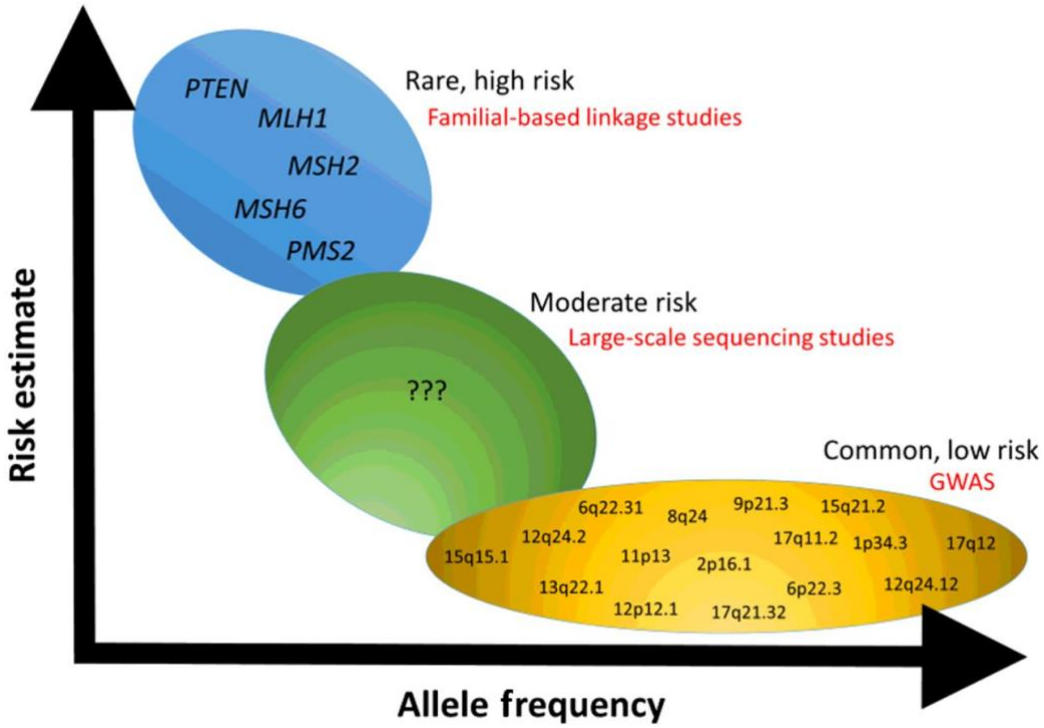

Figure 5. *Genetic model suggested for endometrial cancer[55]. The figure is reproduced with permission*

1.5.4 TERT-CLPTMIL - cancer risk region

The *TERT* and *CLPTM1L* genes are located on chromosome 5p15. *TERT* encodes the catalytic subunit of the enzyme telomerase reverse transcriptase, which is important to remember when considering that most cases of cancer in humans involve an increase in cellular proliferation associated with telomerase activity [58]. Telomerase permits the replication and proliferation of cancer cells in an uncontrolled way and can even enhance their ability to infiltrate tissue and metastasize to other organs[59]. *CLPTM1L*, though not as thoroughly studied, has been implicated as serving in an antiapoptotic role in pancreas and lung cancer [60, 61]. While many studies suggest an association between various cancers (i.e., brain, breast [62], lung[63, 64], prostate[65, 66]) and single-nucleotide polymorphisms (SNPs) in the *TERT-CLPTM1L* region on chromosome 5p15[67], such an association has yet to be discovered concerning endometrial cancer.

1.6 MOLECULAR FACTORS IN ENDOMETRIAL CANCER

Since the field of carcinogenesis remains largely enigmatic, much research remains to be done on the molecular mechanisms underlying endometrial carcinoma. It is known that the process of tumorigenesis entails modification of energy metabolism, as well as a number of other mechanisms. The "Hallmarks of Cancer" as set out by Hanahan and Weinberg [68] serve to explain and organize carcinogenesis. As such, they include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion, and metastasis as different traits that the tumor may acquire over time. Recent additions to the list include the capacity to reprogram energy metabolism and evade immune surveillance.

The genome instability associated with tumors enables them to acquire the properties listed above through mechanisms that activate mutations such as point mutations, deletions, and insertions/translocations, with potential impact on oncogenes like *KRAS*. Because of the genomic instability associated with malignancies, tumor suppressor genes such as *PTEN* may become inactivated, thereby undermining their caretaker role in genomic maintenance (DNA repair mechanisms, apoptosis, or cell senescence).

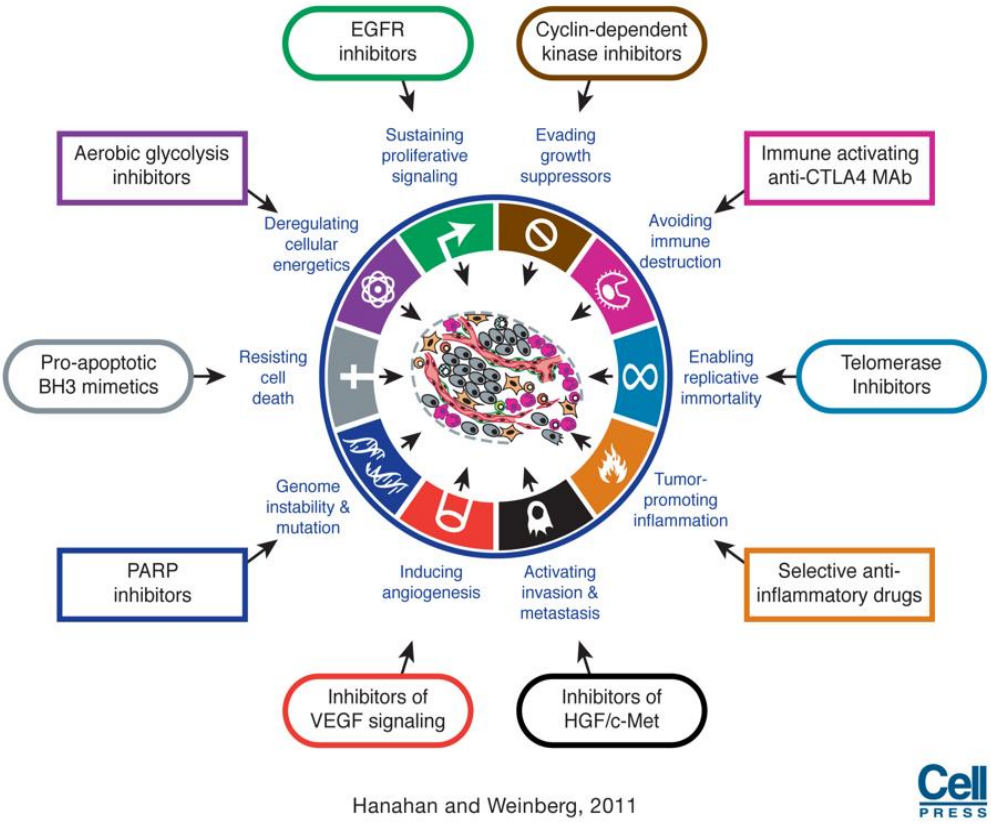

Figure 6. *Hallmarks of Cancer [68] The figure is reproduced with permission*

Molecular changes within cancer cell mitochondria may be impacted by such metabolic modifications, thereby resulting in retrograde signal pathways from mitochondria to nucleus [68]. Researchers have found ways to characterize different types of EC according to their various molecular alterations. A number of molecular characteristics present in EC have been identified, including DNA ploidy, hormone receptors, oncogenes, apoptosis-associated gene, cancer suppressor genes, and mismatch repair genes. Such markers have allowed more accurate sub-classification, which is helpful in models that predict risk, as well as for individualized therapy [9, 69-71]. In addition, molecular data help to classify EC into two subtypes. Type I EC is distinguished by diploidy, hormone sensitivity, and microsatellite instability. At the molecular level, Type I EC is associated with dysregulation of the *PI3K/PTEN/AKT* molecular pathway, loss of *PTEN* gene functionality, as well as mutation and hyperexpression of upstream tyrosine kinase growth factor receptors. Overall, these changes result in modified uncontrolled cell survival and proliferation. However, the Cancer Genome Atlas Research Network (CGARN) found that EEC can be microsatellite-stable with a causal relationship to *CTNNB1* mutation, or MSI with *MLH1* promoter methylation, including associations with *PTEN, TP53, KRAS*.

Type II tumors, on the other hand, are characterized by aneuploidy and distinguished by modification of the *CDK2A, TP53,* and *ERBB2* genes [72]. According to the CGARN, most serous and approximately 25% of grade 3 endometrioid tumors demonstrate high somatic copy number alteration (SCNA) and low mutation rates, which is consistent with aneuploidy. In addition to the *TP53* mutations, 22% showed mutations in *FBXW7* and *PPP2R1A [9]*. there was also loss of E-cadherin expression and overexpression of HER2 [73]. Downregulation of E-cadherin is of special importance in the complex EC invasion mechanism that involves a sophisticated chain of biological events, including various modifications in cell adhesion and motility. The CGARN classification system [9], revealed genomic characteristics shared in common among type I, type II, serous, and the grade 3 endometrioid designations of EC, thereby emphasizing the importance of gaining a deeper understanding of the different molecular mechanisms underlying EC. Finally, novel molecular marker(s) will need to be identified to improve characterization, classification and understanding of EC, thereby leading to the ultimate improvement of patient treatment and survival for those faced with this aggressive cancer.

1.6.1 Mitochondria

Mitochondria are of crucial importance to cell metabolism. Otto Warburg was awarded the Nobel Prize in Medicine and Physiology in 1931 for his work on cell respiration that described a glycolysis-dominated metabolic state characterized by lactate production, as is also the case in many cancers. He proposed the theory that such cancers may primarily be caused by dysfunctional metabolism, a theory ultimately known as "the Warburg effect." This idea has become accepted as one important characteristic of cancer, rather than being an exclusive feature [68]. The consumption of glucose by aerobic glycolysis despite the availability of oxygen is one important metabolic characteristic of tumors, in contrast to normal cells, which primarily depend on oxidative phosphorylation (OXPHOS) [68]. Studies have implicated both altered glycolytic and oxidative capacity with the shorter survival associated with ovarian carcinomas [74].

Research is currently underway concerning the mechanisms by which tumor cells reprogram various metabolic pathways to satisfy their unique bioenergetic requirements. In order to satisfy the large energy requirements needed for rapid proliferation of tumor cells they must maintain an available supply of biosynthetic precursor macromolecule building blocks.

1.6.1.1 Peroxisome proliferator-activated receptor γ (PPARγ) coactivator 1 (PGC1α)

The last decade has seen advances in identifying a group of cofactors relating to transcriptional factors, known as transcriptional coactivators. One such coactivator is called peroxisome proliferator-activated receptor-gamma (PPARγ) coactivator 1 (PGC 1α), which is crucial to the regulation of the complex mechanisms related to mitochondrial biogenesis and metabolic pathways, specifically in relation to cellular energy and homeostasis.

In the normal situation, PGC1α promotes expression of various nuclear genes to stimulate mitochondrial biogenesis, which prioritizes OXPHOS over glycolysis in cellular metabolism [75]. Various cancers, including breast, colon [75], and ovary [76], have been observed to have lower levels of PGC1α expression, though how level of PGC1α expression relates to tumor progression and resistance to chemotherapy is not yet well understood.

1.6.1.2 VDAC

Voltage-dependent anion channel type 1 (VDAC1) is a constitutional protein associated with the outer mitochondrial membrane, where it helps to regulate mitochondrial import and export of ions and metabolites, including ATP and NADH. The regulatory role of VDAC further extends to apoptosis involving interactions among different proteins and other factors, such as

hexokinase (HK), B-cell lymphoma-2 family (Bcl-2), and glyceraldehyde- 3-phosphate dehydrogenase (GAPDH) [77]. Although expression of VDAC in tumors is still insufficiently understood, especially concerning endometrial cancer, elevated VDAC levels relative to normal fibroblasts have been observed in a number of cancer cell lines. High VDAC gene expression has been correlated with poor outcomes in early-stage non-small cell lung cancer [78]. A seemingly incongruous observation is that lower expression of VDAC *in vitro* is associated with resistance to apoptosis, even cisplatin-mediated apoptosis [79-81].

1.6.1.3 TFAM

Mitochondrial transcription factor A (TFAM) helps to regulate the number of copies and structure of mitochondrial DNA (mtDNA), a process necessary for efficient transcription of mtDNA genes. In addition, TFAM helps to regulate mitochondrial biogenesis, a process indirectly regulated by PGC1 through its regulation of NRF1-2 [76, 82]. Decreased TFAM protein expression was discovered in a multiresistant epithelial ovarian carcinoma by Gabrielson et al. [76].

1.7 PROGNOSTIC FACTORS

Since it is known that endometrial cancer has a direct correlation with obesity and high BMI, it begs the question of whether dietary habits play a confounder role or act as individual risk factors. Because interactions between nutrients are often complex, the role of diet becomes difficult to assess with various concerns such as possible diet-related mediation of endogenous estrogen, which may affect risk of EC.

1.7.1 Dietary habits

A number of studies have aimed at finding correlations between dietary habits and EC, generally with inconsistent findings. The effect of nutrients concerning EC requires an understanding of how insulin and insulin-like growth factor (IGF)-1 influence pathogenesis in this disease. Cancer cells demonstrate overexpression of insulin receptors which, even in the presence of normal glucose levels, may alter the ability of such cells to obtain an adequate amount for growth [49]. Another mechanism involves insulin and IGF-1 activation of cancer cell proliferation indirectly through insulin receptor-mediated activation of MAPK and phosphatidylinositol-3 kinase.

