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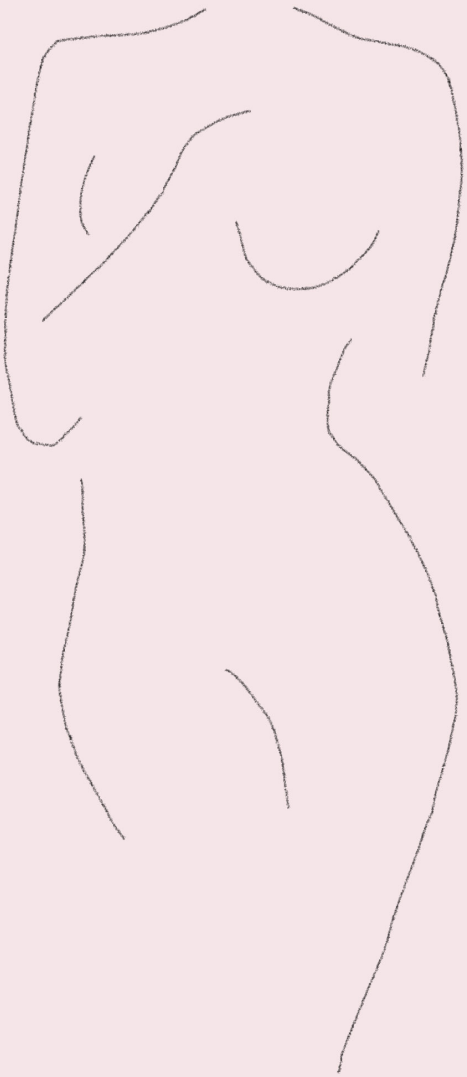

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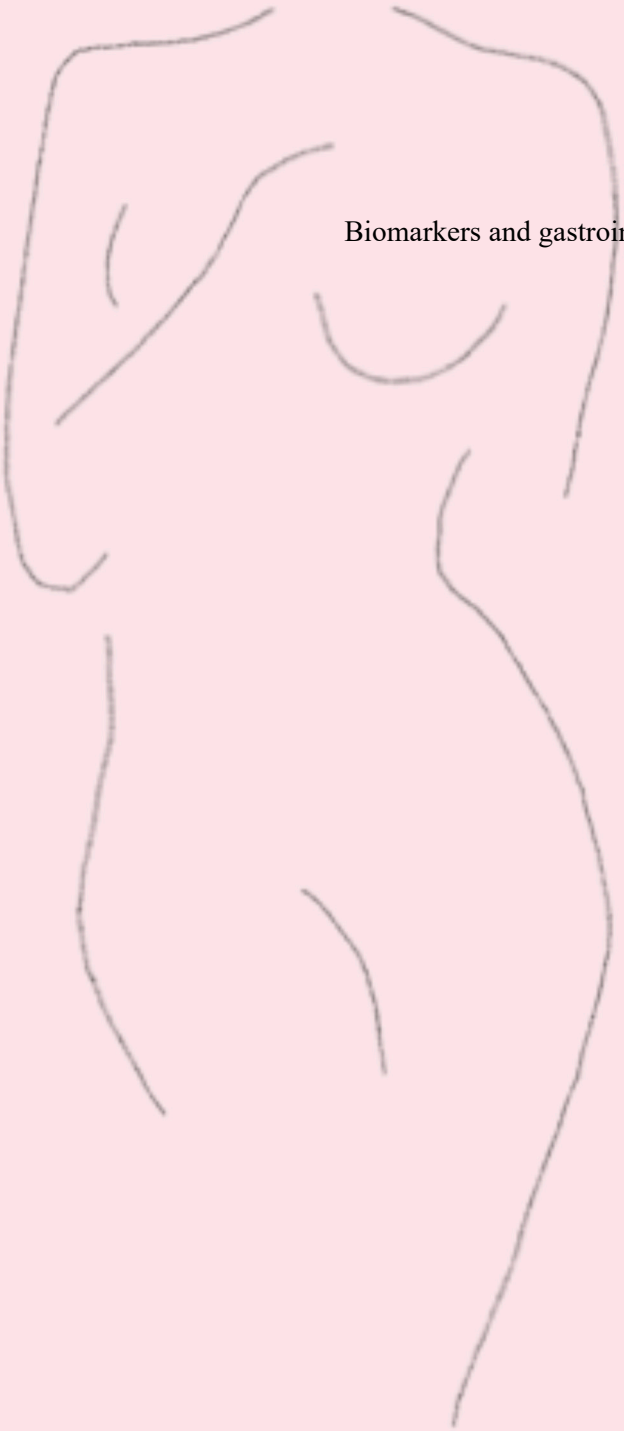

Biomarkers and gastrointestinal symptoms in endometriosis

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Agnes Petersson

LUND
UNIVERSITY

DOCTORAL DISSERTATION
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Abstract:

Endometriosis is a highly prevalent gynecological disease that often causes gastrointestinal symptoms. Given the diagnostic delay of several years, more effective methods for diagnosis are crucial to reduce the disease burden for these women.

This thesis investigated differences between endometriosis and IBS by comparing sociodemographic factors, lifestyle habits, gastrointestinal symptoms and biomarkers. Two cohorts of patients with endometriosis were included: 172 women with surgically confirmed diagnoses and 81 diagnosed by transvaginal ultrasound. Women from the general population and healthy controls served as controls, and women with IBS were used for comparisons of gastrointestinal symptoms and autoantibodies. Questionnaires regarding sociodemographic factors, lifestyle habits and symptoms were completed. Blood and fecal samples were collected. The gut microbiota, polygenic risk scores and autoantibodies were analyzed and evaluated as potential biomarkers for endometriosis.

Differences in sociodemographic and lifestyle factors between endometriosis and IBS were limited. Gastrointestinal symptoms were more aggravated in IBS and different initial triggering factors were identified. Both alpha- and beta diversity of the gut microbiota were higher in controls than endometriosis patients. The abundances of several bacteria differed between the groups. Some associations between PRS and localization of endometriosis and hormone treatment were observed. Thyroid-stimulating hormone receptor antibodies (TRAb), both IgG and IgM, were increased in endometriosis compared with controls, in one study. None of the other analyzed autoantibodies were elevated, indicating that the results were not caused by cross-reactivity. However, the results of higher TRAb IgG levels could not be confirmed when analyzed using updated clinical methods.

These results show that, compared with controls, women with endometriosis have an aberrant microbiota. TRAb is a potential biomarker for endometriosis, but current tests in the clinic cannot be used to detect elevated levels in endometriosis. In future research, further evaluation of potential biomarkers, including TRAb and the gut microbiota, would be valuable.

Key words: Endometriosis, gastrointestinal symptoms, gut microbiota, PRS, TRAb, biomarkers

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To my family

Table of Contents

Abstract

Endometriosis is a highly prevalent gynecological disease that often causes gastrointestinal symptoms. Given the diagnostic delay of several years, more effective methods for diagnosis are crucial to reduce the disease burden for these women.

This thesis investigated differences between endometriosis and IBS by comparing sociodemographic factors, lifestyle habits, gastrointestinal symptoms and biomarkers. Two cohorts of patients with endometriosis were included: 172 women with surgically confirmed diagnoses and 81 diagnosed by transvaginal ultrasound. Women from the general population and healthy controls served as controls, and women with IBS were used for comparisons of gastrointestinal symptoms and autoantibodies. Questionnaires regarding sociodemographic factors, lifestyle habits and symptoms were completed. Blood and fecal samples were collected. The gut microbiota, polygenic risk scores and autoantibodies were analyzed and evaluated as potential biomarkers for endometriosis.

Differences in sociodemographic and lifestyle factors between endometriosis and IBS were limited. Gastrointestinal symptoms were more aggravated in IBS and different initial triggering factors were identified. Both alpha- and beta diversity of the gut microbiota were higher in controls than endometriosis patients. The abundances of several bacteria differed between the groups. Some associations between PRS and localization of endometriosis and hormone treatment were observed. Thyroid-stimulating hormone receptor antibodies (TRAb), both IgG and IgM, were increased in endometriosis compared with controls, in one study. None of the other analyzed autoantibodies were elevated, indicating that the results were not caused by cross-reactivity. However, the results of higher TRAb IgG levels could not be confirmed when analyzed using updated clinical methods.

These results show that, compared with controls, women with endometriosis have an aberrant microbiota. TRAb is a potential biomarker for endometriosis, but current tests in the clinic cannot be used to detect elevated levels in endometriosis. In future research, further evaluation of potential biomarkers, including TRAb and the gut microbiota, would be valuable.

Populärvetenskaplig sammanfattning

Endometrios är en gynekologisk sjukdom som orsakas av kronisk inflammation till följd av att livmoderslemhinna växer utanför livmodern. Det kan förekomma i form av till exempel cystor på äggstockarna eller påväxt på bukväggen, urinblåsan eller tarmarna. Endometrios är en sjukdom som drabbar upp till var tionde kvinna i fertil ålder, vilket innebär att cirka 250 000 kvinnor i Sverige är drabbade. Sjukdomen är godartad men kan orsaka besvärliga symptom i form av smärtor, mag-tarmbesvär, menstruationsrubbningar och ofrivillig barnlöshet. Orsaken till uppkomsten och utvecklingen av endometrios är inte helt kartlagd.

Det finns idag inget godkänt blodprov som kan visa om man har sjukdomen. Tidigare har en definitiv diagnos krävt undersökning med titthålskirurgi men idag har riktlinjerna ändrats till att i första hand använda ultraljud, och i vissa fall MRI. Eftersom symptomen för endometrios kan variera mycket mellan olika patienter och ofta misstas för mensvärk, IBS eller andra sjukdomar, är fördröjningen till rätt diagnos vanligtvis lång.

Syftet med den här avhandlingen var att karakterisera mag-tarmbesvär och sociodemografiska drag hos patienter med endometrios samt undersöka potentiella biomarkörer för sjukdomen. Totalt i avhandlingen har 172 kvinnor med kirurgiskt diagnostiserad endometrios och 81 kvinnor med ultraljudsverifierad endometrios deltagit. Samtliga har svarat på frågeformulär och lämnat blodprover, medan en del även lämnat avföringsprover. Som jämförelse har patienter med IBS, friska kontroller och kontroller från den allmänna befolkningen använts.

I delarbete 1 har vi jämfört sociodemografiska faktorer och magtarmsymptom hos patienter med endometrios och IBS. Skillnaderna i sociodemografi och livsstil visade sig vara begränsad mellan de två grupperna. Patienterna med IBS hade mer symptom vad gäller smärta, diarré, förstoppning, uppspändhet, illamående och inverkan på det dagliga livet än patienterna med endometrios. Det visade sig även finnas tydliga skillnader i vad som initialt triggat i gång symptomen där menstruationsdebut var vanligast vid endometrios medan stress, infektion eller antibiotikabehandling var vanligare vid IBS.

