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Genetic variation in the sex hormone metabolic pathway and endometriosis risk: an evaluation of candidate genes

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Abstract

OBJECTIVE—To evaluate the relationship between common genetic variation in genes involved in the biosynthesis and signaling of estrogen and progesterone and endometriosis risk.

DESIGN—Genetic polymorphism analysis.

SETTING—Population-based case-control study conducted in Group Health Cooperative enrollees in Western Washington.

PATIENT(S)—Women with newly diagnosed, surgically confirmed endometriosis between 1996 and 2001 (n=256), and age and reference year matched female controls without a history of endometriosis (n=567).

INTERVENTIONS(S)—None.

MAIN OUTCOME MEASURE—We evaluated the relationship between common genetic variation and endometriosis risk, using gene-based tests and single variant analysis of genetic polymorphisms in *ESR1*, *ESR2*, *PGR*, *CYP17A1*, *CYP19A1*, *HSD17B1*, *HSD17B2*, *CYP11A1*, *CYP11A2*, *COMT* and *GSTM1*.

RESULTS—The most consistent gene-based association with endometriosis risk was for *CYP19A1* (p-value = 0.02). We did not find evidence for consistent significant associations between previously reported candidate SNPs in sex hormone-related genes and endometriosis risk.

CONCLUSION—In summary, we report increased endometriosis risk with *CYP19A1* gene-based tests; replication of the association between endometriosis and this gene or gene region is necessary in a larger study population.

Keywords

Population-based; case-control; endometriosis; genetic polymorphisms; sex hormone metabolic pathway; candidate genes

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INTRODUCTION

Endometriosis, a leading cause of infertility, is one of the most common gynecologic disorders, affecting approximately 5.5 million North American women each year (1). Symptoms of endometriosis include heavy menstrual flow, inter-menstrual bleeding, dysmenorrhea and dyspareunia. Although the etiology and pathology of endometriosis are not completely understood, endometriosis is commonly regarded as a complex trait, caused by the interplay between genetic and environmental factors. The involvement of genetic factors is supported by concordance among monozygotic twins and higher rates of endometriosis found among relatives of endometriosis cases; the prevalence of endometriosis was three to nine times higher in first degree relatives of affected individuals compared to first degree relatives of controls (2–9).

As the disease occurs mostly in women of reproductive age and regresses after menopause or oophorectomy, the development of endometriosis is likely dependent on sex steroid hormones, specifically estrogen. Additional support for the estrogen-dependent nature of endometriosis is evidenced by endometriotic lesion regression in some women after treatment with estrogen-suppressing drugs (including progesterone) or gonadotropin-releasing hormone (GnRH) agonists and relapse of lesions after discontinuation of treatment (10). Studies have evaluated endometriosis risk associated with single nucleotide polymorphisms (SNPs) in genes involved in the biosynthesis of and response to estrogen and progesterone, including: *CYP17A1*, *CYP19A1*, *ESR1*, *ESR2*, *PGR*, *HSD17B1*, and *HSD17B2*, SNPs in genes involved in synthesis of catecholesterogen, including: *CYP1A1* and *CYP1A2*, and SNPs in genes involved in inactivation of estrogen, catecholesterogen or its products including *COMT* (11–13). Most of these studies, however, focused on selected candidate SNPs in individual genes, rather than a more comprehensive assessment of common genetic variation of potentially important hormone-related genes in the entire pathway.

In a population-based case-control study, we evaluated eleven sex hormone-related genes. We used a tagSNP approach to characterize seven of the eleven genes (*ESR1*, *ESR2*, *PGR*, *CYP17A1*, *CYP19A1*, *HSD17B1* and *HSD17B2*) and selected candidate SNPs for the remaining four genes (*CYP1A1*, *CYP1A2*, *COMT* and *GSTM1*).

MATERIALS AND METHODS

Study population

Women's Risk of ENdometriosis (WREN) is a population-based case-control study of cases with incident endometriosis and frequency-matched control subjects selected from within Group Health Cooperative (GH), a large mixed-model healthcare organization in the Pacific Northwest. Full details of our study design have been previously reported (14).

Briefly, the source population for this study was GH enrollees in western Washington. Eligible cases were female 18–49 year-old GH enrollees with an incident endometriosis diagnosis (International Classification of Disease, 9th Revision (ICD-9) diagnostic codes 617.0–617.5, 617.8 and 617.9; excluding individuals with ICD-9 code 617.0, uterine endometriosis, who actually had adenomyosis as determined by pathology report) between April 1, 1996 and March 31, 2001. For comparison, control subjects were selected from a list of 18–49 year-old female GH enrollees during the same time period as the diagnoses of cases. Controls were frequency matched to cases on 5-year age interval and reference year, had intact uteri, and no history of surgically confirmed endometriosis. All subjects provided informed consent and the GH Institutional Review Board approved all study protocols.

An interview ascertaining potential endometriosis risk factors was completed by 73% (n=340) of eligible cases and 73% of eligible controls (n=741). The interview was a structured interviewer-administered questionnaire that included questions regarding demographics, employment, prior medical conditions, family and personal history of endometriosis, lifestyle factors, and reproductive factors including menstrual history, pregnancy history, contraceptive methods, and hormone use. As a result of information captured in the interview, 12 cases and 14 controls with a prior history of surgically confirmed endometriosis were excluded from analysis. For these analyses cases were further limited to the 313 subjects with surgically-confirmed definite or probable endometriotic disease, that is, disease associated with substantial symptoms or causing structural or functional damage (15). Women meeting this endometriotic disease definition are likely to be symptomatic and may therefore be more inclined to seek medical care, thus helping to decrease the likelihood of incomplete case ascertainment (15).