A 2017 study subjected 27 different studies published between 1995 and 2015 to meta-analysis [83]. Authors could show that healthy dietary patterns, as understood by high intake of vegetables, fruits, low-fat dairy, olive oil, fish, soy, whole grains, and poultry, decreased the risk of EC when compared with a traditional western dietary pattern, as defined by high intake of red/processed meat, sweets, high-fat dairy products, potatoes, high-fat gravy, refined grains, and a lower intake of fruits and vegetables.

One case-control study from Australia examined dietary patterns consisting of foods that rank high on the glycemic index (GI) and/or foods associated with a high glycemic load (GL) [84]. The GI ranking of carbohydrate-containing food is determined based on 2-hour blood glucose level after consumption, indicating that insulin response correlates directly with GI ranking. In part, GL depends on the specifics of GI and yields a value reflecting the quantity and quality of carbohydrate contained by a specific food. Circulating blood insulin levels correlate directly with digestion of carbohydrates and can be evaluated using both GI and GL measurements [85]. The authors of this study report an association between intake of high GI foods and an increase in risk of EC.

1.7.1.1 Acrylamide and cancer risk

Acrylamide is an organic industrial chemical, a vinyl monomer that has been commercially available since 1950. The International Agency for Research on Cancer identified acrylamide as a "probable" carcinogen in 1994, based on animal experiments [86]. A 2002 study alerted to the presence of acrylamide in foods and how cooking technique affected its formation [87]. The association between acrylamide intake and gynecological cancer is thought to be due to alteration of sex hormone levels and attendant ability to increase estradiol [52]. A systemic review by Adani et al. found an association between acrylamide intake and increased risk of endometrial cancer[86].

1.7.1.2 Alcohol intake

Prior studies have addressed the association between alcohol and EC [88]. A large prospective study of 68 067 women aged 34-59 years conducted by Je.Y et al. [89] between 1980 and 2010 examined how long-term alcohol intake affects the risk of developing EC. Findings indicate that light alcohol consumption is inversely related to risk of developing EC, while a different study showed that women who consumed small amounts of alcohol were at lower risk, while high consumption increased risk [90].

1.7.2 Physical activity

Physical activity affects the risk of developing cancer and cancer progression through several mechanisms, including decreases in insulin levels, estrogen and various sex hormones, as well as by improving immune function, and decreasing obesity [91]. Various studies, including several meta-analyses, have been undertaken to explore the relationship between physical activity and risk and progression of EC. Results have been conflicting, with some studies supporting an inverse relationship between exercise and risk of EC, while others were unable to confirm this finding. Some of these studies have distinguished between different intensities of exercise, varying from regular intensive workouts to household activities [31]. According to some of these studies, when reduced physical activity occurs during menopause with an increase in BMI, the potential impact on sex hormone levels may contribute to an increased risk of EC [92]. Ultimately, the majority of these systemic reviews were unable to demonstrate benefit from regular exercise as a protective mechanism against EC [93-96].

1.8 RISK ASSESMENT

Over the past decade it has become routine to conduct a preoperative risk assessment, despite the lack of accepted definition for “low” or “high” risk relating to endometrial cancer. Risk assessment is divided into two sections: preoperative, to assess for risk of lymph node metastasis and plan for lymphadenectomy surgery if needed, and postoperative, to assess risk of recurrence and identify patients who could benefit from adjuvant therapy. The addition of such risk assessment may help to identify patients at high risk of recurrence and thereby improve outcome and overall survival should staging wrongly suggest a better prognosis. Various assessment systems have been developed over time; PROTEC 1, presented in Table 2, is one example [97].

Table 2. PROTEC1 Risk classification

Risk	EC type	FIGO stage	Grade	Myometrial invasion	Age
Low	EEC	Stage IA	G1		
Intermediate	EEC	Stage I	G1	≥50%	
Intermediate	EEC	Stage I	G2 or G3	<50%	
High Intermediate			G1-2	>50%	Age >60
High Intermediate			G3	<50%	Age >60
High	EEC	Stage III -IV			
High	Serous/ Clear cell	Any			

Another example is the COG-99 study[98] who divided patients according to the following classification:

Table 3. COG-99 risk classification

Risk	EC type	FIGO stage	Grade	Age	Risk factors *
Low	EEC	Stage Ia	G1-2		
Low-Intermediate				Age<50	< 2 risk factors
Low - Intermediate	EEC			50-69	< 1 risk factor
Low - Intermediate	EEC			>70	No risk factors
High Intermediate					3 risk factors
High Intermediate				50-59	>1 Risk factor
High	EEC	Stage III -IV			
High	Serous/ Clear cell	Any			

* G2 or G3, LVSI, Myometrial invasion >50%

Currently, one prominent risk classification system is ESMO [7], table 4. which takes into account factors such as FIGO staging, age, depth of myometrial invasion, tumor type, differentiation grade and lymphovascular space invasion (LVSI). Because lymphadenectomy is often followed by lifelong consequences that negatively impact quality of life among endometrial cancer survivors, the procedure should only be undertaken on selected patients. For this reason, the procedure is primarily limited to high-risk patients, but may be considered on patients at intermediate risk, in part to improve staging accuracy [1].

In Sweden, as for today, preoperative risk assessment encompasses two approaches based on the following prognostic risk factors: histological type, FIGO grade and DNA ploidy. About 25% of patients emerge as being at high risk. A designation of preoperative high risk is associated with the following: non-endometrioid type, Figo grade 3, deep myometrial invasion, or cervical stromal invasion suspected by ultrasound or MRI, as well as clinical suspicion of cervical stromal invasion. Preoperative low risk: none of the above.
However, in the future, the vision is that sentinel node biopsy will replace the pre-operative assessment.

The next step, as mentioned above, is to conduct postoperative risk assessments in order to select patients who are appropriate for adjuvant therapy in order to optimize their treatment to improve survival and reduce recurrence. The 2016 ESMP-ESGO-ESTRO consensus conference adopted this risk classification system for EEC [7].

Table 4. ESMO risk classification

Risk	Stage	Grade	Myometrial invasion	LVSI
Low	I	G1-2	<50%	negative
Intermediate	I	G1-2	≥50%	negative
High-Intermediate	I	G3	<50%	neg/pos
High-Intermediate	I	G1-2		positive
High	I	G3	≥50%	neg/pos
High	II-IV			
High	NEEC			

Factors associated with high risk of metastasis, recurrence and decreased survival include deep myometrial invasion (≥50%) in combination with poor histological differentiation [99, 100].

Table 5. proportion of patients with positive lymph nodes in pelvis area (% from Annual Swedish rapport)

Myometrial invasion	Grade 1	Grade 2	Grade 3
No invasion	1	7	16
<50%	2	6	10
≥50%	11	21	37

1.9 TREATMENT

1.9.1 Surgery

The gold standard of surgical treatment is hysterectomy and bilateral salpingo-oophorectomy, which can be carried out either through laparotomy or by minimally invasive surgery, depending on staging. When disease is diagnosed at an early stage, minimally invasive surgery and laparotomy are equally effective [101].

1.9.2 Lymphadenectomy

About 25% of EC patients demonstrate lymph node metastasis or lymphovascular space invasion (LVSI) [102]. Lymphadenectomy serves a therapeutic role and is also used for staging, though it should only be undertaken based on solid indications due to associated potential long-term complications [103]. The 2015 consensus conference [1] concluded that at least ten lymph nodes should be removed and that the procedure should include pelvic and para-aortic lymphadenectomy. The critical anatomy stretches from the inferior mesenteric artery up to the renal vessels [1]. The importance of recognizing sentinel lymph nodes has become generally accepted practice internationally over the past few years, including in Sweden. The presence of a positive sentinel lymph node or lack thereof can impact patient survival or prevent unnecessary lymphadenectomy. The accuracy of sentinel nodes in predicting metastasis has been well demonstrated. As such, a small number of patients preoperatively assessed to be at low risk will nevertheless prove to have a positive sentinel node, while other patients assessed as being at high risk will occasionally be sentinel node-negative, and thereby able to avoid lymphadenectomy and its potential complications [104, 105]. There is approximately a 3% risk that the sentinel node technique will miss metastatic disease, as shown through various studies[106].

1.9.3 Adjuvant therapy

Both risk group and risk assessment of recurrence determine whether adjuvant radiation therapy, chemotherapy, or a combination thereof is to be used. The choice is based on the classification system adopted at the 2016 ESMO-ESGO-ESTRO consensus conference [7]. The benefits of adjuvant therapy may include a positive impact on morbidity and improved overall survival among EC patients [107].

1.9.3.1 Radiotherapy

External beam radiation therapy (EBRT) benefits EC patients at intermediate risk through a reduction in risk of regional recurrence, though improved overall survival has not been demonstrated.

The PROTEC-2 study compared the effects of EBRT with vaginal brachytherapy (VBT) on patients of high to intermediate risk and evaluated long-term outcomes. They concluded that VBT should be the standard of care to treat high-risk (HR) endometrial cancer, while EBRT should be standard treatment in patients with unfavorable risk factors such as the p53 mutation and LVSI [108].

The currently ongoing PORTEC-4 trial is randomizing women with stage I-II EC and those otherwise at HR to be treated with either VBT or EBRT based on molecular profiling. Patients with unfavorable risk factors will be treated with ERBT, while those with favorable risk factors will receive VBT[108].

1.9.3.2 Chemotherapy

Some randomized controlled trials have demonstrated benefit from the addition of chemotherapy to ERBT among HR patients [109]. Chemotherapy has proven beneficial for treatment of early-stage EC disease among patients with unfavorable risk factors, as well as for those in more advanced stages [109]. One meta-analysis that focused on progression-free survival (PFS) compared one group of patients receiving a combination of ERBT and chemotherapy with a second group that did not receive adjuvant chemotherapy and found improved PFS in the combination therapy group (78% vs 69%, respectively, in the two groups ($p=0.009$))[110]. However, combination therapy for late-stage disease was not associated with either a lengthening of relapse-free survival or improved PFS [111].

2 AIMS OF THE THESIS

The overall aim of this thesis was to evaluate associations between a variety of risk factors – genetic and environmental – and endometrial cancer to ascertain how they influence prognosis, recurrence, and survival.
The specific aims were:

Study I

To evaluate the prevalence of familial uterine cancer in Stockholm, Sweden. To explore the existence of hereditary uterine cancer related to Lynch syndrome or Cowden syndrome. To investigate the presence of a possible new cancer syndrome in patients with uterine cancer and a family history of other cancers.

Study II

This association study is designed to determine whether the *TERT-CLPTM1L* region is a novel endometrial risk locus. The study represents a collaborative effort with other research groups worldwide.

Study III

To investigate expression of specific mitochondrial proteins such as the transcriptional coactivator Peroxisome proliferator-activated receptor gamma coactivator 1 (PGC1α) and the Voltage-dependent anion channel type 1 (VDAC1) in endometrial cancer and to ascertain their association to prognosis, recurrence, and survival.

Study IV

To assess how dietary habits and physical activity affect prognosis, recurrence and survival in endometrial cancer, using new machine learning models.

3 PATIENTS AND METHODS

3.1 SETTING

The cohorts in our study originate from both retrospective and prospective data that were collected as part of an extensive project: RENDOCAS (Registry of Endometrial Cancer in Sweden). This study focuses on mapping heredity, identifying disease-causing genes in women with endometrial cancer, and developing early diagnostic markers for uterine cancer. We began collecting data in 2010 and invited women who had been diagnosed and surgically treated for endometrial cancer in Stockholm County, Sweden, beginning in 2008 to participate in the study and continued to invite women to participate prospectively with newly diagnosed endometrial cancer until March 2012. Participants (index patients) were asked to complete questionnaires addressing: 1) personal history of other cancer diseases, parity, comorbidity, medications, 2) family history of cancer, 3) dietary habits, physical activity, lifestyle (se supplementary). All participants had been treated with hysterectomy and bilateral salpingo-oophorectomy, with or without lymphadenectomy. Tumors were staged and graded according to FIGO 2009 staging Criteria [5]. Histopathological reports for all patients were verified through the medical records system. Diagnoses among family members were confirmed through the Swedish Cancer Registry.

3.2. DATA SOURCE

3.2.1 The Swedish cancer registry

The Swedish cancer register was founded in 1958 and is managed by the National Board of Health and Welfare. Its fundamental aim is to register and monitor cancer incidence and survival. Healthcare providers are obligated to report each new case of diagnosed cancer to the relevant regional cancer center. The registry contains personal patient data, as well as medical data. Data may include personal identification number, sex, age, and demographic details, along with detailed medical data, including tumor size and histological type [112].

3.3 DEMOGRAPHIC DATA ON THE STUDY POPULATION

Table 6. Study population

Characteristics	No/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)		
Through the serosa	7/481	(1.5)		
Relapse	17/481	(3.5)		

3.4 PARTICIPANTS

Study I

The study included 481 patients with confirmed diagnosis of EC who underwent hysterectomy and bilateral oophorectomy, with or without lymphadenectomy. Of the 890 patients invited to participate, 481 (54%) agreed. All participants provided written informed consent and had a blood sample drawn for DNA analysis, in line with standard procedure at the Department of Clinical Genetics, Karolinska University Hospital. Histopathological analysis of the uterine cancer was recorded for each patient. In addition, participants completed a questionnaire that included data on personal and family history of cancer.

Study II

A collaborative effort known as the Endometrial Cancer Association Consortium (ECAC) enrolled 5591 women of European ancestry with a histologically confirmed diagnosis of endometrial cancer. The studies conducted in 11 separate centers in Western Europe, North America and Australia. A total of 28 984 healthy women of European ancestry and known age at time of sampling were chosen from studies in the above countries to serve as a control group.

The Swedish contribution to this multicenter study is represented by the Registry of Endometrial Cancer in Sweden (RENDOCAS), a hospital-based register of consecutively occurring EC cases. As part of the ECAC effort, our study enrolled 262 EC patients who had undergone surgical treatment at Karolinska University Hospital, Solna, Sweden, between 2008 and 2011. The following material and data were collected for each patient: DNA sample isolate from peripheral blood, histopathological analysis of the tumor, and detailed family history. Participants also completed a questionnaire focused on additional factors known to be associated with EC, including BMI, DM, HRT, and parity.

