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Polymorphisms in the vascular endothelial growth factor gene and the risk of familial endometriosis

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Vascular endothelial growth factor (*VEGF*) is an endothelial cell-specific angiogenic protein suspected to be involved in the pathogenesis of endometriosis by establishing a new blood supply to the human exfoliated endometrium. Several transcription factor-binding sites are found in the *VEGF* 5′-untranslated region and variation within the region increases the transcriptional activity. Six previous studies which tested between one and three single nucleotide polymorphisms (SNPs) in samples comprising 105–215 cases and 100–219 controls have produced conflicting evidence for association between the SNPs in the *VEGF* region and endometriosis. To further investigate the reported association between *VEGF* variants and endometriosis, we tested the four *VEGF* polymorphisms (−2578 A/C, rs699947; −460 T/C, rs833061; +405 G/C, rs2010963 and +936 C/T, rs3025039) in a large Australian sample of 958 familial endometriosis cases and 959 controls. We also conducted a literature-based review of all relevant association studies of these *VEGF* SNPs in endometriosis and performed a meta-analysis. There was no evidence for association between endometriosis and the *VEGF* polymorphisms genotyped in our study. Combined association results from a meta-analysis did not provide any evidence for either genotypic or allelic association with endometriosis. Our detailed review and meta-analysis of the *VEGF* polymorphisms suggests that genotyping assay problems may underlie the previously reported associations between *VEGF* variants and endometriosis.

Keywords: endometriosis; *VEGF*; polymorphism; meta-analysis

Introduction

The precise aetiology of endometriosis is still not clearly defined, although transplantation theory has been widely accepted (McLaren, 2000). The establishment of a new blood supply in the human exfoliated endometrium requires vascular proliferation and differentiation. From the known angiogenic factors, vascular endothelial growth factor (*VEGF*) has emerged as likely being involved in the pathogenesis of endometriosis (Smith, 1997; Donnez *et al*., 1998).

VEGF is an endothelial cell-specific angiogenic protein that appears to play an important role in a variety of oestrogen target tissues in regulating endometrial angiogenesis at a local level (Girling and Rogers, 2005). *VEGF* expression was observed in serum, peritoneal fluid and endometrial cell cultures in women with endometriosis (Print *et al*., 2004; Lin and Gu, 2005; Oliveira *et al*., 2005). A large number of studies have investigated and observed that *VEGF* messenger ribonucleic acid and protein were significantly higher in women with endometriosis, which supported a key role for *VEGF* in the pathological angiogenesis in endometriosis (Shifren *et al*., 1996; Kupker *et al*., 1998; Fasciani *et al*., 2000; Tan *et al*., 2002; Khan *et al*., 2003; Matalliotakis *et al*., 2003b; Gilabert-Estelles *et al*., 2007). A single family in two generations with four members who have histologically proven endometriosis showed that the circulating levels of *VEGF* were higher than the healthy control group, indicating a role for *VEGF* in disease susceptibility (Matalliotakis *et al*., 2003a; Simpson *et al*., 2003).

The human *VEGF* gene (*VEGFA*, OMIM 192240) is located on chromosome 6p12. Several transcription factor-binding sites are found in the *VEGF* 5′-untranslated region (5′-UTR) and variation within the region increases the transcriptional activity (Fukumura *et al*., 1998; Lambrechts *et al*., 2003). Single nucleotide polymorphisms (SNPs) in the *VEGF* 5′ or 3′-UTR have been reported to be associated with endometriosis in different populations. In Chinese patients, the T allele of the *VEGF* gene −60 T/C (rs833061) polymorphism was associated with a higher risk of endometriosis (Hsieh *et al*., 2004). Study of the *VEGF* +405 G/C (rs2010963) polymorphism in a Korean population showed that the SNP was associated with the risk of advanced stage endometriosis (Kim *et al*., 2005). The analysis of both SNPs in an Indian population identified a haplotype associated with endometriosis (Bhanoori *et al*., 2005). In addition, the analysis of *VEGF* −460 T/C (rs833061), +405 G/C (rs2010963) and +936 C/T (rs3025039) polymorphisms in 147 endometriosis cases and 181 controls found a positive association between stages III–IV disease and the *VEGF* +936 T allele in a Japanese population (Ikuhashi *et al*., 2007). In contrast, there was no association between the *VEGF* +936 C/T (rs3025039) polymorphism and endometriosis in a Korean population (Kim *et al*., 2008). Recently, the first reported study in a Caucasian population of +405 G/C (rs2010963) in 203 Italian women affected with endometriosis and 140 controls reported a weak association of the C allele with endometriosis (Gentilini *et al*., 2008). Moreover, the −2578 A/C polymorphism (rs699947) located in the *VEGF*