Following the interview, study participants were asked to donate 20 mL of blood or an oral rinse sample; of the 313 cases and 727 controls, described above, 256 (81.8%) cases and 567 (78.0%) controls provided a blood sample or oral rinse. Laboratory analysis of genetic variants described in this paper was completed for all participants that provided a sample; therefore the final analytic subset included 256 cases and 567 controls. Leukocyte DNA from venous blood samples (251 cases and 567 controls) and buccal epithelium genomic DNA from oral rinses (5 cases) were extracted by salt precipitation at Fred Hutchinson Cancer Research Center, Seattle, WA.

SNP selection

TagSNPs were selected using the LD Select algorithm through the Genome Variation Server at SeattleSNPs (CEPH collection, HapMap phase 2) (16), specifying a minor allele frequency $\geq 5\%$ and an r^2 of 0.80. We selected tagSNPs for the entire gene region plus 1KB 5' and 3' for *ESR2* (20 tagSNPs), *PGR* (18 tagSNPs), *CYP17A1* (5 tagSNPs), *CYP19A1* (18 tagSNPs), *HSD17B1* (3 tagSNPs) and *HSD17B2* (10 tagSNPs). Due to budgetary constraints and the size of the *ESR1* gene (> 140 kb, 61 bins), we selected 14 tagSNPs from bins containing two or more SNPs. When possible we preferentially selected a SNP from a bin if that SNP had previously been evaluated as a risk factor for endometriosis. We selected two additional SNPs (candidate SNPs) from these genes that had been evaluated in a meta-analysis of genetic polymorphisms in sex steroid synthesis by Guo, one in *PGR* (rs1042838) and one in *CYP17A1* (rs743572) (11). We also genotyped two SNPs in *ESR1* (rs3853250 and rs3853251) that were in nearly complete LD ($> 95\%$) with two SNPs evaluated by Guo (rs2234693 and rs9340799), since these SNPs could not be genotyped on the selected platforms (11). We included in our analyses unpublished WREN study data on six candidate SNPs from four genes involved in sex steroid catabolism [*CYP11A1* (rs464903 and rs1048943), *CYP11A2* (rs762551 and rs2069514), *COMT* (rs4680), *GSTM1* (null polymorphism)].

Genotyping

Of the SNPs genotyped, 90.9% percent of selected SNPs (80 out of 88) were successfully genotyped (greater than 95% call rate) using the SNPlex™ Genotyping System at the Sequencing and Genotyping Core, University of California Los Angeles or the OpenArray™ Genotyping System at the Functional Genomics Lab, Center for Ecogenetics and Environmental Health, University of Washington. Six additional SNPs were included in the analysis; genotypes for five of the six SNPs were determined separately using the polymerase chain reaction (PCR)-RFLP method. Specific primers, probes, and annealing temperatures are available on request. All laboratory personnel were blinded to case-control status. Randomly selected samples (10%) were identified for duplicate testing and were

integrated into the genotyping plates. Among these samples, the level of agreement with blinded duplicates ranged between 94% and 100%.

Statistical Analysis—Hardy-Weinberg equilibrium was assessed in Caucasian control subjects for each SNP. Among the 86 SNPs included in analyses, the allele frequency for six SNPs differed significantly from the expectation of Hardy-Weinberg Equilibrium among self-described Caucasian controls (p-values: *ESR1*: rs2982896 = 0.01, rs2207396 = 0.01; *CYP19A1*: rs1143704 = 0.04, rs1062033 < 0.01, rs2899470 = 0.01; *ESR2*: rs1255998 = 0.02).

Gene-based analyses: A principal components analysis (17) approach was used to test for an overall association between endometriosis and the SNPs genotyped within the six genes with more than two SNPs. The necessary number of principal components needed for each gene was determined using an 80% explained variance criterion and the Markov chain Monte Carlo (MCMC) method was used to impute missing values for SNPs with <5% missing values (18). Once the necessary principal components were determined, multivariable logistic regression models were constructed to assess the significance of each gene. All p-values reported were two sided; analyses were conducted using SAS statistical software (SAS Institute, Cary, NC, version 9.0).

SNP-based analyses: Although our main analyses were gene-based, we also evaluated individual SNPs in relation to endometriosis risk since they can be more powerful at detecting an association if the associations are driven by a single-tagged SNP or candidate SNP. These analyses provide an easier comparison with other studies using different tag SNPs or evaluating candidate SNPs. Unconditional logistic regression was used to estimate odds ratios (ORs) and corresponding 95% confidence intervals (CI), for independent associations between individual SNPs in the 11 genes and endometriosis risk. The major allele designation (M) was based on the most common allele in the Caucasian controls. The per allele risk of endometriosis and the p-value for a one degree of freedom trend test were calculated using a log additive model where the individual SNP was coded as 0, 1, or 2 for the number of minor alleles. Models were adjusted for matching factors (5-year age group and reference year) as well as race.

RESULTS

Demographic and health characteristics of the study population are presented in Table 1 for the subset of cases and controls included in this analysis. Briefly, the study population was primarily Caucasian. Cases and controls were similar with regards to education and body mass index (BMI), while endometriosis cases were more likely to be current alcohol users than controls.

Gene-based analyses

Variation in *CYP19A1* (p-value = 0.02) was statistically significantly associated with endometriosis risk. Variation in *HSD17B2* (p-value = 0.08) was of borderline statistical significance. The remaining genes were not associated with endometriosis risk (Table 2).