Study III

For study III, a cohort of 148 patients were selected from the previous group. Of these cases, 126 (85%) represented EC type I and 22 (15%) EC type II. Upon hysterectomy, tissue samples, about 1 cm square, were obtained from both the tumor and adjacent macroscopically healthy tissue from the same patient. Ultimately, we collected a total of 135 such sample pairs.

Study IV

Study IV enrolled the 481 patients from study I who completed a questionnaire on topics related to activities of daily life, dietary habits, alcohol consumption, and smoking, after which these data were subjected to machine learning.

3.5 METHODS

Study I

All participants were contacted for a telephone interview for the purpose of constructing a pedigree, based on questionnaire information and interview confirmation. All pedigrees included information concerning first-degree relatives (FDRs), second-degree relatives, and first cousins. Current age or age at death, type of cancer, and age at diagnosis were recorded for all relatives with cancer. Most cancer diagnoses among relatives were histologically verified through the Swedish Cancer Registry, medical records, and/or death certificates with written consent from the patient or, if deceased, from the closest living relative.

All pedigrees were analyzed for the possible presence of a potentially hereditary endometrial cancer syndrome. Participants meeting the Amsterdam II criteria for Lynch syndrome or the National Comprehensive Cancer Network (NCCN) testing criteria v1. 2009 [113] for Cowden syndrome, and who were therefore at risk for either of these syndromes were thereby identified. These patients were offered genetic counseling at the Department of Clinical Genetics, and mutation screening for causative genes was carried out according to standard procedure. These patients were informed about surveillance programs and advised about further investigation of family members.

All pedigrees were evaluated for cancer in close relatives, with a special focus on putative hereditary uterine cancer and colorectal, breast, and ovarian cancer. Relative frequencies of different cancers were compared with those in Sweden's general cancer population in 1970 and 2010. Families with at least two cases of uterine cancer (including the index patient) were compared with families having only one case in order to identify possible cancer syndrome characteristics. The International Classification of Diseases Revision 7 (ICD-7) was used to classify all cancers. ICD 8 was used to classify neoplasms involving lymphatic and hematopoietic tissues.

Study II

The Collaborative Oncological Gene–environment Study (COGS), involving a method known as Illumina iSelect high-density genotyping array (iCOGS Genotyping array) with 211 155 SNPs, was used for this study to analyze germline DNA extracted from blood. This study selected 113 genotyped and 283 imputed SNPs (total 396 SNPs) from a 200kb region on the short arm of chromosome 5 (5p15) that contains the *TERT–CLPTM1L* candidate region.

Study III

Study III included all patients from whom paired tissue samples were obtained (benign and malignant). Histological records were obtained from the pathology reports in the medical records system, including information such as: histological type of tumor, tumor characterization according to the International Federation of Gynecology and Obstetrics (FIGO) staging system, histological grade, and myometrial invasion. Evaluation concerning hormone receptor status, p56, and ploidy were conducted at the Karolinska Hospital pathology laboratory.

All paired samples were formalin-fixed on paraffin-embedded tumor blocks, sliced into 4 µm-thick sections and subjected to routine immunohistochemistry staining using the Vectastain Elite ABC kit. The slide sections were incubated with the primary antibodies Anti-Ki-67 and anti-PGC1α for 30 minutes at room temperature and subsequently with the secondary antibody (horse anti-rabbit (BA-1000) or horse anti-mouse (BA-2000), both Vector Laboratories), before adding the avidin-biotinylated peroxidase complex. The stained results were evaluated by blinded observation. All slides were independently evaluated by assessment of the entire tumor area (malignant) and of the epithelial cells in control tissues (benign).

Evaluation of immunohistochemistry. All slides were individually evaluated by two observers who were blinded as to clinical outcome. The evaluation encompassed both epithelial cells in healthy tissue and cancerous cells in tumor tissue. Samples were observed for positive PGC1α and VDAC1 immunoreactivities and measured for percent of positively stained cells. The scoring system described above was used to semi-quantitatively categorize positively stained cells on a scale of 0 to 3 (0 <1%; 1 - 1-25%; 2 – 25-50%; 3 - >50%); the same scoring system was used to determine staining intensity 0-3 (0-negative; 1 – weak; 2 – Moderate; 3 – Strong). The immunoreactivity score is the result of these two parameters

(scoring systems) considered together. The tumor (T) and normal (N) tissue, T/N immunoreactivity was trichotomized as T/N<1, T/N = 1, or T/N >1.

Study IV

All participants in the study were given two questionnaires for completion. The questionnaires addressed topics relating to activities of daily life, dietary habits, alcohol consumption, and smoking. In addition, comorbidities, medications, parity, and hormonal therapy were also addressed. Following processing and data extraction, the relevant variables were grouped into different categories. Medical records were reviewed from which clinical data were extracted, including histopathological data concerning tumor characterization, follow-up for recurrence, and survival. Figure 7 includes a sampling from the total of nearly 180 variables.

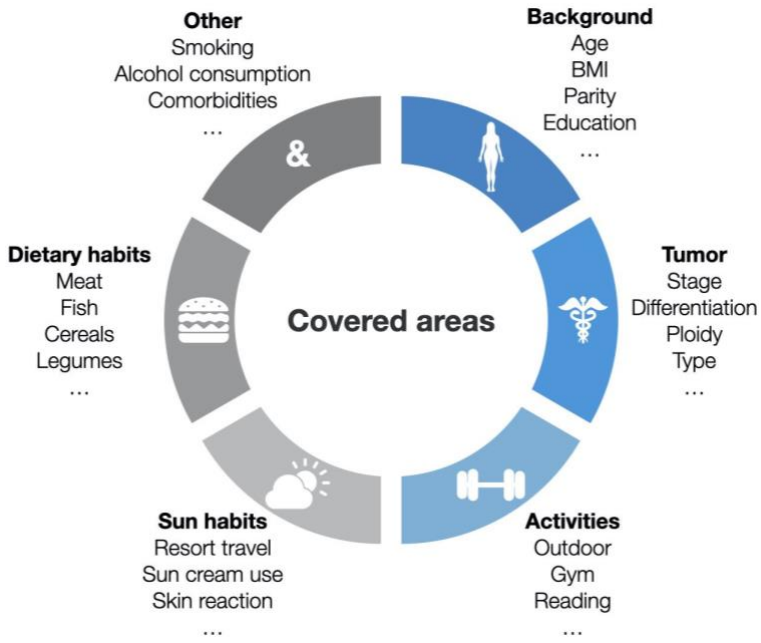

Figure 7. *The different categories covered by the questioners with several variables as an example.*

3.6 STATISTICAL ANALYSIS

Chi-square test: The chi-square test is used to assess data that follow a nominal scale or ordinal scale and tests the differences in binary outcomes between two or more independent groups.

Monte Carlo simulation: Monte-Carlo significance test procedures [114] compare the observed data with random samples generated by following the hypothesis being tested.

Wilcoxon signed-rank test: a nonparametric test that can be used to determine whether two dependent samples were selected from populations having the same distribution. Two samples can be compared to assess whether their population means ranks differ. It provides an alternative to the t-test when the distribution of the difference between means of two samples cannot be assumed to be normally distributed.

Admixture maximum likelihood (AML) method: This is a method developed to analyze multiple, common variants for association with a specific trait. It is an approach that developed in order to estimate both the proportion of associated SNPs and their size of effect [115].

Bayesian: Bayesian has different models but it's a mathematical procedure applied to update the probability for a hypothesis. It can be used to confirm AML result.

Kaplan-Meier analysis: The Kaplan-Meier provides a graphical means for illustrating survival or time-to-event analysis. The Kaplan-Meier curve shows the probability that a specific event will occur within a defined time interval. The horizontal axis represents the time from enrollment corresponding to the time at which participants are considered to be at-risk for the outcome of interest, while the vertical axis represents the estimated probability of survival or possible other outcomes. Each downward step in the lines represents an event (outcome), and each small vertical tick represents a censored observation. Censoring means that the total survival time for that subject cannot be accurately determined, which occurs when an individual drops out of the study, is lost to follow-up, or the study ends before the subject experiences the event of interest; over time, fewer people remain at risk. Survival curves can be compared using the HR or through the log-rank test.

Spearman´s rank: Spearman's correlation coefficient measures the strength and direction of association between two ranked variables.

Kruskal-Wallis H test: is a rank-based nonparametric test used to determine whether statistically significant differences occur between two or more groups of an independent variable on a continuous or ordinal dependent variable.

Mann-Whitney U tes*t:* The Mann-Whitney U test is used to compare differences between two independent groups when the dependent variable is either ordinal or continuous, but not normally distributed.

Cox regression: Also known as proportional hazards regression, is used to investigate the relationship of predictors and the time to the event through a hazard function. Cox regression is time-dependent and provides an estimate of the hazard ratio (HR), which reflects the relative risk of an event occurring over a given timeframe, or the relative event rate. It provides an assessment of rate, referring to number of new cases of an outcome per population at risk per unit of time. An HR>1 means that the event is more likely to occur, while HR<1 means that the predictor is likely to have a protective effect. When HR equals 1, the predictor likely does not influence the hazard of the event.

Logistic regression: It is used to predict the probability of a certain prognosis or investigate the relationship between different variables and outcome. We can obtain odds ratio (OR) Its used when the dependent variable is categorical. The OR is the impact of the variables independently, meaning it helps overcome confounders. *Conditional logistic* regression is commonly used for case-control data in order to match data. *Unconditional logistic regression* is used when the data is not matching, than one can compare conditional to unconditional results.

Random Survival Forest: The Random Survival Forest (RSF) is an extension of the Random Forest model, introduced by Breiman in 2011. RSF is an attractive alternative approach to the Cox proportional hazards (PH) models since PH could be restricted by the assumption that proportional hazards are violated. Survival tree methods are fully non-parametric, flexible, and can easily handle high-dimensional covariate data. RSF processes the data and builds a model capable of further analyzing the variables in order to evaluate how informative a specific variable actually is, referred to as Variable Importance (VIMP). VIMP assesses the

change in prediction accuracy should a particular variable be excluded from the model; the highest-ranked variables decrease prediction power the most. Minimal depth evaluates the variables in terms of how close they split nodes nearest to the root node. Since VIMP and minimal depth are calculated differently, we ranked our variables using both.

Study I

Using the Swedish Cancer Registry to obtain data for a reference population, our study assessed the relative rates of cancers diagnosed in the family members of our index patients and compared them with those of the cancer population in Sweden. From the latter, we limited our data collection to 1970 and 2010 to be used as a basis for comparison with our material so as to compensate for any differences in incidence over time. We used binomial distribution to calculate 95% confidence intervals (CIs). We converted site-specific CIs from numbers to proportions by dividing the number of cancers among family members at a specific site by the total number of cancers among these family members. Chi-square tests were run on tables containing categorical data to check for heterogeneity. P-values were then calculated by using Monte Carlo simulation in the chi-square tests. Shifts in distribution between groups were studied through the Wilcoxon rank-sum test and used for ordered outcomes.

Study II

Initially we employed the admixture maximum likelihood (ADM) test to examine the association between EC and various SNPs. This global test was run against the null hypothesis that none of the genotyped SNPs within the region are associated with endometrial cancer. We used unconditional logistic regression, with a per-allele (1df) model, that was based on expected genotype dosages for imputed SNPs to obtain an estimate of associations among various SNPs and EC. To ascertain independently associated SNPs, we then applied forward and backward stepwise logistic regression. Secondary analysis was undertaken in regard to the most significant independent SNPs to test for specific associations with endometrioid and non-endometrioid EC. The samples derived from iCOGS were used as a basis to calculate pairwise linkage disequilibrium measures. In addition, we subjected all genotyped and imputed SNPs in the region to simultaneous analysis using the Bayesian–inspired penalized maximum likelihood approach in order to identify the optimal subset for disease prediction. Lastly, all data were compared with gene expression analysis.

Study III

The Wilcoxon signed-rank test was used to compare expression of PGC1α and VDAC 1 in the malignant and benign paired tissue samples. The cohort was stratified according to the Wilcoxon test so as to compare expression of PGC1α at different stages. The Kaplan-Meier and log-rank test was used to assess survival as correlated with expression of PGC1α and VDAC1, while the Spearman's rank test was used to investigate the correlation between tumor characteristics and each of the genes PGC1α, TFAM, and p53, respectively. Since this study primarily dealt with non-normally distributed data, we used the Mann-Whitney U when comparing two groups and the Kruskal-Wallis for comparing several groups.

Study IV

The Mann-Whitney U test was used to compare continuous variables between unpaired samples, while, because of the non-normal distribution of continuous variables, the $\chi 2$ test was used for categorical variables. Next, the Cox proportional hazards model was used to evaluate the association between survival or relapse time and various predictor values. P-value was set at 0.05. Due to the many variables involved, as well as the attendant risk for multicollinearity, which may result from multiple testing, we applied Random Survival Forest (RSF), an extension of Breiman's learning method, for right-censored data [116]. RSF was used to analyze time to event, which was either death or relapse. The ForestSRC [117] and the ggRandomForest packages [118] were used to visualize the data. The function of RSF is to process the variables and construct a model in which two primary measurements are evaluated to determine how informative each separate variable actually is, referred to as Variable Importance (VIMP) and minimal depth.

The Random Forest SRC package handles the missing values by imputing them through *adaptive tree imputation*. Notably, exploratory data analysis shows that most features in our study had fewer than 1% missing data points.

Study I analyses were performed using R(R core team 2012), study III using IBM SPSS 25.0, MAC OS and study IV using R studio 1.2 and Anaconda for Mac OS.