Bakteriefloran i tarmen har identifierats som en bidragande faktor i många sjukdomar och associationer har setts även till endometrios. Därför tittade vi i delarbete 2 på vilka skillnader i tarmflora som finns mellan patienter med endometrios och den allmänna befolkningen. Vi kunde se att mångfalden av bakterier var högre i befolkningen än hos de med endometrios. Flera olika bakterier visade olika riklig förekomst mellan grupperna.

I delarbete 3 undersökte vi patienter med endometrios avseende deras genotyp och genetisk riskpoäng, så kallad polygenic riskscore (PRS), beräknades. Vi undersökte associationer mellan PRS och kliniska fynd såsom typ av endometrios, symptom

och behandling. Det fanns vissa associationer mellan PRS och spridning av endometrios, involvering av magtarmkanalen samt hormonbehandling.

I tidigare studier har man sett att nivåerna av antikroppar mot sköldkörtelreceptorn som kallas TRAK IgG verkar vara förhöjda i blodet hos patienter med endometrios. I delarbete 4 tittade vi på ett större antal antikroppar inom samma familj för att se att de förhöjda nivåerna inte kunde förklaras av en korsreaktion vid analysen, vilket vi kunde bekräfta inte var fallet. I delarbete 5 ville vi bekräfta de tidigare resultat som visat att TRAK IgG är förhöjda vid endometrios, vilket gjordes genom att analysera prover från nya patienter på två olika laboratorier. Jämfört med tidigare hade analysmetoderna ändrats och resultaten kunde inte bekräftas. Tolkningen är att analysmetoderna inte är tillräckligt känsliga för att användas i detta syfte.

Ytterligare forskning behövs för att utreda om fynden i avhandlingen verkligen skiljer sig hos patienter med endometrios och kan användas i kliniken. Att hitta en kliniskt användbar biomarkör skulle vara till stor nytta för patienter med endometrios då det kan leda till att kvinnor med hög sannolikhet för endometrios snabbt kan identifieras och remitteras för vidare utredning.

List of Papers

Paper I

Agnes Petersson, Bodil Roth, Ligita Jokubkiene, Povilas Sladkevicius, Bodil Ohlsson, Comparison of sociodemographic factors, lifestyle, and gastrointestinal symptoms between patients with endometriosis and IBS. Submitted.

Paper II

Agnes Svensson, Louise Brunkwall, Bodil Roth, Marju Orho-Melander and Bodil Ohlsson, Associations Between Endometriosis and Gut Microbiota. Reprod Sci, 2021. 28(8): p. 2367-2377.

Paper III

Agnes Svensson*, Koldo Garcia-Etxebarria*, Anna Åkesson, Christer Borgfeldt, Bodil Roth, Malin Ek, Mauro D'Amato and Bodil Ohlsson, Applicability of polygenic risk scores in endometriosis clinical presentation. BMC Womens health, 2022. 22 (1): p. 208.

* Shared first authorship.

Paper IV

Agnes Svensson, Bodil Roth, Linnea Kronvall and Bodil Ohlsson, TSH receptor antibodies (TRAb) - A potential new biomarker for endometriosis. Eur J Obstet Gynecol Reprod Biol, 2022. 278: p. 115-121.

Paper V

Agnes Petersson, Bodil Roth, Charlotte Becker and Bodil Ohlsson, Elevated levels of TRAb IgG autoantibodies are not recognized in endometriosis by current clinical methods. Submitted.

Related articles by the author

Agnes Petersson, Bodil Roth, Ligita Jokubkiene, Povilas Sladkevicius and Bodil Ohlsson, Differences in circulating AXIN1 between endometriosis and IBS are influenced by the tests used. A cross-sectional study. Submitted.

Author's contribution to the papers

Paper I

Conceptualization of the project. Data processing. Statistics. Interpretation of data. Writing, original draft. Reviewing and editing including all communication with the journals.

Paper II

Writing, original draft. Reviewing and editing.

Paper III

Statistics. Interpretation of data. Writing, original draft. Reviewing and editing including all communication with the journals and reviewers.

Paper IV

Conceptualization of the project. Data processing. Statistics. Interpretation of data. Writing, original draft. Reviewing and editing including all communication with the journals and reviewers.

Paper V

Conceptualization of the project. Data processing. Statistics. Interpretation of data. Writing, original draft. Reviewing and editing including all communication with the journals.

Abbreviations

AUC	Area under the curve
BD	Blood donor
BMI	Body mass index
BSA	Bovine serum albumin
hCG	Human chorionic gonadotropin
ECLI	Electro-chemiluminescence immunoassay
ELISA	Enzyme-linked immunosorbent assay
FSH	Follicle-stimulating hormone
FSHR	Follicle-stimulating hormone receptor
GI	Gastrointestinal
GnRH	Gonadotropin-releasing hormone
GWAS	Genome wide association study
IBS	Irritable bowel syndrome
IBS-SSS	Irritable bowel syndrome severity scoring system
IQR	Interquartile range
LH	Luteinizing hormone
LHR	Luteinizing hormone receptor
MOS	Malmö Offspring Study
MRI	Magnetic resonance imaging
PRS	Polygenic risk score
ROC	Receiver operating characteristic
RU	Relative units
SD	Standard deviation
TRAb	Thyroid-stimulating hormone receptor antibody
TSH	Thyroid-stimulating hormone
VAS-IBS	Visual analogue scale for irritable bowel syndrome

Introduction

Endometriosis

Endometriosis is a benign gynecological disease characterized by the presence of endometrial-like cells and stroma located outside the uterus. Lesions are most commonly found on the pelvic peritoneum, the ovaries and in the rectovaginal septum [1]. The prevalence of endometriosis varies across studies and depends on the diagnostic methods. Estimates typically range from 2 to 10% within the female population. Recently, a systematic review estimated that the overall prevalence was 18% [2].

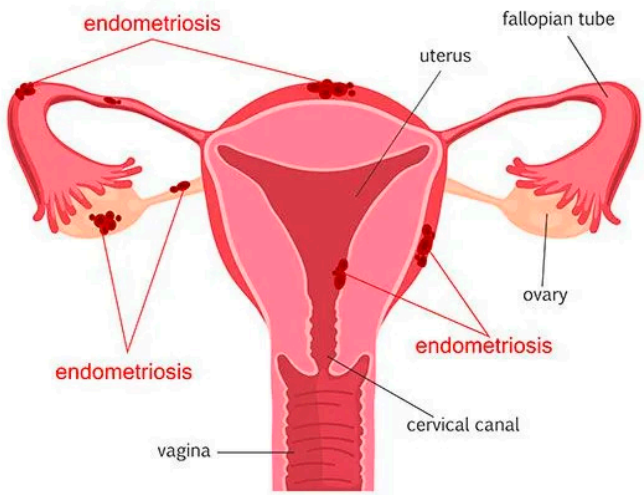

Figure 1. Female internal reproductive organs with possible localizations of endometriotic lesions. Image source: Adobe Stock.

Symptoms and presentation of the disease

In general, pain is the most apparent symptom of endometriosis. It usually begins with severe menstrual cramps at the beginning of the menstrual phase. For some patients, the number of days with pain increases, leading to constant pain and chronic pain syndrome due to pain sensitization. Endometriosis can also cause symptoms such as deep dyspareunia, back pain, and symptoms associated with the bladder and bowel [3]. GI symptoms have been reported in 90% of women with endometriosis. Since only 7.5% of the women had established endometriosis located to the bowel, the GI symptoms seem to be mainly independent of the localization of lesions [4]. What causes GI symptoms in patients with endometriosis is not fully understood. Visceral hypersensitivity has been found to be common in endometriosis patients, which could intensify pain and explain why symptoms often not are proportionate to disease extent [5]. Inflammatory activity caused by endometriosis lesions, comorbidity with IBS and endometriosis lesions involving the bowel are other explanations presented [6]. This disease is a common cause of infertility, which can be observed in 25% of women with endometriosis [7].

Diagnosis

For many years, laparoscopic visualization with histopathological confirmation has been considered the gold standard for the diagnosis of endometriosis. However, recent guidelines recommend a nonsurgical diagnosis based on anamnesis, physical examination and medical imaging [8]. This recommendation is based on the recognition that surgery not only involves risks but can also lead to long diagnostic delays. Several studies have reported an overall diagnostic delay of 4–10.4 years from the onset of symptoms to diagnosis [9, 10]. The three subtypes of endometriosis include superficial disease, deep infiltrative disease, and endometriomas, where the first is difficult to detect with imaging techniques [11]. Since 2022, transvaginal ultrasound has been considered gold standard in diagnosing endometriosis [8]. Transvaginal ultrasound can be used to identify endometriomas and deep endometriosis involving the bowel, bladder or ureter. MRI is not recommended as a primary investigation in patients with suspected endometriosis. However, it may be useful for assessing the extent of deep endometriosis. In patients with normal findings upon clinical examination, ultrasound and MRI, the possibility of endometriosis should not be excluded if a clinical suspicion remains [12].

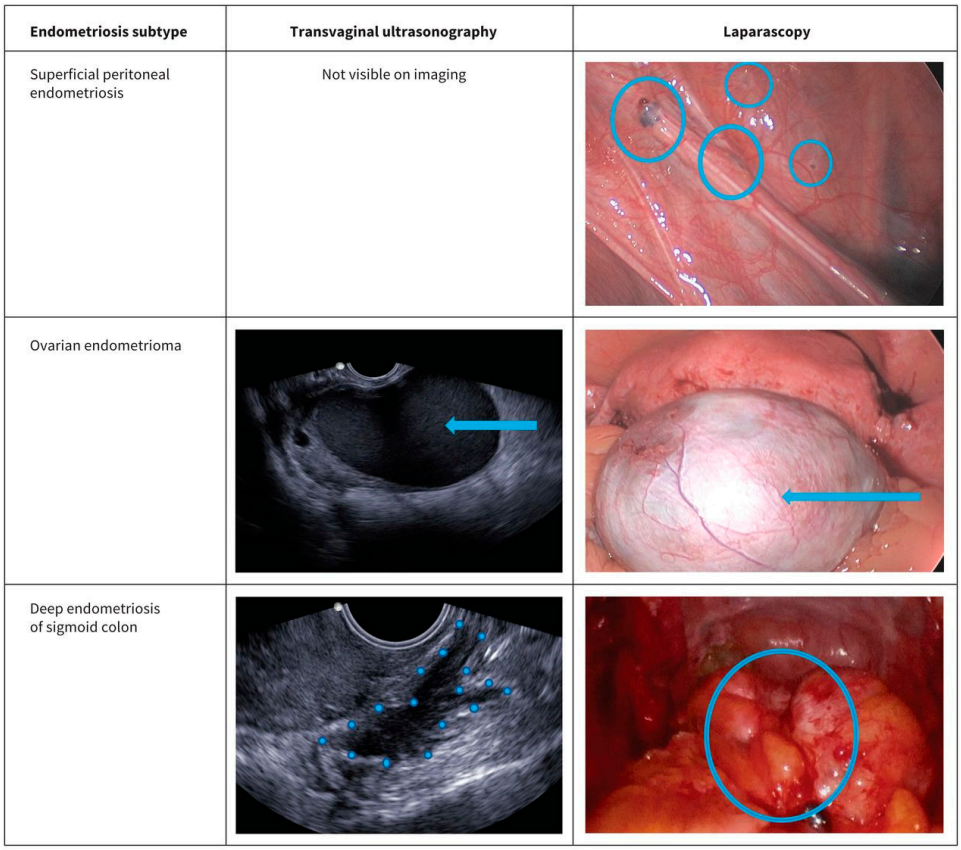

Figure 2. Imaging and laparoscopic appearance of endometriosis subtypes. Reproduced from Allaire et al. CMAJ. 2023: E363-E371.

