GENETIC AND ENVIRONMENTAL INFLUENCES ON ENDOMETRIOSIS IN SWEDISH TWINS

Rama Saha

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GENETIC AND ENVIRONMENTAL INFLUENCES ON ENDOMETRIOSIS IN SWEDISH TWINS

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By

Rama Saha
MD

Principal Supervisor:
Associate professor
Lena Marions, MD, Ph.D.,
Karolinska Institutet
Department of Clinical Science and Education
Södersjukhuset

Opponent:
Associate professor
Annika Strandell, MD, Ph.D.,
University of Gothenburg
Sahlgrenska Academy
Department of Obstetrics and Gynecology

Co-supervisor:
Professor
Per Tornvall, MD, Ph.D.,
Karolinska Universitet
Department of Clinical Science and Education
Södersjukhuset

Examination Board:
Professor
Paul Dickman, Ph.D.,
Karolinska Universitet
Department of Medical Epidemiology and Biostatistics

Associate professor
Arne Rådestad, MD, Ph.D.,
Karolinska Institutet
Department of Clinical Science and Education
Danderyds Sjukhus

Adjunct professor
Helena Åkerud, MD, Ph.D.,
Uppsala University
Department of Immunology, Genetic and Pathology
To all women worldwide who suffer from endometriosis, the painful condition not always recognized or believed

Don’t focus on the problem alone, look for the solution
ABSTRACT

Background: Endometriosis is a common gynecological condition with a substantial economic burden on the society. It is known that both genetic and environmental factors are contributing to the phenotypic development of the disease but the mechanisms of their coexistence in the disease process are poorly understood. Endometriosis is assumed to be caused by multiple genes, however, no candidate gene has been found so far to be associated with endometriosis. Further, genome-wide association studies have found several loci to be associated with endometriosis, but the particular genetic variants have not been described yet. Thus, little is known about heritability so far. Furthermore, the possible types of environmental factors are yet to be investigated. Associations between reproductive, lifestyle factors and endometriosis have been investigated, while the significance of these factors in the development of endometriosis is not well established.

Methods: The study population comprised of all female twins in the Swedish twin registry, aged 20–65 years in Study I and II and aged 20–60 years in Study III. Study I estimated the prevalence of endometriosis and the influence of heredity on occurrence of endometriosis by genetic modeling. The associations between reproductive, lifestyle factors and endometriosis were investigated in Study II. In Study III we examined the validity of self-reported endometriosis and endometriosis-related questions with the data on endometriosis in the in-patient registry (IPR).

Results: The prevalence of endometriosis among Swedish twins was estimated to be 4.3%. The heritability of endometriosis was 47% and the remaining effect of 53% was explained by unique environment. A history of late age at menarche and higher parity with two or more children showed an inverse association with the risk to develop endometriosis, while, infertility was strongly associated with endometriosis. Women who used oral contraceptive pills solely for contraception showed no significant association with endometriosis. Body mass index, level of education, coffee consumption, smoking and alcohol consumption did not show any association with endometriosis. Good agreement was found between self-reported endometriosis and data on endometriosis in IPR and the predictive ability of self-reported endometriosis having an endometriosis diagnosis in IPR was increased when there was information about age and infertility.

Conclusions: In conclusion, the collective findings from this thesis suggest a strong genetic influence on phenotypic manifestation of endometriosis. Infertility and endometriosis are strongly associated with each other, however, the causality and whether they have a common genetic origin remains unknown. Self-reported data on endometriosis may be useful in clinical and epidemiological studies.
LIST OF SCIENTIFIC PAPERS


Heritability of endometriosis

*Fertility and Sterility.* 2015;104(4):947.

II. Saha R, Kuja-Halkola R, Tornvall P, Marions L

Reproductive and lifestyle factors associated with endometriosis in a large cross-sectional population sample

*Journal of Women’s Health.* Posted online. DOI:10.1089/jwh.2016.5795

III. Saha R, Marions L, Tornvall P

The validity of self-reported endometriosis and endometriosis-related questions in a Swedish female twin cohort

*Fertility and Sterility.* Posted online.

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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>A</td>
<td>Additive genetic</td>
</tr>
<tr>
<td>AIC</td>
<td>Akaike information criteria</td>
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<tr>
<td>AUC</td>
<td>Area under the curve</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>C</td>
<td>Shared environment</td>
</tr>
<tr>
<td>CASP8</td>
<td>Caspase-8 precursor</td>
</tr>
<tr>
<td>CDKN2BAS</td>
<td>Cyclin-dependent kinase inhibitor 2B antisense RNA</td>
</tr>
<tr>
<td>CGAS</td>
<td>Candidate Genes Association Studies</td>
</tr>
<tr>
<td>CI</td>
<td>Confidential interval</td>
</tr>
<tr>
<td>COC</td>
<td>Combined Oral Contraceptives</td>
</tr>
<tr>
<td>CPP</td>
<td>Chronic pelvic pain</td>
</tr>
<tr>
<td>Df</td>
<td>Degree of freedom</td>
</tr>
<tr>
<td>DZ</td>
<td>Dizygotic</td>
</tr>
<tr>
<td>E</td>
<td>Unique environment</td>
</tr>
<tr>
<td>GREB1</td>
<td>Growth regulation by estrogen in breast cancer 1</td>
</tr>
<tr>
<td>GTPase 3</td>
<td>Guanosine triphosphate 3</td>
</tr>
<tr>
<td>GWAS</td>
<td>Genome-wide association studies</td>
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<tr>
<td>ICD</td>
<td>International classification of diseases</td>
</tr>
<tr>
<td>ID4</td>
<td>Inhibitor of DNA binding 4</td>
</tr>
<tr>
<td>IEC</td>
<td>Immune evasion cluster</td>
</tr>
<tr>
<td>IPR</td>
<td>In-patient registry</td>
</tr>
<tr>
<td>IL1A</td>
<td>Interleukin 1-alpha</td>
</tr>
<tr>
<td>LL</td>
<td>Log likelihood</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnet Resonance Imaging</td>
</tr>
<tr>
<td>MZ</td>
<td>Monozygotic</td>
</tr>
<tr>
<td>NIAAA</td>
<td>National Institute of Alcohol Abuse and Alcoholism</td>
</tr>
<tr>
<td>OC</td>
<td>Oral contraceptive</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>PCB</td>
<td>Polychlorinated biphenyl</td>
</tr>
<tr>
<td>PIN</td>
<td>Personal Identification Number</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
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</tr>
<tr>
<td>RANTES</td>
<td>Regulated on activation normal T cell expressed and secreted</td>
</tr>
<tr>
<td>RND3-RBM43</td>
<td>RNA binding motif protein 43</td>
</tr>
<tr>
<td>ROC</td>
<td>Receiver Operating Characteristics</td>
</tr>
<tr>
<td>SALT</td>
<td>Screening Across the Life Span Twin</td>
</tr>
<tr>
<td>SAT</td>
<td>Saturated model</td>
</tr>
<tr>
<td>sICAM-1</td>
<td>Soluble Intercellular Adhesion Molecule-1</td>
</tr>
<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
</tr>
<tr>
<td>STAGE</td>
<td>Swedish Twin Study of Adult’s Genes and Environments</td>
</tr>
<tr>
<td>STR</td>
<td>Swedish Twin Registry</td>
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<tr>
<td>TGF-β</td>
<td>Transforming Growth Factor beta</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor alfa</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
</tr>
<tr>
<td>VEZT</td>
<td>Vezatin</td>
</tr>
<tr>
<td>WNT4</td>
<td>Wingless-related MMTV integration site 4</td>
</tr>
</tbody>
</table>
1 INTRODUCTION

Endometriosis is a chronic, inflammatory and estrogen-dependent disease (1). The prevalence of endometriosis is not exactly known since the disease requires surgical confirmation, but it is estimated to be 2-10% in women of reproductive age and up to 50% among infertile women (1-3).

The exact etiology and pathogenesis of endometriosis remain largely unknown. However, it is suggested to be a complex multifactorial disease where both genes and environment contribute to the development of the disease (3-8).

Studies have indicated familial accumulation (5, 6, 8) and there is a greater risk, 3–15 times in first degree relatives of women with endometriosis compared to healthy controls (4, 7-9). Based on these findings researchers have performed studies to identify potential candidate genes and also to identify susceptibility loci using Genome-Wide Association Studies (GWAS) approach in populations of European descent (10, 11) and in Japanese populations (12, 13).

A number of menstrual and reproductive factors are considered to be associated with endometriosis (14-21). Environmental and lifestyle factors are also suggested as risk factors or markers of risk for endometriosis (22-27).

Questionnaires with self-reported diagnosis are often used for epidemiological studies. Validation of self-reported diagnosis with medical records or in-patient registries is important especially for endometriosis where surgery is required for diagnosis. Good agreement has been shown between self-reported data and in-patient registry data on other questions than endometriosis (28, 29). To our knowledge, there is no study published on validation of self-reported endometriosis and endometriosis-related questionnaire with in-patient registry data.

