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Common Variation in the *CYP17A1* and *IFIT1* Genes on Chromosome 10 Does Not Contribute to the Risk of Endometriosis

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Abstract: Endometriosis is a complex disease involving multiple susceptibility genes and environmental factors. Our previous studies on endometriosis identified a region of significant linkage on chromosome 10q. Two biological candidate genes (*CYP17A1* and *IFIT1*) located on chromosome 10q, have previously been implicated in endometriosis and/or uterine function. We hypothesized that variation in *CYP17A1* and/or *IFIT1* could contribute to the risk of endometriosis and may account for some of the linkage signal on chromosome 10q. We genotyped 17 single nucleotide polymorphisms (SNPs) in the *CYP17A1* and *IFIT1* genes including SNP rs743572 previously associated with endometriosis in 768 endometriosis cases and 768 unrelated controls. We found no evidence for association between endometriosis and individual SNPs or SNP haplotypes in *CYP17A1* and *IFIT1*. Common variation in these genes does not appear to be a major contributor to endometriosis susceptibility in our Australian sample.

INTRODUCTION

Endometriosis is a complex disease involving multiple susceptibility genes and environmental factors [1-4]. Estimates of the population prevalence indicate that endometriosis affects 8–10% of women of reproductive age [2, 5], but the reasons for establishment and progression of endometriosis remain uncertain.

Our previous studies on endometriosis identified a region of significant linkage on chromosome 10q26 [6]. The peak linkage signal is located at 148.75 cM between markers D10S587 and D10S1656 and the 95% confidence interval (CI) spans a region of 8.5 megabase pairs (Mbp). Gene mapping of plausible candidates may help to define the mechanisms contributing to the genetic susceptibility of endometriosis. Two candidate genes on chromosome 10q, which have previously been implicated in endometriosis and uterine function, are cytochrome P450, family 17, subfamily A, polypeptide 1 (*CYP17A1*, MIM #609300) and interferon-induced protein with tetratricopeptide repeats 1 (*IFIT1*, MIM #147690) [7-9].

Human endometrium is highly responsive to hormonal stimuli during the menstrual cycle with estrogen and progesterone influencing maturational and functional changes in the endometrium. The *CYP17A1* gene lies at 104.5 Mbp, on the shoulder of our linkage peak on chromosome 10 and encodes the cytochrome P450c17α enzyme that is involved in estrogen biosynthesis and metabolism [10]. The gene is expressed in human follicles, corpora lutea and endometrial carcinoma cells [11-13]. A number of studies on *CYP17A1* variation suggest it could be a genetic biomarker for hormone related diseases [7, 14, 15]. One single nucleotide polymorphism (SNP, rs743572) at the -34 bp position relative to the start codon in the 5'UTR promoter region of *CYP17A1* has been commonly studied. The SNP was thought to be associated with a Sp-1 binding site that would lead to higher gene expression in male baldness [16]. In patients with endometrial cancer, a marked decrease in the A2 allele of *CYP17A1* was seen when compared with normal controls [7]. A subsequent study on 119 endometriosis cases and 108 normal controls demonstrated that the *CYP17A1* allele was associated with an increased risk of endometriosis in a Chinese population [8], but lack of association between endometriosis and the *CYP17A1* polymorphism was observed in UK and Japanese populations [17].

The *IFIT1* gene is located at 91.1 Mbp, close to the *CYP17A1* locus just outside the 95% confidence region for our linkage peak. However the linkage peak is broad and there is evidence for linkage and association with endometriosis in Puerto Rican families at marker D10S677 [18], which is located at 113.34 cM (95.95 Mb) close to the *IFIT1* locus. During early pregnancy, *IFIT1* is highly expressed upon stimulation with interferon tau (*IFNT*), a type I interferon produced by the conceptus trophectoderm [9, 19]. As an endometrial gene, *IFIT1* is believed to respond to hormonal stimulation and may have a functional role for uterine support of peri-implantation conceptus survival, growth, and implantation [9]. Although *IFIT1* has been studied in the sheep model, it is unknown if variants in *IFIT1* contribute to risk of human endometriosis.

We previously excluded association between endometriosis and candidate genes under the linkage peak including empty spiracles homeobox 2 (*EMX2*), phosphatase and tensin homolog (*PTEN*) and fibroblast growth factor receptor 2 (*FGFR2*) in our Australian sample [20, 21]. To continue to search for the gene or genes contributing to the linkage peak, we examined variation in *IFIT1* and/or *CYP17A1* to determine whether those genes contribute to the risk of endometriosis and may account for some of the linkage signal on chromosome 10q.