SNP-based analyses

Figure 1 summarizes the association between each SNP and endometriosis (p-trend per minor allele). One SNP in *ESR2* (rs944052), one SNP in *HSD17B1* (rs1424151) and 3 SNPs in *CYP19A1* (rs1004982, rs1870049, and rs936307) were statistically significantly associated with endometriosis (p-trend < 0.05), however, these results did not reach statistical significance based on the Bonferroni corrected p-value (p-trend = 0.0006 =

0.05/86). Study findings were not substantially different in analyses restricted to Caucasian participants only (results not shown). A summary of the magnitude of the association for each SNP and endometriosis risk is provided in Supplementary Table 1. (Available online)

DISCUSSION

We evaluated 11 sex hormone-related genes, 7 using a tagging approach and 4 using candidate SNPs only, in a population-based case-control study. The strongest evidence for an association with endometriosis was for common variation in *CYP19A1*. In humans, aromatase, encoded by *CYP19A1*, is the key enzyme synthesizing the conversion of androstenedione to estrone and testosterone to estradiol; it is involved in the final and rate-limiting step of estrogen synthesis and has been associated with circulating estrogen levels (19, 20). Given its key role in estrogen biosynthesis, it has been hypothesized that the presence of aromatase in endometriotic lesions plays an important role in the production of estradiol, a key factor for endometrial tissue proliferation (21, 22). Recently, the absence of aromatase activity in endometriotic lesions has been reported, suggesting that aromatase produced in the endometriotic lesions may not be as important as previously hypothesized (23, 24). However, aromatase is produced in a number of other human tissues, including adipose tissue, follicular cells and skin; therefore, it is possible that aromatase produced by other tissues may also contribute to increases in estradiol production and account for the positive proliferative impact on the endometriotic lesion in the absence of aromatase activity from within the lesion (23).

While SNPs in *CYP19A1* have been associated with sex-steroid hormone levels and other estrogen-dependent diseases, including breast cancer and endometrial cancer (25–28), the results of previous studies of the associations between various *CYP19A1* polymorphisms and endometriosis have been inconsistent (13). Eight studies of *CYP19A1* polymorphisms in relation to endometriosis risk have been published to date (12, 29–35). *CYP19A1* polymorphism associations with endometriosis have been reported for a single amino acid change in exon 3 (rs700518 Val80Val) (34), a SNP in the 3'-UTR region (rs10046) in high LD ($r^2 = 0.89$) with rs700518 (34) and with a variable number tandem repeat (VNTR) (TTTA)_n (31, 35), and a 3 base-pair insertion-deletion (TCT) (30), both in intron 4. However, these findings were not confirmed in additional study populations (rs700518 (33), rs10046 (12, 29, 33), VNTR (29, 30, 33), TCT (29, 35)). In the current study we did not genotype these polymorphisms directly, instead we genotyped a SNP (*CYP19A1* rs1143704) in complete LD ($r^2 = 1.0$) with rs700518, a SNP (*CYP19A1* rs2899470) in LD ($r^2 = 0.78$) with rs10046, and a SNP (*CYP19A1* rs16964258) in LD ($r^2 = 0.70$) with rs700519. None of these *CYP19A1* polymorphisms were associated with endometriosis risk in our study.

Although none of the *CYP19A1* SNPs that have been associated with endometriosis risk in the literature were evaluated directly in our study, we report an increased risk of endometriosis with 3 intronic SNPs in *CYP19A1* (rs1870049, rs1004982 and rs936307), as well as an overall gene-based association. To our knowledge no other study has reported increased endometriosis risk with these three SNPs. Additional studies of *CYP19A1* are warranted given the current finding, the equivocal results of previous studies, and the hypothesized role of aromatase and estradiol in endometriotic tissue proliferation.

A recent genome-wide association study (GWAS) among Caucasian women in Australia and the UK identified and replicated a locus at 7p15.2 associated with endometriosis (36). There was little evidence of an association with SNPs or genes on chromosome 15, the location of the *CYP19A1* gene. Among the reported candidate associations for endometriosis as summarized by Montgomery and colleagues (13), *PGR* on chromosome 11 was the only gene with SNPs associated with endometriosis in the Painter et al. GWAS, however, the

results for SNPs in *PGR* were not confirmed in the replication stage (36). Polymorphisms in *PGR* were not significantly associated with endometriosis risk in our study or another GWAS in Japanese women (37).

The *ESR1* gene encodes ER- α which mediates the major estrogenic effects on classic estrogen target tissues while *ESR2* encodes ER- β which has anti-proliferative effects and may oppose the actions of ER- α in the endometrium. A recent meta-analysis cited an increased risk of endometriosis associated with the minor allele (C) of *ESR1* (rs2234693) (11). Since the meta-analysis additional studies have reported positive (38), null (39–42) and inverse (43) associations with this polymorphism. Fewer studies have evaluated the minor allele (G) of *ESR1* (rs9340799) and endometriosis risk; overall the studies reported a lack of association (38, 39, 41). In the current study we did not genotype these two *ESR1* candidate SNPs directly, instead we genotyped a SNP (*ESR1* rs3853250) in complete linkage disequilibrium (LD) ($r^2 = 1.0$) with rs2234693, and a SNP (*ESR1* rs3853251) in strong LD ($r^2 = 0.957$) with rs9340799. Neither of these *ESR1* polymorphisms were associated with endometriosis risk in our study.