Biomarkers

A biomarker is defined as a specific characteristic, often biological, that is measured as an indicator of a physiological process or a pathological condition or to assess the effects of an intervention or treatment. Several markers, including glycoproteins, angiogenetic factors, oxidative stress markers, inflammatory proteins, hormone-related factors, miRNA markers, DNA markers and the microbiota, have been tested as potential biomarkers for endometriosis [13]. A Cochrane study from 2016, including 54 studies, concluded that currently no biomarker candidates can be considered diagnostic tools for endometriosis in clinical practice [14]. Most biomarkers were assessed in only single studies, and a meta-analysis could be performed for only PGP 9.5 and CYP 19. Currently, biomarkers are not recommended for diagnosing endometriosis [15].

Treatment

Many different guidelines have been published to help clinicians treat endometriosis. The main goal is to improve pain symptoms, limit the growth of the lesions and increase fertility. First-line treatments for suspected or verified symptomatic endometriosis include combined oral contraceptives and progesterone. Second-line treatments include gonadotropin-releasing hormone agonists (GnRH agonists) and intrauterine devices (IUDs) [16]. Hormonal treatment is often combined with analgesics such as paracetamol, nonsteroidal anti-inflammatory drugs (NSAIDs) and, in some cases, opioids [17].

In patients who do not respond to conservative treatment, surgery is an option. If possible, laparoscopic surgery is always preferred before laparotomy [18]. Conservative surgery, which aims to preserve fertility, includes the excision or ablation of lesions, division of adhesions and pelvic nerve interruption. Definitive surgery, which generally involves hysterectomy with or without oophorectomy, is thought to be more effective over time; however, this procedure is no guarantee of pain relief [19].

Complementary therapies such as acupuncture and transcutaneous electrical nerve stimulation (TENS) have both been shown to reduce chronic pelvic pain and deep dyspareunia in women with deep endometriosis [20]; however, further studies are needed to elucidate their roles in the clinic.

Pathogenesis and pathophysiology

The etiology and pathology of endometriosis are not fully known. In 1927, Sampson presented the theory of retrograde menstruation, which is still widely supported [21]. He proposed that blood containing endometrial cells was passed backward to the pelvic cavity through the fallopian tubes during menstruation. However, 90% of women with patent tubes have evidence of blood in their peritoneal fluid during perimenstrual period, indicating that retrograde menstruation is a very common physiological event [22]. The fact that only a minority of women with retrograde menstruation develop endometriosis suggests that other mechanisms are involved in lesion development, and several different theories have been proposed [23]. The coelomic metaplasia theory states that cells lining the visceral and abdominal peritoneum differentiate in situ into endometrial tissue. Another theory is the Mullerian rest theory, which states that residual cells migrating from the embryologic Mullerian duct develop into endometriotic lesions when stimulated by estrogen [23]. Additionally, other theories posit that endometrial tissue originates from the differentiation of stem cells, which are disseminated from the bone marrow [24].

The hereditability of endometriosis has been estimated to be approximately 50% based on twin studies [25, 26]. Genome-wide association studies (GWASs) can be

used to identify genetic variants underlying a disease. Endometriosis GWASs have identified several genomic regions and variants associated with the endometriosis risk [27]. These regions are related to estrogen-induced cell growth, cell differentiation, intracellular adhesion, hormone receptors, inflammatory cytokines, and cell damage. In addition, epigenetic modifications play a definite role in the development of endometriosis [28].

The gut microbiota and endometriosis

The gastrointestinal (GI) tract is a complex system characterized by the symbiosis of gut mucosal cells, the immune system, food molecules and microorganisms. It is a dynamic environment, and the microbiota is constantly changing. The development of 16S ribosomal RNA (rRNA) sequence identification has provided insights into the diversity of the gut microbiota. An analysis of 16S rRNA sequences allows the identification of species and determination of operational taxonomic units (OTUs). The 97% sequence identity of 16S rRNA is often considered a good approximation to species [29]. Bacteria are classified into groups and subgroups according to kingdom, phylum, class, order, family, genus and species (Figure 3). Over 1500 species of bacteria belonging to over 50 different phyla reside in the intestines [30]. A culture-independent analysis revealed that the gut microbiota is dominated by Bacteroidetes and Firmicutes, followed by Actinobacteria, Fusobacteria, Proteobacteria, Tenericutes and Verrucomicrobia [31, 32].

Sequencing of the 16S rRNA revealed that the vast majority of bacteria belong to three bacterial groups: Bacteroides, Clostridia cluster IV and Clostridia cluster XIVa [33]. Clostridia are gram-positive rods in the phylum Firmicutes [34]. We are colonized with commensal Clostridia from early infancy throughout life, and they participate in maintaining well-functioning metabolic, physiologic and immune processes in our intestines. Clostridia strongly contribute to maintaining a normal gut function but are also involved in the development of dysbiosis. Some Clostridia are pathogenic, such as *Clostridium perfringens* and *Clostridium tetani* in cluster I and *Clostridium difficile* in cluster XI. However, most of the Clostridia in our GI tract are commensals [34].

The gut microbiota plays major roles in the maintenance of health and the development of disease [35]. The gut microbiota, through the inflammatory and metabolic changes it induces, has been shown to affect conditions both inside and outside the GI tract. Strong evidence is available for an association between an imbalance in the microbiota composition, known as dysbiosis, and diseases such as arthritis, inflammatory bowel disease (IBD) and colon cancer [36, 37]. Previous studies in animal models and patients with endometriosis have shown dysbiosis in the gut [38]. The gut microbiota has been shown to affect estrogen levels and estrogen-dependent diseases [39, 40]. Systemic levels of estrogen in postmenopausal women are strongly associated with fecal microbiome richness and

fecal levels of Clostridia [25]. Higher estrogen levels stimulate epithelial proliferation in the female reproductive tract and have been shown to drive diseases such as endometriosis and endometrial cancer [41].

The gut microbiota may also affect other mechanisms involved in the pathogenesis of endometriosis. Recent studies have shown that the gut microbiota is a major regulator of inflammatory processes outside the GI tract [42]. For example, the gut microbiota affects the activity level of IL-17 producing CD4+ T lymphocytes [43]. The levels of IL-17 are significantly higher in patients with mild endometriosis than in those with moderate/severe endometriosis or healthy women, suggesting that IL-17 plays a role in the pathogenesis of endometriosis [44]. Due to the impact of immunological changes in patients with endometriosis and the impact of the gut microbiota on immune responses, researchers have hypothesized that the gut microbiota is involved in the pathogenesis of endometriosis [11].

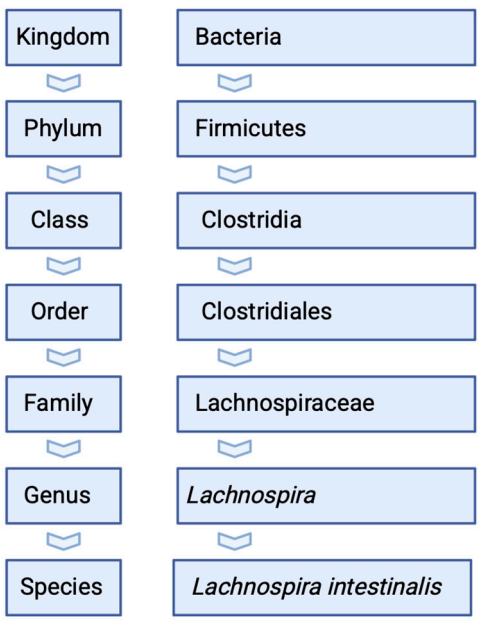

Figure 3. Example of bacterial taxonomic classification.

Polygenic risk scores

The interest in risk models has increased over the years and genetic risk variants for various diseases are being discovered through GWASs [45, 46]. GWASs is used in genetics research and test thousands of genetic variants to identify those who are statistically associated with a disease. Since single risk loci usually have a low impact on disease risk, combining the effects of multiple risk variants has become a way to predict the risk more accurately [47, 48]. One commonly used score is the polygenic risk score (PRS), which combines allelic variations of single nucleotide polymorphisms (SNPs) derived from GWASs [49]. In endometriosis, approximately 26 % of the polygenic risk is explained by SNPs [50]. There are several GWASs for endometriosis, reporting genetic variants involved in sex steroid hormone pathways and development of the female reproductive tract [51]. PRS derived from GWASs has been associated with endometriosis, and the subtypes ovarian, infiltrating and superficial [52].

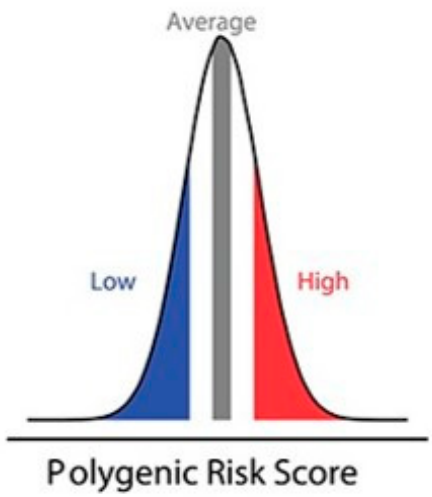

Figure 4. Representative density plot of a population according to the polygenic risk score. The figure is labelled according to the lowest (0–20%), population average (40–60%) and highest (80–100%) quintiles of genetic risk.

Thyroid disease and endometriosis

Thyroid disease occurs more frequently in women than in men, which correlates with the autoimmune nature of many thyroid diseases. Different thyroid disorders can disturb menstruation and ovulation [53]. Hyperthyroidism can cause oligomenorrhoea, whereas hypothyroidism can manifest as menorrhagia or oligomenorrhoea, infertility or miscarriage. Several autoimmune disorders, including thyroid disorders such as Hashimoto's thyroiditis and Graves' disease, have been reported by some authors to be associated with endometriosis [54-57]. However, compared with that in the general population, the prevalence of thyroid disorders in patients with endometriosis is not increased according to one study [58].