Table 7. Overview of studies I-IV

Variables	Study I	Study II	Study III	Study IV
Type of study	Cohort	Cohort	Cohort	Cohort
No. Of participants	481	5591 (262 SE)	148(135)	481
Setting	KS	ECAC	KS	KS
Source of information	Medical charts Questionnaire	Medical charts Blood samples	Medical charts IHC	Medical charts Questionnaire
Outcome assessment	Familial uterine cancer and Frequency of LS/CS	Genetic risk loci	PGC1α VDAC1 expression in EC	Dietary, alcohol and physical activity on EC outcome
Data analysis	Chi-square Monte Carlo Wilcoxon rank	Logistic regression AML Bayesian	Wilcoxon rank Kaplan Meier Spearman´s rank Kruskal-Wallis Mann.-Whitney U	Mann-Whitney U Chi-square Cox proportional hazard Random survival forest

KS – Karolinska University Hospital, ECAC - Endometrial Cancer Association Consortium, IHC – Immunohistochemistry, LS – Lynch syndrome, CS – Cowden syndrome, AML Admixture Maximum Likelihood

3.7 ETHICAL CONSIDERATION

The 1964 Declaration of Helsinki addresses medical research principles where human material and data are involved. One of the key important statements is as follows: *"It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent." [119].*

The principles outlined in the Declaration aim to protect personal integrity in the processing of personal data. More recently, pursuant to the Swedish Personal Data Act, research can only be conducted in Sweden if ethical permission is granted by one of the six ethical boards.

Ethical perspectives

The studies are based on data collected from patients who provided consent to participate in the study. Data were obtained from medical records, including histopathological analyses of tissue samples. The tumor slides in study III were not sectioned for IHC staining until after completion of clinical pathological analyses. Patients completed questionnaires concerning lifestyle, environmental factors, and family history. Blood sampling was undertaken, a simple procedure with few attendant and mild risks, such as local hematoma or at worst, phlebitis.

Our genetic studies (study I) posed an interesting ethical dilemma regarding both index patients and their family members. Though study patients provided consent to participate in this study, which included genetic testing, they may not have been entirely aware of the potential consequences of being diagnosed with a genetic disorder entailing increased risk of other (non-endometrial) cancers, which could result in attendant worry and concern with a negative impact on quality of life and mental health. However, in most cases such concerns may be mitigated by the ability of the Department of Clinical Genetics to offer enrollment in surveillance programs aimed at early detection of tumors in the specific organs at risk. A second dilemma concerns the relatives of our study patients, since they did not provide consent to participate in the study. Should a hereditary cancer syndrome be discovered, they would be contacted by the index patient and informed of their potential increase of risk for cancer, in which case they too can be offered carrier testing and, if necessary, be included in preventive screening programs. Situations may arise in which the index patient refuses to contact relatives. As researchers, we would be left with the knowledge that these family members may be at increased risk for developing a preventable cancer illness that could be diagnosed at an early stage, but would be unable to use that information to directly contact

at-risk individuals. This would not be an uncommon dilemma when investigating families at the Department of Clinical Genetics, which has extensive experience of such issues.

Studies II-IV concern patients who are not thought to be either positively or negatively impacted. However, these studies could potentially improve the prognosis of women diagnosed with endometrial cancer in the future by identifying molecular markers or dietary risk factors that could contribute to early diagnosis and improved treatment strategies for individual patients.

Ethical approval for studies I-IV: The regional ethical committee at Karolinska Institutet, Stockholm, Sweden, approved the study protocol (DNR 2010/1536-31/2), dates of approval November 1st 2010.
Ethical approval for study II: QIMR Berghofer-HREC – P1051
Ethical approval for study III and IV: The regional ethical committee at Karolinska Institutet, Stockholm, Sweden approved the study protocol (No. 2006/649-31/4), dates of approval June 20th 2006.

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4. RESULTS

4.1 STUDY I

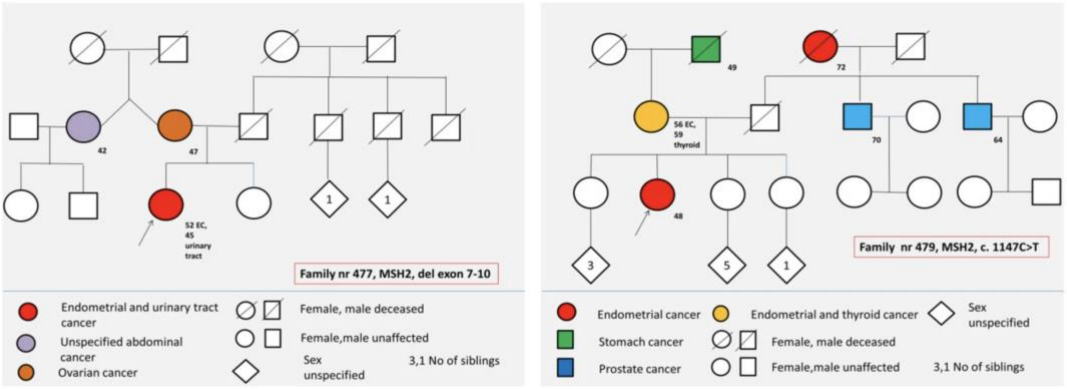
Among relatives of our index patients, we uncovered 1316 diagnoses of cancer, 73 (6%) of which were endometrial cancer. When comparing these findings with the figures from the general population in 1970 and 2010, we found the proportion of EC among our study population to be relatively higher than among the general population in these two comparative years (4% and 3%, respectively). Overexpression of EC also occurred at a higher rate among first-degree relatives, with or without second-degree relatives, in relation to the general population. Family members of index patients also demonstrated overrepresentation of stomach/unspecified abdominal cancer as well as laryngeal cancer.

In contrast, the relatives of index patients did not demonstrate overrepresentation of breast cancer (16%), colon (8%), rectal (3%), and ovarian (25). In fact, such family members showed a lower occurrence of cancers of the pancreas, rectum, urinary tract, lip/tongue/mouth, endocrine glands (excluding thyroid), peritoneum, pharynx, small intestine, eye, mediastinum, nose, as well as non-Hodgkin's lymphoma and myelofibrosis.

Presence of hereditary cancer syndromes

In order to explore possible hereditary cancer syndromes such as LS, we constructed pedigrees for our index patients, 9 (2%) of whom fulfilled the Amsterdam II criteria. Median age of those patients was 58 (range 39-80). Of these, 7/9 were found to have a deleterious mutation in mismatch repair genes, while 4 had a mutation 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*) and 3 patients had a mutation in *MLH1*(c.546-2A> G; c.790+1G>c and deletion of exon 1-3). One patient among these 7 came from a known LS family. We found no *MSH6* mutations in any patient and none of the index patients fulfilled the NCCN criteria for Cowden syndrome.

To summarize, our study revealed LS in 6 of the study families, see Figure 8. Assessment of pedigrees resulted in 9 families that fulfilled the Hereditary Breast and Ovarian Cancer (HBOC) criteria. However, because the index patients declined screening, three families did not undergo mutation screening, while six families were screened for BRCA 1 and BRCA 2 mutations, though none were found.



Pedigrees of two families that were diagnosed with Lynch syndrome. *MSH 2*, del exon 7–10 and *MSH2*, c1147C > T mutations were diagnosed as part of the study. Note that there was no known colorectal cancer in any of the families.

Figure 8. *Pedigrees of two LS families conducted during our study*

Family history of cancer

At least 59% of our index patients reported a family history of cancer with at least one relative having this diagnosis, while 24% had at least two relatives.

When exploring the link between EC and ovarian, breast, and colorectal cancer, we found that 17% of our index patients were shown to have family members with breast cancer, 12% with colorectal, and 6% ovarian cancer.

Table 8 presents the distribution of cancer among relatives of index patients.

Table 8. Family history of cancer among 481 index cases

Heredity	No	%
At least 3 first-degree-relatives with any cancer	38	8
At least 2 first-degree-relatives with any cancer	113	24
At least 1 first-degree-relatives with any cancer	285	59
At least 1 first-degree-relatives with any cancer OR At least 2 relatives* with breast cancer	82	17
At least 1 relative* with endometrial cancer	64	13
At least 1 first-degree-relatives with endometrial cancer	33	7
At least 1 first-degree-relatives with colorectal cancer OR At least 2 relatives* with colorectal cancer	57	12
At least 1 relative* with ovarian cancer	29	6
At least 1 relative* with any cancer diagnosed at <50 years old	137	29

When assessing the association between EC among our index patients and their relatives with EC, we found that 64 patients (13%) had at least one relative with EC. We found no differences in patient characteristic among these 64 patients compared with the other 417 index patients who had no relatives diagnosed with EC, with the exception of statistical significance in the number of patients diagnosed with EC before age 50 in the first group (p<0.001). Table 9. Two LS families were found among those patients.

Table 9. Comparison of the characteristics and variables in families with and without relatives with endometrial cancer

		Uterine cancer in family n= 64		No Uterine cancer in family n= 417		
		Median	**Range**	**Median**	**Range**	**P-value**
Age(years)		65	(36,87)	67	(34,95)	0.082*
Body mass index		26.6	(17.6,43.4)	26.2	(17.7, 55.1)	0.466*
		No	**Row %**	**No**	**Row %**	
Histology	Endometrioid	52	81	342	82	0.102**
	Serous or mixed	4	6	52	12	
	Clear cell	3	5	6	1	
	Sarcoma	5	8	15	4	
	Hyperplasia	0	0	2	0	
Figo stage	1A	45	70	271	65	0.367**
	1B	11	17	84	20	
	2	2	3	32	8	
	3A	4	6	12	3	
	3B	0	0	7	2	
	3C	0	0	2	0	
	4	0	0	3	1	
	4B	2	3	5	1	
Relapse	No	61	95	403	97	0.726**
	Yes	3	5	14	3	
Ploidy	Aneuploidy	13	22	108	28	0.338**
	Diploid	47	78	274	72	
Multiple cancers	No	52	81	354	85	0.478**
	yes	12	19	63	15	
Uterine cancer <50 years of age	No	57	89	389	93	0.300**
	Yes	7	11	28	7	
Relatives with cancer <50 years of age	No	34	53	310	74	0.001**
	Yes	30	7	107	26	

***** Wilcoxon rank sum test. ** Chi.sq test

Presence of multiple cancer in index patients

When evaluating our index patients for multiple cancers, we found 75 (16%) with at least one additional cancer, 9 of whom had 3 different cancer diagnoses.

Breast cancer - In all, 34 were diagnosed with breast cancer, 31 of whom were identified prior to being diagnosed with EC; only 2 were diagnosed with EC first, and 2 others were diagnosed at the same time. The median time interval between these two diagnoses in the former group was 7 years. Only 12 index patients were treated with selective estrogen receptor modulators as part of their breast cancer treatment regimen. Histological findings among patients treated with Tamoxifen did not differ from those of the study population.

While 7 of the patients with breast cancer also had an additional cancer diagnosis, including colorectal, myeloma, skin cancer, salivary gland, and malignant melanoma, no pattern of significance was found.

Colorectal cancer - In all, 14 patients had an additional diagnosis of colorectal cancer, 12 of whom were diagnosed prior to EC, 1 at the same time, and one afterwards. The median time interval between these cancer diagnoses was 6 years. In addition, 2 of the 14 patients were also diagnosed with urinary tract or skin cancer.

Ovarian cancer – In 4 index patients, the diagnosis was made simultaneously.

Moreover, 4 of our index patients with multiple cancer diagnoses also had Lynch syndrome.

Table 10. Multiple tumours in 75 of 481index cases

Additional Cancer	Of patients with multiple tumors	Of total patients' population	No. in group with other tumors	Other tumors
Breast	34/75 (45%)[1]	34/481 (7%)	7/34	CRC+myeloma, CRC+skin, CRC(nr 2) salivary gland, malignant melanoma, skin
Colorectal	14/75(19%)	14/481 (3%)	6/14	BC+myeloma, BC+skin, BC (nr 2), urinary tract, skin
Ovarian	4/75 (5%)	4/481 (0.4%)	0/4	
Other[2]	34/75 (45%)	34/481 (7%)	7/34	Skin (nr 3), myeloma, salivary gland, malignant melanoma, urinary tract

CRC – Colorectal cancer, BC – Breast cancer

[1]12/34 Had therapy with selective estrogen receptor modulator

[2]11skin cancer, 4 lymphoma, 4 cervix cancer, 5 malignant melanoma, 2 Urinary tract, 2 myeloma, 1 Kidney, 1 CNS, 1 lung, 1 salivary, 1 vocal cord, 1 extra adrenal paraganglioma.

4.2 STUDY II

SNP data related to the 5p15 region were generated for 4401 cases and 28 758 controls. Using the costume-designed Illumina iSelsect (iCOGS) array we genotyped our samples. The iCOGS includes 118 genotyped SNPs. We then imputed the additional genotypes using 1000 Genome project data as a reference.

When performing an admixture maximum likelihood (AML) test against the null hypothesis claiming that none of the genotyped SNPs within the *TERT –CLPTM1L* region are associated with EC, significant evidence was obtained that at least one SNP is associated (P = 0.0001) with EC. A total of 61 SNPs out of 396 (113 genotypes + 283 imputed) that generated P values <0.05 (as compared with <20 expected by chance) were able to be identified through single-SNP association testing.

Using forward stepwise logistic regression, additional testing uncovered three imputed SNPs (rs7705526 (in SNP set 1, set of 12 SNPs), rs13174814 (SNP set 2, set of 4 SNPs) and rs62329728 (SNP set 3, set of 10 SNPs)) each of which was backed by evidence of independent association with EC with a respective OR of 1.11, 0.87, and 1.27 based on unconditional analysis (table 11). Further support that these SNPs represent independent risk factors for EC comes from the weak linkage disequilibrium (LD) between them. rs7705526 is located in an LD region that has previously been found to be associated with other cancers, including ovarian and breast, where it can be found in the first intron of *TERT*. All SNPs were subjected to a Bayesian-inspired penalized maximum likelihood approach in order to define the optimal subset that can be used to predict disease, a step that further supports the evidence that SNP set 1 and 2 play a role in EC.