A limited number of studies have evaluated *ESR2* polymorphisms and endometriosis risk, and only one study to date reported an increased risk with endometriosis (44). The remaining studies reported a lack of association between *ESR2* polymorphisms and endometriosis risk (42, 45). We evaluated 20 tagSNPs in the *ESR2* gene and overall reported no association with endometriosis.

Structurally and functionally intact progesterone receptors are essential to mediate the anti-proliferative effects of progesterone on the growth of endometrial tissue (46). A number of studies have evaluated endometriosis risk associated with a 306-bp insertion of the PV/HS-1 Alu subfamily in intron G of the *PGR* gene, commonly referred to as “PROGINS” (47–49). PROGINS is defined as a combination of three mutations in nearly complete LD ($r^2 > 0.95$): the 306-bp Alu-insertion mentioned, a silent point mutation in exon 5 (rs1042839) and a single amino acid change in exon 4 (rs1042838). An early study of the PROGINS Alu-insertion reported a two-fold increased risk of endometriosis with the minor allele (T2) (47). Additional studies have reported a lack of association between PROGINS polymorphisms and endometriosis risk (43, 48–52). The lack of association between *PGR* rs1042838 and endometriosis in the current study is consistent with the findings of these latter studies.

The *CYP17A1* gene has both 17 α -hydroxylase and 17,20-lyase activities and is a key enzyme in the steroidogenic pathway that controls two successive early steps of endogenous androgen and estrogen biosynthesis by converting pregnenolone and progesterone to precursors of androgen and estrogen. The minor allele variant (C) of *CYP17A1* (rs743572) has been associated with elevated serum estrogen and progesterone levels in premenopausal women (53). A number of studies have evaluated the *CYP17A1* rs743572 polymorphism and endometriosis risk, however, only one has reported an increased risk of endometriosis with the minor allele (54), while the remaining studies showed no association (30, 34, 49, 55–57). Our individual SNP result is consistent with previous reports of a lack of association between *CYP17A1* rs743572 and endometriosis risk.

CYP11A1 and *CYP11A2* may be related to endometriosis risk through metabolism of environmental pollutants (including PCB and dioxin) or their role in catechol estrogen metabolism (58). Catechol estrogens have also been shown to stimulate prostaglandin synthesis in the uterus, which may play an important role in the pathogenesis of endometriosis (59). In a review of the published literature, the heterozygous or homozygous variant genotypes of *CYP11A1* (rs4646903) was associated with increased endometriosis risk [summary OR: 1.4; 95% CI: 1.0, 2.1] (58), while additional studies have reported no

association (56, 60). Our individual SNP result is consistent with a lack of association between *CYP1A1* rs464903 and endometriosis. Tsuchiya and colleagues reported that the minor allele (C) of *CYP1A1* (rs1048943) was not associated with endometriosis risk; consistent with our finding of a lack of association between rs1048943 and endometriosis (61).

The enzyme COMT catalyzes the O-methylation of catechol estrogens into inactive metabolites, which display selective estrogen activity in human tissues (62). Studies of *COMT* polymorphisms to date have been limited and have shown no association between the minor allele (A) of *COMT* (rs4680) and endometriosis risk (56, 63, 64). The lack of association between *COMT* rs4680 and endometriosis risk in the current study is consistent with the published data.

GSTM1 encodes an enzyme that functions in the detoxification of electrophilic compounds, carcinogens, therapeutic drugs, environmental toxins and products of oxidative stress, via conjugation with glutathione. To date, a large number of studies have examined the role of the *GSTM1* null genotype and endometriosis risk (summarized in a meta-analysis by Guo) (65). The pooled summary OR from the most recent meta-analysis suggested that women with the null genotype had nearly twice the risk of developing endometriosis compared to women with other genotypes [summary OR (95% CI): 2.0 (1.3, 3.0)] (65). Additional studies since the meta-analysis have reported positive associations with endometriosis risk (60, 66, 67) as well as a null association (68). While we did not find a statistically-significant association between the *GSTM1* null deletion and endometriosis risk, our results were not inconsistent with the meta-analysis findings as our point estimate (OR=1.3) was within the confidence interval of that analysis' summary OR.

Our study has several limitations. First the likelihood of false positives was high due to the large number of statistical tests performed. However, for six of the eleven genes evaluated we relied on gene-based p-values, and for the remaining 5 genes we evaluated fewer than three SNPs per gene. Ultimately we chose to report uncorrected p-values since the bulk of our findings supported no association with endometriosis risk; we did, however, provide the Bonferroni corrected p-value as a reference in Figure 1.

Six of the tagSNPs analyzed in this study had Hardy-Weinberg p-values less than 0.05, indicating that there might be population stratification or genotyping errors in these SNPs that were not detected by our other QC measures. However, the other QC measures for these SNPs did not suggest genotyping errors. Ultimately, we elected not to use Hardy-Weinberg p-values as an exclusionary factor when deciding whether or not to include SNPs in our analysis.

It is plausible that some of our controls may have had undiagnosed endometriosis. We limited our case group to women with definite or probable endometriotic disease, and it is unlikely that participants with this extent of disease were included in the control group. The prevalence of undiagnosed, symptomatic endometriosis in the control group is likely to be less than 2%, resulting in a very modest number of cases being misclassified as controls (15).

Our study strengths include the population-based design and the use of the GH study population which is a well-enumerated source population for epidemiologic studies. Cases and controls were members of the same healthcare delivery system, eliminating most issues pertaining to disparity of access to medical care. In addition, the GH population had a race, income, and educational profile similar to other western Washington State residents (69), and minor allele frequencies similar to the HapMap CEPH European population. (<http://www.ncbi.nlm.nih.gov/>) Furthermore, by using tagSNPs to investigate associations

with endometriosis risk, we achieved moderate coverage of common variation in these genes, increasing the likelihood of observing any true association.