5′-UTR region and close to SNP −460 T/C (rs833061) has been implicated with increased breast cancer risk in number of studies (Jin *et al*., 2005; Jacobs *et al*., 2006; Schneider *et al*., 2007). This finding may be taken as further support for a possible role of *VEGF* variants in endometriosis given the strong evidence from epidemiological data supporting a link between endometriosis and breast cancer (Mostoufizadeh and Scully, 1980; Brinton *et al*., 2005; Melin *et al*., 2007).

Since several studies testing between one and three SNPs consisting of 105–215 cases and 100–219 controls have produced conflicting evidence for association between SNPs in the *VEGF* region and endometriosis, we genotyped four *VEGF* polymorphisms (−2578 A/C, rs699947; −460 T/C, rs833061; +405 G/C, rs2010963 and +936 C/T, rs3025039) to evaluate the association between endometriosis and these polymorphisms in our large Australian case–control sample.

Materials and Methods

Study participants

The project was approved by the Human Research Ethics Committee of the Queensland Institute of Medical Research and the Australian Twin Registry. One woman with surgically confirmed endometriosis was selected from each of our 958 Australian families as previously described (Zhao *et al*., 2007). The woman with the most severe stage of disease was chosen. Disease severity was assessed retrospectively from medical records using the revised American Fertility Society (rAFS) (1985) classification system. Fifty-nine percent of the cases were classified with minimal to mild endometriosis (rAFS stage I/II). The remaining 41% of the cases were classified with moderate to severe (rAFS stage III/IV) endometriosis and were more likely to have ovarian endometriosis. The controls were 959 unrelated women who had volunteered for a twin study of gynecological health (Treloar *et al*., 1999). Controls were selected from women who self-reported that 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?' (Treloar *et al*., 1999). 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. No evidence of endometriosis was reported in the 27% of control women who reported having a hysterectomy and/or laparoscopy, thus supporting the low risk for endometriosis in our control sample. The mean ages (+SD) of the cases and controls at the time of data collection were 35.82 ± 8.87 years (range = 17–65) and 45.60 ± 11.98 (range = 29–90) years, respectively. The majority (>90%) of our samples were Caucasian (Treloar *et al*., 2002, 2007). Genomic DNAs were extracted (Miller *et al*., 1988) and diluted to a working concentration of 2.5 ng/ml. The case and control DNAs were randomly placed in 384-well PCR plates.

SNP selection

Four highly plausible candidate SNPs in the *VEGF* gene were selected and genotyped, including three 5′-UTR SNPs (−2578 A/C, rs699947; −460 T/C, rs833061 and +405 G/C, rs2010963) and one 3′-UTR SNP (+936 C/T, rs3025039) (Table I). All SNP sequences were downloaded from the Chip Bioinformatics database (http://snpper.chip.org/) and the sequences were cross checked in the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) and Sequenom RealSNP databases (https://www.realsnp.com/) before assay design.

SNP genotyping

Multiplex assays were designed using the Sequenom MassARRAY Assay Design software (version 3.0). SNPs were genotyped using Sequenom iPLEX™ chemistry on a MALDI-TOF Compact Mass Spectrometer (Sequenom Inc., San Diego, CA, USA). The 2.5 μl PCR reactions were performed in standard 384-well plates using 12.5 ng genomic DNA, 0.8 U of *Taq* polymerase (HotStarTaq, Qiagen, Valencia, CA, USA), 500 μmol of each dNTP, 1.625 mM of $MgCl_2$ and 100 nmol of each PCR primers (Bioneer, Korea). Standard PCR thermal cycling conditions and post-PCR extension reactions were carried out as previously described (Zhao *et al*., 2007). The iPLEX reaction products were desalted by diluting samples with 15 μl of water and adding 3 μl of resin. The products were spotted on a SpectroChip (Sequenom Inc, San Diego, CA, USA), and data were processed and analysed by MassARRAY TYPER 3.4 software (Sequenom Inc.).