Finally, data obtained from the cancer genome atlas (TCGA) found increased expression of both *TERT and CLPTM1L* RNA in EC tissue compared with normal tissue.

Table 11. The 3 SNPs showing independent associations with endometrial cancer

SNP	**Position (bld37)**	**A1/A2**	**Frequency of A1**	**Imputation Information score**	**Uncondonditional analysis**		**Conditional analysis**	
					OR (95% CI)	***P* value**	**OR (95% CI)**	***P* value**
Rs7705226	1,285,974	C/A	0.33	0.89	1.11 (1.06, 1.17)	7.7E-05	1.08 (1.02, 1.14)	9.7E-03
Rs13174814	1,299,859	G/C	0.25	0.98	0.87 (0.82, 0.93)	4.9E-06	0.89 (0.84, 0.95)	1.7E-04
Rs6239728	1,356,890	G/A	0.06	0.82	1.27 (1.14, 1.43)	2.2E-05	1.24 (1.11, 1.39)	1.8E-04

4.3 STUDY III

We used immunohistochemistry to study expression of PGC1α and VDAC1 among EC patients with a median age of 70.0 (Range 65.2-77.0) at time of diagnosis. Of these, hormone replacement therapy had previously been prescribed to 67 of the total of 146 (44.9%) patients. Median BMI was 26.3 (23.7-30.1) and parity - 2 (1-3). Table 12 presents tumor characteristics.

Table 12. Tumour characteristics (n=148)

Characteristics	**No (%)**
Histology	
Endometroid	125 (84.5)
Serous or mixed	15 (10.1)
Clear cell	7 (4.7)
Stage	
1	103 (69.6)
2	25 (16.9)
3	17 (11.1)
4	3 (2.0)
Grade	
1	39 (26.4)
2	60 (40.5)
3	49 (33.1)
Depth of myometrial invasion	
None	10 (6.8)
<50%	72 (49.0)
≥50%	63 (42.9)
Through the serosa	2 (1.4)
Relapse	25 (16.9)

The subtypes demonstrated the expected distribution of hormone biomarkers. There was a 20-fold higher percentage of Ki67 positive cells in malignant tissue than in benign tissue. A significant correlation was observed between Ki 67 expression and shorter time to relapse ($p<0.001$) (Figure 9)

PGC1α expression in EC: PGC1α expression was found to be decreased in malignant tissue compared with benign tissue ($p<0.001$) (Figure 10).

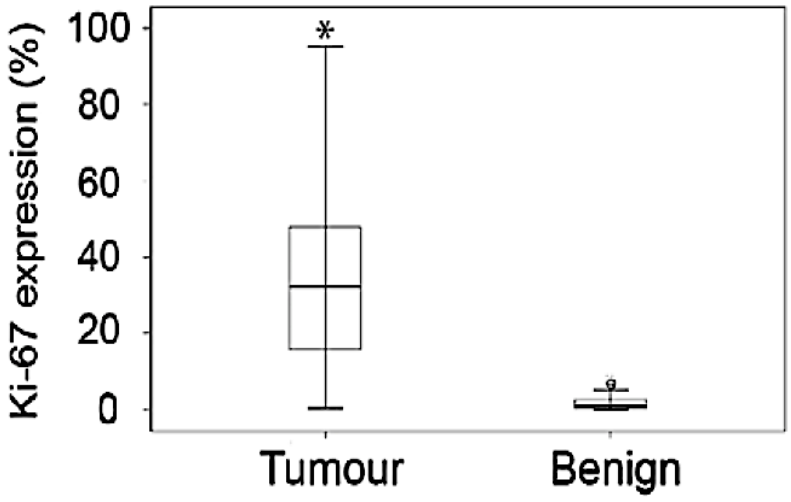


***Figure 9**. Ki-67 expression. The Ki-67 index, or percentage of tumour cells with a positive nucleus in immunohistochemistry was significantly higher in tumour cells [31.1 (14.6, 47.9) vs 1.00 (0.38, 2.60), P<0.0001;Wilcoxon signed rank test]*P<0.05*

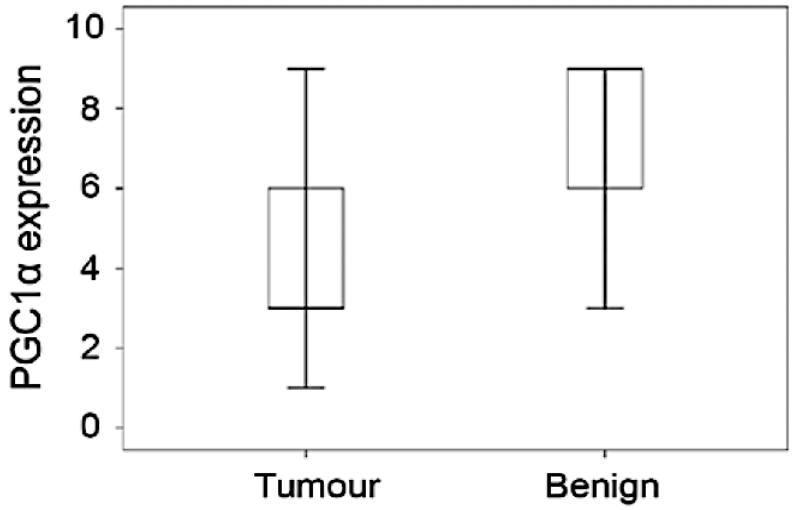


***Figure 10.** PGC1α expression. Immunohistochemical staining and scoring for PGC1α demonstrated significantly lower expression in tumour than in benign tissue [3.0 (3.0, 6.0) vs.9.00(6.00, 9.00); P<0.0001; Wilcoxon signed rank test]. Expression levels (y-axis) are based on the proportion of positive cells and the intensity of staining. PGC1α, peroxisome proliferator activated receptor γ coactivator 1.*

The Mann-Whitney U test uncovered no association between PGC1α expression and tumor subtype (p=0.113;), nor was any association found between PGC1α expression and invasion, stage, or tumor grade (Table 13). No significant correlation was demonstrated between PGC1α expression and tumor size, nor was any association found between PGC1α expression and relapse or mortality (p=0.345 and 0.758 respectively; Log-rank test).

Perhaps somewhat surprisingly, the lower PGC1α expression observed in stage I FIGO patients was associated with a tendency toward shorter time to death.

Table 13. Tumour characteristics

Characteristics	No	PGC1α score, median (IQR 25,75)	p-value
Invasion			
0	9	4.00 (3.00, 6.00)	0.294
1	73	4.00 (3.00, 6.00)	
2-3	65	3.00 (3.00, 6.00)	
Stage			
1	103	3.00 (3.00, 6.00)	0.773
2	25	6.00 (3.00, 6.00)	
3-4	20	6.00 (3.00, 6.00)	
Grade			
1	39	3.00 (3.00, 6.00)	0.903
2	60	3.50 (3.00, 6.00)	
3	49	6.00 (3.00, 6.00)	
Tumor size*(mm)			
≤30	47	3.50 (3.00, 6.00)	0.090
>30	43	3.00 (3.00, 6.00)	
Histology			
Type I	125	3.00 (3.00, 6.00)	0.113
Type II	22	6.00 (3.00, 6.00)	

*Data from the 90/148 sample which size was documented. PGC1α expression is shown as a semiquantitative score based on the categorization of percentage of positively stained cells and the maximum staining intensity. The Kruskal-Wallis test was used for comparing several groups, and the Mann-Whitney U test for comparing two groups.

TFAM - Analysis of this mitochondrial transcription factor TFAM showed a significant decrease in expression in malignant tissue (p=0.016).

VDAC1 – VDAC1 expression was significantly lower in tumor tissue than in benign tissue (Fig 11). Intermediate VDAC1 expression also weakly correlated with shorter time to relapse (P=0.03, $Chi^2$6.81; Log-rank test)(Fig.12), though the correlation became non-significant after age adjustment.

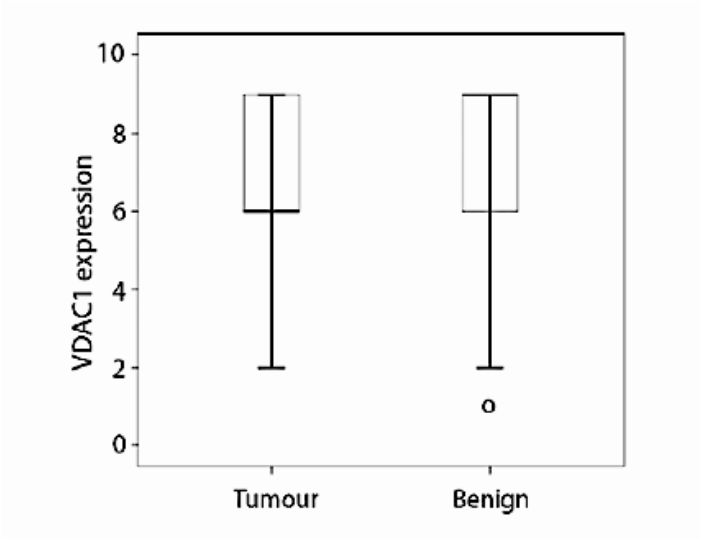


Figure 11. *VDAC1 expression. Immunohistochemical staining and scoring for VDAC1 demonstrated significantly lower expression in tumour tissues than in benign tissue [6.0 (6.0, 9.0) vs. 9.00(6.00, 9.00); P=0.005; Wilcoxon signed ranked test] Expression scores (y-axis) are based on the proportion of positive cells and the intensity of staining. VDAC1, voltage-dependent anion channel type 1.*

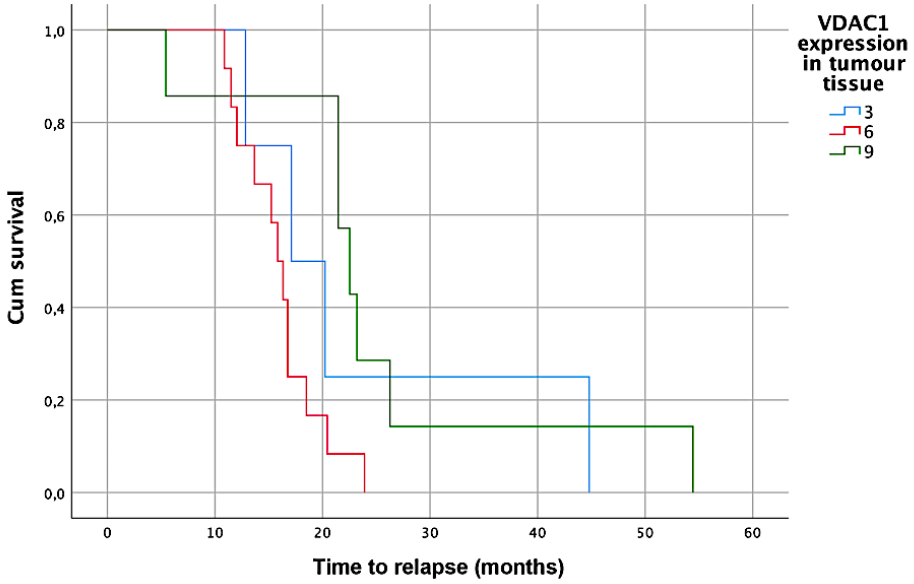


Figure 12. *A weak correlation of VDAC1 expression and shorter time to relapse was observed. P=0.03, Chi^{2}6.81; Log-rank test*

4.4. STUDY IV

In all, 890 questionnaires were sent, of which 481 were completed and returned (drop-out rate 46%). Median patient age was 67.00 years [IQR 61.75 - 75.00], BMI - 26.50 [IQR 23.47 - 30.45]. Table 14 summarizes patient characteristics.

Table 14. Background clinical and tumours characteristics of the study group

Background characteristics	**No (%)**
Education	
< High school	152 (36.5)
High school	108 (26.0)
> High school	139 (33.4)
Diabetes	
Yes	46 (11.1)
No	369 (88.7)
Ever use of lipid-lowering therapy	
Yes	92 (22.1)
No	320 (76.9)
Ever use of hormone replacement therapy	
Yes	208 (50.0)
No	203 (48.8)
Smoking	
Yes	181 (43.5)
No	204 (49.0)
Tumor characteristics	
Stage	
IA	279 (67.1)
IB	80 (19.2)
II	29 (7.0)
IIIA	13 (3.1)
IIIB	8 (1.9)
IVA	1 (0.2)
IVB	6 (1.4)
Type	
Endometrioid adenocarcinoma	382 (91.8)
Serous carcinoma	25 (6.0)
Clear cell carcinoma	9 (2.2)
Grade	
G3	82 (19.7)
G2	151 (36.3)
G1	183 (44.0)
Ploidy	
Aneuploid	111 (27.3)
Diploid	295 (72.7)

Relapse

Our most recent follow-up was on February 1, 2019 when 54 women were found to have experienced relapse of EC (13.0 %), with a 20-month median time-to-relapse [IQR 12,75-29,00]. As expected, relapse and decreased overall survival were associated with advanced age, aneuploidy, non-EEC tumor, and poor histological differentiation. See figure 13 table 15.