Since endometriosis is considered a chronic inflammatory process, the increased prevalence of autoimmune thyroid disorders could be linked to the immune dysregulation in patients with endometriosis [59]. Moreover, thyroid dysfunction may affect the development of endometriosis. Thyroid hormone action in humans is mediated by receptor binding. Binding sites for thyroid hormones have been found in different human tissues, including the brain, heart, liver, lung, kidney and pancreas [60]. The thyroid-stimulating hormone (TSH) receptor mRNA and protein are highly expressed in the ovarian surface epithelium in humans. TSH thereby stimulates the endometrium to produce thyroid hormones, with function as a site for extrathyroidal hormone production [61, 62]. The development of multicystic ovaries during profound hypothyroidism has been reported [63], and a mouse study showed that endometriotic implants grow in the presence of increased thyroid hormone levels [58]. A previous study reported that the serum levels of thyroid-stimulating hormone receptor antibody (TRAb) IgG exceed the detection limit of 0.3 IU/L in 93.0% of patients with endometriosis compared with 7.9% in the general population [64]. Only TRAb levels under or in grey-zone values were associated with endometriosis, not levels above the cut-off value for thyroid disease.

Irritable bowel syndrome

IBS is a disease of the gut–brain interaction (DGBI), in which recurrent abdominal pain is associated with defecation or a change in bowel habits [65]. Estimates of the global prevalence vary from 1% to 25%, with a pooled prevalence of 3.8% [66]. Prevalence rates are higher for women than for men, and individuals younger than 50 years are more commonly affected [67].

Diagnostic criteria for IBS

IBS is clinically diagnosed according to the Rome IV criteria [68]. The prevalence is lower according to the updated criteria of Rome IV (3.8%) compared with the previously used Rome III (9.2%). Differences in Rome III and Rome IV criteria are presented in Table 1. According to Rome IV, the diagnosis is made if a patient has experienced abdominal pain ≥1 day/week in the last 3 months, related to at least two of the following characteristics: related to defecation, associated with a change in the frequency of stool, and associated with a change in the form of stool. The disease is divided into four subtypes based on the predominant pattern of bowel habits: constipation-predominant IBS (IBS-C), diarrhea-predominant (IBS-D), mixed IBS (IBS-M) and unspecified IBS (IBS-U). The subtype is determined by the Bristol stool form scale [69].

Table 1. Diagnostic criteria for IBS according to Rome III and Rome IV.

Rome III	Rome IV
Recurrent abdominal pain or discomfort for at least 3 days per month in the last 3 months, associated with 2 or more of the following criteria:	Recurrent abdominal pain, on average, at least 1 day per week in the last 3 months, associated with 2 or more of the following criteria:
1. Improvement with defecation	1. Related to defecation
2. Onset associated with a change in frequency of stool	2. Associated with a change in frequency of stool
3. Onset associated with a change in form (appearance) of stool	3. Associated with a change in form (appearance) of stool
Criteria fulfilled for the last 3 months with symptom onset at least 6 months prior to diagnosis	Criteria fulfilled for the last 3 months with symptom onset at least 6 months prior to diagnosis

Extraintestinal symptoms in IBS

Although IBS is characterized by abdominal pain and altered bowel habits, extraintestinal manifestations are common in this group of patients. The prevalence of extraintestinal syndromes or symptoms have been shown to be much higher in IBS than in healthy controls or in patients with organic GI diseases. About 50% of patients with IBS have some sort of additional somatic or mental symptom [70]. The most reported extraintestinal symptoms in patients with IBS are back pain, pelvic pain, fatigue, fibromyalgia, headache, sleep difficulties and urogenital symptoms [71]. Pelvic pain causes many patients with IBS to seek gynaecological care, without any findings of gynaecological diagnoses, and several studies have shown IBS to be associated with gynaecological symptoms such as dyspareunia and dysmenorrhea [72]. GI symptoms in IBS vary over the phases of the menstrual cycle, with worsening of constipation during the luteal phase and overall increasing symptoms during the menstrual phase [73]. Chronic fatigue is most common in females and younger patients with IBS, and impacts GI symptoms, psychological well-being and quality of life [74]. The prevalence of IBS is estimated to be 35-92% in patients diagnosed with chronic fatigue syndrome [75, 76]. Results show that the more extraintestinal symptoms and psychiatric comorbidity patients with IBS have, the more IBS symptoms they have and the harder it gets to successfully treat their GI symptoms. Patients with IBS attend healthcare twice as much as controls, and most of their healthcare visits are caused by extraintestinal symptoms [77].

Pathogenesis and pathophysiology

The pathogenesis and pathophysiology of IBS are complex and still not fully known. It is considered a functional disorder, since no structural or biochemical abnormalities have been identified. IBS is a heterogenous disorder, and the pathogenesis appears to be multifactorial. Several potential disease-contributing factors have been identified, and research has focused on gut–brain signalling, the gut microbiota, visceral hypersensitivity, disturbed intestinal motility, immunological factors, psychological factors, and food hypersensitivity [78]. Depression and anxiety affect up to one-third of patients with IBS, and results indicate that there are bidirectional gut–brain and brain–gut pathways [79, 80] (Figure 5). In approximately half of the patients, IBS seems to be developed primarily, suggesting that disturbance in the gut function is contributing to the development of the mood disorder [81].

Several environmental factors are associated with IBS, such as stress, food intolerance, antibiotic treatment and GI infection [82, 83]. Disturbance in intestinal motility, with increased or decreased gut transit time and irregular bowel contractions, is described in some patients with IBS [84]. The role of microbiota in IBS is debated, but alterations in the gut microbial composition have been found

compared with healthy subjects. Lower microbial diversity in the gut has been found in patients with IBS [85]. A reduction in abundance of Lactobacillus and Bifidobacterium, and an increase in potential pathogenic bacteria such as Escherichia coli, have been found in patients with IBS compared with healthy subjects [86]. Also, an increased ratio of Firmicutes/Bacteroides has been reported [87]. Post-infectious IBS (PI-IBS) is a phenomenon where IBS symptoms arise after an acute gastroenteritis, and the risk of developing IBS after a gastrointestinal infection has been shown to significantly increase [88]. Suggested pathophysiologic mechanism for PI-IBS are altered gut motility, increased intestinal permeability, intestinal inflammation and increased proinflammatory cytokines [89].

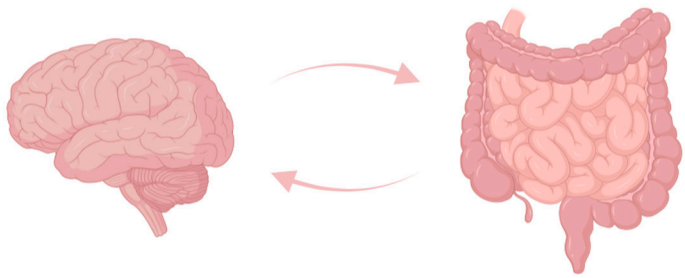

Figure 5. Bidirectional gut–brain interaction in IBS. Image created with BioRender.com.

Management of IBS

Lifestyle alterations can alleviate both GI and extraintestinal symptoms in patients with IBS. This motivates first line-treatment, including advice regarding diet, increased physical activity, sleep, stress management and smoking, which has been shown to be efficient in up to 50% of patients [90]. The UK National Institute for Health and Care Excellence (NICE) present current clinical dietary guidelines for patients with IBS. The guidelines recommend regular meals, and restriction of caffein, fizzy drinks, alcohol, resistant starch and high-fiber food [91]. If adequate symptom relief is not achieved by these recommendations, further dietary management should be given by healthcare professionals. Dietary advice includes single food avoidance and exclusion diets such as a low FODMAP (fermentable oligosaccharides, disaccharides, monosaccharides and polyols) diet.

For those with insufficient effects of lifestyle alterations, more advanced treatment strategies, including medical, behavioral and dietary therapies, should be considered [91]. Pharmacological treatment of IBS is focused on identifying the dominant GI symptoms and, accordingly, finding treatment options that improve the symptoms. A challenge is that the predominant symptoms can vary over time, and treatment must therefore be personalized. For patients with IBS-C, bulking agents and osmotic laxatives are most often used, whereas IBS-D patients are treated with antidiarrheal

drugs such as loperamide. Antidepressants, such as selective serotonin reuptake inhibitors and tricyclic antidepressants, are believed to decrease the degree of abdominal pain associated with IBS via centrally mediated antinociceptive pathways. For more temporary abdominal pain, antispasmodics can relax smooth muscle and affect GI motility [92]. Other medical treatments include antibiotics, probiotics, prosecretory agents and 5-HT3 receptor antagonists [82]. In patients with IBS, psychological comorbidities are common and can aggravate GI symptoms [93]. For these patients, cognitive behavioral therapy and gut-directed hypnotherapy have been well studied and shown to be effective [94, 95].

Overlaps between endometriosis and irritable bowel syndrome

Symptomatology

Endometriosis and IBS have a significant overlap in symptom presentation, and consequently the diseases may coexist or be misdiagnosed, leading to diagnostic delays, unnecessary investigations and inadequate treatment. Examples of symptoms which can be found in both diseases are abdominal pain, bloating, diarrhea, constipation and dyspareunia. To differentiate between the two diseases in clinical practice is a challenge due to the overlap in symptomatology and lack of clinically useful biomarkers.

A recent meta-analysis reported that the odds of IBS were three times higher in patients with endometriosis compared with healthy controls [96]. All studies included in the analysis showed a positive association of IBS and endometriosis. The prevalence rate of IBS in women with endometriosis ranged from 10.6 to 52%. An increased probability of being diagnosed with IBS is seen in endometriosis patients both with and without bowel involvement [97].

Pathophysiology

The two diseases share several potential pathophysiological mechanisms, and multiple theories have been proposed. An immunological linkage has been suggested, with altered levels of inflammatory cytokines in the peritoneal cavity and increased mast cell activation found in both conditions. In endometriosis, activated mast cells have been shown near nerve endings in the abdomen and pelvis, and in IBS they have been found near the bowel mucosa [98]. Pro-inflammatory cytokines promote the chronic low-grade inflammations which can be observed in both conditions. Other pathophysiological mechanisms described in both endometriosis

and IBS are visceral hypersensitivity, dysbiosis of gut microbiota and altered intestinal permeability [5, 99, 100]. As previously mentioned, it has been described that both patients with endometriosis and patients with IBS might experience visceral hypersensitivity. Having a diet including FODMAPs cause luminal distension, which can be painful in patients with visceral hypersensitivity. In IBS, a low FODMAP diet is known to decrease GI symptoms and is one of the main recommended dietary managements [91]. Also, a majority of patients with endometriosis report improvement in bowel symptoms with a low FODMAP diet [101].

Another theory is that endometriosis and IBS have an increased association due to a hormonal connection, involving GnRH-containing neurons, and LH-receptors within the pelvic organs and the ENS [102, 103]. GI symptoms, both in patients with IBS and patients with endometriosis, have been reported to fluctuate over the menstrual cycle with worsening during menstruation, indicating that female sex hormones impact the symptoms [73, 104].