Since endometriosis is a common gynecological disease with high prevalence and with huge economic burden on society that is theoretically estimated to be between 0.8 million and 12.5 billion euros yearly (30). Increased knowledge about the etiology, pathology and potential risk factors or markers of risk for endometriosis is important to be able to reduce the morbidity among those affected. This could improve the quality of life and as well as to reduce the financial burden.
2 BACKGROUND

2.1 ENDOMETRIOSIS

2.1.1 Definition and epidemiology

Endometriosis is defined by the presence of endometrial glands and stroma in sites outside
the uterus and adenomyosis is defined by the presence of endometrial glands and stroma
within the muscle wall of the uterus. Endometriosis can also cause cysts in the ovaries, called
endometrioma or “chocolate cysts”. The prevalence of endometriosis is estimated to be 2-10% among fertile women. Of all women with endometriosis 2-4% is estimated to affect
postmenopausal women, probably due to exogen estrogen therapy (31, 32). The incidence of
endometriosis has been suggested to be higher in Asian than in Caucasian women and lowest
in African women. These results are uncertain, due to confounding factors such as
socioeconomic status and the different availability of health care facilities (33, 34).

2.1.2 History of endometriosis

Endometriosis is often believed to be a disease of the modern, industrialized society of the
1900s. The increased occurrence has been attributed to industrial or commercial
environmental pollutants (such as dioxins with estrogen-like properties), or postponement of
childbearing to later age. However, symptoms associated with endometriosis have been
amply documented in medical texts more than 4,000 years ago (35).
Historically the symptoms of endometriosis were poorly understood since it is an internal
disease. Its recognition as a disease by the medical profession came about in 1860 when a
detailed monograph was published by Austrian pathologist, Carl von Rokitansky (36). In
1908, the surgeon Thomas Cullen described the complete morphological and clinical picture
of endometriosis and adenomyosis for the first time. In 1921, J.A Sampson first reported on
ovarian endometrioma which he described as perforating hemorrhagic (chocolate) cysts of
the ovary (37). In 1927, he formally described endometriosis when he presented a paper
identifying 13 patients in whom the presence of endometrial tissue was observed during
abdominal surgery (38). Sampson also provided the first theory of retrograde menstruation as
the main etiological factor in the development of the disease.
With the advent of laparoscopy, it became evident that many of the macroscopic appearances
of active endometriosis, (67-71% of cases) were found to be endometrial glands and stroma
on histological inspection of peritoneal biopsies (39).
Surgical treatment of endometriosis commenced in the early 1900's, after advancements in
anesthesia had rendered surgery relatively safe. Conservative surgery was introduced in
1960s and there was development in the use of specialized instruments and laser in 1980s.
Hormone therapies were not available until the mid-1900s. Large dose estrogen was the first
line of therapy used in 1948 by Karnaky (40), but the negative side effects were severe and
the success rates relatively low. Combined estrogen-progestogen (pseudo-pregnancy)
treatment was introduced by Kistner in 1958 (41). The synthetic androgen Danazol was
introduced in 1976 and in late 1980s gonadotrophin releasing hormone (GnRH) was introduced.

2.1.3 Pathogenesis

The etiology and pathogenesis of endometriosis have not been fully understood, but different theories have been proposed. It is likely that several theories are involved for all aspects of the disease. It is described as a heterogeneous disease with different manifestations like peritoneal, deeply infiltrating, ovarian and extrapelvic endometriosis and each manifestation may have its’ own etiology.

The embryonic remnants theory

In the late 19th century, von Recklinghausen presented the embryonic remnants theory which proposed an activation of remnants from the Müllerian tract that later developed to endometriosis in the pelvic region (42, 43). This mechanism possibly explains endometriosis of the recto-vaginal septum (44).

Implantation theory

Sampson proposed in 1927 that the endometrial effluent is disseminated into the abdominal cavity by retrograde transport through the fallopian tubes at the time of menstruation (45). This theory is widely accepted and explains why endometriosis is extremely unusual in women with amenorrhea. Perhaps, the strongest argument against this explanation is that retrograde menstruation occurs in the majority of women, while endometriosis develops only in 2-10 percent. Obviously, other factors also play a role in pathogenesis (31, 45, 46). Superficial lesions of endometriosis in peritoneal, serosal and ovarian surfaces can be explained by this theory.

The lymphatic and vascular metastatic theory

The lymphatic and vascular metastatic theory suggests a dissemination of endometrial cells through lymphatic and blood vessels and explains the development of endometriosis outside the pelvis as in the lungs, pleura and kidneys as well as in other distant places like skeletal muscle, peripheral nerves and brain (47-49).

Metaplastic transformation theory

Another important theory proposed that endometriosis develops through the metaplastic transformation of coelomic cells lining the pelvic peritoneum, called coelomic metaplasia (50). This theory is supported by the fact that both endometrial and peritoneal cells are derived from the same embryonal structure (coelomic-wall epithelium) (31). Ovarian endometriosis and possibly even rectovaginal endometriosis occur due to metaplastic transformation (51, 52). The occurrence of endometriosis in women with Mayer-Rokitansky-Küster-Hauser Syndrome, who do not have a uterus, could be explained by this theory (53).
Theory of immunology, inflammation and endocrine disruption

A defect in the immune system is considered to be the basis for some women suffering from endometriosis (54, 55). Macrophages are believed to play a central role in regulating both cellular and humoral inflammatory activity since they can synthesize and release a variety of cytokines and growth factors (56). The natural killer cells in women with endometriosis express for instance a lower cytotoxic activity compared to healthy women, which could contribute to a lower ability to identify and destroy displaced endometrial fragments (57). Further, it is believed that endometrial fragments can escape detection by releasing adhesion molecules including soluble intercellular adhesion molecule-1 (sICAM-1). Cytokines such as tumor necrosis factor alfa (TNF-α), interleukin IL-1, IL-6, IL-8, IL-18 (58), regulated on activation normal T cell expressed and secreted (RANTES) (59), transforming growth factor beta (TGF-β) and vascular endothelial growth factor (VEGF) are also considered to contribute to the development of endometriosis.

Subtypes of endometriosis

The main theories explaining different subtypes of endometriosis are described below.

Peritoneal endometriosis

Peritoneal endometriosis is superficial lesions presented over the serosal, ovarian and peritoneal surfaces. These lesions are partly explained by Sampson’s theory of retrograde menstruation (45).

Deeply infiltrating disease

Deep infiltrating disease of the rectovaginal septum was first proposed by Sampson in 1922 (60). Three hypotheses have been described later. It was first suggested by Cullen that lower uterine adenomyosis directly extends to the rectovaginal septum (43). Further, Vercellini et al., suggested that lesions originate from secondary infiltration of peritoneal endometriosis (61). The third hypothesis suggests that lesions probably arise from metaplastic process of müllerian rest and have a different entity to peritoneal lesions (51).

Ovarian endometrioma

Three theories have been proposed so far. The first hypothesis by Hughesdon in 1957 suggested that superficial lesions on the ovarian cortex become inverted and invaginated (62). Another hypothesis is that the lesions are derived from functional ovarian cysts (63, 64). The third hypothesis is the metaplastic process of the mesothelial inclusions resulting in the formation of endometrioma (50, 53).
2.1.4 Clinical findings and diagnosis

Endometriotic lesions are mainly located in the sacrouterine ligaments, the ovaries, the vesico-uterine pouch and the pouch of Douglas, while the cells can also implant on the wall of the bladder and the intestines. Lesions respond to steroid hormones and usually bleed monthly at the time of menstruation. Further, an inflammatory reaction occurs leading to pain, adhesions and fibrosis. The disease can spread superficially or can invade the deeper tissues like recto-vaginal septum.

Painful menstruations (dysmenorrhea), pain during intercourse (dyspareunia) (65-68) and chronic pain (68, 69) in the lower abdomen and pelvic region are the main symptoms of endometriosis. In rare cases endometriosis can grow through the wall of the bladder or bowel, causing hematuria or melena at the time of menstruation. Infertility is often associated with endometriosis (2, 68). There is no clear link between endometriosis severity or localization and subfertility/infertility (70). It has been discussed that adhesion formation and altered motility of fallopian tube (71), phagocytosis of sperm (72), ovulatory dysfunction, defects in fertilization or implantation and embryo toxicity are causal (73).

The prediction and diagnosis of endometriosis based on symptoms alone may be weak and incomplete. Clinical examination helps in diagnosis and treatment of the disease. Examination includes inspection of the vagina using a speculum, bimanual and rectovaginal palpation (74, 75). One should also inspect posterior fornix of the vaginal wall (74). Immunological biomarkers such as CA125 in plasma, urine or serum are not recommended for diagnosis of endometriosis (76). Transvaginal or transrectal ultrasound or Magnet Resonance Imaging (MRI) is better than clinical examination in diagnosis of endometrioma and/or deep endometriosis (74, 75). However, it is not possible to detect endometriosis lesions of less than 3 mm with the help of MRI (77, 78). Transvaginal sonography is a useful tool for the identification of rectal endometriosis (79) and ovarian endometrioma (80). MRI is a good method for diagnosis of endometriosis in the ovaries (81) and can detect and characterize different types of deep endometriosis (75). It is important to exclude ureter, bladder and bowel involvement by additional imaging.

Diagnostic laparoscopy is beneficial for ruling out endometriosis in women with signs and symptoms of the disease (82). Laparoscopy with or without histological verification has been widely used for the diagnosis of endometriosis, even if it can result in both false negative and false positive findings (83, 84). Laparoscopy along with histology, as a diagnostic tool, is believed to be both safe and successful, from the data currently available. Histological verification constitutes a good basis for further processing (85).

There is wide variation in diagnosis and management of endometriosis worldwide which causes delayed diagnosis and suboptimal care. Reports from Europe show an overall diagnostic delay of 4-10 years (30, 86, 87).