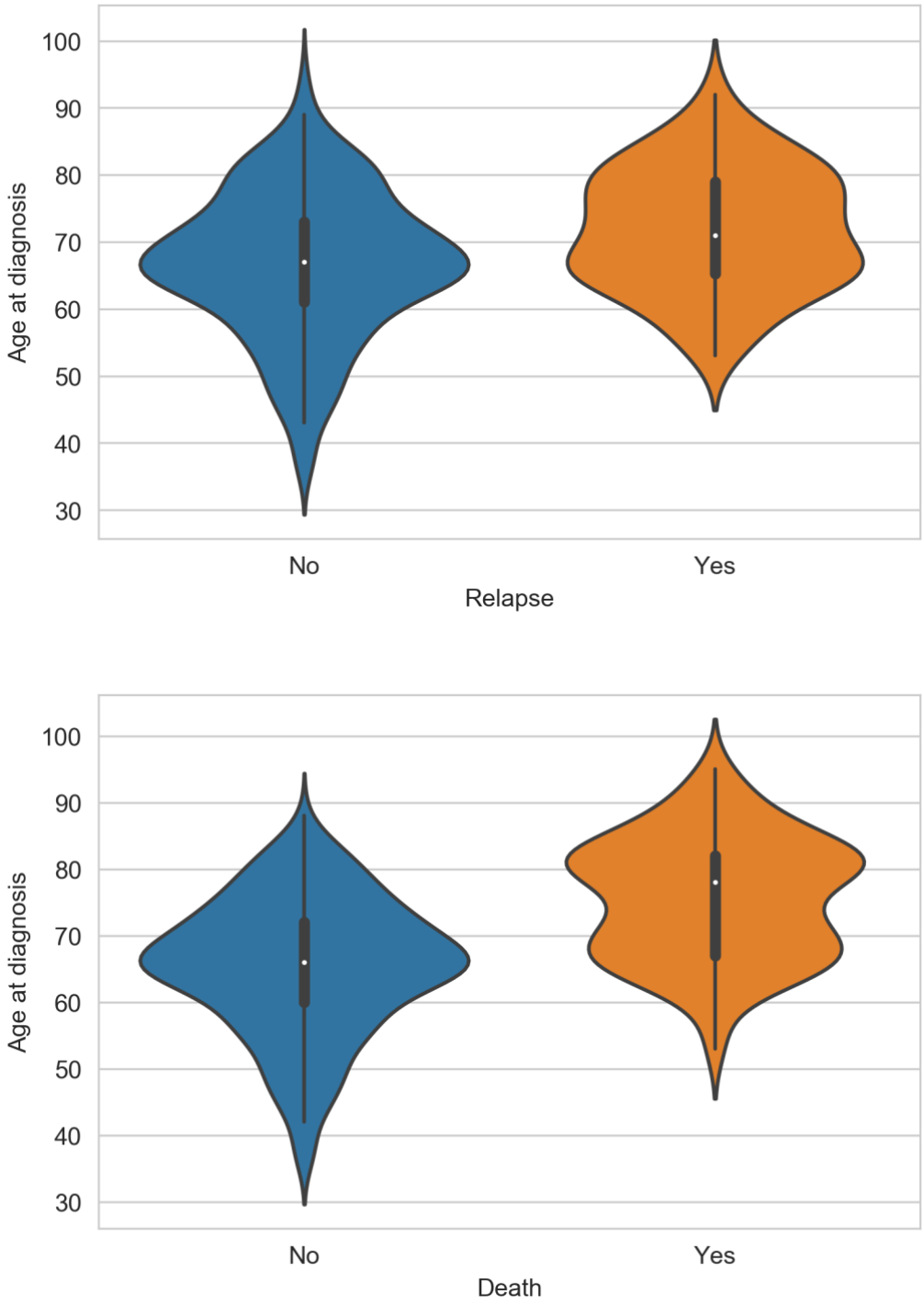


Figure 13. *Comparison of the age distribution between women who had a relapse or died and those who did not (p=0.02 and p<0.0001 respectively).*
Median is marked with white dot, while interquartile range - with black bars around

Table 15. Relapse and survival between patients with different tumor characteristics

	Relapse No (%)	**Death** No (%)
Stage**		
IA	17 (6.09%)	36 (12.90%)
IB	20 (25.00%)	23 (28.75%)
II	5 (17.24%)	8 (27.59%)
IIIA	8 (61.54%)	8 (61.54%)
IIIB	1 (12.50%)	4 (50.00%)
IVA	1 (100%)	1 (100%)
IVB	2 (33.33%)	3 (50.00%)
Type of EC*		
Endometrioid	43 (11.26%)	67 (17.54%)
Serous carcinoma	7 (28.00%)	10 (40.00%)
Clear cell carcinoma	4 (44.44%)	6 (66.67%)
Grade of EC*		
G1	14 (7.65%)	27 (14.75%)
G2	21 (13.91%)	30 (19.87%)
G3	19 (23.17%)	26 (31.71%)
Ploidy**		
Diploid	25 (8.47%)	44 (14.92%)
Aneuploid	26 (23.42%)	36 (32.43%)

*$p < 0.05$; **$p < 0.0001$ (According to $\chi 2$ test)

In this group, mean OS was 45 months [IQR 29.00-74.00] and a total of 83 patients died (20.0%).

The study continues by applying the progression-free survival (PFS) model to analyze all variables as predictors. Relapse and death from any cause were designated as the main events, while OS and time-to-relapse were designated as the time-to-event characteristics. Note that when PFS was considered in relation to relapse, both death and OS period were excluded (and *vice versa*), in order to avoid misrepresenting these significant variables. Next, VIMP and minimal depth were calculated for all variables and sorted in descending order. Table 16 lists the top 10 features.

Table 16. Features sorted in descending order of importance based on VIMP and minimal depth assessments

Death		Relapse	
VIMP	**Minimal depth**	**VIMP**	**Minimal depth**
Stage	Age at diagnosis	Stage	Stage
Age at diagnosis	Stage	Ploidy	Fried potatoes
Tumor type	Tumor type	Tea black final	Prunes
Ploidy	Age discontinued (alcohol)	Prunes	Ploidy
Other soft drinks soda	Other soft drinks soda	Skin reaction	Tumor type
Prunes	Liver pate	Fried potatoes	Other soft drinks soda
Other fruit	Coffee unfiltered	Differentiation	Fruit yoghurt sour milk
Age discontinued (alcohol)	Fried potatoes	Tumor type	Beetroots
Differentiation	Ploidy	Meat toppings	Age at diagnosis
Physical activity	Physical activity	Sun behavior	Liver pate

Certain variables rank highly both according to VIMP and minimal depth. The focus was mainly on relapse and death as the main events. Variable features in order of importance for relapse include: stage, ploidy, tumor type, prunes, and fried potatoes. Variable features in order of importance for death include: age, stage, tumor type, ploidy, other carbonated soft drinks final, physical activity, and age at which alcohol consumption was discontinued. Age and tumor characteristics are well-known modifiers of PFS and OS in EC and these were taken into account in the exploratory data analysis of our study. For this reason, we would also like to turn our attention to other variable features, such as dietary habits, alcohol consumption and physical activity.

Concerning this group of variables, we ran the Cox proportional hazard models; Table 16 presents the analysis of the patterns obtained. To summarize, fried potatoes increased the risk of EC, while Prunes had no impact on risk of EC relapse or death. Even after adjustment for BMI, age, and smoking status, the hazard remained. Similarly, carbonated soft drinks increased risk of death [HR=3.262; CI 95%, 1.834-5.800] and likewise, the hazard remained after adjustment for confounders.

In contrast, physical activity had an inverse impact on these risks. A 7.3% reduction [CI 95%, 0.892-0.964] was associated with each incremental unit of Metabolic Equivalent (MET/day) of physical activity. The positive impact of physical activity persisted even after adjustment for age and BMI, but not for tumor stage (Table 17).

Table 17. Hazard ratios of developing EC relapse or death according to specific dietary factors and physical activity

		Unadjusted		Adjusted	
Covariate		**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**
Fried potatoes	death	8.624 (2.217-33.555)	0.002	4.442 (1.094-18.040) *	0.037
	relapse	6.003 (1.059-34.010)	0.043	4.585 (0.896-23.457) *	0.067
Other soft drinks soda final	death	3.262 (1.834-5.800)	< 0.0001	2.127 (1.169-3.867) *	0.013
	relapse	2.243 (0.983-5.119)	0.055	1.563 (0.751-3.255) *	0.233
Physical activity	death	0.927 (0.892-0.964)	< 0.0001	0.951 (0.911-0.993) **	0.022
	relapse	0.966 (0.918-1.1016)	0.183	1.003 (0.953-1.056) **	0.913

* adjusted for age, BMI, tumour stage and smoking status
**adjusted for age, BMI

Stratification based on age at time of discontinued alcohol consumption was carried out and considered in relation to five categories: “never drinker”, “30-50 years”, “51-70 years”, “71-99 years”, “continuous drinker”. By applying Kaplan-Meier analysis we were able to see a significant difference among those categories. (log-rank test, chi2=24.237; p<0.0001). Figure 14.

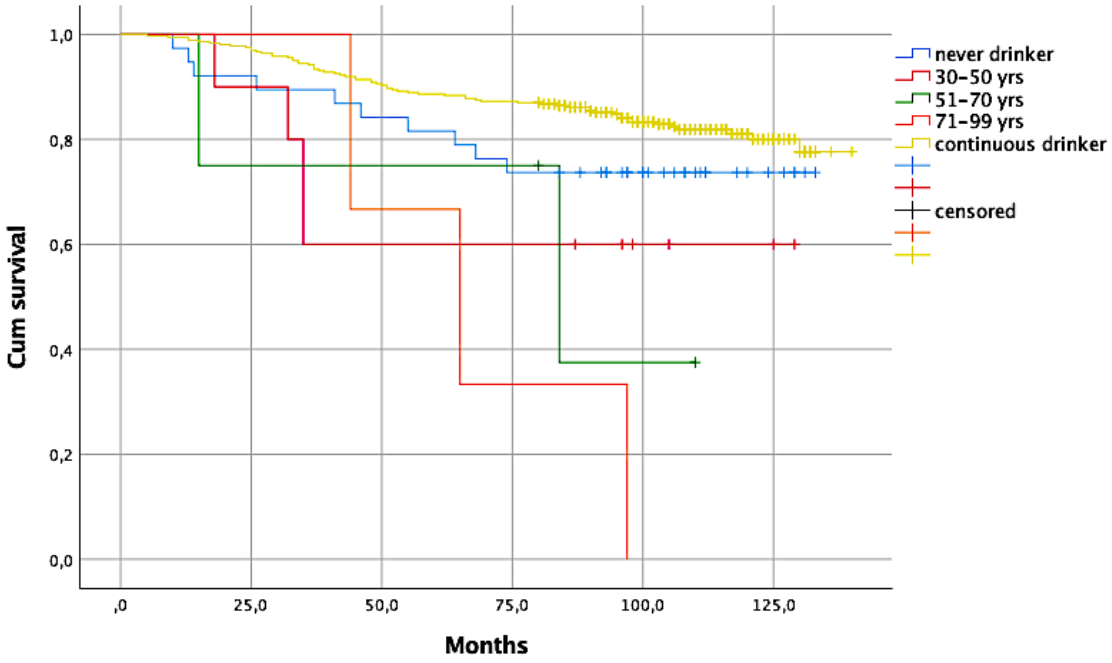


Figure 14. *Survival distribution in EC patients with different alcohol consumption patterns*

Remarkably, continuous drinkers have a better prognosis than those who consume no alcohol whatsoever. However, there were relatively few people in the age groups that completely stopped alcohol consumption at a given point in time, which we believe may have an impact on the final results. Moreover, neither frequency of consumption nor quantity consumed impacted the likelihood of EC relapse or death among current drinkers.

There was a significant correlation between eating deep fried potatoes and consumption of bologna, sausage, and bacon (Spearman's rho=0.339, $p<0.001$ and Spearman's rho 0.285, $p<0.001$, respectively], which is likely evidence for a certain dietary trend. Similarly, soft drink consumption showed a positive correlation with eating white bread with jam (Spearman's rho=0.212, $p<0.001$ and Spearman's rho 0.199, $p<0.001$, respectively]. Although this last correlation is weak, it is interesting to note that these foods can be grouped into "high-fat" or "high-carbohydrate" dietary patterns.

5. DISCUSSION

Endometrial cancer is a common and complex gynecological disease that is currently of high relevance due to its increasing incidence. This thesis focuses on genetic susceptibility and prevalence of EC among LS families with an emphasis on mitochondrial activity and other risk factors, both known and unknown, that may impact this disease.

5.1 STUDY I

Is endometrial cancer a genetic disease?

Our investigation of EC among selected groups of patients in Sweden, establishment of family history of cancer, and construction of pedigrees brings new insight into genetic predisposition to EC.

In our study, 13% (64 patients) of index patients had at least one family member diagnosed with EC, more than half of them (7%) among first-degree relatives. Of these, 30 patients (47%) had family members who were diagnosed early in life, under age 50. Multiple cancers may be indicative of a hereditary cancer syndrome.

In the modern medical era of advanced cancer genomics, the ability to recognize a possible familial cancer syndrome or genetic susceptibility can be crucial for cancer prevention.

While there are known genetic mutations that affect the risk of breast and ovarian cancer (BRCA 1 and 2)[47] the genetic predisposition for EC other from LS or the rare Cowden syndrome, can be highly beneficial. In this regard, a number of studies have found that women with at least one first-degree relative with a history of EC are themselves at increased risk of developing this disease [51, 120-122].

In 2015, Win et al. [123] conducted a systemic review and meta analysis of 16 studies, including six cohort and ten case control studies. Of these, 13 studies reported an association between EC in the patient and a family history of EC among her first-degree relatives. Both genetic risk factors and confounding environmental risk factors, such as obesity, DM, and hormonal therapy, pose a challenge.

An almost threefold risk of developing EC was found among first degree relatives of patients diagnosed with type I EC, as reported by Seger et al. [120]. The group also found more than a twofold risk among second-degree relatives to EC patients when comorbidity factors such as obesity were present, which suggests synergy between environmental factors and genetic

predisposition. The median BMI among our study participants was 26.6, with no significant difference between groups.

Endometrial cancer and other cancers

Among the family members of the index patients in our study, we found an increased relative proportion of laryngeal, stomach/unspecified abdomen, and skeletal cancers. To date, no known common genetic factor has been discovered that can explain a relationship to those cancers. But interestingly, a study focused on acrylamide [124] related dietary intake of this substance to a risk of developing other cancers such as EC, laryngeal, and stomach cancers. The high rate of skeletal cancer may possibly be in error and attributable to incorrectly diagnosing metastases as a primary cancer.