Hypersensitivity

The experience of pain is a physiological response to activation of nociceptive pathways. The nociceptive system can be sensitized by functional, inflammatory or chemical factors, leading to pain hypersensitivity. Both peripheral and central neurons can be involved in sensitization. Central hypersensitivity is normally reversible if the stimulus ceases. However, in some individuals, genetic and emotional factors appear to interact with afferent input and lead to irreversible increases central pain sensitivity [105]. Visceral hypersensitivity refers to an increased pain sensation experienced in the visceral organs, which is affected by the bidirectional communication between the GI tract and the brain, often referred to as the brain–gut axis. Influences such as psychological traits, genetic predisposition and stress response system impact the brain–gut axis and can modulate the perception of visceral pain. The organization of the enteric nerve system (ENS) is in close proximity to the visceral organs and there is a neurogenic afferent convergence within the central nervous system. The crosstalk between visceral organs is physiological but enables cross organ sensitisation, which means that pain in one organ can cause symptoms in other organs [106]. Visceral hypersensitivity and central sensitisation in IBS have been demonstrated with lower pain thresholds for rectal distension, referred pain, skin hypersensitivity and muscular hyperalgesia [107, 108]. In endometriosis, intensity of pain has been reported to be independent of disease extent [109], and the patients seem to have lower thresholds for pain related to central sensitization mechanisms. Pain provocation by rectal balloon dilation, detected lower pain thresholds in patients with endometriosis compared with controls, implying that visceral pain hypersensitivity is common in endometriosis [5].

Aims

The overall aim of this thesis was to investigate potential biomarkers for endometriosis. The specific aims of the included papers are as follows:

Paper I

The primary aim was to compare sociodemographic factors and GI symptoms between patients with endometriosis and those with IBS.

Paper II

The primary aim was to investigate the gut microbiota in patients with endometriosis compared with that in people from the general population. The secondary aim was to examine differences in microbiota abundance within the endometriosis cohort, dependent on disease localization, GI symptoms, and treatment.

Paper III

The primary aim was to examine whether the PRS for endometriosis and different clinical presentations of the disease were associated. The secondary aim was to investigate the associations of the PRS for endometriosis with the levels of different inflammatory proteins and TRAb.

Paper IV

The primary aim was to examine the prevalence of autoantibodies in patients with endometriosis with the purpose of evaluating the potential of TRAb IgG as a diagnostic marker for endometriosis.

Paper V

The primary aim was to confirm that the concentrations of TRAb IgG are truly elevated in patients with endometriosis compared with controls from the general population and patients with IBS by performing routine clinical analyses.

Materials and methods

Study population

Endometriosis patients

Women with endometriosis were identified at the Department of Gynecology at Skåne University Hospital, Malmö, Sweden. The first cohort, which had been previously recruited, was identified by a search of medical records in the County of Region Skåne according to the International Classification of Diseases and Related Health Problems (ICD-10, N80). Recruitment occurred between March 2013 and July 2014 and between September 2016 and March 2017. The inclusion criteria were a definite diagnosis of endometriosis, confirmed by laparotomy or laparoscopy, an ability to comprehend the Swedish or English language and an age of 18–70 years. The exclusion criteria were an uncertain diagnosis of endometriosis, multiple or severe somatic or psychiatric comorbidities, a diagnosis of inflammatory bowel syndrome (IBD) and current pregnancy. A total of 605 patients were identified between 2013 and 2017. Among those, 307 declined to participate, 72 had moved from the region, 32 had significant comorbidities, 18 had an uncertain diagnosis, and four denied a diagnosis, leaving 172 women included. In Paper I, 32 women were excluded because of having a diagnosis of IBS, leaving 140 women to be included for clinical analysis.

The second cohort of patients was recruited between February 2022 and March 2023. Patients who were diagnosed with endometriosis by transvaginal ultrasound at the Department of Gynecology at Skåne University Hospital, Malmö, Sweden, were asked to participate in the study. Patients were systematically examined by ultrasound examiners experienced in identifying endometriosis according to the International Deep Endometriosis Analysis (IDEA) group recommendations [110]. The diagnostic method was changed from the first inclusion period due to updated guidelines [8]. The inclusion criteria were a diagnosis of endometriosis, confirmed by ultrasonography, and comprehension of the Swedish or English language. The exclusion criteria were the same as those in the first cohort. During the inclusion period, 96 patients fulfilled the inclusion criteria and were asked to participate in the study. Of those, 15 declined to participate, leaving 81 women to be included. In

Paper I, seven women were excluded because of having a diagnosis of IBS, leaving 74 women to be included for clinical analysis (Table 2).

IBS patients

Patients with IBS were recruited during two periods to participate in a dietary trial. During the first inclusion period, which took place from 2018 to 2019, patients were recruited from primary care centers (PCCs) and the Department of Gastroenterology at Skåne University Hospital, Malmö. Patients with IBS were identified by a search of the medical records in the County of Region Skåne according to ICD-10, K58.0 and K58.9. The inclusion criteria were a symptom score >175 on the IBS-SSS, age 18–70 years, and ability to understand the Swedish language. The exclusion criteria were alcohol or drug abuse, severe somatic or psychiatric diseases, a severe food allergy, eating disturbances, having a low-FODMAP diet, LCHF, and a gluten-free or vegan diet. In total, 697 patients were contacted. Among them, 145 were willing to participate. Later, 22 did not meet the inclusion criteria and 18 declined to participate, leaving 105 included participants. All men (n=23) and one patient with a diagnosis of endometriosis were excluded from this study, leaving 81 women who were ultimately included.

The second inclusion period took place from 2022 to 2024. Patients were identified by a search of the medical records in the County of Region Skåne according to the ICD-10, K58.1 (IBS-D), K58.2 (IBS-C), K58.3 (IBS-M), and K58.8 (IBS-U), diagnosed from 2019 to 2022. A total of 744 patients were randomly selected and contacted by letter or phone. Of these, 58 were willing to participate. From social media, 218 patients with an IBS diagnosis signed up to participate. Later, 6 did not meet the inclusion criteria, and 66 declined to participate. All men (n=21) and one patient with a diagnosis of endometriosis were excluded. Only patients (n=118) who had been included before August 2023 were included in Study I. In total, 199 women with IBS were included in Study I. In Study V, patients were randomly selected from the second inclusion period for an analysis of TRAb levels in Gothenburg (n=50) or Malmö (n=50), of whom 24 were analyzed in both departments (Table 2). Celiac disease was excluded in all IBS patients by analyzing the levels of transglutaminase antibodies.

Controls

Malmö Offspring Study

The Malmö Diet and Cancer Study (MDCS) consists of 28,098 individuals from the general population, enrolled between 1991 and 1996. From the MDCS 6103 participants were randomly selected and included in the Malmö Diet and Cancer Cardiovascular Cohort (MDC-CC). Offspring of the subjects in the MDC-CC were

invited to participate in the Malmö Offspring Study (MOS) [111]. In the present study, controls were randomly recruited from a previously selected cohort from MOS to study GI symptoms in the general population [112]. In Study II, each patient was matched with three controls according to sex (female), age (± 730 days), body mass index (BMI) (±2 BMI units), and smoking status. Only participants from the MOS who had answered a questionnaire and provided stool samples were included in the matching process. Those who were diagnosed with celiac disease, lactose intolerance, IBD or IBS were excluded from the matching process. In total, 198 women served as controls [median age 37 (32–44) years]. In Study IV, the control group for the analysis of TRAb IgG levels consisted of 100 and 114 MOS participants, respectively. From the initial selected MOS cohort [112], women under the age of 60 years who had both answered questionnaires and provided blood samples were recruited as controls for GI symptoms and circulating biomarkers [64, 113].

Healthy controls

The control group for the analysis of antibodies against FSH, FSHR, hCG, LH, LHR, TSH and TRAb IgA/IgM in Study IV consisted of 50 healthy, female blood donors from Malmö, who were randomly asked to participate as controls.

In Study V, healthy controls, consisting of health care workers, relatives of health care workers and medical students practicing at SUS, Malmö, aged 18–70 years, were recruited to participate by personal invitations or advertisement. The exclusion criterion was having an acute or chronic illness or significant GI symptoms. In total, 74 controls were recruited, of whom 50 women were randomly selected for Study V.

Reference values from the Department of Clinical Chemistry in Malmö were used for TRAb IgG, TSH, T3, FT3, T4 and FT4 levels in Study IV. Reference values from the Division of Clinical Chemistry in Malmö and the Departments of Clinical Chemistry at Sahlgrenska University Hospital for TRAb IgG levels were used in Study V.

Table 2. Table of patients and controls included in Papers I–V.

	Paper I	Paper II	Paper III	Paper IV	Paper V
Endometriosis	214, excluded all with concomitant IBS	66, first cohort	172, first cohort	172, first cohort	121, first and second cohorts
IBS	199				76
MOS, general population		198, excluded those with organic GI diseases and IBS		100/114 excluded those with organic GI diseases and IBS	
Healthy blood donors				50	
Healthy hospital staff/relatives/ students					50

MOS: Malmö Offspring Study

Study design

All studies included in this thesis were cross-sectional. Study I compared sociodemographic factors between patients with endometriosis and patients with IBS. Study II compared the gut microbiota in patients with endometriosis and people from the MOS. Study III investigated PRS in patients with endometriosis. Study IV compared antibodies in patients with endometriosis with people from the MOS and healthy controls. Study V compared TRAb levels in patients with endometriosis and patients with IBS and healthy controls. Study participants answered a study questionnaire regarding sociodemographic factors, lifestyle habits, and medical history, completed the VAS-IBS and provided blood samples. For Study II, all participants also provided stool samples.

Questionnaires

Clinical data survey

All participants, except healthy blood donors, answered questions regarding their education, occupation, marital status, smoking habits, alcohol habits, physical activity, medical history, and pharmacological treatments. Healthy blood donors answered only a brief questionnaire in which they stated that they were healthy and used no medications. All participants in the MOS answered the lifestyle questionnaire in a web-based form [111]. All the endometriosis patients answered a previously developed questionnaire addressing their endometriosis-associated symptoms and GI symptoms, including the onset of symptoms, triggering factors and treatment. All IBS patients answered a similar questionnaire addressing their GI symptoms, including onset, triggering factors and treatment [64].

The Visual Analogue Scale for Irritable Bowel Syndrome

GI symptoms in patients and controls (except blood donors) were quantified using the VAS-IBS. The VAS-IBS is a questionnaire that was initially developed to measure GI symptoms in patients with functional bowel disease. It has been psychometrically validated for use prospectively [114, 115], and it has been validated in an Asian cohort [116]. The severity of seven different symptoms over the last two weeks were estimated: abdominal pain, diarrhea, constipation, bloating and flatulence, nausea and vomiting, psychological well-being, and the influence of intestinal symptoms on daily life. Each symptom was measured on a continuous scale from 0 to 100 mm, where 0 represents no symptoms and 100 represents a lot of symptoms. The scales were inverted from the original version [114]. Reference values are available for healthy volunteers [117].