2.1.5 Treatment

Pharmaceutical treatment

Endometriosis is a complex disease and the condition sometimes requires several areas of expertise for good therapeutic results with regard to pain. Medical treatment is often the first choice but can also be used in combination with surgical treatment when there is incomplete
removal of endometriosis lesions and also to prevent recurrence of the disease after surgery. Gestagens, Combined Oral Contraceptives (COC), and GnRH-agonists are the hormonal treatments for endometriosis used since the 1960’s (88-90). Analgesics are used simultaneously with hormonal treatment.

**Surgical treatment**
Surgery is usually performed due to drug-resistant pain, high suspicion of or previously verified endometriosis. The surgical trend for endometriosis is now more towards laparoscopic or minimal invasive surgery. In the 1970’s a diagnostic laparoscopy was more common and surgical procedures were mainly performed by laparotomy, but since the 1980’s more and more surgical procedures have been performed laparoscopically and even some as day surgery.

### 2.1.6 Twin and family studies

Increased concordance in monozygotic (MZ) twins (3) and familial accumulation of endometriosis (5, 8, 91) have been demonstrated in twin and family studies. Previous studies have reported a 3-15 fold higher risk in first-degree relatives of women with endometriosis compared to controls from the general population (4, 7, 9, 92). Studies have been conducted to establish the heritability of endometriosis, i.e., the contribution of genetic factors. The largest twin-based study from Australia reported a two fold increase in endometriosis risk MZ compared to dizygotic (DZ) twin pairs and the genetic component contributing to the phenotypic variability of endometriosis was about 49-51% (3, 93). Details of related studies are presented in Table 1 and Table 2.

#### Table 1 Twin studies in women with endometriosis

<table>
<thead>
<tr>
<th>Author</th>
<th>Study design</th>
<th>Number of twin pairs and singleton twins</th>
<th>Number of cases or prevalence</th>
<th>Genetic variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treloar et al 1999</td>
<td>Cohort A questionnaire survey</td>
<td>910 MZ pairs 521 DZ pairs 234 singleton</td>
<td>Prevalence 7.2%</td>
<td>51%</td>
</tr>
<tr>
<td>Nyholt et al 2009</td>
<td>Cohort A questionnaire survey</td>
<td>815 MZ pairs 457 DZ pairs</td>
<td></td>
<td>49%</td>
</tr>
<tr>
<td>Hadfield et al 1997</td>
<td>Cohort A questionnaire survey</td>
<td>16 MZ pairs</td>
<td>14 pairs concordant 2 pairs discordant</td>
<td></td>
</tr>
<tr>
<td>Moen et al 1994</td>
<td>Cohort A questionnaire survey with interview</td>
<td>8 MZ pairs</td>
<td>75%</td>
<td></td>
</tr>
</tbody>
</table>
### Table 2: Studies on familial aggregation of endometriosis

<table>
<thead>
<tr>
<th>Author</th>
<th>Design</th>
<th>Number of cases and controls</th>
<th>Incidence</th>
<th>Risk rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stefansson et al 2002</td>
<td>Population-based cohort, compared with matched controls</td>
<td>750 cases</td>
<td></td>
<td>5 fold increased risk for sisters 1,5 fold increased risk for first cousin</td>
</tr>
<tr>
<td>Matalliotakis et al 2008</td>
<td>Case control</td>
<td>485 cases 197 controls</td>
<td>9,5% in first-degree relatives 1% in controls</td>
<td>10 fold increased risk in first-degree relatives</td>
</tr>
<tr>
<td>Kennedy et al 1995</td>
<td>Cohort A questionnaire survey</td>
<td>230 cases in 100 families with at least two affected members</td>
<td>19 mother-daughter pairs, 1 set of cousins and 56 sister pairs, 5 families with 3 affected sisters, 1 family with 5 affected sisters, 18 families with ≥ 3 affected members</td>
<td>Familial tendency</td>
</tr>
<tr>
<td>Moen et al 1993</td>
<td>Case control</td>
<td>515 cases 149 control</td>
<td>Mothers: cases 3,9%, control 0,7% Sisters: cases 4,8%, control 0,6%</td>
<td>7 fold increased risk in mothers and sisters</td>
</tr>
<tr>
<td>Lamb et al 1986</td>
<td>Cohort</td>
<td>43 cases</td>
<td>34,9% of mothers, 21,2% of sisters were affected</td>
<td>7 fold increased risk in first-degree and 2 fold increased risk for second-degree relatives</td>
</tr>
</tbody>
</table>
2.1.7 Genetic determinants

Endometriosis is recognized to have heritability and scientists are making great effort to search for specific susceptibility genes (number and location) for the occurrence of endometriosis. Two main approaches have been used: First, candidate gene studies which are “hypothesis-driven” and test one or more genetic variants which might be biologically relevant. The second approach is “hypothesis-free” with two variants. The first variant is GWAS, where scientists search the whole genome for rare and common genetic variants and the other variant is linkage mapping search for the disease causing variants in families.

Candidate-gene studies in endometriosis

Endometriosis may be caused by multiple genes as in other complex diseases. These studies are based on prior biological mechanisms considered to be responsible for the disease. They include cytokines/inflammation, steroid-synthesizing enzymes, hormone receptors, other enzymes and metabolic systems, growth factor system, adhesion molecules, matrix enzymes, apoptosis, cell-cycle regulation and oncogenes, human leukocyte antigen system and immune components. The search for genes started with “Candidate Gene Association” Studies (CGAS). Many candidate genes were investigated with small number of variants and with small number of cases and controls. An earlier review by Montgomery et al., (94) of seventy six studies until 1 April, 2008 reported no clear association of endometriosis with any gene variants. Further, another review by Rahmioglu et al., (95) which included studies from April 1 2008 to April 1 2012 concluded that no candidate gene was associated with endometriosis. Most of the single nucleotide polymorphism (SNP) studies failed to show any association with endometriosis due to factors like low statistical power and insufficient knowledge about both the biological mechanism of the disease as well as biological functions of the genes. A very large sample size is necessary to achieve convincing results. For example, a recent study which identified a common variant in Caspase-8 precursor (CASP8) associated with risk for breast cancer included 18,000 cases and 22,000 controls (96).

Hypothesis-free genetic studies in endometriosis

There are two approaches to examine the underlying genetic etiology. First is GWAS studies based on the hypothesis that common diseases are caused by genetic variants i.e. SNPs, which are common in the general population. Uno et al., (13) reported the first endometriosis GWAS in Japanese individuals and identified a significant association between endometriosis and the locus located in the cyclin-dependent kinase inhibitor 2B antisense RNA (CDKN2BAS) gene on chromosome 9. Painter et al., (11) who performed the first endometriosis GWAS on European ancestry identified a genome-wide significant intergenic locus on chromosome 7. Nyholt et al., (97) in a meta-analysis of the Japanese, Australian and UK GWAS data sets confirmed three previously identified loci and identified four new loci situated near Vezatin (VEZT), in an inter-genic region on chromosome 2, near the inhibitor of
DNA binding 4 (ID4) gene and near Cyclin-dependent kinase inhibitor B- antisense (CDKN2B-AS). A large two-staged GWAS included a replication sample of endometriosis including 2,019 cases and 14,471 controls with only surgically-confirmed cases with >95% European ancestry could not replicate the immune evasion cluster (IEC) signal on chromosome 7 and two genome-wide significant signals near wingless-related MMTV integration site 4 (WNT4) and an inter-genic signal of Rho family guanosine triphosphate 3 (GTPase 3) - RNA binding motif protein 43 (RND3-RBM43) (10). A recent meta-analysis reported genome-wide association of SNPs in six loci with endometriosis among European and Japanese ancestry near WNT4, VEZT, CDKN2B-AS1, ID4 and in growth regulation by estrogen in breast cancer 1 (GREB1) (98). The result of a replication study in an Italian population confirms a locus near VEZT which was significantly associated with endometriosis and was the strongest among the SNPs identified by GWAS (99). The same study with more severe forms of the disease confirmed an association of SNPs in four out of five genetic loci with endometriosis (99). A very recent study further investigated eight interleukin 1-alpha (IL1A) SNPs using a sample of 3908 cases and 8568 controls of European and Japanese population and concluded that there was significant evidence of IL1A locus in endometriosis susceptibility which was stronger in moderate-to-severe endometriosis and also supports the suggestion for a link between inflammatory responses and the pathogenesis of endometriosis (100). The second approach is linkage mapping focused on genetic investigation of families with multiple affected individuals based on detecting disease-causing variants in a family, but these variants are rare in the general population. Many families with multiple affected women are required to perform these studies. The international endogene study which investigated 1,176 affected sib-pairs from Australia and the United Kingdom identified a region of significant linkage on chromosome 10q26 (101). Another region was identified on chromosome 7q13-15 using a subset of 248 families with 3 or more affected members.