Statistical analysis

SNP genotypes were tested for departures from Hardy–Weinberg equilibrium (HWE) in the 959 controls using Haploview version 4.0 (Barrett *et al*., 2005). Departures from HWE often indicate technical problems with SNP assays. The PLINK program is a toolset ideal for population and family-based association analysis and was used to test for association between endometriosis and individual SNPs (Purcell *et al*., 2007). The global significance level was derived from multiple tests and values ≤ 0.05 were considered to be statistically significant. Pairwise linkage disequilibrium (LD), haplotype frequencies and blocks were determined by Haploview using the default method of Gabriel *et al*. (2002).

We also conducted a literature-based review of all relevant association studies of these *VEGF* SNPs (−460 T/C, rs833061; +405 G/C, rs2010963; +936 C/T, rs3025039) in endometriosis and performed a meta-analysis of all available data. Our search strategy involved an online query of PubMed for publications up to 1 April 2008, using the search terms 'endometriosis' plus '*VEGF*' or 'polymorphism'. Papers included were those with information on both case and control selection, showed allelic and/or genotypic results for named germ-line polymorphisms and were published only in the English language. Tabulated meta-analysis Mantel–Haenszel results were obtained using the MYSTAT program (http://www.qimr.edu.au/davidD/). A meta-analysis standard forest plot was generated using the MIX program (version 1.61) (Bax *et al*., 2006) (http://www.mix-for-meta-analysis.info).

Genetic analyses for a co-dominant model (genotypic), dominant model (i.e. one or two copies of the associated variant provide the same increase in risk) and recessive model (i.e. two copies of the associated variant are required to increase risk) were performed on all available genotype data. For each SNP, pair-wise comparisons were performed between three genotypes for the co-dominant model, between major allele genotypes and combined minor allele and heterozygous genotypes for the dominant model and between minor allele genotypes and combined major allele and heterozygous genotypes for the recessive model.

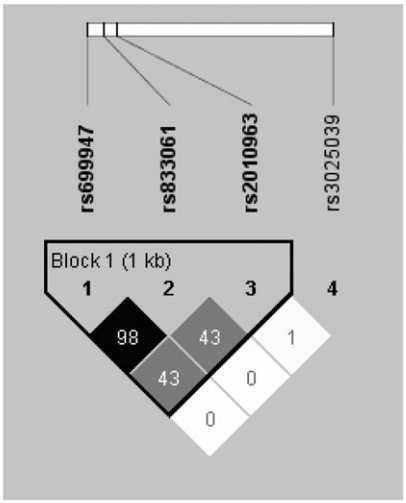
Results

We genotyped four *VEGF* SNPs in 958 unrelated endometriosis cases and 959 unrelated controls. Genotype completion rate in all samples

Table I. Association analyses of *VEGF* polymorphisms genotyped in 958 endometriosis cases and 959 controls.

SNP name	dbSNP ID	Minor allele frequency		Allelic association		
		Cases	Controls	χ^2	*P*-value	OR (95% CI)
−2578C<A	rs699947	0.492	0.504	0.559	0.455	1.05 (0.92–1.19)
−460C<T	rs833061	0.498	0.508	0.382	0.537	1.04 (0.92–1.18)
+405C<G	rs2010963	0.312	0.297	1.061	0.303	1.08 (0.94–1.24)
+936T<C	rs3025039	0.157	0.146	1.051	0.305	1.10 (0.92–1.31)

SNP name: as it appears in publications. dbSNP ID, database SNP identification.