Irritable bowel syndrome-severity scoring system

In Paper I and V, IBS patients and healthy controls completed the IBS-SSS regarding abdominal pain, abdominal distension, satisfaction with bowel habits, and the impact of bowel habits on daily life. IBS-SSS estimated the symptoms using visual analogue scales (VAS) scores ranging from 0 mm to 100 mm, and the number of days with abdominal pain over the previous 10 days was reported to ensure that the patient fulfilled the inclusion and exclusion criteria. The maximum achievable score is 500. Scores <75 indicate the absence of disease, scores ranging from 75–174 indicate mild disease, scores ranging from 175–299 indicate moderate disease, and scores ≥300 indicate severe disease [118].

Laboratory methods

Paper II

Analysis of the gut microbiota

Stool samples were collected from all patients and controls in their homes and stored frozen in sterile tubes until analysis. After arrival at the laboratory, the samples were stored at –80 °C. Microbial DNA was extracted at GATC Biotech in Germany using a QIAamp Column Stool Kit. The V1–V3 regions of the 16S ribosomal RNA were pairwise amplified and sequenced using the HiSeq Illumina platform at GATC Biotech, Constance, Germany. The sequences were binned together into operational taxonomic units (OTUs) using QIIME and classified at the genus level by matching with the Greengenes reference database [119]. Bacteria that occurred in only <10 samples were excluded, leaving 58 bacteria included in the comparison between patients and controls and 62 bacteria in calculations within the endometriosis cohort.

Paper III

DNA sample sequencing

DNA samples were genotyped using the Global Screening Assay, on an Illumina iScan high-throughput screening system at the Institute of Clinical Molecular Biology (Christian-Albrechts-University, Kiel, Germany). The GenCell algorithm implemented in Illumina GenomeStudio software was used to obtain the alleles from the raw intensity data.

Papers IV and V

Immunological analyses

Antibodies against FSH, FSHR, hCG, LH, LHR, TSH and TRAb IgA/IgM in serum were analyzed using ELISAs. Microtiter plates were coated with FSH, FSHR, hCG, LH, TSH, or TRAb IgA/IgM in phosphate-buffered saline (PBS) and LHR in carbonate buffer (pH 9.2) and incubated at 4 °C overnight on a shaker. The plates were washed with PBS containing 0.05% tween (PBST) three times, blocked with bovine serum albumin (BSA), and incubated at room temperature for 1 h on a shaker. Mouse anti-FSH, rabbit anti-TSH IgG and mouse anti-TSHR IgG were serially diluted with 1% BSA–0.05% PBST. Antibodies were detected by adding HRP-conjugated anti-human, rabbit anti-mouse or goat anti-rabbit antibodies. Washing and an incubation at room temperature were repeated between each step. The color reaction was induced by adding a peroxidase substrate system, and the

absorbance was directly read at 450 nm. The absorbance was translated to a concentration in relative units (RUs). Serum from controls was used to construct a frequency table with a 97.5% positive cutoff value.

TSH, T3, FT3, T4, FT4 and TRAb IgG levels were analyzed at the Department of Clinical Chemistry in Malmö, according to standardized methods used in the clinic. Serum TSH, T3, FT3, T4 and FT4 levels were analyzed using a competitive immunoassay with direct chemiluminescence technology according to the Atellica-IM method. An analysis of serum TRAb IgG levels was conducted using a competitive electrochemiluminescence immunoassay (ECLI) detection technique based on ruthenium derivate. As stated in the laboratory protocol, TRAb IgG levels >1.7 IU/L were considered positive, and levels of 1.2–1.7 IU/L were considered grey zone. Until 2016, the detection level in the laboratory was ≥0.3 IU/L, and the functional level was 0.8 IU/L. In 2017, the laboratory raised the detection level to ≥1.0 IU/L, due to low sensitivity at low levels. In Study V, TRAb IgG levels were analyzed at the Department of Clinical Chemistry at Sahlgrenska University Hospital in Gothenburg, which is able to obtain lower values than the Department of Clinical Chemistry in Malmö. The detection level in the laboratory was ≥0.26 IU/L, and the functional level was 0.8 IU/L, and the intra-assay CV was 12% at low concentrations.

Data Categorization

The education level was categorized into graduated primary school, graduated secondary school, or graduated university. In Paper I, graduation from university was replaced by at least one year of university studies. Occupation was divided into working full time, working 51–99% of the time, working 1–50% of the time, sick leave, retired, unemployed, or studying. Marital status was categorized into living alone, married/partners living together, and other, e.g., partners not living together or living with individuals others than their partner. In Paper I, smoking was divided into never smokers, former smokers, present irregular smokers, and regular smokers. Alcohol intake was divided into <1 standard glass per week, 1–4 standard glasses per week, 5–9 standard glasses per week, and >10 standard glasses per week. Physical activity per week was categorized into never, <30 minutes, 30–60 minutes, 60–90 minutes, 90–120 minutes, and >120 minutes. In Paper I, BMI was categorized as <25, 25–29.9, and ≥30 kg/m^2 according to the World Health Organization (WHO) standard [120]. In Papers II, III, IV and V, smoking was divided into currently smoking or not currently smoking. Alcohol was divided into < 1or ≥1 standard glass of alcohol per week. Physical activity was divided into <1 hour or ≥1 hour of activity that led to breathlessness per week. Hormone treatment included estrogen, progesterone and GnRH agonists, and was divided into current treatment or no current treatment. Previous habits or treatments were not considered.

The localization of endometrios was divided into isolated ovarian lesions or spread to any other location and involvement of the bowel or not.

Statistical methods

Statistical analyses were performed using the IBM SPSS® statistical computer package versions 26 & 28 for Windows. Variables were tested for a normal distribution via visualization in a histogram and the Kolmogorov–Smirnov test. Comparisons between groups were performed using the Mann–Whitney U test (Paper IV and V) or Kruskal–Wallis (Paper V) when the distribution was skewed. For correlations, Spearman's rank correlation test was used (Paper IV). Fischer's exact test was used for categorical variables (Papers I, IV and V). Binary logistic regression was used in Paper I to estimate odds ratios and 95% confidence intervals (CIs). In Paper IV, receiver operating characteristic (ROC) curves, with areas under the curves (AUCs) and 95% CIs, were calculated for TRAb IgG and IgM levels. The values are presented as medians (interquartile ranges (IQRs)), means ± standard deviations (SDs) or numbers (percentages (%)). $p < 0.05$ was considered statistically significant.

In Paper II, alpha diversity was tested to analyze the diversity of genera among samples using the Shannon diversity index. Alpha diversity was calculated using *diversity,* and an analysis of variance (ANOVA) was performed. Beta diversity was calculated to detect differences in the microbiota composition among the groups using the Bray–Curtis dissimilarity index. V*egdist* was used to calculate beta diversity. Further significant differences in the dissimilarity index were tested with *Adonis*, within the R package *vegan*.

Genetic analyses

Quality control

In Paper III, genotyping data were quality controlled (QC) by removing samples and markers using the following pipeline: exclusion of samples with ≥15% missing rates; exclusion of markers with noncalled alleles; exclusion of markers with missing call rates >0.05; exclusion of samples with ≥5% missing rates; exclusion of related samples (PI-HAT >0.1875); exclusion of samples whose genotyped sex could not be determined; exclusion of samples with high heterozygosity rates (more than three times the SD of the mean); only autosomal SNPs were retained; removal of markers with Hardy–Weinberg equilibrium P-value $<1x10^{-5}$; removal of markers whose P-value for the difference in missingness between cases and controls was $<1x10^{-5}$; and removal of samples that

were outliers, identified using principal component analysis (deviation of more than 6 times the interquartile range). In total, 140 samples passed QC.

Calculation of Polygenic risk score

The results from a genome-wide association study on endometriosis [51], available from the GWAS catalog GCTS004549 [46], were used for the calculation of the PRS. The 13 SNPs available in our data and with p-value $<5 \times 10^{-8}$ were applied. The weighted and unweighted PRSs were calculated as it is implemented in PLINK software (version 1.9) [121].

Principal component analysis

Four principal components were calculated for each patient to control for population stratification. The genotyped data were pruned to obtain SNPs with no linkage disequilibrium using PLINK software [122], and SNPs from high-LD regions were excluded. FlashPCA was subsequently used to calculate the principal components of the SNP data.

Ethical considerations

All studies were conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Board of Lund University. Approval numbers were as follows: for the MOS population 2012/594; for endometriosis patients 2012/564, 2016/56 and 2016/375; for IBS patients 2017/171, 2017/810 and 2021/05407–01; and for healthy controls 2020/02432 and 2021/00049. For Study II, III, IV and V, the Swedish Biobank approved the use of fecal and blood samples, respectively, and the Swedish Authority for Privacy Protection approved the genetic analyses (approval number 1565–2012). All the subjects provided written, informed consent before inclusion in the studies and were informed about their right to withdraw their consent at any time after inclusion.

None of the study participants were exposed to any medical risks associated with the studies in the present thesis. All examinations by vaginal ultrasonography or laparoscopy were performed for diagnostic purposes, and patients with endometriosis were asked to participate in the study after the diagnosis was confirmed. Since personal data regarding health and genetic information are handled, a potential integrity risk exists. The data in all the studies were transferred to coded datasets to minimize this risk. The potential benefits of these studies outweigh the potential risks and are considered justifiable.

Results

Baseline characteristics

In Paper I, differences in socioeconomic factors and lifestyle factors between women with endometriosis and those with IBS were found to be limited. Patients with endometriosis were younger ($p<0.001$) and more often studying ($p=0.006$) than patients with IBS. No differences were identified in education, marital status, smoking status, alcohol consumption or physical activity. The prevalence of hypo- and hyperthyroidism did not differ between endometriosis patients (8.4% and 1.4%) and IBS patients (9.0% and 1.0%). Hormonal treatment and analgesic treatment such as NSAIDs and opioids were more common in patients with endometriosis (40.7% vs. 20.6% and 18.7% vs. 9.0%, respectively). Patients with IBS used more proton pump inhibitors (20.6% vs. 4.2%), laxatives (16.6% vs. 3.3%), and antidiarrheic drugs (7.5% vs. 1.4%) (Table 3).

Patients with IBS reported more severe GI symptoms on VAS-IBS than did those with endometriosis regarding abdominal pain ($p<0.001$), diarrhea ($p<0.001$), constipation ($p<0.001$), bloating and flatulence ($p<0.001$), vomiting and nausea ($p<0.042$), the influence of intestinal symptoms on daily life ($p<0.001$), and psychological well-being ($p<0.003$), after adjustment for confounders. A total of 15% of the patients with endometriosis reported no GI symptoms. In endometriosis, 47.2% of the women said that they were able to differentiate between abdominal pain from endometriosis or from the GI tract.

An initial triggering factor for GI symptoms was reported by 21.5% of the endometriosis patients and 27.1% of the IBS patients. A significant difference in what initially triggered the disease was observed between the groups. Menarche was the most common trigger of endometriosis, and stress, infection or antibiotic treatment were the most common triggers of IBS.