To summarize, genes have a strong influence on the development of endometriosis. Many genes or variants with small effects are suggested to have possible involvement. Previous studies on European and Japanese population have identified several loci, but the particular genetic variants causal to endometriosis are unknown so far. Further, to date, only a fraction of the heritability of endometriosis is explained.
Figure 1 Factors of importance in Endometriosis
2.1.8 Risk factors or markers of risk

Besides heredity, few other established risk factors associated with endometriosis have been reported. Factors like early age at menarche, shorter menstrual cycle length, prolonged menstrual flow, nulliparity, increased age and increased peripheral body fat have been suggested as risk factors or markers of risk for endometriosis (1, 14-16, 18-21, 102, 103). An association between oral contraceptive pill (OC) and endometriosis is still unclear. Previous published studies have reported diverse association between OC and endometriosis (104-106). Infertility has been estimated to affect up to 50% of women with endometriosis (2, 17). Whether infertility is causally associated with endometriosis or it is a consequence of endometriosis is not known so far. Endometriosis and infertility might have the same causal factor which needs to be investigated. Coffee consumption has been shown to increase the risk of endometriosis (22) and to have no association (107). Smoking has shown either a protective effect against endometriosis by decreasing estrogen levels (108, 109) or to have no association (110, 111). An increased risk for endometriosis with alcohol consumption was reported by a meta-analysis of 15 studies (26), however, it was not possible to evaluate whether exposure preceded the outcome.

Previous quantitative genetic analyses have estimated that environmental factors also play a contributing role in risk of endometriosis, but evidence on the type of environmental contribution remains unknown. It is known that endometriosis is more common in urban than rural settings, most likely due to environmental contamination (112). Polychlorinated biphenyl (PCB) is an organic chlorine compound and is similar in structure and mode of toxic action as dioxin. Some studies suggest an association with certain PCBs and endometriosis (23, 27, 113), while other studies have failed to find a clear association (114, 115). It is proposed that the diet has a role in the development of endometriosis but very few studies have investigated this. One study conducted in Northern Italy, reported a significant reduction in the risk of endometriosis with high intake of green vegetables and fresh fruit. In contrast, an increased risk for endometriosis was associated with beef and other red meat and ham consumption (25). The environmental factors involved in the occurrence of endometriosis are still unclear.

2.1.9 Studies on questionnaire

Epidemiological and clinical studies are sometimes performed based on questionnaire including self-reported diagnosis. To estimate the accuracy and reliability of the self-reported diagnoses validity studies are necessary. To our knowledge, only few studies have been published to date on the agreement of self-reported diagnoses with a diagnosis in the in-patient registries. Published studies on validity between self-reported diagnoses and medical records or hospital discharge diagnoses have shown diverse agreement (3, 28, 29, 103, 116-119).
3 AIMS

The overall objective of this thesis was to estimate the influence of heredity and the impact of reproductive and lifestyle factors on the occurrence of endometriosis and the validity of self-reported endometriosis and endometriosis-related questions among Swedish female twins.

Specific aims

- To estimate genetic and environmental influences on the occurrence of endometriosis and the prevalence of endometriosis among Swedish twins (Study I)

- To investigate the relations between reproductive and lifestyle factors with endometriosis in Swedish twins. (Study II)

- To examine the validity of self-reported endometriosis and to improve the reliability of questionnaires by including endometriosis-related questions. (Study III)
4 SUBJECTS, STUDY DESIGN AND METHODS

All three papers in this thesis are epidemiological studies, using large Swedish population-based registers. Linkages between the registers were performed by the 10-digit Personal Identification Number (PIN) which is unique to each citizen living in Sweden.

4.1 Population based register

4.1.1 The Swedish Twin Registry (STR)

The Swedish Twin registry (STR) is an extraordinary resource for the researcher. In the late 1950s the registry was first established to study the association of lifestyle factors on cancer and cardiovascular diseases and also the genetic influences on the respective disease. So far, the STR is the largest twin registry in the world. All twins born in Sweden since 1886 are included. The registry comprises of more than 194 000 twins and more than 75 000 twin pairs (120). Between 1998-2002 one cross-sectional data, the Screening Across the Lifespan Twin (SALT) and during 2005-2006 another cross-sectional data, the Swedish Twin Study of Adults’ Genes and Environments (STAGE) collections has been undertaken. By using twin database hereditary and environmental factors can be investigated.

SALT is a computer-assisted telephone interview based data collection performed during the period 1998-2002 and consists of twins born between 1886 and 1958, aged 40 years or older at the time of interview. Response rates were 65% for those twins born between 1886 and 1925, and 74% for those twins born between 1926 and 1958 (121).

STAGE is a web-based data collection performed during the period 2005-2006 and consists of twins born between 1959 and 1985, aged 20 to 40 years at the time of interview. Non-responders were interviewed over phone. The response rate was 59.6% (122).

Zygosity determination

Intra-pair similarities in childhood were considered in assigning zygosity. Twins were classified as monozygotic (MZ) if both the individuals of a pair replied “alike” and were classified as dizygotic (DZ) if both individuals of a pair replied “not alike”. They were classified as undetermined (XZ) if the twins replied differently, or if only one twin of the pair replied to the question. The zygosity classification was validated using 13 DNA markers with 98% or higher accuracy (120).

Both the studies SALT and STAGE include the same set of questions. The entire questionnaire contained approximately 1300 questions, in 34 sections and included 161 questions related to the woman’s health and 8 questions specific for endometriosis.
QUESTIONS USED FOR ALL THREE STUDIES OF THIS THESIS

Table 3 Endometriosis and endometriosis-related questionnaire used by the Swedish twin registry

1. Have you ever been diagnosed with endometriosis, also called chocolate cysts?

2. How old are you at interview?

3. What is the highest education you have undergone / are undergoing, how many years in total?

4. Weight and height were reported at interview and the measures were used to calculate body mass index.

5. How old were you when you had your first menstrual period?

6. Number of children?

7. Do you regularly use oral contraceptive pills as contraceptive?

8. Do/did you experience severe menstrual pain?
   a. Do you take strong painkillers because of pain?
   b. Have you been absent from work due to pain?
   c. Do you take oral contraceptive pills because of menstrual pain?

9. Do you experience pelvic pain in between menstrual periods?

10. Do you experience painful intercourse?

11. Have you been investigated or treated for infertility?

12. How many cups of coffee do you drink on average per day (do not drink/ drink sometimes, 1-2 cups, 3-4 cups or 5 or more cups per day)?

13. Cigarette smoking and use of snuff were calculated according to an algorithm based on survey questions, whether they smoked or used snuff regularly, sometimes, or did not use?

14. Exposure of alcohol was assessed using a variable with weekly consumption of alcohol units (i.e., number of drinks per week)
4.1.2 The National Swedish In-patient Registry (IPR)

The national Swedish in-patient registry (IPR) is preserved by the National Board of Health and Welfare and includes data on all hospital admission in Sweden. It started in 1964 and includes data for in-patient care in public hospitals. The registry covered 60% of in-patient care in 1969, 85% in 1983 and almost 100% coverage of in-patient care since 1987. Visit to day surgery clinics and outpatient clinics were included since 1997 and 2001 respectively. Data on the name of the hospital the patient was admitted, date of discharge, discharge diagnoses, surgical procedures performed are preserved and are available for the researcher. The discharge diagnoses in IPR are coded according to the International Classification of Diseases 8, 9 and 10 (ICD 8-10).

The discharge diagnoses for endometriosis used in all three studies are for ICD 8 the codes are 625.30-625.33.625.38 and 625.39, for ICD 9, the codes are 617A-617G and 617X, and for ICD10, the codes are N80.0-N80.9.

4.1.3 Medical records

Medical records are preserved at archives in respective hospitals and private outpatient clinic according to the Personal identification number (PIN). After receiving permission from the Regional Ethics Committee, and the patients’ consent, medical records could be retrieved.

4.1.4 The Personal Identification Number (PIN)

The National Tax Board maintains PIN since 1947, which is a ten-digit unique number for all citizens residing in Sweden since 1947. The entire Swedish health care system is covered by the Swedish PIN and linkages can be performed between different populations and medical registers (123). The ten digits represent in order, the year, month and day of birth followed by a four digit control number. For anonymity PIN were replaced by random index numbers by the authorities before delivery to the researchers.
4.2 An overview of the studies

**Table 4 An overview of all studies**

<table>
<thead>
<tr>
<th>Study</th>
<th>Objective</th>
<th>Study design</th>
<th>Cases</th>
<th>Statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study I</td>
<td>To estimate the relative contribution of genetic and environmental influence on endometriosis, prevalence of endometriosis and validation of self-reported endometriosis with medical records</td>
<td>Retrospective population-based cross-sectional cohort</td>
<td>1,228 Aged 20-65 years</td>
<td>Descriptive statistics, t-test, Twin analysis, Chi-square test</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3595 MZ and 3601 DZ female twin pairs</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>13,978 single female twins</td>
<td></td>
</tr>
<tr>
<td>Study II</td>
<td>To examine the relations between reproductive and lifestyle factors and endometriosis</td>
<td>Retrospective population-based cross-sectional cohort</td>
<td>1,228 Aged 20-65 years</td>
<td>Descriptive statistics, Logistic regression, Conditional logistic regression</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study III</td>
<td>To investigate the validity of self-reported endometriosis and endometriosis-related questions with data on endometriosis in IPR</td>
<td>Retrospective population-based cross-sectional cohort</td>
<td>1,168 Aged 20-60 years</td>
<td>Descriptive statistics, Logistic regression, Receiver operating characteristics</td>
</tr>
</tbody>
</table>
4.3 Twin methodology