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MATERIALS AND METHODS

Participants and Sample Collection

The project was approved by the Human Research Ethics Committee of the Queensland Institute of Medical Research and the Australian Twin Registry. Women with surgically confirmed endometriosis were selected from each of 768 Australian affected sister pair families as previously described [20]. The sister with the most severe stage of disease was chosen for genotyping. Disease severity was assessed retrospectively from medical records using the revised American Fertility Society (rAFS) classification system [22]. Sixty one percent of cases were classified with minimal to mild endometriosis (rAFS stages I/II). The remaining 39% of cases with moderate to severe (rAFS stages III/IV) endometriosis were more likely to have ovarian endometriosis. A total of 645 cases (84%) were diagnosed at laparoscopy; the remaining cases were mostly diagnosed at hysterectomy, or in a small number of cases at laparotomy or during another procedure.

The controls were 768 unrelated women who had volunteered for a twin study of gynaecological health [2]. Controls were selected after consideration of the competing issues of ascertainment bias from clinic controls and presence of undiagnosed cases. They were selected from women who self-reported they had never been diagnosed with endometriosis and were therefore considered to be at low risk of having endometriosis. Twins had been asked simply 'have you had endometriosis?'[2]. Additional information from medical records was used where available. Women were also asked whether they had ever had a laparoscopy and/or a hysterectomy and the reasons for each. About 14% of control women reported having a hysterectomy and/or laparoscopy. No evidence of endometriosis was reported at any of these procedures in our control sample [20]. The mean ages (± SD) of the cases and controls at the time of data collection were 35.6 ± 9.1 years (range = 17-65) and 45.7 ± 12.2 (range = 29-90) years respectively. Genomic DNAs were extracted [23], and diluted to a working concentration of 2.5 ng/µl. The case and control DNAs were randomly placed in 384-well PCR plates.

SNP Selection

We selected 7 SNPs in the *CYP17A1* gene based upon the allelic association results in endometriosis [8] and allele frequency information from the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/). Ten SNPs were selected in the *IFIT1* gene on the basis of the allelic frequency information and SNP distribution across the gene. The chosen *CYP17A1* SNP list comprised two promoter, two intronic, two coding exonic and one 3'UTR SNPs. One 5'UTR promoter, seven intronic and two coding exonic SNPs were chosen in the *IFIT1* gene. All SNP sequences were downloaded from the Chip Bioinformatics database (http://snpper.chip.org/) and the sequences were cross checked in NCBI and Sequenom RealSNP databases (https://www.realsnp.com/) before assay design.

Genotyping

Multiplex assays were designed using the Sequenom MassARRAY Assay Design software (version 3.0). SNPs were typed using Sequenom iPLEX™ chemistry on a MALDI-TOF Mass Spectrometer. The 2.5 µL PCR reactions were performed in standard 384-well plates using 10 ng genomic DNA, 0.5 unit of Taq polymerase (Qiagen, Valencia, CA), 500 µmol of each dNTP, and 100 nmol of each PCR primers. Standard PCR thermal cycling conditions and post-PCR extension reactions were carried out as described previously [24]. The iPLEX reaction products were desalted by diluting samples with 15µl of water and adding 3µl of resin. The products were spotted on a SpectroChip (Sequenom), and data were processed and analysed in a Compact Mass Spectrometer by MassARRAY Workstation (version 3.4) software (Sequenom).

Statistical Analysis

The genotypes were inspected and results were tested for departures from Hardy-Weinberg equilibrium (HWE) separately for cases and controls using Haploview version 3.32 (Whitehead Institute for Biomedical Research, USA). The PLINK program (http://pngu.mgh.harvard.edu/purcell/plink/) was used to test association between endometriosis and individual SNPs. Global p-values were obtained for each marker or each haplotype by performing 10,000 permutation tests. Haplotype frequencies, linkage disequilibrium (LD) estimates and analysis were determined by Haploview [25] using the default method of Gabriel [26]. A global p-value <0.05 was considered to be statistically significant.

We performed power calculations for our case-control study assuming a disease (endometriosis) prevalence of 10% using the Genetic Power Calculator [27]. Power calculations were based on 768 unrelated cases and 768 unrelated controls using a significance threshold (α) of $P = 0.01$.