The majority of patients with both endometriosis (51.6%) and IBS (87.9%) had tried various dietary changes due to GI symptoms. Among those patients, 73.3% with endometriosis and 72.0% with IBS experienced an improvement in their symptoms ($p=1.000$).

Table 3. Patient characteristics, pharmacological treatment and gastrointestinal symptoms differing significantly between patients with endometriosis and patients with IBS.

	Endometriosis	IBS	P-value
Age (years)	38 (33–43)	43 (33–55)	<0.001
Alcohol intake per week, glasses n (%)			
<1	134 (62.6)	95 (47.7)	
1–4	66 (30.8)	76 (38.2)	0.022
5–9	11 (5.1)	24 (12.1)	0.004
≥10	2 (1.0)	4 (2.0)	0.684
Drugs n (%)			
NSAIDs	40 (18.7)	18 (9.0)	0.007
Opioids	20 (9.3)	0	<0.001
Laxatives and bulking agents	7 (3.3)	33 (16.6)	<0.001
Loperamide	3 (1.4)	15 (7.5)	0.003
Hormonal treatment	87 (40.7)	41 (20.6)	<0.001
Abdominal pain	40 (9–72)	50 (34–65)	<0.001
Diarrhea	11 (0–48)	52 (10–73)	<0.001
Constipation	28 (0–65)	54 (10–75)	<0.001
Bloating and flatulence	50 (15–76)	76 (62–88)	<0.001
Vomiting and nausea	6 (0–35)	14 (2–40)	0.042
Intestinal symptoms' influence on daily life	35 (5–77)	71 (57–83)	<0.001
Psychological well-being	32 (6–62)	47 (20–64)	0.003

IBS, irritable bowel syndrome. Gastrointestinal symptoms during the last 2 weeks were measured by the visual analogue scale for irritable bowel syndrome (VAS-IBS). The values are presented as numbers and percentages or medians and interquartile ranges (IQRs). A p-value <0.05 was considered to indicate statistical significance.

Gut microbiota

In Paper II, we investigated the gut microbiota in patients with endometriosis compared with that in people from the general population. According to the ANOVA results, the alpha diversity was significantly higher in the control group than in the endometriosis patient group ($p=4.9e^{-0.5}$). The Adonis test revealed that the beta diversity was also higher in the control group than in the endometriosis group, however the R^2 value was low (0.02) (Table 4).

The abundance of 19 gut bacteria at genus level differed between the endometriosis patients and the controls (Table 4). After correction for multiple testing, with a false discovery rate (FDR) of 0.05, the number was reduced to 12 bacteria belonging to the classes Bacilli (N=1), Bacteroidia (N=4), Clostridia (N=4), Coriobacteriia (N=2) and Gammaproteobacteria (N=1). Two bacteria in the Bacteroidia class and two in the Clostridia class were more abundant in patients than in controls, whereas two different bacteria in the Bacteroidia and Clostridia classes were more abundant in

controls than in patients. The genera in the Bacilli and Coriobacteriia classes were less abundant, whereas the genus in Gammaproteobacteria was more abundant in patients than in controls.

No significant differences in microbiota abundance were observed after FDR adjustment within the endometriosis cohort when stratified based on disease location, symptoms or hormone treatment.

Patients who had received antibiotic treatment in the last six months were excluded, and after adjustment for the FDR, only three bacteria with a significant difference in abundance between patients and controls were detected, namely, *Lachnospiria*, *Oscillospira* and a genus in the order Bacteroidales.

Figure 6. Altered gut microbiota. Image source: Adobe Stock.

Table 4. Summary of organic findings in Papers II and IV.

	Endometriosis patients	Controls
Alpha diversity	Lower	Higher
Beta diversity	Lower	Higher
g__Paraprevotella; f__Paraprevotellaceae; o__Bacteroidales; c__Bacteroidia	Lower	Higher
g__Adlercreutzia; f__Coriobacteriaceae; o__Coriobacteriales; c__Coriobacteriia	Lower	Higher
g__f__o__Bacteroidales; c__Bacteroidia	Lower	Higher
g__Lachnospira; f__Lachnospiraceae; o__Clostridiales; c__Clostridia	Lower	Higher
g__Oscillospira; f__Ruminococcaceae; o__Clostridiales; c__Clostridia	Higher	Lower
g__f__Coriobacteriaceae; o__Coriobacteriales; c__Coriobacteriia	Lower	Higher
g__Bacteroides; f__Bacteroidaceae; o__Bacteroidales; c__Bacteroidia	Higher	Lower
g__Parabacteroides; f__Porphyromonadaceae; o__Bacteroidales; c__Bacteroidia	Higher	Lower
g__f__o__SHA98; c__Clostridia	Lower	Higher
g__f__Enterobacteriaceae; o__Enterobacteriales; c__Gammaproteobacter	Higher	Lower
g__Turicibacter; f__Turicibacteraceae; o__Turicibacterales; c__Bacilli	Lower	Higher
g__Coprococcus; f__Lachnospiraceae; o__Clostridiales; c__Clostridia	Higher	Lower
g__f__o__YS2; c__4C0d2	Lower	Higher
g__f__o__RF32; c__Alphaproteobacteria	Lower	Higher
g__f__Peptostreptococcaceae; o__Clostridiales; c__Clostridia	Lower	Higher
g__f__Barnesiellaceae; o__Bacteroidales; c__Bacteroidia	Lower	Higher
g__f__Halanaerobiaceae; o__Halanaerobiales; c__Clostridia	Lower	Higher
g__f__o__RF39; c__Mollicutes	Lower	Higher
g__f__Lachnospiraceae; o__Clostridiales; c__Clostridia	Higher	Lower
TRAb IgM levels	Higher	Lower
TRAb IgG levels (Study IV)	Higher	Lower

Polygenic risk score

The primary aim of Paper III was to examine whether the PRS for endometriosis development and different clinical presentations of the disease were associated. The results revealed that in the third quartile of both the weighted PRS and unweighted PRS, fewer patients had spread endometriosis than in the lowest quartile (OR: 0.252; 95% CI: 0.081–0.782, p=0.017; OR: 0.182; 95% CI: 0.052–0.0630, p=0.007) and highest quartile (OR: 0.409; 95% CI: 0.136–1.288, p=0.111; OR: 0.245; 95% CI: 0.077–0.781, p=0.017). An inverse association between the second quartile of the weighted PRS and endometrial involvement of the GI tract was observed (OR: 0.158; 95% CI: 0.026–0.949, p=0.044). The third quartile of the unweighted PRS was associated with lower use of hormone therapy (OR: 0.250; 95% CI: 0.075–0.829, p=0.023). However, both the sensitivity and specificity for all the clinical outcomes were low. No associations between PRS and any of the analyzed circulating inflammatory proteins or TRAb were observed.

Antibodies (Papers IV and V)

Sera from 172 endometriosis patients had previously been analyzed for TRAb IgG levels according to standardized methods at the Department of Clinical Chemistry, Malmö, with a detection limit of ≥1.0 IU/L. The results revealed that 29.1% of the endometriosis patients had TRAb IgG levels above the detection limit of 1.0 IU/L, whereas 2.6% of the controls from the general population did ($p<0.001$).

Prior to 2016, the detection limit was ≥0.3 IU/L. Serum samples from 128 of the 172 endometriosis patients were also analysed for TRAb IgG levels prior to the change in the detection limit. These results showed that 94.5% of the endometriosis patients had TRAb levels over ≥0.3 IU/L, whereas 7.9% of the controls had TRAb levels greater than 0.3 IU/L in the MOS ($p<0.001$). ROC curves revealed an area under the curve (AUC) of 0.940 for TRAb, with a detection limit ≥0.3 IU/L, and an AUC of 0.602, with a detection limit of 1.0 IU/L. The serum levels of TRAb IgM were also increased in patients with endometriosis compared with blood donor controls ($p<0.001$).

The concentrations of TRAb IgG did not correlate with age, disease duration, thyroid hormone levels, TSH levels or GI symptoms. As expected, Graves' disease was associated with higher levels of TRAb IgG ($p=0.002$). No difference in TRAb IgG concentrations was observed between endometriosis patients treated with and without hormonal therapy ($p=0.554$), those with isolated ovarian endometriosis ($p=0.394$) or those with endometriosis involving the bowel ($p=0.123$). A difference in TRAb IgG levels was not observed between controls from the MOS who had IBS (n=25, 21.9%; $p=0.655$) or reported GI symptoms in the past two weeks (n=30, 26.3%; $p=0.885$) and to those without a diagnosis or GI symptoms.

The prevalence of autoantibodies against FSH, FSHR, hCG, LH, LHR or TSH was not increased in patients with endometriosis compared with blood donor controls. The titers of FSHR IgG ($p=0.008$), FSHR IgM ($p<0.001$) and TSH IgA ($p=0.029$) were lower in patients than in blood donor controls.

When serum TRAb IgG levels were analyzed in two different clinical laboratories in 2023, its levels were not confirmed to be elevated in patients with endometriosis compared with heathy controls and patients with IBS. When analyzed in Gothenburg with a detection limit of 0.26 IU/L, the number of patients with detectable serum levels of TRAb did not differ between the endometriosis patients (n=10, 8.3%) and the controls (n=2, 4%) ($p=0.512$) or between the endometriosis patients and the IBS patients (n=3, 6%) ($p=0.758$). The concentrations of TRAb in the serum did not differ between the endometriosis patients and the controls ($p=0.260$) or between the endometriosis patients and the IBS patients ($p=0.725$). Concordant results were found when the serum was analyzed in Malmö, with a detection limit of 0.8 IU/L. TRAb was not more commonly detected in endometriosis patients (n=4, 4.9%) than in controls (n=4, 8.0%) ($p=0.710$) or IBS

patients (n=4, 8.0%), (p=0.710). The concentrations did not differ between the endometriosis patients and the controls (p=0.524) or between the endometriosis patients and the IBS patients (p=0.585).

Discussion

General discussion

Sociodemographic factors, lifestyle and gastrointestinal symptoms

A main finding from Study I was that women with IBS reported more severe GI symptoms when estimated with a self-rating questionnaire than women with endometriosis did. Significant differences were observed in abdominal pain, diarrhea, constipation, bloating and flatulence, vomiting and nausea, the influence of intestinal symptoms on daily life, and psychological well-being. Similar results have been reported in a previous study [123]. The results indicate that rating of symptoms with a validated questionnaire, in combination with a thorough anamnesis, is valuable in clinical practice, to find which patients who should be further examined for endometriosis. Although patients with IBS reported higher levels of abdominal pain, patients with endometriosis were more often treated with analgesic drugs. None of the patients with IBS were treated with opioids, whereas 9.3% of those with endometriosis were treated with opioids. The difference might depend on fluctuations in pain during the menstrual cycle and on-demand treatment with analgesics. Notably, in this study, we did not know what phase of the menstrual cycle the patients were experiencing while estimating their symptoms using the VAS-IBS, which reflects only symptoms over the last two weeks. Additionally, in this study, 37.9% of patients with endometriosis used hormonal treatment, which efficiently relives the symptoms of many patients. Treatment with opioids in patients with IBS is not recommended since it aggravates GI dysfunction [124]. The opioid prescription in endometriosis should also be questioned, since this group of patients have a greater risk for chronic opioid use, and the benefits seems to be limited [125].