In summary, we report increased endometriosis risk with nucleotide variation in *CYP19A1*. For the other 10 genes evaluated the results from the present study do not provide evidence that common genetic variation in these genes is associated with endometriosis risk. Evaluation of common genetic variation in the *CYP19A1* gene or gene region in larger studies or consortial efforts is warranted to attain adequate power to establish or rule out a putative association in the hormone pathway.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Reference List

1. Eskenazi B, Warner ML. Epidemiology of endometriosis. *Obstet Gynecol Clin North Am.* 1997; 24:235–58. [PubMed: 9163765]
2. Kennedy S, Bennett S, Weeks DE. Affected sib-pair analysis in endometriosis. *Hum Reprod Update.* 2001; 7:411–8. [PubMed: 11476354]
3. Kennedy S. Is there a genetic basis to endometriosis? *Semin Reprod Endocrinol.* 1997; 15:309–18. [PubMed: 9383840]
4. Simpson JL, Elias S, Malinak LR, Buttram VC Jr. Heritable aspects of endometriosis. I. Genetic studies. *Am J Obstet Gynecol.* 1980; 137:327–31. [PubMed: 7377252]
5. Stefansson H, Geirsson RT, Steinthorsdottir V, Jonsson H, Manolescu A, Kong A, et al. Genetic factors contribute to the risk of developing endometriosis. *Hum Reprod.* 2002; 17:555–9. [PubMed: 11870102]
6. Treloar S, Hadfield R, Montgomery G, Lambert A, Wicks J, Barlow DH, et al. The International Endogene Study: a collection of families for genetic research in endometriosis. *Fertil Steril.* 2002; 78:679–85. [PubMed: 12372440]
7. Treloar SA, O'Connor DT, O'Connor VM, Martin NG. Genetic influences on endometriosis in an Australian twin sample. *Fertil Steril.* 1999; 71:701–10. [PubMed: 10202882]
8. Lamb MR, Taylor S, Liu X, Wolff MS, Borrell L, Matte TD, et al. Prenatal exposure to polychlorinated biphenyls and postnatal growth: a structural analysis. *Environ Health Perspect.* 2006; 114:779–85. [PubMed: 16675437]
9. Moen MH, Magnus P. The familial risk of endometriosis. *Acta Obstet Gynecol Scand.* 1993; 72:560–4. [PubMed: 8213105]
10. Olive DL. Medical therapy of endometriosis. *Semin Reprod Med.* 2003; 21:209–22. [PubMed: 12917790]
11. Guo SW. Association of endometriosis risk and genetic polymorphisms involving sex steroid biosynthesis and their receptors: a meta-analysis. *Gynecol Obstet Invest.* 2006; 61:90–105. [PubMed: 16244490]
12. Huber A, Keck CC, Hefler LA, Schneeberger C, Huber JC, Bentz EK, et al. Ten estrogen-related polymorphisms and endometriosis: a study of multiple gene-gene interactions. *Obstet Gynecol.* 2005; 106:1025–31. [PubMed: 16260521]