RESULTS

Seven SNPs in the *CYP17A1* gene and ten SNPs in the *IFIT1* gene were typed in 768 endometriosis cases and 768 unrelated controls. All SNPs were in Hardy–Weinberg equilibrium. The minor allele frequencies of the *CYP17A1* SNPs ranged from 0.230 to 0.414 in cases and from 0.200 to 0.443 in controls. The minor allele frequencies of the *IFIT1* SNPs ranged from 0.058 to 0.484 in cases and from 0.072 to 0.498 in controls (Table **1**). The minor allele frequency for the key SNP (rs743572) in the *CYP17A1* gene was 0.386 and 0.397 in cases and controls, respectively. There was no significant difference in allele frequency between cases and controls for this key SNP (Table **1**). SNP rs2486758, at the -362 bp position relative to the start codon in the 5'UTR promoter region of *CYP17A1* gene showed nominal evidence of association ($P < 0.05$). However, the difference in allele frequency between cases and controls was small and the effects were not significant after correcting for multiple testing of all SNPs.

The positions of the SNPs genotyped in the *IFIT1* gene and the *CYP17A1* gene are shown in Fig. (**1a**). A linkage disequilibrium plot of SNPs and common haplotype blocks for the both genes are also shown in Fig. (**1b**,**c**). We found no evidence for association between endometriosis and individual SNPs in either *IFIT1* or *CYP17A1* for either the allelic or the genotypic association tests (Table **1**).

Stratification of cases according to stage of disease (469 Stage A cases and 768 controls) gave a best point-wise P-value of 0.02 for SNP rs619824 in the *CYP17A1* gene, but the global result correcting for multiple tests was non-significant ($P = 0.58$). Analysis of 296 cases diagnosed with

Table 1. Association Analysis of 17 SNPs Across the *IFIT1* and *CYP17A1* Gene Locus Genotyped in 768 Endometriosis Cases and 768 Controls

dbSNP ID	SNP Position	Gene(s)	Role	Alleles	MAF-Cases	MAF-Controls	χ^2	*P* Value	OR
rs304478	chr10:91140902	*IFIT1*	Promoter	G>T	0.456	0.463	0.139	0.709	0.972
rs303218	chr10:91142573	*IFIT1*	Intron	G>A	0.175	0.186	0.620	0.431	0.929
rs303217	chr10:91143679	*IFIT1*	Intron	T>C	0.470	0.469	0.002	0.968	1.003
rs303216	chr10:91145166	*IFIT1*	Intron	C>T	0.169	0.182	0.858	0.354	0.915
rs303215	chr10:91146811	*IFIT1*	Intron	T>C	0.170	0.182	0.754	0.385	0.921
rs304484	chr10:91149303	*IFIT1*	Intron	G>T	0.231	0.244	0.770	0.380	0.928
rs304485	chr10:91149890	*IFIT1*	Intron	T>A	0.484	0.498	0.578	0.447	0.946
rs303212	chr10:91151335	*IFIT1*	Intron	T>C	0.232	0.241	0.357	0.550	0.950
rs303211	chr10:91152477	*IFIT1*	Coding exon	G>A	0.058	0.072	2.581	0.108	0.790
rs303210	chr10:91152657	*IFIT1*	Coding exon	C>T	0.169	0.182	0.822	0.365	0.916
rs619824	chr10:104571278	*CYP17A1*	3' UTR	G>T	0.414	0.443	2.633	0.105	0.888
rs3740397	chr10:104582665	*CYP17A1*	Intron (boundary)	C>G	0.375	0.396	1.404	0.236	0.916
rs4919687	chr10:104585238	*CYP17A1*	Intron (boundary)	G>A	0.347	0.359	0.539	0.463	0.946
rs6163	chr10:104586914	*CYP17A1*	Coding exon	C>A	0.376	0.395	1.058	0.304	0.926
rs6162	chr10:104586971	*CYP17A1*	Coding exon	G>A	0.389	0.403	0.573	0.449	0.945
rs743572	chr10:104587142	*CYP17A1*	5' UTR	T>C	0.386	0.397	0.376	0.540	0.954
rs2486758	chr10:104587470	*CYP17A1*	5' UTR	T>C	0.230	0.200	3.870	0.049	1.189

MAF: minor allele frequency.
dbSNP ID: database SNP identification.
UTR: untranslated region.

stage B and 768 controls, showed no significant differences between cases and controls for any SNPs typed in the study.