A new breast cancer syndrome?

Almost half of the index patients in our study were diagnosed with multiple cancers, 45% with both EC and breast cancer, which is higher than expected compared with other studies. Prior studies have suggested an association between these two cancers, regardless of family history. A broad national study [125] including 37,583 women found no association between EC and family history of breast cancer, but did find a significant increase in risk of EC among women with a breast cancer diagnosis. The explanation may lie in use of medications such as unopposed estrogen as HRT, or adjuvant use of Tamoxifen for breast cancer, especially since Tamoxifen is known to increase the risk of EC [126] in postmenopausal women [127, 128]. Even after adjusting for age and BMI, the latter risk persists, suggesting that it is predominantly due to Tamoxifen intake [126].

While HRT combination therapy with dose-appropriate continuous progesterone administration may help protect against EC, treatment with unopposed estrogen or combined with insufficient progesterone is believed to increase this risk [129].

Concomitant endometrial cancer and ovarian cancer.

Conflicting results concerning a search for familial correlation between EC and ovarian cancer have been reported by various studies. In line with our own data, a comprehensive meta-analysis of nine studies showed no such association [123]. Ovarian cancer among family members of our index patients was present in 5% of the study population.

However, Type II EC is also overrepresented among patients with BRCA 1/2 [130]. Uccella et al. reported that in most cases, the combination of ovarian and EC cancers can be explained by metastasis from the endometrium to the ovaries[131].

Concomitant endometrial and colorectal cancer

Among our index patients with multiple primary cancers, 17% had both colorectal cancer and EC, though only in two women was there an association with LS. Despite our expectations of finding an association between EC and CRC, stemming from the well-known association seen in LS, no overrepresentation of CRC emerged. The finding of concomitant CRC in 17% of our index patients is considerably higher than the figure reported by Uccella et al. [131], who found only 3%. In addition, Uccella et al. found that LS is the major risk factor for the association with CRC. In contrast, Delin et al. [132] reported findings more similar to our results, at 11% CRC. When compared with the general population, the risk of EC patients for developing a secondary primary malignancy is elevated and correlates with a higher degree of histological differentiation [133].

Endometrial cancer and Lynch syndrome.

Nine families (1.9%) in our study met the Amsterdam criteria, 7 of which had a verifiable LS mutation – the *MSH2* mutation was found in 4 families, while 3 families had the *MLH1* mutation. No case with the *MSH6* mutation was found in our study population, which may be because such cases are less likely to meet the Amsterdam II criteria [134, 135]. Otherwise, the distribution of mutations is in line with overall knowledge concerning a variety of mutations [136]. Since the Amsterdam II criteria are thought to be 60%-80% sensitive, the 1.5% of LS cases found in our study likely represent an underestimate [137]; it would be especially easy to miss the MSH6 mutation.

5.2 STUDY II

The genome-wide association study (GWAS) is developed in order to identify heritability of complex genetic diseases in which many gene variants are causative[138]. All genetic material varies from individual to individual and a large part of the variation is in a single nucleotide variants[139]. Variations in a single nucleotide that exist in more than 1% of the population are called single nucleotide polymorphism, SNP[140]. The hypothesis behind GWAS is that common disorders are most probably determined by a common genetic variants, and SNPs are the most common type of variation studied by GWAS.

To date, GWAS have identified 16 different risk regions associated with EC [55-57]. Additional studies have analyzed expression of trait loci and successfully identified a number of genes and genetic pathways involved in EC tumorigenesis. Furthermore, mendelian randomization methodology has confirmed that risk factors considered to be causal for EC, including BMI and early menarche, may actually be inherited with genetic implications

concerning risk for EC [55]. [55]. To differentiate between high-risk gene mutations, such as those seen in LS (*MLH1*, *MSH2*, or *MSH6*), The principle emerging from GWAS is that even though the risk of an individual genetic variation is only low or modest, the impact of those variations together could explain the risk for familial EC[54, 56, 141]. Many recent, GWAS studies provide support for the fact that common genetic variation that can influence the susceptibility to EC[142]. Common polygenic factors are assumed to increase the risk for EC, and could account for about 28% of the familial risk of EC[55]. The 16 identified genetic regions could explain approximately 7% of the polygenic risk[143].

Our study shows for the first time an association between genetic variants in the *TERT-CLPTM1L* region and risk for EC. We investigated this association, using the iCOGS array and genotype imputation, to study the risk of EC in a population of European descent. We identified 3 independent risk SNPs within the region. Wang et al. [67] notably in a significantly smaller study, investigated almost 2000 SNPs in the 5P15 region, no support emerged for the association that we found to EC. Prior to our study, GWAS did identify a risk association between the *HNF1B* locus and EC. Meanwhile, Sprudel et al. provided convincing evidence for an association between EC and SNP rs4430796, located near the *HNF1B* gene on chromosome 17q [53], which was later confirmed in a follow-up study investigating how altered *HNF1B* gene expression affects risk of EC [54, 144].

Interestingly, some of those SNPs and risk regions were also associated with the risk of other hormone-sensitive cancers such as prostate [145], breast [146], and ovarian [147].

Bafilgil et al [143] did not find an association to the *TERT* region but they did not include the same SNPs as ours in their analysis of polygenic risk score (PRS).

Fine-mapping of genomic regions has its limitations due to the potential presence of multiple causal variants. However, we followed a previous suggestion [148] to use Bayesian inference instead of P-value and found an association with 2 of 3 SNPS, SNP 1 and 2. Thus, the results suggest an association between presence of SNPs 1 and 2 and risk of developing EC.

The risk allele rs7705526 (set 1) is located within the *TERT* gene. Increased TERT promoter activity in both ER negative and ER positive breast and ovarian cancer has been reported by Bojesen et al. [147], as well as in EC [149]. Similarly, Cheng et al[150] has found association between rs7705526 in the *TERT-CLPTM1L* region and risk for EC.

The rs13174814 (set 2) is located in the *TERT* promotor region. It has been shown to be associated with risk of gastrointestinal stromal cancer[151]. The rs13174814 has proven to affect binding of both *RAD21*, a gene encoding for repair protein, and *CTCF*[152, 153]. Prior studies have demonstrated decreased expression of both RAD21 and CTCF with EC [154]. *CTFC* is thought to be a tumour suppressor, a chromatin remodeling and transcriptional factor[155].

The third SNP associated with risk of cancer is rs62329728, which is located in the upstream/promoter region of *CLPTM1L*. Evidence for an association between *CLPTM1L* and tumorigenesis is lacking, but in pancreatic cancer it is known to be associated with chromosomal instability [61]. [61]. Our analysis suggests differences in expression of *CLPTM1L* among different subtypes of EC; both *TERT* and *CLPTM1L* expression are increased in malignant tissues when comparing with the paired benign tissue sample.

5.3 STUDY III

One hallmark of tumor pathogenesis is metabolic alteration [156]. An important key regulator of mitochondrial biogenesis and function is transcriptional coactivator PGC1α, which has been well studied, especially in healthy tissue, and is now considered to play a key role specifically as a regulator and promoter of oxidative metabolism [75]. However, its relationship to cancer is still under debate [157].

Because PGC1α is central to mitochondrial biogenesis and crucial for metabolic programming and regulation, the question should be asked whether its function is reduced in cancer cells. In fact, evidence is growing supporting its role in tumorigenesis for a variety of hormone-related cancers [158]. But paradoxically, PGC1α is overexpressed in both breast and ovarian cancers [159].

PGC1α plays a complex part in its role as a master regulator and may potentially interact differently with various tissue-specific transcription factors in terms of outcome and function in relation to different types of cancer [160]. Our study found that expression of PGC1α is decreased in EC tissue compared with its benign counterpart, but no correlation between expression levels and tumor aggressiveness was found. Although we had expected to find a discrepancy in expression level because of the synergism suggested between estrogen and EC, expression was equally low regardless of EC tumor type [158].

Interestingly, studies by both Ren et al. and Cormio et al. [161, 162] came up with results that contradicted those of our study. Ren et al., however, based their findings on a small cohort (n=15), and their expression levels in cancer were compared with a healthy control group, rather than a paired tissue sample. Moreover, Cormio et al. used a different technique, western blotting, to assess protein expression in heterogeneous extracts, while our study evaluated specific cancer cells. Results similar to ours, with lower expression of PGC1α, were also demonstrated in other cancers, including clear cell ovarian cancer [76], colon [163], and breast [164].

The flux of respiratory substrates occurs through the voltage-dependent anion channel, VDAC, which is localized to the outer membrane of the mitochondria [165], for which reason VDAC expression serves as an indicator for mitochondrial activity.

As in the case of PGC1α, VDAC expression was found to be lower among cancer cells compared with benign tissue. In line with the findings concerning PGC1α expression, here too, no discrepancy was seen between tumor types. Results concerning VDAC expression are inconsistent in different cancers and from different studies [166, 167]. In addition, there is no correlation between expression levels of VDAC and PGC1α, one example being clear cell ovarian cancer [76]. Taken together, these inconsistencies indicate that the role and function of VDAC is highly dependent on specific cellular context, for which reason it has no value as an indicator of mitochondrial function.

However, our finding of the correlation between low PGC1α expression and shorter time to death may hold potential clinical value, especially given that this observation was mainly noted in the FIGO stage I group, where patients were treated exclusively by surgery. Thus, should lower expression of PGC1α correlate with an increased risk for EC recurrence, a change in therapeutic strategy would be warranted.

5.4 STUDY IV

Diet has been clearly linked with modifying risk of cancer in general and gynecological cancer specifically. Other important risk factors include obesity and insulin resistance, which cause an increase in estrogen related to a mechanism by which aromatization of androgens to estrogens occurs in adipose tissue [1, 168]. The hyperinsulinemia resulting from a high glycemic index diet has been implicated in EC tumorigenesis [169], while the popular

ketogenic diet, on the other hand, has been associated with a beneficial effect in EC patients and has therefore been recommended for adjuvant therapy in this disease, as well as for other cancers [85, 170].

Knowing this, we thought to search for a more specific dietary factor that could affect risk or influence disease progression in EC. To this end we used a machine learning approach known as Random Survival Forest to study dietary habits and activities of daily living among EC patients. We uncovered two factors – consumption of fried potatoes and carbonated soft drinks – that link to an increased risk of EC relapse and death, and one factor, physical activity, that improved patient prospects.

The rate of EC recurrence has been linked to well-known individual and clinical parameters, including age, obesity, and tumor type. Moreover, it has been well demonstrated that endometrial proliferation is dependent on the balance of female sex hormones. In addition to such previously recognized risk parameters, our study showed that lifestyle habits, such as dietary preferences, also have an influence on ultimate risk of relapse.

Fried food – rich in Acrylamide

High acrylamide concentrations are known to be found in starchy foods that are prepared using high-temperature methods such as frying and roasting [171, 172]. Acrylamide is a vinyl monomer. Already in 1994 Acrylamide has been considered as "probably carcinogenic to humans" by International Agency for Research on Cancer Working Group. This decision was based on experimental studies in which animals were subjected to significantly higher acrylamide loads than those found in regular foods. In a 2019 statement, the FDA noted that acrylamide continues to be found in various foods, although levels had dropped somewhat since the last update, especially in foods such as crackers and potato chips [173]. In Sweden, the National Food Agency has declared that acrylamide exposure is approximately 30-40 μg/per person per day, well below hazard level. Nevertheless, acrylamide has been implicated as a possible endocrine disrupter capable of altering hormonal balance even in small quantities [174-176], as suggested by one experiment on female rats that developed endometrial hyperplasia and uterine adenocarcinoma in response to glycidamide (epoxide metabolite of acrylamide) [177]. Since acrylamide intake is associated with alterations in sex hormone levels, this mechanism is thought to underlie the association with gynecological cancer, specifically through an increase in estradiol [86, 178]. A recent meta-analysis on dose-response pertaining to acrylamide intake found an increased risk, albeit small, of

developing EC [179]. The negative effects attributable to frying did not pertain to boiled or mashed potatoes, thereby suggesting that the specific cooking method was responsible for the results, which is consistent with the lower acrylamide levels found in boiled or steamed foods.

Cigarette smoking

In the same meta-analysis [86], found that the correlation between high risk of developing EC and acrylamide intake was linear and stronger among never smokers than among smokers, an expected result since a number of studies have demonstrated a protective effect on risk of developing EC attributable to the antiestrogenic properties associated with smoking [180-182]. Nevertheless, among patients in our study the risk of death persisted even after adjustment for age, tumor stage, and smoking status and notably, smoking status *per se* had no effect on relapse or survival rate.

Sugar-sweetened beverages (SSB)

Our study found that consumption of carbonated soft drinks increases the risk of death among patients with EC. Insulin levels increase in response to consumption of sugar-sweetened beverages (SSBs). Insulin impacts EC in several known ways [183, 184]. First, insulin inhibits production of sex hormone-binding globulin (SHBG), which normally binds to circulating sex hormones [185]. This decrease in SHBG results in higher levels of bioactive free estrogen, which in turn induces endometrial proliferation [186]. Next, insulin may indirectly exert an apoptotic and mitogenic effect [187, 188] via insulin growth factor-1 (IGF-1) which increases in the blood [189, 190] in response to elevated insulin. Inoue-Choi et al. [ref] assessed consumption of sugary foods and beverages (including SSBs) in 23,039 postmenopausal women and found a positive association between SSB intake and increased risk of type I EC, an association that persisted even after adjusting for BMI, physical activity, smoking status, and history of diabetes [191]. Our findings regarding consumption of SSBs are in line with many prior studies, though it must be kept in mind that the ultimate results vary, likely due to differences in study design and variation in beverage sugar levels within the group. A recent dose-response meta-analysis found that when large (>50,000 participants) and prolonged (≥10 years follow-up) studies are included [192], there is an association between EC and carbohydrate intake.