The methodological background for twin studies is that MZ twins are genetically identical (share 100% of the genes), whereas DZ twins share about 50% of the genes (124). The higher proband-wise concordance and the within-pair correlations in MZ twin pairs compared to DZ twin pairs indicate genetic influence. By using twin methodology, a unique opportunity to examine the importance of hereditary factors for endometriosis is provided. Historically twin studies were described first in 1875. Sir Francis Galton published an article entitled “The history of twins, as a criterion of the relative powers of nature and nurture” (125). The twin methodology has been widely used since then to perform quantitative estimation of genetic and environmental contributions on complex diseases. Structural equations relating to the observed disease can be written based on biometric genetic theory (126). In the classic twin model, the variance of an observed phenotype can be decomposed into three variance components: additive genetic factors (A), shared environmental factors (C), and non-shared environmental factors (E). Additive genetic effect is the total effect of the individual alleles at all loci which influence the disease. Shared environmental factors are non-genetic influences that make the twins similar, and non-shared environmental factors refer to non-genetic influences that make the twins different. Non-additive genetic effect is the interaction between alleles at the same locus (dominance, D) or on different loci (epistasis). The contribution of these latent factors can be estimated by comparing the similarity between MZ and DZ twins. Figure 2 illustrates the classic ACE model for analyzing twin data.
The genetic correlation between twin pairs is 1 for MZ twins and 0.5 for same sex DZ twins. Under the equal environment assumption, the shared environment correlation is 1 for both MZ and DZ twins. The non-shared environment correlation is 0, by definition. If the ratio of twin pair correlations between MZ and DZ twin pair is 2:1 and almost no influence of shared environmental factors, then additive genetic influence is suggested (127). A ratio of more than 2:1 suggests the possibility of genetic nonadditivity. Possibility to detect nonadditivity in the classic twin study is low (128). Heritability is defined as the proportion of phenotypic variance among individuals attributable to genetic factors (124). It should be remembered that heritability does not refer to the genetic composition, rather, genetic contribution to the difference between individuals in a particular population.
4.4 Design and analysis in study I

Study population

A total of 38,154 female twins were retrieved from STR. Females over 65 years of age (n=9332) were excluded because they did not receive the question on endometriosis. Further, females with unknown zygosity (n=452) were also excluded. After exclusion, a total of 28,370 female twins were identified and out of them 1228 responded positively. Finally 3595 MZ and 3601 DZ female twin pairs, and 13,978 single female twins were included in the twin analysis.

Validation of self-reported endometriosis data

We sent letter to all women with self-reported endometriosis and were still alive for their consent to retrieve their medical records. Of the 1228 women 18 were not alive and of the 1210 women 737 gave consent. To obtain a medical record, we then sent a letter to all hospitals and private clinics. Of the total 737, we received 442 medical records from 44 hospitals and 5 private gynecological outpatient clinics. Information on diagnosis of endometriosis by surgery, histology or also clinically (medical history, clinical examination and sonography) were scrutinized and recorded in a structured protocol.

Statistical methods

Summary statistics was used to estimate mean and ± standard deviation of age. Proband-wise concordance was estimated by using 2×2 contingency tables for MZ and DZ twin pairs. Correlation of liability (tetrachoric or within-pair correlation) was also estimated for each zygosity group. To estimate the heritability of endometriosis, quantitative genetic models were used under the assumptions of the classical twin designs. Akaike Information Criterion (AIC) was used to determine the best-fitting model. Statistical analysis was conducted using STATA IC 12 (StataCorp 2011. Stata Statistical Software: Release 12, College Station. Tx: StataCorp LP) and the package OpenMx, version 1.4–3060, in the statistical software R, version 3.1.1 (R Foundation for Statistical Computing, Vienna, Austria).

4.5 Design and analysis in study II

Study population

The cohort consisted of total 28,822 female twins including 3,595 MZ and 3,601 DZ female twin pairs aged 20-65 years at interview. Among 28,822, 1,228 reported positively to the question about endometriosis diagnosis, which was validated with medical records.
Exposure variables

Potential risk factors or markers of risk considered were age at menarche, level of education, body mass index (BMI, kg/m$^2$), parity, OC, infertility, coffee consumption, cigarette smoking, snuff, alcohol consumption. Risk factors or markers of risk were assessed by questionnaire extracted from the STR.

Statistical methods

Descriptive statistical analysis was used to estimate the characteristics of subjects including response rate of potential risk factors or markers of risk for subjects with and without endometriosis. Further, logistic regression analyses with cluster robust standard errors were performed to calculate the odds ratios (OR) with 95% confidence intervals (CI). Conditional logistic regression analyses for co-twin control were performed for the statistically significant risk factors or markers of risk and were considered as a sensitivity test.

Statistical analysis was conducted using STATA IC 12 (StataCorp 2011. Stata Statistical Software: Release 12, College Station. Tx: StataCorp LP)

4.6 Design and analysis in study III

Study population

A total of 28,822 female twins aged 20-65 years at the time of interview replied the question on endometriosis in STR. We excluded women who were older than 60 years of age, because IPR covered almost 100% since 1987. After exclusion, the final figure for total population was 26,898 and 1168 replied positively to the question on endometriosis. We linked STR with IPR to identify cases who received endometriosis diagnoses during discharge from the hospital. We identified 602 cases of endometriosis from IPR.

Statistical methods

First, we calculated the descriptive information of self-reported endometriosis and endometriosis-related questions. Secondly, we calculated the measures of agreement like sensitivity, specificity and area under the curve (AUC) between self-reported data and data on endometriosis in IPR.

To determine the variables which were independently associated with overall agreement, logistic regression analysis was performed. Further, sensitivity and specificity of self-reported endometriosis, age and infertility were plotted on a receiver operating characteristics (ROC) curve to determine the prediction of endometriosis diagnosis in IPR.

Statistical analysis was conducted using STATA IC 12 (StataCorp 2011. Stata Statistical Software: Release 12, College Station. Tx: StataCorp LP)
4.8 ETHICAL REFLECTION

Swedish law permits research on human subjects only if the potential scientific value of the research project in question far exceeds the risks that the study participants could be exposed to (Swedish Riksdag lag 2003:460). In contrast to experimental and interventional studies, observational studies in general have a lower risk of inflicting harm on participants involved. In the current thesis, all data has been extracted from the STR, for which prior ethical approval was obtained. Ethical approval for SALT and STAGE in STR have number FEK KI Diary number 94-280, FEK KI Diary number 00-132 and FEK CI 03-224 respectively. All studies were reviewed and approved by the Regional Ethics Committee in Stockholm, Sweden (diary number 2009/1676-31/2).

All potential participants received written information about the study. Written informed consent was taken from those women who wished to participate, before their medical records were retrieved. This indicates the voluntary nature of the study.

All personal information was kept strictly confidential. Data accounts were anonymous at the group level and no individual data has been presented. No patient identifier information was used in the statistical analysis either. All patient files were kept locked and only study personnel had access to them. Database access was limited to the study biostatistician and myself.
5  RESULTS

5.1 Study I - Heritability of Endometriosis

We received a total of 442 medical records. Out of these, we could confirm the self-reported endometriosis diagnosis in 360 cases (360/442=82%), which was considered to have acceptable validity. Of 360 validated cases 287 were diagnosed surgically (287/360=80%) and 73 (73/360=20%) clinically. The prevalence was estimated to be 4.3% (95% CI: 4.1, 4.6) and there was no significant difference in prevalence between MZ compared with DZ twins (p-value =0.23). The proband-wise concordance and the within-pair correlations were estimated to be higher in MZ twin pairs (0.21, 0.47) than in DZ twin pairs (0.10, 0.20), which indicates that there is genetic influence. We found the AE model allowing for additive genetic and environmental effects to be the best model. The heritability of endometriosis was 47% and the unique environmental effect was 53% (Table 5).
Table 5: Parameter estimates and model fit statistics of genetic and environmental effects on endometriosis among Swedish female twins

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameter estimates (95% CI)</th>
<th>Fit of model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>C</td>
</tr>
<tr>
<td>SAT&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE&lt;sup&gt;g&lt;/sup&gt;</td>
<td>47%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>(20%,57%)</td>
<td>(0%,22%)</td>
</tr>
<tr>
<td>AE&lt;sup&gt;h&lt;/sup&gt;</td>
<td>47%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(36%,57%)</td>
<td></td>
</tr>
<tr>
<td>CE</td>
<td>35%</td>
<td>65%</td>
</tr>
<tr>
<td></td>
<td>(26%,43%)</td>
<td>(57%,74%)</td>
</tr>
<tr>
<td>E</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>95% CI: 95% profile likelihood confidence interval

<sup>b</sup>LL: -2 log likelihood

<sup>c</sup>df: Degrees of freedom

<sup>d</sup>p-value: Significance of difference in -2 log likelihood compared to the saturated model

<sup>e</sup>AIC: Akaike information criterion

<sup>f</sup>SAT: Saturated model

<sup>g</sup>ACE: Additive genetic (A), shared environment (C), unique environment (E)

<sup>h</sup>AE: Was the best–fitting and most parsimonious model according to AIC
5.2 Study II - Reproductive and lifestyle factors associated with endometriosis in a large cross-sectional population sample

The response rate to the questionnaire was very high except for the question about snuff, where the non-response was 63%. We found that late age at menarche was associated with a decreased risk for endometriosis with an OR of 0.75 at 14 years and an OR of 0.63 at 15 years and parity of two or more children had an inverse association with endometriosis (OR 0.70). There was a significant association between infertility and endometriosis with an OR of 5.04. We found no evidence of association between regular use of OC, BMI, daily consumption of coffee and smoking with endometriosis (Table 6).
Table 6 Odds ratios for probable risk factors or markers of risk for endometriosis