Haplotype (-2578/-460/+405)	Frequency	Chi Square	P Value
ACG	0.501	0.555	0.4561
CTC	0.303	1.222	0.2689
CTG	0.191	0.216	0.6418

Figure 1: Haplotype analyses for the 5′-UTR contiguous *VEGF* SNPs. The top figure shows LD patterns of *VEGF* 4 SNPs estimated as r^2. There was strong LD between SNPs −2578 A/C(rs699947) and −460T/C(rs833061) (white, $r^2 = 0$; shades of grey, $0 > r^2 < 1$; black, $r^2 = 1$). Haplotype frequencies and haplotype association analyses were estimated utilising Haploview program as shown in the bottom of the figure.

was 99.4%. To estimate genotyping error rate due to undetected technical issues, SNP −460 T/C (rs833061) was typed twice on 768 DNAs independently at different times using Sequenom h-ME or iPLEX chemistry. The dropout rate was 1.04% using Sequenom h-ME chemistry compared with 0.26% with the Sequenom iPLEX chemistry. Of the 1516 completed genotypes, there were only two discordant genotypes providing a low error frequency of 0.13% similar to that observed in our previous study (Zhao *et al*., 2005).

SNP genotypes were tested for departures from HWE for controls and all SNPs were in HWE. The minor allele frequencies of the four polymorphisms (−2578 A/C, −460 T/C, +405 G/C and +936 C/T) located in the *VEGF* gene 5′ or 3′-UTR regions were 0.492, 0.498, 0.312 and 0.157 in the endometriosis cases and 0.504, 0.508, 0.297 and 0.146 in controls, respectively (Table I). We did not find any evidence for association between endometriosis and the four individual SNPs (Table I). Polymorphisms −2578 A/C (rs699947) and −460 T/C (rs833061) are in strong LD ($r^2 = 0.983$) in our samples (Fig. 1). Haplotype analyses for the 5′-UTR contiguous *VEGF* SNPs also did not provide any evidence for association with the risk of endometriosis (Fig. 1).

Because the reports of *VEGF* +405 G/C (rs2010963) and +936 C/T (rs3025039) polymorphisms were associated with the risk of endometriosis at stage III-IV disease in Korean and Japanese populations (Kim *et al*., 2005; Ikuhashi *et al*., 2007), we investigated the effects of disease stages (Table II). We found no evidence for association between individual *VEGF* SNPs and endometriosis in either rAFS stages I–II disease or rAFS stages III–IV disease (Table II).

Six association studies on the relationship between endometriosis and the three *VEGF* polymorphisms (−460 T/C, rs833061; +405 G/C, rs2010963; +936 C/T, rs3025039) were identified from a literature search. The reported genotype frequencies for these studies are shown in Table III. Genetic analysis was performed for each of these SNPs. For SNP −460 T/C (rs833061), the combined *P*-values for the co-dominant, dominant and recessive model were 0.63, 0.34 and 0.83, respectively. For SNP +405 G/C (rs2010963), the combined *P*-values for the co-dominant, dominant and recessive model were 0.5, 0.75 and 0.24, respectively. For SNP +936 C/T (rs3025039), the combined *P*-values for the co-dominant, dominant and recessive model were 0.09, 0.036 and 0.89, respectively. Allelic analyses from the given studies are shown in Table IV.

Forest plots of the association of the three VEGF polymorphisms with endometriosis are shown in Fig. 2. The odds ratios (ORs) for SNP −460 T/C (rs833061) in the five studies ranged from 0.61 to 1.10 (Fig. 2A) and there was no sign of heterogeneity ($Q = 4.2$, $P = 0.38$). Similarly, the ORs for SNP +405 G/C (rs2010963) ranged from 0.6 to 1.48 (Fig. 2B) with no evidence of heterogeneity across the five estimates ($Q = 9.3$, $P = 0.054$). The three studies of SNP +936 C/T (rs3025039) producing ORs ranging from 0.97 to 1.42 also indicated no evidence of heterogeneity ($Q = 1.20$, $P = 0.55$). The Mantel–Haenszel fixed effect model yielded a pooled OR of 0.946 (95% CI = 0.82–1.09), 1.046 (95% CI = 0.91–1.21) and 1.134 (95% CI = 0.92–1.40) for SNPs −460 C/T (rs833061), +405 G/C (rs2010963) and +936 T/C (rs3025039), respectively. We found no evidence for association between endometriosis and the *VEGF* polymorphisms −460 T/C (rs833061), +405 G/C (rs2010963) or +936 C/T (rs3025039) in the total combined data set (Fig. 2). Because our controls are older than our cases, to explore any possible age effect on endometriosis (i.e. endometriosis risk increases with age), we compared allele frequencies between our 958 (age range = 17–65) cases and 868 (age range = 29–64) controls at the time of data collection. Analogous to the total data set, none of the tested *VEGF* SNPs produced a $P < 0.05$ using the age-restricted control set. Association was also not found for these SNPs within the stage of disease strata in the combined data sets (data not shown).