13. Montgomery GW, Nyholt DR, Zhao ZZ, Treloar SA, Painter JN, Missmer SA, et al. The search for genes contributing to endometriosis risk. *Hum Reprod Update*. 2008; 14:447–57. [PubMed: 18535005]
14. Marino JL, Holt VL, Chen C, Davis S. Shift work, hCLOCK T3111C polymorphism, and endometriosis risk. *Epidemiology*. 2008; 19:477–84. [PubMed: 18379422]
15. Holt VL, Weiss NS. Recommendations for the design of epidemiologic studies of endometriosis. *Epidemiology*. 2000; 11:654–9. [PubMed: 11055625]
16. Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA. Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. *Am J Hum Genet*. 2004; 74:106–20. [PubMed: 14681826]
17. Gauderman WJ, Murcray C, Gilliland F, Conti DV. Testing association between disease and multiple SNPs in a candidate gene. *Genet Epidemiol*. 2007; 31:383–95. [PubMed: 17410554]
18. Schafer, JL. *Analysis of Incomplete Multivariate Data*. London: Chapman & Hall; 1997.
19. Haiman CA, Dossus L, Setiawan VW, Stram DO, Dunning AM, Thomas G, et al. Genetic variation at the CYP19A1 locus predicts circulating estrogen levels but not breast cancer risk in postmenopausal women. *Cancer Res*. 2007; 67:1893–7. [PubMed: 17325027]
20. Stocco C. Aromatase expression in the ovary: hormonal and molecular regulation. *Steroids*. 2008; 73:473–87. [PubMed: 18321551]
21. Bulun SE, Imir G, Utsunomiya H, Thung S, Gurates B, Tamura M, et al. Aromatase in endometriosis and uterine leiomyomata. *J Steroid Biochem Mol Biol*. 2005; 95:57–62. [PubMed: 16024248]
22. Bulun SE. Endometriosis. *N Engl J Med*. 2009; 360:268–79. [PubMed: 19144942]
23. Colette S, Donnez J. Endometriosis. *N Engl J Med*. 2009; 360:1911–2. [PubMed: 19403912]
24. Colette S, Lousse JC, Defrere S, Curaba M, Heilier JF, Van LA, et al. Absence of aromatase protein and mRNA expression in endometriosis. *Hum Reprod*. 2009; 24:2133–41. [PubMed: 19493871]
25. Justenhoven C, Hamann U, Schubert F, Zapatka M, Pierl CB, Rabstein S, et al. Breast cancer: a candidate gene approach across the estrogen metabolic pathway. *Breast Cancer Res Treat*. 2008; 108:137–49. [PubMed: 17588204]
26. Haiman CA, Stram DO, Pike MC, Kolonel LN, Burt NP, Altshuler D, et al. A comprehensive haplotype analysis of CYP19 and breast cancer risk: the Multiethnic Cohort. *Hum Mol Genet*. 2003; 12:2679–92. [PubMed: 12944421]
27. Setiawan VW, Doherty JA, Shu XO, Akbari MR, Chen C, De VI, et al. Two estrogen-related variants in CYP19A1 and endometrial cancer risk: a pooled analysis in the Epidemiology of Endometrial Cancer Consortium. *Cancer Epidemiol Biomarkers Prev*. 2009; 18:242–7. [PubMed: 19124504]
28. Yang HP, Gonzalez BJ, Li Q, Platz EA, Brinton LA, Sherman ME, et al. Common genetic variation in the sex hormone metabolic pathway and endometrial cancer risk: pathway-based evaluation of candidate genes. *Carcinogenesis*. 2010; 31:827–33. [PubMed: 20053928]
29. Lamp M, Peters M, Reinmaa E, Haller-Kikkatalo K, Kaart T, Kadastik U, et al. Polymorphisms in ESR1, ESR2 and HSD17B1 genes are associated with fertility status in endometriosis. *Gynecol Endocrinol*. 2010
30. Kado N, Kitawaki J, Obayashi H, Ishihara H, Koshiba H, Kusuki I, et al. Association of the CYP17 gene and CYP19 gene polymorphisms with risk of endometriosis in Japanese women. *Hum Reprod*. 2002; 17:897–902. [PubMed: 11925378]
31. Arvanitis DA, Goumenou AG, Matalliotakis IM, Koumantakis EE, Spandidos DA. Low-penetrance genes are associated with increased susceptibility to endometriosis. *Fertil Steril*. 2001; 76:1202–6. [PubMed: 11730751]
32. Tsuchiya M, Nakao H, Katoh T, Sasaki H, Hiroshima M, Tanaka T, et al. Association between endometriosis and genetic polymorphisms of the estradiol-synthesizing enzyme genes HSD17B1 and CYP19. *Hum Reprod*. 2005; 20:974–8. [PubMed: 15640252]
33. Hur SE, Lee S, Lee JY, Moon HS, Kim HL, Chung HW. Polymorphisms and haplotypes of the gene encoding the estrogen-metabolizing CYP19 gene in Korean women: no association with advanced-stage endometriosis. *J Hum Genet*. 2007; 52:703–11. [PubMed: 17687621]

34. Vietri MT, Cioffi M, Sessa M, Simeone S, Bontempo P, Trabucco E, et al. CYP17 and CYP19 gene polymorphisms in women affected with endometriosis. *Fertil Steril*. 2008
35. Altmae S, Haller K, Peters M, Saare M, Hovatta O, Stavreus-Evers A, et al. Aromatase gene (CYP19A1) variants, female infertility and ovarian stimulation outcome: a preliminary report. *Reprod Biomed Online*. 2009; 18:651–7. [PubMed: 19549443]
36. Painter JN, Anderson CA, Nyholt DR, Macgregor S, Lin J, Lee SH, et al. Genome-wide association study identifies a locus at 7p15. 2 associated with endometriosis. *Nat Genet*. 2011; 43:51–4. [PubMed: 21151130]
37. Uno S, Zembutsu H, Hirasawa A, Takahashi A, Kubo M, Akahane T, et al. A genome-wide association study identifies genetic variants in the CDKN2BAS locus associated with endometriosis in Japanese. *Nat Genet*. 2010; 42:707–10. [PubMed: 20601957]
38. Hsieh YY, Wang YK, Chang CC, Lin CS. Estrogen receptor {alpha}-351 XbaIG and -397 PvuIIC-related genotypes and alleles are associated with higher susceptibilities of endometriosis and leiomyoma. *Mol Hum Reprod*. 2007; 13:117–22. [PubMed: 17121748]
39. Kim SH, Choi YM, Jun JK, Kim SH, Kim JG, Moon SY. Estrogen receptor dinucleotide repeat polymorphism is associated with minimal or mild endometriosis. *Fertil Steril*. 2005; 84:774–7. [PubMed: 16169423]
40. Renner SP, Strick R, Oppelt P, Fasching PA, Engel S, Baumann R, et al. Evaluation of clinical parameters and estrogen receptor alpha gene polymorphisms for patients with endometriosis. *Reproduction*. 2006; 131:153–61. [PubMed: 16388018]
41. Xie J, Wang S, He B, Pan Y, Li Y, Zeng Q, et al. Association of estrogen receptor alpha and interleukin-10 gene polymorphisms with endometriosis in a Chinese population. *Fertil Steril*. 2009; 92:54–60. [PubMed: 18687423]
42. Lamp M, Peters M, Reinmaa E, Haller-Kikkatalo K, Kaart T, Kadastik U, et al. Polymorphisms in ESR1, ESR2 and HSD17B1 genes are associated with fertility status in endometriosis. *Gynecol Endocrinol*. 2011; 27:425–33. [PubMed: 20586553]
43. Govindan S, Ahmad SN, Vedicherla B, Kodati V, Jahan P, Rao KP, et al. Association of progesterone receptor gene polymorphism (PROGINS) with endometriosis, uterine fibroids and breast cancer. *Cancer Biomark*. 2007; 3:73–8. [PubMed: 17522428]
44. Wang Z, Yoshida S, Negoro K, Kennedy S, Barlow D, Maruo T. Polymorphisms in the estrogen receptor beta gene but not estrogen receptor alpha gene affect the risk of developing endometriosis in a Japanese population. *Fertil Steril*. 2004; 81:1650–6. [PubMed: 15193490]
45. Lee GH, Kim SH, Choi YM, Suh CS, Kim JG, Moon SY. Estrogen receptor beta gene +1730 G/A polymorphism in women with endometriosis. *Fertil Steril*. 2007; 88:785–8. [PubMed: 17336962]
46. Fang Z, Yang S, Lydon JP, DeMayo F, Tamura M, Gurates B, et al. Intact progesterone receptors are essential to counteract the proliferative effect of estradiol in a genetically engineered mouse model of endometriosis. *Fertil Steril*. 2004; 82:673–8. [PubMed: 15374713]
47. Wieser F, Schneeberger C, Tong D, Tempfer C, Huber JC, Wenzl R. PROGINS receptor gene polymorphism is associated with endometriosis. *Fertil Steril*. 2002; 77:309–12. [PubMed: 11821088]
48. Lattuada D, Somigliana E, Vigano P, Candiani M, Pardi G, Di Blasio AM. Genetics of endometriosis: a role for the progesterone receptor gene polymorphism PROGINS? *Clin Endocrinol (Oxf)*. 2004; 61:190–4. [PubMed: 15272913]
49. De Carvalho CV, Nogueira-de-Souza NC, Costa AM, Baracat EC, Girao MJ, D'Amora P, et al. Genetic polymorphisms of cytochrome P450c17alpha (CYP17) and progesterone receptor genes (PROGINS) in the assessment of endometriosis risk. *Gynecol Endocrinol*. 2007; 23:29–33. [PubMed: 17484509]
50. Treloar SA, Zhao ZZ, Armitage T, Duffy DL, Wicks J, O'Connor DT, et al. Association between polymorphisms in the progesterone receptor gene and endometriosis. *Mol Hum Reprod*. 2005; 11:641–7. [PubMed: 16126772]
51. Near AM, Wu AH, Templeman C, Van Den Berg DJ, Doherty JA, Rossing MA, et al. Progesterone receptor gene polymorphisms and risk of endometriosis: results from an international collaborative effort. *Fertil Steril*. 2011; 95:40–5. [PubMed: 20719308]