<table>
<thead>
<tr>
<th>Potential risk factors</th>
<th>OR (95% CI)</th>
<th>OR* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at menarche, year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;11</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>12</td>
<td>0.79 (0.65,0.97)</td>
<td>0.82 (0.66,1.01)</td>
</tr>
<tr>
<td>13</td>
<td>0.81 (0.67,0.98)</td>
<td>0.83 (0.67,1.01)</td>
</tr>
<tr>
<td>14</td>
<td>0.72 (0.59,0.88)</td>
<td>0.75 (0.60,0.93)</td>
</tr>
<tr>
<td>15, or more</td>
<td>0.63 (0.51,0.79)</td>
<td>0.63 (0.50,0.81)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;18.5</td>
<td>0.78 (0.52,1.17)</td>
<td>1.30 (0.82,2.22)</td>
</tr>
<tr>
<td>18.5-24.9</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>25-29.9</td>
<td>1.20 (1.05,1.37)</td>
<td>1.49 (0.93,2.37)</td>
</tr>
<tr>
<td>30 or more</td>
<td>1.15 (0.92,1.44)</td>
<td>1.36 (0.82,2.26)</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>1</td>
<td>1.38 (1.15,1.64)</td>
<td>0.86 (0.70,1.05)</td>
</tr>
<tr>
<td>2 or more</td>
<td>0.99 (0.86,1.15)</td>
<td>0.70 (0.59,0.83)</td>
</tr>
<tr>
<td>OC as contraceptive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonuser</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Regular user</td>
<td>0.81 (0.70,0.95)</td>
<td>0.88 (0.74,1.04)</td>
</tr>
<tr>
<td>Infertility</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Yes</td>
<td>6.34 (5.56,7.23)</td>
<td>5.04 (4.35,5.83)</td>
</tr>
<tr>
<td>Coffee/day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sometime</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>1-2 cups</td>
<td>1.20 (1.01,1.41)</td>
<td>1.10 (0.91,1.32)</td>
</tr>
<tr>
<td>3-4 cups</td>
<td>1.28 (1.09,1.51)</td>
<td>1.04 (0.88,1.24)</td>
</tr>
<tr>
<td>5 cups or more</td>
<td>1.47 (1.21,1.79)</td>
<td>1.09 (0.88,1.39)</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsmoker/sometime</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Regular smoker</td>
<td>1.29 (1.15,1.46)</td>
<td>1.11 (0.97,1.27)</td>
</tr>
<tr>
<td>Alcohol intake (drinks/week), unit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-user</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>&gt;0-&lt;4.5</td>
<td>0.90 (0.76,1.07)</td>
<td>0.94 (0.78,1.13)</td>
</tr>
<tr>
<td>&gt;4.5-9</td>
<td>0.71 (0.57,0.89)</td>
<td>0.88 (0.70,1.12)</td>
</tr>
<tr>
<td>&gt;9 or more</td>
<td>0.76 (0.56,1.03)</td>
<td>0.91 (0.65,1.28)</td>
</tr>
</tbody>
</table>

OR: Odds ratio, CI: Confidence interval, OC: Oral contraceptive pill

*Adjusted for age at interview (20-65years), age at menarche, body mass index, parity, OC as contraceptive, infertility, coffee, smoking and alcohol intake
Within-pair analyses as sensitivity analyses confirmed the inverse association between parity of two or more children (OR 0.31) and endometriosis and the association between infertility (OR 2.65) and endometriosis.

5.3 Study III – Validity of self-reported endometriosis and endometriosis-related questions in a Swedish female twin cohort

The response rate was 100% for self-reported endometriosis, age at interview, infertility and OC pill. On the contrary, the non-response rates were about 30% for severe dysmenorrhea, pelvic pain and dyspareunia (Table 7). We found high specificity for all variables except for severe dysmenorrhea while sensitivity was low (Table 8).
Table 7 Response of self-reported endometriosis and endometriosis-related questions in STR\textsuperscript{a} with endometriosis diagnosis in IPR\textsuperscript{b} among women aged 20-60 years

<table>
<thead>
<tr>
<th>Questionnaire in STR\textsuperscript{a}</th>
<th>Endometriosis in IPR\textsuperscript{b} n (%)</th>
<th>No endometriosis in IPR\textsuperscript{b} n (%)</th>
<th>Missing values n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age at interview, year</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-30</td>
<td>8 (1.33)</td>
<td>4 835 (18.4)</td>
<td>0</td>
</tr>
<tr>
<td>31-40</td>
<td>52 (8.6)</td>
<td>4 826 (18.4)</td>
<td>0</td>
</tr>
<tr>
<td>41-50</td>
<td>213 (35.4)</td>
<td>7 962 (30.28)</td>
<td>0</td>
</tr>
<tr>
<td>51-60</td>
<td>329 (54.65)</td>
<td>8 673 (33.0)</td>
<td>0</td>
</tr>
<tr>
<td><strong>Self-reported endometriosis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>372/602 (61.8%)</td>
<td>796/26 296 (3.0)</td>
<td>0</td>
</tr>
<tr>
<td>No</td>
<td>230/602 (38.2)</td>
<td>25 500/26 296 (97.0)</td>
<td>0</td>
</tr>
<tr>
<td><strong>Severe dysmenorrhea</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>123/602 (20.4)</td>
<td>5680/26 296 (21.6)</td>
<td>8014 (30.5%)</td>
</tr>
<tr>
<td>No</td>
<td>91/602 (15.1)</td>
<td>12 990/26 296 (49.4)</td>
<td></td>
</tr>
<tr>
<td><strong>Chronic pelvic pain</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>53/602 (8.8)</td>
<td>2029/26 296 (7.7)</td>
<td>8014 (30.5%)</td>
</tr>
<tr>
<td>No</td>
<td>161/602 (26.7)</td>
<td>16 641/26 296 (63.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Dyspareunia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>34/602 (5.7)</td>
<td>752/26 296 (2.9)</td>
<td>8014 (30.5%)</td>
</tr>
<tr>
<td>No</td>
<td>180/602 (3.0)</td>
<td>17 918/26 296 (68.1)</td>
<td></td>
</tr>
<tr>
<td><strong>Infertility</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>167/602 (27.7)</td>
<td>1900/26 296 (7.2)</td>
<td>0</td>
</tr>
<tr>
<td>No</td>
<td>435/602 (72.3)</td>
<td>24 392/26 296 (92.8)</td>
<td>0</td>
</tr>
<tr>
<td><strong>Oralpill as contraceptive</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>99/602 (16.5)</td>
<td>5297/26 296 (20.1)</td>
<td>0</td>
</tr>
<tr>
<td>No</td>
<td>503/602 (83.6)</td>
<td>20 999/26 296 (79.9)</td>
<td>0</td>
</tr>
</tbody>
</table>

\textsuperscript{a}STR: Swedish Twin Registry, \textsuperscript{b}IPR: Swedish National Inpatient Registry
### Table 8 Measures of agreement between self-reported and national register data on endometriosis in a Swedish twin cohort

<table>
<thead>
<tr>
<th>Questions in STR(^a)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>ROC(^b) area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Self-reported endometriosis</td>
<td>61.8%</td>
<td>97.0%</td>
<td>0.79</td>
</tr>
<tr>
<td>Severe dysmenorrhea</td>
<td>57.5%</td>
<td>69.6%</td>
<td>0.64</td>
</tr>
<tr>
<td>Chronic pelvic pain</td>
<td>24.8%</td>
<td>89.1%</td>
<td>0.57</td>
</tr>
<tr>
<td>Dyspareunia</td>
<td>15.9%</td>
<td>96.0%</td>
<td>0.56</td>
</tr>
<tr>
<td>Infertility</td>
<td>27.7%</td>
<td>92.8%</td>
<td>0.60</td>
</tr>
<tr>
<td>Oral pill as contraceptive</td>
<td>16.4%</td>
<td>79.9%</td>
<td>0.48</td>
</tr>
</tbody>
</table>

\(^a\)STR: Swedish Twin Registry, \(^b\)ROC: Receiver operating characteristics
Our result showed that the prevalence of endometriosis increases with age until 40 years in self-reported data and until 50 years in IPR.

Predictive ability of self-reported endometriosis having a confirmed endometriosis diagnosis in IPR was good with an AUC of 0.79. Additional information about age and infertility increases the predictive ability to AUC of 0.89 (Figure 3).

**Figure 3** Receiver operating characteristics for prediction of Endometriosis diagnosis in the in-patient registry
6 DISCUSSION

6.1 Interpretation of findings and implications

Despite the high prevalence of endometriosis, there is limited knowledge of its etiology as well as risk factors or markers of risk. This thesis aimed to acquire more knowledge about the influence of genetic and other factors of importance for example, age at menarche, parity, OC, coffee consumption, smoking and alcohol consumption on the occurrence of endometriosis.

6.1.1 Study I - Genes have a strong influence on endometriosis

Findings

In paper I, we found that an additive genetic effect contributes about 50% to endometriosis etiology, while the remainder was explained by unique environmental factors. Further, we confirmed self-reported endometriosis with medical records in 82% cases and the prevalence of endometriosis was estimated to be 4.3%.

Interpretation

Diversity in diagnostic methods and delay in diagnosis makes it difficult to estimate the true prevalence. The prevalence estimate in our study is slightly higher than previous reports from non-twin samples in the USA (2.5–3.3%) and the UK (1.8%) (129-131), but lower than reports from the Australian twin sample (7.2%) (3). The explanation might be differences in selection criteria and sample size. The interpretation of the results on prevalence depends on the representativeness of the twin population compared with the Swedish female population. Studies have suggested that the results of twin studies can be generalized to singleton populations (132-134). The validity of self-reported endometriosis diagnosis in this study was acceptable.