Our study found that carbonated soft drinks significantly increased risk of death, but have no association with relapse of EC. One finding of notable interest is that the rate of relapse and death was unaffected by substitution of light carbonated soft drinks containing lower sugar levels or artificial sweeteners, perhaps because OS data include all-cause mortality, not just EC-related deaths. Given parameters such as old age and attendant inevitable comorbidities, SSB consumption may also have a considerable impact on other conditions, such as metabolic syndrome, and thereby via these mechanisms contribute to an increased mortality rate. This serves as a reminder that caution should be observed when speculating about the impact of various predictors on cancer-specific mortality.

Physical exercise

Beyond beneficial effects on obesity, diabetes, and cardiovascular disorders, regular physical activity reduces risk of developing several malignancies, including EC [193]. A meta-analysis by Moore et al. showed that active women were at 30% less risk of developing EC compared with their non-active counterparts [94]. A systematic review reported similar results and found that women who exercise regularly are at a 20-40% lower risk of developing EC [194]. Our study found no effect of physical activity on relapse of EC, but did show an association with decreased risk of death. Once again, due to the presence of multiple health issues among elderly patients, the effects of physical activity are ultimately related to a variety of mechanisms. Examples of benefits from physical activity include but are not limited to, normalization of insulin levels, decrease in inflammatory markers and reactive oxygen species, modulation of the immune system and, obviously, weight loss [195-197]. This last benefit results in depletion of adipose tissue, which causes less aromatization, and thereby decreased conversion of androgens into the bioactive estrogens that otherwise promote endometrial proliferation.

Alcohol

Due to complicated and unequal distribution of data in our specifically related patient categories, as well as a paucity of relevant patients, we are unfortunately unable to draw any firm conclusions concerning the effects of discontinuing alcohol consumption on PFS and OS in patients with EC. When assessing the average quantity of alcohol consumed in relation to patient outcomes, we found no statistically significant differences. More parameters related to alcohol consumption need to be assessed in order to unveil potential hidden patterns.

The questionnaires

Some inaccuracies in our data may stem from errors made by patients when completing the questionnaires. Nevertheless, because patients were given adequate time to fill out the questionnaires, there were very few missing data, which is a testament to the reliability of this approach. Many studies focus on investigating food products or activities that may impact risk of developing EC. Our study, however, took a retrospective approach in studying patients who had already undergone surgical treatment for EC. Therefore, due to differences in study perspectives, some outcomes may not be the same. Our results, however, are aligned with previous major findings, implying at least some commonality in how cancers initially arise and progress.

6. METHODOLOGICAL CONSIDERATION

6.1 STUDY DESIGN

Study I-IV were retrospective observational cohort studies in which data were collected from questionnaires and medical records, and subsequently confirmed through patient interviews. Observational studies are somewhat limited by the quality of evidence obtained compared with that in a randomized study, though we partially compensated for this by having a large cohort. Nevertheless, the three studies in this thesis share one major strength: data were collected from electronic medical records, in which information was recorded by pathologists following the same structural procedures. Study I sourced its information from the well-controlled data of the Swedish cancer registry, while the general population served as our control group. One and the same person systematically conducted all phone interviews, thereby decreasing risk of selection bias and recall bias. However, we were, of course, dependent on how precisely healthcare providers entered relevant information into the medical records system.

In our opinion, study IV was strengthened by the relatively large number of participants, lengthy monitoring periods, and an extensive set of assessed parameters. Moreover, the Random Survival Forest is considered to be an effective approach to data analysis involving high dimensional datasets with limited survival data. Despite this, it must be acknowledged that certain confounding factors may have escaped our scrutiny, even if they potentially influenced findings.

One limitation of study I was family pedigree construction based solely on patient-provided information, obviously entailing a risk of recall bias. To counter this, we confirmed the information provided using the Swedish cancer registry.

Study III may be limited by a lack of external validation since the paired control tissue samples were obtained from the same patient, and in retrospect it would be impossible to exclude the possibility that healthy tissue in close proximity to the tumor tissue could have harbored subtle precancerous molecular changes.

Recall bias is a likely limitation of study IV since patients were asked to complete questionnaires concerning lifestyle factors which they may have remembered imperfectly, or perhaps did not want to report accurately.

6.2 SYSTEMATIC AND RANDOM ERRORS

Both systematic and random errors may be associated with epidemiological studies. The risk of systematic errors, for example, is higher in observational studies, where selection bias, information bias, and confounding factors may intervene.

6.2.1 Selection bias

Given the study population, the exposure-outcome association should be fairly consistent. Where exposure and outcome differ, it is likely that the sample under study did not accurately reflect the target population, known as selection bias.

Selection bias in our studies, primarily studies, I II, and IV should be low since our data were collected from consecutive patients with EC who had surgery at Karolinska Hospital. However, concerning study III, one group of patients had to be chosen from a larger cohort, in which all patients with type II, recurrent disease/death were selected and matched with a control group.

6.2.2 Information bias

Another name for information bias is misclassification. When evaluating the relationship between exposure and outcome, it is important to avoid inaccurately representing or classifying either exposure or outcome. When misclassification is evenly distributed across the entire study group, it is referred to as random or non-differential, but when unevenly distributed across the study group, it is considered to be differential. Non-differential misclassification is associated with results that reduce the differences between groups and underestimate the true association, while differential misclassification may result in a false association in either direction.

One common cause of differential misclassification is patients lost to follow-up, which did not occur in our study, as follow-up among all cohorts was sustained. However, studies I and III were limited by another type of differential misclassification, namely recall bias. Recall bias in study I results from either lack of information, or misleading information, provided by patients regarding their family history. A parallel situation occurs in study III, where recall bias could be due to possible inaccuracies obtained from the information in the questionnaires.

6.2.3 Confounding

Confounding, a well-known challenge in epidemiologic research, is a third problem that may adversely impact the association between exposure and outcome. Although confounding cannot be eliminated from observational studies, its impact can be reduced through stratification, matching, restriction, or by multivariable analysis. In randomized studies, confounders can be controlled through equal distribution within the study population. Due to the large quantity of data, many confounding factors may occur in the studies. We attempted to overcome these by applying statistical analyses and stratification to a variety of relevant factors.

6.2.4 Random error

Random errors may occur when variability within the data occur by chance. The risk of random errors decreases as the study population increases. Confidence intervals (CI) of 95% are generally used to calculate risk which means that the observed association has a 95% probability of not being due to chance as long as the CI does not include 1.00 and that no systematic errors have occurred. A wide CI translates to lower precision, whereas a narrow CI indicates less variability in the data.

P-value is a number between 0 and 1 that indicates how compatible the study results are with the null hypothesis. A p-value <0.05 is considered significant; the lower the P-value, the stronger the evidence against the null hypothesis.

We did calculate p-Value in order to avoid random error.

6.3 EXTERNAL VALIDITY

External validity is a reflection of how well the study results could be generalized to other populations. Internal validation refers to the probability that an observed situation is correct, the higher the internal validation, the more generalizable the results to other populations. Internal validation is optimized by reducing or completely eliminating bias. The degree of similarity between populations is highly relevant to the concept of external bias. An example of internal validation is that in Study III, there we blindly to clinical outcome evaluated the samples by two independent observers. The degree of similarity between the populations is extremely relevant in the discussion of an external bias. In study I for example we compered our population to the registry of both 1970 and 2010 in order to validate our result and compensate for the time deferens.

7. CONCLUSION

Study I:

- Overrepresentation of EC among first and second-degree relatives and first cousins of endometrial cancer patients compared with the general population.
- Multiple cancers and young age of onset in families with EC strengthen the case for existence of an additional hereditary EC syndrome.
- The prevalence of LS was about 2%.
- Only one of seven mutation-verified LS families had been previously diagnosed.
- Importance of appropriate diagnosis and referral for genetic counseling and better follow-up of individuals at high risk of EC.

Study II:

- A novel risk locus for EC has been uncoverd.
- Three novel independent varients within 5p15 locus that increase the risk developing EC.
- Over expression of *TERT* among EC patients, suggesting a potential role in tumourogenesis.
- About 0.5% of the relative risk for familal cancer may be attributable to our findings.

Study III:

- Downregulation of PGC1α was observed in endometrial cancer tissue in both types.
- Downregulation of VDAC1 protein levels indicating an altered mitochondrial function in the cancer tissue compared with benign tissue. Also found in both types.
- An interesting tendency toward an association between lower PGC1α expression among Stage I FIGO and shorter time to death. This could be of high clinical value as low risk patients are treated exclusively with surgery.

Study IV:

- Using the Random Survival Forest algorithm, we observed that fried potato consumption significantly increases the risk of EC relapse and death.
- This effect persists after adjustment for age, BMI, stage, and smoking status.
- Sugar-sweetened beverage consumption significantly reduces OS, but has less impact on EC relapse.
- Physical activity is associated with a lower risk of death among patients with EC; the effect persists after adjustment for age and BMI.
- Our findings suggest that dietary modification may be beneficial in patients with EC; moderate-vigorous physical activity helps to reduce risk of recurrent EC.

8. FUTURE PROSPECTIVES

- Further studies on constructing a family history of cancers to assess for a possible endometrial cancer syndrome are underway, including application of next-generation sequencing in a study population with positive family history.
- To further elucidate the functional effects of *TERT/CLPTM1L* variants on EC and other hormone-related cancers, additional biological studies will be required on these variants. Future gene studies concerning the role of other multicancer loci would also seem appropriate.
- GWAS using resequencing may have the potential to identify additional low frequencey causal variants.
- Further develop a new model for the personalized risk assesment using PRS in combination with life style and clinical measures.
- Studies aimed at understanding the exact nature and role of PGC1α in endometrial cancer are needed, since findings so far are contradictory. A larger cohort study is suggested; the same applies to VDAC.
- Should lower expression of PGC1α in EC correlate with increased risk of recurrence, it could find clinical use as a marker.
- Different molecular markers that can be assessed during endometrial biopsy need to be further studied so as to optimize treatment and thereby reduce recurrence and increase OS.
- Due to the nature of questionnaires, they may be associated with a risk of bias. To eliminate such bias, a prospective study on dietary modification could be conducted on EC patients to evaluate the correlation between dietary habits and recurrent EC, as well as OS.

SAMMANFATTNING PÅ SVENSKA

Livmoderskroppcancer (endometriecancer) är en av de vanligaste gynekologiska cancerformerna och den sjätte vanligaste cancerdiagnosen hos kvinnor totalt sett. Incidensen har ökat under de senaste decennierna och år 2020 drabbades 1400 kvinnor i Sverige av sjukdomen.

Överlevnaden i livmoderkroppscancer är god tack vare att symtomen av sjukdomen debuterar tidigt i förloppet, vilket gör att de flesta som insjuknar upptäcks i ett tidigt skede. Idag är femårsöverlevnaden i sjukdomen cirka 85%.

Livmoderkroppscancer är en komplex sjukdom som har många olika riskfaktorer. Sjukdomar som fetma, diabetes och högt blodtryck är bland de vanligaste och de mest välkända riskfaktorerna. Även höga östrogennivåer, både externt intag eller kroppsegen produktion kan bidra till utveckling av livmoderkroppscancer.

Ärftliga cancersyndrom som oftast beror på mutationer i DNA:s reparationsgener, exempelvis Lynch Syndrom, har visat sig ha en hög risk för utveckling av livmoderkroppscancer. Hos Lynch Syndrom patienter har man sett en kraftigt ökad risk för tjocktarmscancer och livmoderkroppscancer med en livstidsrisk på ca 50% att utveckla sjukdomen.

I studie I ville vi studera om det kan finnas andra ärftliga faktorer förutom redan kända ärftliga cancersyndrom, som ökar risken för utveckling av livmoderkroppscancer hos vissa patienter. I vår studiepopulation sågs en ökad risk för insjuknande i livmoderkroppscancer hos individer med första eller andragradssläktingar som också haft sjukdomen jämfört med normalpopulationen, samt att de insjuknade i en tidigare ålder. Bland dessa individer kunde inga kända cancersyndrom verifieras.

I studie II ville vi studerade om TERT-CLPTM1L genen är associerad med ökad risk för endometriecancer. Studiemetoden var en Genom Wide Association Study (GWAS). Vi hittade en association mellan tre tidigare ej kända riskloci som ligger i närheten eller i själva området för TERT-CLPTM1L och ökad endometriecancer förekomst. Enligt våra estimeringar är dessa tre locus associerade med ungefär 0. 5% av alla fall med familjär endometriecancer.

I studie III ville vi studera uttrycket av de specifika mitokondrieproteinerna PGC1α och VDAC1 i tumörvävnad. Mitokondrier kallas oftast för cellens kraftverk eftersom de är

ansvariga för cellens energiproduktion. Mitokondrierna har därför en huvudroll i cancerceller, då de driver och effektiviserar deras höga energiproduktion. Vi ville därför se om det fanns en koppling mellan nivåerna av PGC1α och VDAC1 och tumörens kliniska karaktär. Vi har kunnat påvisa att båda proteinerna hade ett ökat uttryck i malign vävnad jämfört med benign vävnad. Vidare, vi observerade icke signifikant korrelation mellan lågt PGC1a-uttryck och kortare tid till döden bland patienter med endometriecancer i stadium 1. Denna korrelation har ett kliniskt värde eftersom dessa patienter endast behandlas med kirurgi. Om ett lägre uttryck av PGC1a är korrelerat med ökad risk för återfall kan en annan terapistrategi vara aktuell.

I studie IV har vi studerat om kost påverkar utvecklingen av livmoderkroppscancer. Patienterna svarade på en enkät med frågor om kostvanor. Patienter med ett högt intag av friterad potatis samt söt kolsyrad dryck hade båda en signifikant ökad risk för återfall och dödlighet i livmoderkroppscancer. Vi rekommenderar därför att man inte bara ser på kostvanor som en riskfaktor att få sjukdomen, utan också som ett verktyg för att optimera utfall av cancerbehandlingen.

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