52. Gimenes C, Bianco B, Mafra FA, Rosset V, Christofolini DM, Barbosa CP. The progins progesterone receptor gene polymorphism is not related to endometriosis-associated infertility or to idiopathic infertility. *Clinics (Sao Paulo)*. 2010; 65:1073–6. [PubMed: 21243274]
53. Feigelson HS, Shames LS, Pike MC, Coetzee GA, Stanczyk FZ, Henderson BE. Cytochrome P450c17alpha gene (CYP17) polymorphism is associated with serum estrogen and progesterone concentrations. *Cancer Res*. 1998; 58:585–7. [PubMed: 9485002]
54. Hsieh YY, Chang CC, Tsai FJ, Lin CC, Tsai CH. Estrogen receptor alpha dinucleotide repeat and cytochrome P450c17alpha gene polymorphisms are associated with susceptibility to endometriosis. *Fertil Steril*. 2005; 83:567–72. [PubMed: 15749482]
55. Asghar T, Yoshida S, Nakago S, Morizane M, Ohara N, Motoyama S, et al. Lack of association between endometriosis and the CYP17 MspA1 polymorphism in UK and Japanese populations. *Gynecol Endocrinol*. 2005; 20:59–63. [PubMed: 15823822]
56. Juo SH, Wang TN, Lee JN, Wu MT, Long CY, Tsai EM. CYP17, CYP1A1 and COMT polymorphisms and the risk of adenomyosis and endometriosis in Taiwanese women. *Hum Reprod*. 2006; 21:1498–502. [PubMed: 16527884]
57. Bozdag G, Alp A, Saribas Z, Tuncer S, Aksu T, Gurgan T. CYP17 and CYP2C19 gene polymorphisms in patients with endometriosis. *Reprod Biomed Online*. 2010; 20:286–90. [PubMed: 20113968]
58. Guo SW. The association of endometriosis risk and genetic polymorphisms involving dioxin detoxification enzymes: a systematic review. *Eur J Obstet Gynecol Reprod Biol*. 2006; 124:134–43. [PubMed: 16289302]
59. Kelly RW, Abel MH. A comparison of the effects of 4-catechol oestrogens and 2-pyrogallol oestrogens on prostaglandin synthesis by the rat and human uterus. *J Steroid Biochem*. 1981; 14:787–91. [PubMed: 6895395]
60. Babu KA, Reddy NG, Deendayal M, Kennedy S, Shivaji S. GSTM1, GSTT1 and CYP1A1 detoxification gene polymorphisms and their relationship with advanced stages of endometriosis in South Indian women. *Pharmacogenet Genomics*. 2005; 15:167–72. [PubMed: 15861041]
61. Tsuchiya M, Tsukino H, Iwasaki M, Sasaki H, Tanaka T, Katoh T, et al. Interaction between cytochrome P450 gene polymorphisms and serum organochlorine TEQ levels in the risk of endometriosis. *Mol Hum Reprod*. 2007; 13:399–404. [PubMed: 17449539]
62. Mannisto PT, Kaakkola S. Catechol-O-methyltransferase (COMT): biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. *Pharmacol Rev*. 1999; 51:593–628. [PubMed: 10581325]
63. Wieser F, Wenzl R, Tempfer C, Worda C, Huber J, Schneeberger C. Catechol-O-methyltransferase polymorphism and endometriosis. *J Assist Reprod Genet*. 2002; 19:343–8. [PubMed: 12168735]
64. Maria CD, Souto TJ, Lima VF, Mendonca AG, Bianco B, Parente BC. COMT polymorphism and the risk of endometriosis-related infertility. *Gynecol Endocrinol*. 2011
65. Guo SW. Glutathione S-transferases M1/T1 gene polymorphisms and endometriosis: a meta-analysis of genetic association studies. *Mol Hum Reprod*. 2005; 11:729–43. [PubMed: 16291859]
66. Roya R, Baludu GS, Reddy BS. Possible aggravating impact of gene polymorphism in women with endometriosis. *Indian J Med Res*. 2009; 129:395–400. [PubMed: 19535834]
67. Hosseinzadeh Z, Mashayekhi F, Sorouri ZZ. Association between GSTM1 gene polymorphism in Iranian patients with endometriosis. *Gynecol Endocrinol*. 2011; 27:185–9. [PubMed: 20504102]
68. Huang PC, Tsai EM, Li WF, Liao PC, Chung MC, Wang YH, et al. Association between phthalate exposure and glutathione S-transferase M1 polymorphism in adenomyosis, leiomyoma and endometriosis. *Hum Reprod*. 2010; 25:986–94. [PubMed: 20147336]
69. Saunders, KW.; Davis, RL.; Stergachis, A. Group Health Cooperative. In: Strom, BL., editor. *Pharmacoepidemiology*. West Sussex, England: John Wiley & Sons, Ltd; 2008. p. 223-39.