The genetic contribution of 47% to the occurrence of endometriosis is consistent with a study performed on an Australian twin sample (3). Increased concordance in MZ twins (3), familial accumulation of endometriosis (5, 6, 8) and 3-15 fold higher risk in first-degree relatives of women with endometriosis (4, 7, 9, 92) have been demonstrated. All these above mentioned studies suggest the genetic influence on endometriosis. Thus, our results using a large population-based twin sample strengthen the previous hypothesis that genes have a strong influence on endometriosis. The remainder 53% was explained by the unique environmental factors, suggesting that they also have an important role in endometriosis. It is reported that endometriosis is more common in urban than rural settings (112). While some studies have reported association between certain dioxins, PCBs and endometriosis (23, 27, 113), other studies did not find any association (114, 115). One study reported a significantly decreased risk of endometriosis with intake of green vegetables and fresh fruits and increased risk with beef and ham consumption (25). Thus, diet also has a role in endometriosis. Although few studies suggest about the role of some environmental factors on endometriosis, the evidence on the types of environmental factors is unknown.

The strengths of this study are the large population-based sample and the validation of self-reported endometriosis diagnosis by medical records.
One weakness was that in STAGE, young women (20-40 years) were included and all women had not received their endometriosis diagnosis yet, which might have contributed to underestimation of the prevalence. Another weakness was that there were women who had no symptoms and were diagnosed only by histology by chance. Hence they were not aware of their diagnosis and did not report positively which could also have underestimated the prevalence. Further, not all women received their diagnosis even if they had symptoms, as many did not seek medical help, assuming that dysmenorrhea is a normal condition. Unfortunately, 18% of medical records did not support endometriosis or had information only on adenomyosis without endometriosis. One probability could be that adenomyosis and endometriosis were previously considered as a single entity. The other probability could be that we did not receive the right medical record for those women.

Implications

Our result strengthens the hypothesis that genes have a strong influence on the development of endometriosis. There is only one similar study published previously, by Treloar et al., (3) in 1999 based on the Australian twin cohort, involving a much smaller group of women (3,300 women, 215 self-reported endometriosis cases). Therefore this study represents an important contribution to the body of evidence for a genetic basis of endometriosis.

6.1.2 Study II – Reproductive and lifestyle factors associated with endometriosis

Findings

The results showed inverse associations between reproductive factors like late age at menarche and higher parity, with endometriosis. On the contrary, infertility was strongly associated while OC showed no statistically significant association with endometriosis. For BMI, coffee consumption, smoking and alcohol consumption, no statistically significant associations were observed.

Interpretation

An inverse association between late age at menarche and endometriosis was found in the current study which is consistent with other studies (18, 20). It is described that women with early menarche have higher levels of serum and urine estradiol concentrations (135, 136) and higher level of urine estrone concentrations (136) during the follicular phase of the menstrual cycle. High levels of estradiol might have an influence on endometriosis (137-139) as estrogen has influence on growth, differentiation and function of eutopic endometrium which might explain why early menarche is associated with endometriosis (17, 140-143). One probable weakness could be uncertainty of the self-reported age at menarche but it would have also influenced the patients without endometriosis. About the inverse association of parity with endometriosis, our speculation is that women with endometriosis are less fertile and hence less prone to have two or more children. This result is in line with the previous studies (17, 19, 21, 144, 145). Strong association between infertility and endometriosis have been described previously (145, 146), but the knowledge about the causality is unclear. Our result did not show any statistically significant
association between OC and endometriosis in the adjusted analysis, which is consistent with some previous studies (15, 105). Previous studies have also reported a protective effect (106, 147) and an association with endometriosis (148, 149). Different results might be due to varying indications and duration of treatment in individuals. The strength of our study is that we only considered women using OC solely as contraceptive purpose. Our result on BMI showed no association with endometriosis which is not in line with other studies since, they have shown either positive or inverse associations (14, 24, 103, 150, 151). The weakness of our study is that women were asked about their BMI at interview and most probably they had a higher BMI then than when they were younger, because BMI increases with age (152). There was no statistically significant association between coffee consumption and endometriosis. A similar result was reported in a previous meta-analysis (107). We did not find any association between smoking and endometriosis, which is consistent with a previous large meta-analysis (110). There was no association between alcohol consumption and endometriosis. One previous meta-analysis showed an increased risk for endometriosis with alcohol consumption (26) but there was a limitation in evaluation as to whether exposure occurred before or after the development of endometriosis.

The strength of this study is the large population-based sample. One weakness is the possibility of reverse causality because it is a cross-sectional study, exposure and outcome was ascertained at the same time point hence we do not know whether exposure preceeded the development of the disease. One limitation for all endometriosis studies is that it is unknown whether asymptomatic women are free from the disease. One drawback could be the different ways of interpretation of a question by the respondents. Further, recall bias is also a problem with questionnaires if the respondents have to reply to some questions like symptoms and signs, lifestyle factors and other personal questions a long time after they had their experience.

Implications

The results from our study about a protective effect of late age at menarche on endometriosis and a strong association between infertility and endometriosis in a large population-based sample strengthen the previous knowledge and findings. Our results on OC showed neither a protective effect nor higher risk for the occurrence of endometriosis. Thus, we can neither oppose or support the use of OC for primary prevention of endometriosis.

6.1.3 Study III – Self-reported endometriosis is moderately acceptable

Findings

The predictive ability of self-reported endometriosis having an endometriosis diagnosis in IPR was good and was even better when additional information about age and infertility was added. Further, our results showed a high specificity for self-reported endometriosis and endometriosis-related questions and prevalence of endometriosis increased with age.
Interpretation

Endometriosis is a common gynecological disease. Good national registers are needed to perform clinical and epidemiological research. To our knowledge, this is the first study on the validity and reliability of self-reported endometriosis and endometriosis-related questions with data on endometriosis in IPR. On the contrary, previous studies have validated self-reported data on endometriosis with medical records with good agreement (3, 153). Our results showed good agreement between self-reported endometriosis in STR and endometriosis diagnosis in IPR and the predictive ability of self-reported endometriosis having an endometriosis diagnosis in IPR increased when there was additional information about age and infertility.

We investigated the frequency of endometriosis among those who responded positively to the questions on symptoms for example, severe dysmenorrhea, pelvic pain, and dyspareunia. In a previous study, prevalence of dysmenorrhea and dyspareunia were 62% and 55% respectively in women with endometriosis (68). However, we investigated the frequency of endometriosis in IPR among those who replied positively to the questions on severe dysmenorrhea and dyspareunia symptom in STR and the results were 20% and 6% respectively. To our knowledge, there is no such study published to date to compare our results on prevalence of endometriosis with history of severe dysmenorrhea and dyspareunia. We found a frequency of 9% endometriosis in IPR among those who responded positively to the question on pelvic pain symptom which is much lower compared to a review including only very small sample sized studies (154). The present study reported that 28% of infertile women received an endometriosis diagnosis in IPR, which is in line with the previous studies (2, 17).

Implications

This study concluded that the self-reported data may be useful in clinical and epidemiological studies, similar to what is described in other studies performed on other diseases (28, 29, 117, 118).
6.2 Methodological considerations

6.2.1 Study design

Cohort studies consist generally of a group of individuals who are followed over time and then the outcome, the occurrence of the disease is recorded. Prospective cohort studies are followed forward in time and then the outcome is recorded, while in retrospective cohort outcome has already occurred and the data on exposure and outcome is retrieved from the registers. Paper I-III are retrospective cohort studies. Individuals were identified from the STR. Data from STR were linked with data from IPR. In STR the exposure and outcome were collected at the same time point, at interview.

6.2.2 Internal validity

Random and systemic errors are the two major types of errors considered in epidemiological studies. Systemic error is also called bias and is divided into selection bias, information bias and confounding. Random errors are the variability in the data and can be decreased with increased sample size.

6.2.2.1 Selection bias

Selection bias refers to a selection of subjects included in the study. A selection bias might have been introduced if the association between exposure and outcome differs between participants and non-participants. Case-control studies are more prone to selection bias than cohort studies since selection bias can be minimized by collecting data prospectively.

Response rates in SALT were 65% and 74% for those twins born between 1886 and 1925 and 1926 and 1958 respectively. In STAGE the response rate was 59.6% for those twins born between 1959 and 1985. A selection bias could have been introduced if for instance more responders came from university hospitals and non-responders from county hospitals because there is variability in diagnosis, treatment regimens and resources between the two.

6.2.2.2 Information bias

An information bias error occurs when a variable is measured wrongly and places the subject in the wrong category and one should consider that misclassification has occurred. Misclassification can occur for both exposure and outcome and it can be differential or non-differential. A misclassification of exposure is differential if it is different for those with and without the outcome and non-differential if it is unrelated to the outcome. The same is true regarding misclassification of the outcome. Differential misclassification can either overestimate or underestimate an effect. Non-differential misclassification estimates the effect which is closer to a no-effect value than the actual effect.

In all three studies information on self-reported endometriosis diagnosis was retrieved from STR and we were not able to distinguish endometriosis from adenomyosis, which allows
for overestimation of the prevalence estimate for endometriosis. Here it is inevitable to introduce a misclassification. In study II we had several exposure variables and the true exposure time might be unclear and it is inevitable to introduce misclassification. However, it will be a non-differential misclassification, since the differences between cases and controls are not likely in this respect.