Discussion

Association has been reported between endometriosis and the *VEGF* 5′ or 3′-UTR polymorphisms (Hsieh *et al*., 2004; Bhanoori *et al*.,

Table II. Association analyses of the *VEGF* polymorphisms in rAFS stage subgroups of endometriosis patients compared with 959 controls.

SNP name	db SNP ID	rAFS stage I/II ($n = 559$)				rAFS stage III/IV ($n = 394$)			
		MAF	χ^2	*P-value*	OR (95% CI)	MAF	χ^2	*P-value*	OR (95% CI)
−2578C<A	rs699947	0.498	0.295	0.587	1.04 (0.90–1.21)	0.492	0.575	0.448	1.07 (0.90–1.26)
−460C<T	rs833061	0.500	0.200	0.655	1.03 (0.89–1.2)	0.505	0.407	0.523	1.06 (0.89–1.25)
+405C<G	rs2010963	0.322	2.078	0.150	1.13 (0.96–1.32)	0.297	0.000	0.984	1.00 (0.83–1.20)
+936T<C	rs3025039	0.157	0.739	0.390	1.09 (0.89–1.35)	0.157	0.617	0.432	1.10 (0.87–1.38)

rAFS, revised American Fertility Society (see 'Study participants' section); MAF, minor allele frequency.

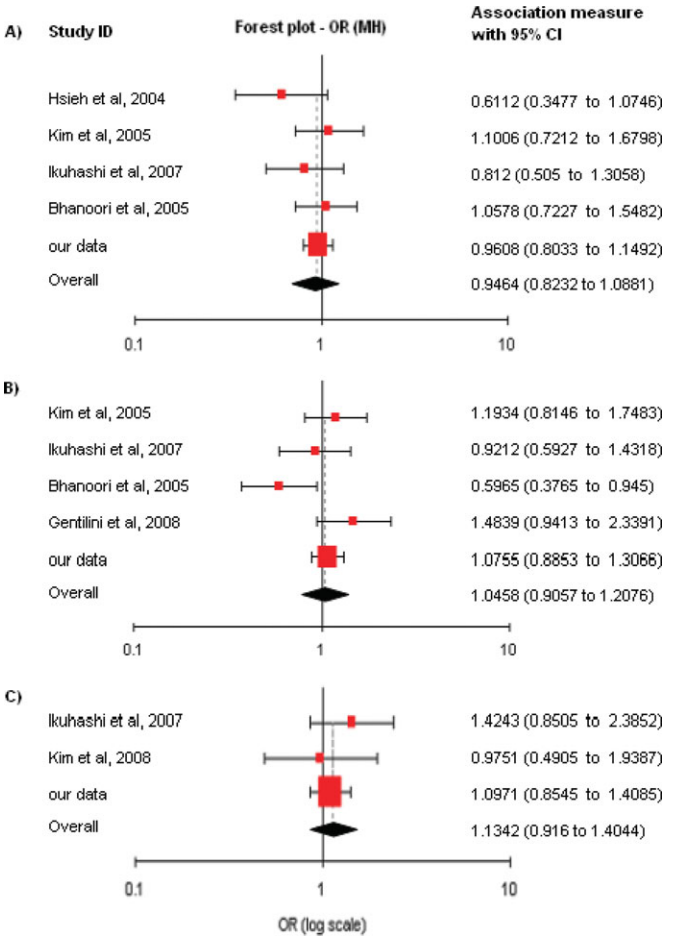


Figure 2: Forest plots show association between the *VEGF* polymorphisms −460 C/T (rs833061, A), +405 C/G (rs2010963, B) and +936T/C (rs3025039, C) with endometriosis in a meta-analysis.
Individual and pooled OR and their 95% confidence intervals were estimated by Mantel–Haenszel (MH) method. The size of the square is proportional to the percent weight of each study in the fixed effect meta-analysis (Note: control data for SNP rs3025039 were not given from the study of Kim *et al.*; therefore, the data present in the forest plot C for Kim's study was estimated from their cases which show no allele frequency different from controls).