Figure 1.

LOD scores for 86 SNPs in 11 sex-hormone-related genes examined in the population-based case-control study of endometriosis, Group Health, Puget Sound Metropolitan Area, 1996–2001. LOD scores are from models using the log-additive parameterization. The solid horizontal line represents the threshold for $p\text{-value} = 0.05$ and the dotted horizontal line represents the threshold for the conservative Bonferroni corrected $p\text{-value} = 0.0006 (= 0.05/86)$.

Table 1

Demographic and health characteristics of endometriosis cases and controls, Group Health, Puget Sound Metropolitan Area, 1996–2001

	Cases	Controls
	N=256	N=567
<i>Age (years)</i>	n* (%)	n* (%)
18–24	21 (8.2)	45 (7.9)
25–34	51 (19.9)	101 (17.8)
35–44	126 (49.2)	291 (51.3)
45–49	58 (22.7)	130 (22.9)
<i>Race</i>		
Caucasian	209 (81.6)	470 (83.0)
African American	8 (3.1)	24 (4.2)
Asian American	13 (5.1)	35 (6.2)
Other	26 (10.2)	37 (6.5)
<i>Income</i>		
<\$35,000	77 (31.0)	153 (27.9)
35,000–49,999	52 (21.0)	117 (21.3)
50,000–69,999	57 (23.0)	125 (22.7)
70,000+	62 (25.0)	155 (28.2)
<i>Education (years)</i>		
<12	9 (3.5)	18 (3.2)
12	44 (17.2)	101 (17.8)
>12	203 (79.3)	448 (79)
<i>BMI</i>		
Underweight (<18.5 kg/m ²)	8 (3.1)	11 (2.0)
Normal (18.5–24.9 kg/m ²)	127 (49.6)	293 (52.0)
Overweight (25–29.9 kg/m ²)	64 (25.0)	145 (25.8)
Obese (>30 kg/m ²)	57 (22.3)	114 (20.3)
<i>Smoking</i>		
Current	53 (20.7)	95 (16.8)
Former	57 (22.3)	133 (23.5)
Never	146 (57.0)	339 (59.8)
<i>Alcohol use</i>		
Current	141 (55.3)	257 (45.3)
Former	42 (16.5)	119 (21.0)
Never	72 (28.2)	191 (33.7)
<i>Parity</i>		
Parous	135 (52.7)	400 (70.7)
Nulliparous	121 (47.3)	166 (29.3)
<i>Average menstrual cycle length (days)</i>		
< 28	68 (26.6)	104 (18.4)

	Cases	Controls
	N=256	N=567
28–34	103 (40.2)	278 (49.1)
35 +	7 (2.7)	12 (2.1)
Not regular in last decade	65 (25.4)	96 (17.0)
No periods in last decade	2 (0.8)	10 (1.8)
<i>OC use</i>		
Current	31 (12.1)	70 (12.4)
Former	196 (76.6)	412 (72.7)
Never	29 (11.3)	85 (15.0)

*
Cell counts may not sum to column total because of missing data

Table 2

Gene-based tests of association between SNPs in sex hormone-related genes and risk of endometriosis (256 case, 567 controls), Group Health, Puget Sound Metropolitan Area, 1996–2001.

Gene	# SNPs	# PC	Gene-based p-value
<i>ESR1</i>	13	4	0.71
<i>ESR2</i>	20	5	0.23
<i>PGR</i>	16	4	0.89
<i>CYP17A1</i>	5	2	0.22
<i>CYP19A1</i>	17	6	0.02
<i>HSD17B2</i>	7	4	0.08

PC = Principle components