The gut microbiota

When this thesis was initiated, only one previous study examined the alterations in the gut microbiota in humans with endometriosis [126]. The main finding of that study was that women with stage 3–4 endometriosis had an Escherichia/Shigella dominant gut microbiome. The hypothesis that endometriosis has an impact on the

gut microbiota has also been supported by several animal studies. A systematic review from 2020 identified in total six studies on the role of the gut microbiota in endometriosis [127]. A study of rhesus monkeys showed that monkeys with endometriosis had a significantly altered gut microbiota profile compared with healthy controls [40]. The monkeys with endometriosis had higher concentrations of gram-negative bacteria and lower concentrations of lactobacilli.

Our study of the gut microbiota in endometriosis patients revealed an overall greater diversity among controls than among patients with endometriosis. Most importantly, the alpha diversity differed, indicating a decreased microbial richness in patients with endometriosis. The beta diversity also differed, although it was only marginally higher in controls than in endometriosis patients.

Since 2020, the field has expanded rapidly and multiple studies investigating the gut microbiota in patients with endometriosis have been published [128]. Consistent findings of an endometriosis–microbiome relationship have been reported. In agreement with our study, repeated studies have shown a lower diversity of the gut microbiota in endometriosis patients than in controls [129-131]. Patients with endometriosis have an increased abundance of pathogens in their peritoneal fluid and a reduction in the abundance of protective microbes in their feces. In contrast, in one study, diversity analyses could not identify any differences between endometriosis and controls [132]. An elevated Firmicutes/Bacteroidetes ratio and reduced abundances of Gardnerella, Lachnospira, Paraprevotella and Sneathia are reported alterations in the gut microbiota of endometriosis patients [133]. A depletion of Ruminococcus has also been identified as a potential biomarker for endometriosis [131]. Increased abundances of Bifidobacterium, Blautia, Dorea, Parabacteroides, and Enterobacteriaceae, mainly Escherichia/Shigella, have also been detected in the gut of patients with endometriosis. A recent study explored the relationships between the gut microbiota and anatomical subtypes of endometriosis and recognized several associations. Different bacteria are associated with either an increased or decreased risk of endometriosis in the ovaries, fallopian tube, pelvic peritoneum, vagina, rectovaginal septum or adenomyosis [134].

Studies on the role of the gut microbiota in the pathogenesis of endometriosis are increasing, and results indicate that the microbiota is related to estrogen metabolism, inflammation, and immunity, contributing to the development of endometriosis [38]. It should be considered that the altered composition of gut microbiota also could depend on the GI symptoms in these patients. One example is gut transit time, which is known to be involved in shaping the microbiota composition [135].

TRAb and endometriosis

An increased prevalence of thyroid disorders in patients with endometriosis has previously been described in several studies [54-57]. In the total endometriosis cohort in this study, the prevalence of hypothyroidism was 8.4%. Among patients with IBS, 9.0% were diagnosed with hypothyroidism.

In 2018, a previous study reported novel findings of significantly elevated levels of TRAb IgG in women with endometriosis compared with controls from the general population [64]. In agreement with previous results, Study IV revealed that 94.5% of women with endometriosis had TRAb IgG levels over the detection limit of 0.3 IU/L, whereas 7.9% of controls did. The levels of TRAb did not differ between endometriosis patients with or without hypothyroidism, but as expected, patients with Graves' disease had high levels of TRAb. An in-house analysis of TRAb IgM levels also revealed increased levels in patients with endometriosis compared with controls. Although the levels of TRAb IgM also were found to be elevated in endometriosis, we only chose to analyze TRAb IgG further. In our first study of TRAb, the ROC curves revealed a larger AUC for IgG than for IgM. Additionally, TRAb IgG is analyzed in routine clinical practice with standardized methods in contrast to TRAb IgM.

The analyses were repeated in a new cohort at two different clinical laboratories to further evaluate whether TRAb IgG levels were truly elevated in patients with endometriosis. The results showed that current clinical laboratory setups for analyzing TRAbs cannot be used to detect elevated levels, as previously described using other methods. The clinical use of a TRAb analysis is to identify thyroid disorders with high sensitivity and specificity. In recent years, the methods have been developed to be more specific for Graves' disease. One critical concern with the initial findings of elevated TRAb levels was whether the suggested increase in TRAb expression among endometriosis patients was caused by cross-reactivity with some other antibodies. TSH and its cognate receptor belong to the glycoprotein hormone family, which also includes the closely related glycoproteins FSH, LH and hCG. Their structural similarities increase the possibility of cross-reactivity [136]. In our study, no differences in the prevalence or levels of any of the analyzed antibodies or their receptors were identified between the endometriosis patients and the controls, indicating that the elevated TRAb levels are not explained by cross-reactivity. Even if no cross-reactivity was detected in this study, the question remains as to whether the TRAbs detected in previous studies were truly elevated.

TSH receptors have been identified in the endometrium and ectopic endometrial tissue [61, 62]. Theoretically, different subclasses of TSH receptors may be expressed in different organs, although no proof of this expression pattern has been published. Additionally, heterogeneity may exist among TRAbs, and TRAbs with different antigenic epitopes have been detected in patients with autoimmune thyroid

diseases [137, 138]. Slightly different TRAbs may be detected in patients with endometriosis than in those with in thyroid disease.

Genetic analyses of endometriosis

In our study, the effects of 13 risk variants were computed into a PRS for endometriosis to assess whether an association with the clinical presentations of the disease existed. The results showed an inverse association between the third quartile of weighted and unweighted PRS and the spread of endometriosis, between the second quartile of the weighted PRS and GI involvement, and between the third quartile of the unweighted PRS and hormone treatment. However, the genetic variants involved in the development of the disease seemed to be of no clinical use for the prediction of the clinical presentation since the sensitivity and specificity were low. This is in line with another study, suggesting that PRS for endometriosis does not capture an increased risk for a specific subtype of endometriosis [52].

Methodological considerations

The study design of all the papers included in this thesis is cross-sectional; therefore, causality could not be conclusively determined. The patients with endometriosis included in Paper II, III and IV had received their diagnosis prior to inclusion in the study, and a majority were already undergoing treatment. Native blood and fecal samples were therefore not available for analysis.

Endometriosis is a heterogenous disease with patients ranging from basically asymptomatic to having severe symptoms [139]. There is a possibility that controls could have undiagnosed endometriosis without prominent symptoms. This risk was reduced by excluding all controls who reported GI symptoms. Theoretically, one way to minimise this risk could have been to examine all study participants with ultrasound, however this was not practically possible in this thesis.

GI symptoms are known to be fluctuating over the menstrual cycle, not the least in patients with endometriosis [104]. For the studies in this thesis, we did not obtain data regarding what phase of the menstrual cycle patients or controls were in. Also, of the patients with endometriosis 37.9% were currently using hormonal treatment, which can affect the menstrual cycle and cause amenorrhea.

The clinical methods used to analyze TRAb IgG are developed to identify thyroid disease. Over the last years, the methods have become more specific for Graves' disease which is positive for diagnostic purposes of thyroid disease. However, the new methods seem to be inferior at identifying the variant of antibodies previously identified in endometriosis. To further investigate the slightly elevated

concentrations of TRAb that in some studies have been identified in patients with endometriosis, analytical methods that are more sensitive in the lower concentrations are needed. When TRAb IgG was analyzed in Gothenburg, the lowest given concentrations were only available for research and not for clinical use due to low sensitivity.

There are many different questionnaires available for research and clinical practice to estimate GI symptoms and quality of life and psychological symptoms. The IBS-SSS is one of the most frequently used to measure for IBS severity and was used in study I and V [140]. However, the IBS-SSS does not measure different bowel symptoms separately. In this thesis, VAS-IBS was also used to estimate GI symptoms, quality of life and psychological well-being. One of the advantages of VAS-IBS is that symptoms are graded on a continuous scale unlike other commonly used questionnaires such as the Gastrointestinal Symptom Rating Scale (GSRS), which VAS-IBS has been validated against [114].

Conclusions

This thesis investigated potential biomarkers for endometriosis. Based on the findings of the included papers, the following conclusions were drawn:

1. Differences in socioeconomic factors and lifestyle factors are limited between women with endometriosis and those with IBS. This finding highlights the diagnostic value of potential biomarkers.
2. Patients with IBS seem to have more severe GI symptoms than those with endometriosis in terms of abdominal pain, diarrhea, constipation, bloating and flatulence, vomiting and nausea, the influence of intestinal symptoms on daily life, and psychological well-being, as evaluated with the VAS-IBS.
3. Both alpha diversity and beta diversity are higher in controls from the general population than in patients with endometriosis.
4. Genetic variants involved in the risk of developing endometriosis cannot be used to explain the clinical presentations of endometriosis via the calculation of PRS.
5. TRAb IgG levels, which were analyzed with previous clinical methods, and TRAb IgM levels, which were analyzed in house, were elevated in patients with endometriosis compared with controls. No signs that the results were caused by cross-reactivity with other antibodies were observed.
6. With the current routine clinical methods used to analyze TRAb IgG levels, elevated TRAb IgG levels could not be detected in patients with endometriosis.

Future perspectives

Several studies have revealed changes in the microbiota of patients with endometriosis, and more research is continuously published. However, a consensus among the results is lacking, which might be explained by the large number and complex composition of bacteria in the gut, methods of microbiota detection, inconsistency in diagnostic criteria and confounders for the microbiota composition. A challenge for further studies is to standardize sample collection and analysis to enable comparisons between studies. A deeper understanding of the gut microbiota and microbiome-derived metabolites would provide a basis for the development of new diagnostic and treatment methods for endometriosis. The side effects of medical and surgical treatments used today could be reduced if interventions that target the microbiota are developed.

The genetic information identified from GWASs of endometriosis is not able to explain the clinical presentation of the disease. For this task, an analysis of genetic variants involved in disease presentation is needed to develop useful PRSs.

The present thesis evaluated TRAb as a potential biomarker for endometriosis. An in-house analysis of TRAb IgM levels and previous methods for clinical analyses of TRAb IgG levels indicated elevated levels in patients with endometriosis. The levels were moderate and required methodological sensitivity at low levels, which current methods cannot provide. The results of elevated levels of TRAb IgG in endometriosis patients could not be reproduced with the current clinical methods. Further research on TRAb and an evaluation of the previous positive findings may be performed in laboratory experimental settings. The roles of TSH receptors and autoantibodies against TSH receptors in the pathophysiology of endometriosis deserve further research.

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