DISCUSSION

Our results do not support an association between endometriosis and common variation in either *CYP17A1* or *IFIT1*. Although both genes are biological candidates for endometriosis and are located near the peak of our linkage signal on chromosome 10, there was no evidence that variants in either gene were associated with endometriosis or contribute to the linkage signal on chromosome 10q.

Genetic studies of *CYP17A1* variants to date have followed a defined biological hypothesis suggesting the 5'UTR promoter region SNP (rs743572) is associated with gene expression [16]. While the T allele (rs743572) was found to be associated with increased risk of endometriosis in a study of Chinese women [14], there was no association between *CYP17A1* variants and endometriosis in studies in Brazilian, UK or Japanese populations [17, 28, 29]. Endometriosis is a sex steroid-dependent disease [30]. *CYP17A1* is involved in estrogen biosynthesis and metabolism so that certain genetic polymorphisms in the gene could be associated with increased risk of developing endometriosis. The differences in the results may relate to study power (sample size) or population differences. We estimated power for our case-control study based on total sample of 768 cases and 768 controls. There is over 80% power to detect allele frequency of 0.05, 0.25 and 0.5 contributing a dominant genotype relative risk (GRR) of 1.7, 1.5 and 1.8, respectively. In contrast, when the sample size changed to total sample of 100 cases and 100 controls, there is only 10% power to detect allele frequency of 0.05, 0.25 and 0.5 contributing the similar genotype relative risk as stated above. These calculations demonstrate our sample has high power to detect novel gene associations of moderate effect. However, because our cases are highly selected in terms of family history, compared to a standard case-control association study, our sample will have considerably more power to detect gene associations.

We examined variation in *CYP17A1* in cases from families contributing to the linkage peak in this region of chromosome 10q and genotyped 7 *CYP17A1* SNPs including rs743572 in 768 endometriosis and 768 controls. We found no evidence for association with either SNP rs743572 in the promoter of *CYP17A1* or the other 6 SNPs in the gene in our Australian sample. Strong linkage disequilibrium (LD) was detected between SNP rs743572 and the other three SNPs in the gene (rs3740397, r^2=0.948; rs6163, r^2=0.958; rs6162, r^2=0.919) in our sample. It is unlikely that any asymptomatic cases present in the control samples would affect the conclusion from this study for a disease with a prevalence of 8-10% [31].

IFIT1 is a hormonally responsive gene expressed in sheep endometrium following stimulation with *IFNT* [9, 19]. To test for association between *IFIT1* variants and human endometriosis we typed 10 common *IFIT1* SNPs in our 768 endometriosis cases and 768 unrelated controls. There was no evidence for association between the individual SNPs and endometriosis. Analysis of the SNPs across the *IFIT1* locus

Fig. (1). Variants typed in the human *IFIT1* and *CYP17A1* genes (**a**) the genomic structure of the *IFIT1* and *CYP17A1* genes showing the location of the 17 SNPs genotyped (**b**) the linkage disequilibrium plot of single nucleotide polymorphism estimated as γ^2 using Haploview (**c**) common haplotypes and association analysis with endometriosis. Shading key: white γ^2=0; shades of grey 0>γ^2<1; black γ^2=1

showed strong linkage disequilibrium, with two haplotype blocks and three major haplotypes accounting for 87% of the chromosomes in our case samples. Our data also show strong linkage disequilibrium (LD) in two blocks covering most of the gene in our samples (Fig. **1b**). We did not find any evidence for association between SNP haplotype frequencies and endometriosis. *IFIT1* may be important in uterine development and function, but common variation is not associated with risk of endometriosis.

In this study, we examined the association between endometriosis and individual common SNPs and haplotypes in the *IFIT1* and *CYP17A1* genes on chromosome 10q in an Australian population including a functional SNP in the *CYP17A1* promoter region. Our data does not provide evidence supporting an association between common variation in the *IFIT1* and *CYP17A1* genes and endometriosis susceptibility. We have previously demonstrated significant linkage to endometriosis on chromosome 10q [6]. Results of the present study demonstrate that variation in the *CYP17A1* and *IFIT1* genes does not explain linkage to endometriosis in this region of chromosome 10. Both genes are good candidates for endometriosis but the linkage region spans approximately 8.5 million DNA base pairs and contains over 50 known genes within the 95% confidence interval for the linkage peak. Many of these other genes can also be considered candidates for endometriosis and variation in one or more of these genes may explain the linkage signal in this region. We conclude that common variants in the *IFIT1* and *CYP17A1* genes do not play a key role in the pathogenesis of endometriosis.

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