### 6.2.2.3 Confounding

Confounding is a problematic factor that is associated with both exposure and outcome but is not an effect of the exposure (Figure 4). The effect of one exposure is mixed with the effect of another exposure which leads to bias. It can cause either an overestimation or an underestimation of the effect. Prevention of confounding can be done by randomization and restriction. With randomization one can control unknown confounders, however, not with restriction. Further, by stratifying the data where the confounder is held constant within each stratum, prevention can be done for confounding. Furthermore, by matching distributions of factors between the cases and controls, prevention of known confounders can be achieved, which can be performed in both cohort and case-control studies. In regression analyses several probable (and measured) confounders can be included simultaneously and adjusted for.

In study II where we investigated the association between different risk factors or markers of risk for endometriosis, we controlled for age and zygosity, and also adjusted all the potential risk factors in the statistical analyses to avoid known confounding.

Figure 4 Theoretical model of a confounder acting on both exposure and outcome
6.2.3 Twin methodological issues

6.2.3.1 Equal environment assumption

Equal environment is one of the fundamental assumptions in twin methodology. It is assumed that the similarity caused by environment is mostly the same for both MZ and DZ twins reared in the same family. The assumption is violated if MZ twins are treated more similarly than DZ twins and the genetic effect will be inflated. Studies have indicated that the assumption is valid for attention deficit hyperactivity disorder (ADHD) and other psychiatric disorders (155, 156).

6.2.3.2 Additive genetic effect

The basic twin model often focuses on additive genetic effects, assuming that the effects of alleles at a locus and across loci are independent and, therefore, additive (124).

6.2.3.3 Non-additive genetic effect

The effect of alleles can interact with other alleles at the same locus (dominance) or at other loci (epistasis) (124). Non-additive genetic effects may be present if MZ correlations are more than half of DZ correlations.

6.2.3.4 Gene-environment interaction/correlation

The basic assumption of twin model is that the influence of genes and environment on a phenotype is independent. Genes and environment have direct effect. Further, there may be an interplay between genes and environment through gene-environment interactions and correlations (157). Individuals of the same genotype may respond differently to environments due to gene-environment interactions. The epigenetic disruption of gene expression plays an important role in the development of endometriosis through interaction with environmental changes (158). Individuals with different genotypes are selectively exposed to different environments due to gene-environment correlation.

In study I quantitative genetic modelling was performed under the assumption of classic twin design which is random mating that result in no gene-environment interaction due to equal environments in MZ and DZ twin pairs (126).

6.2.3.5 Generalizability from twins to singletons

Twins may differ from singletons regarding lifestyle characteristics and psychological development (159). In one study from UK compared certain diseases and prevalence of lifestyle characteristics between twins in the St Thomas’ Hospital UK adult twin registry consists of 600 MZ and 1,400 DZ female twin pairs and population-based singleton 1,003 women aged between 45 to 65 years reported that there were few or no differences regarding prevalence or variances for height, bone mineral density in the hip, osteoarthritis in the knee and hip, systolic blood pressure, anti-hypertensive pharmaceutical treatment,
menopausal status, history of hysterectomy and oophorectomy, and for overall tobacco and current alcohol consumption (132). Another study from Finland comparing twins with singletons consisting of 122 twins and 5455 singletons in the National Epidemiological Child Psychiatric study, reported no significant difference in the mean scores for hyperactivity (134). An Australian study reported that female twins are representative of the Australian population regarding age, general level of education, and marital status (133). Several studies have suggested that the results of twin studies can be generalized to singleton populations (132-134), but the interpretation of results of twin studies should be made carefully with special consideration to the nature of the sample.

6.2.4 Generalizability

All three studies in this thesis used data from Swedish female twin population. The magnitude of heritability of endometriosis and the associations of other variables with endometriosis were mostly similar to other previous studies. Anyhow, our findings are not exactly the same as other countries and thus, generalizations from the results of these studies should be made with caution.
7 CONCLUSIONS

- Our results suggest that both genetic and environmental factors have influence on the complex etiology of endometriosis. The prevalence of endometriosis was estimated to be 4.3%. Self-reported endometriosis could be validated with medical records in 82% of cases.

- Infertility was found to be strongly associated with endometriosis but the knowledge about the causality is unclear. This finding motivates further research on causality and whether they have a common genetic origin.

- Inverse association between late age at menarche and endometriosis was observed and may be considered as having a protective effect on endometriosis. Parity with two or more children was inversely associated with endometriosis and we speculate that women who have endometriosis are less fertile and are thus less prone to have two or more children. These findings highlight the need to increase awareness in clinical practice.

- Use of oral contraceptives (OC) solely for contraceptive purpose did not show any significant association with endometriosis. Based on the results of this study, we can neither oppose or support the use of OC for primary prevention of endometriosis.

- Good predictive ability of self-reported endometriosis having a confirmed endometriosis diagnosis in the IPR was found in our study. The predictive ability increases with additional information on age and infertility. Thus, self-reported data on endometriosis may be useful for clinical and epidemiological studies when register data are not available.
8 FUTURE PERSPECTIVES

8.1 Can we find genes that cause endometriosis?

It has been suggested that genes have a strong influence on the development of endometriosis. Previous studies on European and Japanese populations have identified several loci, but the definite genetic variants causal to endometriosis are not yet defined. It has been suggested that several gene variants with small effects are likely to be involved in the etiology of endometriosis. Studies have suggested a role for gene-gene and gene-environment interactions in the occurrence of endometriosis (160, 161), but the results are in a very preliminary stage. To assess gene-gene and gene-environment interactions robustly without a strong prior hypothesis, well-designed studies in even larger samples than used for GWAS studies are necessary. Endometriosis research groups worldwide need to acquire similar strategies and work collectively to combine data to identify genes contributing to the disease. Future studies should also focus on including cases with detailed endometriosis subtypes. Successful evidence for genes associated with endometriosis may open up the possibility for functional and biological studies which may further develop better diagnosis and treatment for this debilitating disease. Further, research in animal models for example the rhesus and the baboon might add knowledge about the genetics of endometriosis.

8.2 Do endometriosis and infertility share a common cause?

Studies have shown up to 50% association of infertility with endometriosis and the vice versa. We do not know so far, whether they are sharing a common cause or which one of them causes the other. They may share a common genetic effect and further studies need to investigate this issue.

8.3 Can we improve the accuracy of self-reported endometriosis diagnosis?

By including highly structured detailed questionnaires focusing on symptoms, signs, subtypes of endometriosis, we may probably improve the accuracy of self-reported endometriosis.

8.4 How can we perform good epidemiological studies on endometriosis?

Previous epidemiological studies have mostly been performed with small sample sizes. They have also been poorly-designed and mostly with retrospective nature. To minimize the limitations we should in the future focus on well-designed prospective studies with more information about confounders. Future studies should also focus on other potential risk factors or markers of risk for endometriosis which were not previously studied for example environmental factors or exposure to drugs. The possibility of linkages between
drug registers and in-patient register should be considered for future studies on exposure to drugs.

The Swedish national registers are gold mines for performing epidemiological studies. We need to establish a national register for endometriosis with hospital discharge diagnoses, diagnoses from day surgery department, clinical diagnoses in the outpatient department and highly structured self-reported questionnaire which women should respond to before clinical and surgical diagnosis and treatment.

Denna avhandling syftar till att dels uppskatta förekomsten av endometrios och ärflichkeitens betydelse (Studie I), dels effekterna av reproduktiva och livsstilsfaktorer på förekomsten av endometrios (Studie II) och slutligen överensstämelsen av självrapporterade endometrios och endometriosrelaterade frågor med endometriosdiagnos i slutenvårdsregistret (Studie III) bland svenska kvinnliga tvillingar. Studiepopulationen utgjordes av alla kvinnliga tvillingar i svenska tvillingregistret, i åldern 20-65 år i Studie I och II och i åldern 20-60 år i Studie III. Prevalensen av endometrios bland svenska tvillingar uppskattades vara 4,3%. Ärflichkeitens av endometrios förklaras med 47% och resten av effekten förklaras av miljöfaktorer. Högre ålder vid menarche och högre paritet med två eller fler barn medförde lägre risk att diagnostiseras med endometrios, medan infertilitet hade ett starkt positivt samband med endometrios. Användning av p-piller enbart som preventivmedel visade inget signifikant samband med endometrios. Faktorer som BMI, utbildningsnivå och andra livsstilsfaktorer som kaffe, rökning och alkohol visade heller inte signifikant samband med endometrios. God överensstämmelse fanns mellan självrapporterade endometrios och endometriosdata i slutenvårdsregistret och sannolikheten att förutsäga en endometriosdiagnos i slutenvårdsregistret ökade när det också fanns information om ålder och infertilitet.

Sammanfattningsvis, resultaten från studierna i denna avhandling tyder på stark genetisk inverkan på utvecklingen av endometrios. Infertilitet och endometrios är starkt associerade till varandra, men inget kausalsamband har kunnat påvisas. Studierna har också visat att enkäter med endometriosspecifika frågor förefaller vara ett tillförlitligt instrument i kliniska och epidemiologiska studier.
First and foremost, I wish to express my sincere thanks to all those amazing women, suffering from endometriosis, who volunteered to share their information to make this project possible. I would also like to express my appreciation for the financial support received from Karolinska Institutet and my thanks to the Swedish Research Council, the Ministry for Higher Education and Astra Zeneca for their support to the Swedish twin registry.

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