2005; Kim *et al*., 2005; Ikuhashi *et al*., 2007; Gentilini *et al*., 2008). In the present study, we have investigated the possible association between endometriosis and the *VEGF* polymorphisms in a large Australian population sample by genotyping *VEGF* candidate SNPs −2578 A/C (rs699947), −460 T/C (rs833061), +405 G/C (rs2010963) and +936 C/T (rs3025039) in 958 unrelated endometriosis cases and 959 unrelated controls. With conflicting association results from several small studies, we herein observed that these polymorphisms are not associated with risk of endometriosis.

Our study constitutes one of the largest available collections of DNA from endometriosis patients and has high power to detect genetic associations (Zhao *et al*., 2006). In addition, given the familial background of our endometriosis cases, compared with sporadic cases, they should further increase the power to detect gene associations. That is, sporadic cases are more likely to be affected by environmental factors and less likely to share genetic risk factors than the familial cases. Thus, our case–control sample has a high power to detect common alleles of moderate to high effect, if they exist. Recently, three independent groups from South India, Korea and Italy have reported an association between the *VEGF* gene 5′-UTR polymorphism +405 G/C (rs2010963) and risk of endometriosis. By comparing their findings, a clear discordance between results was revealed since the +405 C allele was associated with endometriosis in study of Kim *et al*. (2005), and the +405 G allele was more often associated with the disease in study of Bhanoori *et al*. (2005). The latest study by Gentilini *et al*. (2008) found that the minor C allele was associated with the disease. However, they failed to observe a biological gradient with disease stages and acknowledged that the small sample size may not have sufficient power to provide reliable subgroup analyses. Although the observed genotype distributions in all studies were in HWE in both cases and controls, the results are contradictory in that the associated alleles and frequencies differ, even across similar Asian populations (Kim *et al*., 2005; Ikuhashi *et al*., 2007). Genetic studies in endometriosis have been reviewed by several groups, showing that published studies are often conflicting due to small sample size in many studies (Di and Guo 2007; Montgomery *et al*., 2008). Given the individual genetic effects underlying complex disease are typically small and may interact with each other and/or environmental factors, their detection requires comprehensive typing of SNPs in large powerful samples (Cardon and Bell 2001; Colhoun *et al*., 2003).

In an attempt to obtain a common estimate of the *VEGF* polymorphisms effect on risk for endometriosis and provide compelling evidence for association, we performed a meta-analysis combining all available genotype and allele frequency data from six published association studies. The analysis of the available data detected a significant deviation from Hardy–Weinberg disequilibrium in a positive association study for polymorphism −460 T/C (rs833061) in a Chinese population (Hsieh *et al*., 2004). The deviation most likely indicates a genotyping assay problem with an erroneous gain/loss of homozygous genotypes. The commonly used polymerase chain reaction–restriction fragment length polymorphism analysis for genotyping is reported to have poor accuracy and reproducibility (Ding and Cantor, 2003) and may underlie this finding. Conversely, effects of sample selection and differences in biological and environmental complexity between samples could also hinder efforts to replicate association in most of the studies which are statistically underpowered. The meta-analysis helps researchers to deal with the diversity of the published data but in general cannot do justice to complex human diseases, which involve multiple genetic and environmental determinants (Collins, 1997). However, in the total combined data, no evidence for association between the *VEGF* polymorphisms genotyped and risk of endometriosis was observed. Therefore, the different results across studies may result from small sample size and/or genotyping technique rather than ethnic differences.

In this study, we examined the association between endometriosis and four *VEGF* polymorphisms and haplotypes in a large Australian population sample. Our data do not provide any evidence supporting an association between these *VEGF* polymorphisms and endometriosis susceptibility. A detailed genotyping review and meta-analyses of the *VEGF* polymorphisms from six studies revealed possible genotyping assay problems for one published positive association and no overall evidence for association between these *VEGF* variants and endometriosis. In this study, we did not use a tag approach to cover all common variations in the *VEGF* gene. Rather, the primary goal of our paper was to assess the evidence for association with endometriosis at four previously examined *VEGF* polymorphisms which have produced conflicting evidence for association. Therefore, our results cannot exclude the possibility that either other common or rare variants in the gene or genomic deletions or insertions in the gene might contribute to the disease susceptibility. Further study of all *VEGF* gene variation in large samples will be required to ultimately confirm and characterise the involvement of *VEGF* gene variation in the pathogenesis of endometriosis.

Table III. Genotypic analyses of genotyped *VEGF* SNPs in endometriosis cases and controls.

SNP name (dbSNP ID)	Study	Sample population	Sample Size		Genotypes (%)								Genotypic model-analysis		
			Case	Control	Case				Control				Co-dominant	Dominant	Recessive
−460C/T (rs833061)					CC	CT	TT	HWE (*P*)	CC	CT	TT	HWE (*P*)	*P*-value	*P*-value	*P*-value
	Hsieh *et al*. (2004)[a]	Chinese	122	131	0(0)	54.1 (44.3)	67.9 (55.7)	0.02	0 (0)	83.1 (63.4)	47.9 (36.6)	0.00005	0.010	0.002	1.000
	Kim *et al*. (2005)	Korean	215	219	18.9 (8.8)	83 (38.6)	113.1 (52.6)	0.91	16 (7.3)	83 (37.9)	120 (54.8)	0.97	0.810	0.650	0.570
	Ikuhashi *et al*. (2007)	Japanese	147	181	7.9 (5.4)	67 (45.6)	72 (49)	0.54	17 (9.4)	84 (46.4)	80 (44.2)	0.87	0.355	0.390	0.170
	Bhanoori *et al*. (2005)	Indian	215	210	46.7(21.7)	112 (52.1)	56.1 (26.1)	0.90	42 (20)	111.9 (53.3)	56.1 (26.7)	0.77	0.895	0.890	0.660
	Our data	Australian	958	959	224.2 (23.4)	502 (52.4)	227 (23.7)	0.51	234 (24.4)	494.8 (51.6)	217.7 (22.7)	0.61	0.806	0.670	0.540
	All		1657	1700									0.630	0.340	0.830
+405C/G (rs2010963)					CC	CG	GG		CC	CG	GG				
	Kim *et al*. (2005)[a]	Korean	215	219	50.1 (23.3)	89 (41.4)	75.9 (35.3)	0.25	28.9 (13.2)	116.1 (53)	74 (33.8)	0.53	0.010	0.740	0.006
	Ikuhashi *et al*. (2007)	Japanese	147	181	22.2 (15.1)	76.6 (52.1)	48.4 (32.9)	0.81	31 (17.1)	93.9 (51.9)	55.9 (30.9)	0.86	0.860	0.710	0.620
	Bhanoori *et al*. (2005)[a]	Indian	215	210	4.1 (1.9)	71 (33)	140 (65.1)	0.53	18.1 (8.6)	79 (37.6)	113 (53.8)	0.86	0.002	0.018	0.002
	Gentilini *et al*. (2008)[a]	Italian	203	140	28.4 (14)	105.6 (52)	69 (34)	0.67	14 (10)	58.8 (42)	67.2 (48)	0.99	0.034	0.009	0.270
	Our data	Australian	958	959	85.3 (8.9)	421.5 (44)	441.6 (46.1)	0.74	73.8 (7.7)	413.3 (43.1)	459.4 (47.9)	0.59	0.560	0.290	0.350
	All		1738	1709									0.500	0.750	0.240
+936T/C (rs3025039)					TT	TC	CC		TT	TC	CC				
	Ikuhashi *et al*. (2007)	Japanese	147	181	11 (7.5)	56 (38.1)	80 (54.4)	0.97	10 (5.5)	53 (29.3)	118 (65.2)	0.72	0.140	0.047	0.470
	Kim *et al*. (2008)	Korean	105	100	2 (1.9)	37 (35.2)	66 (62.9)	0.63	N/A	N/A	N/A				
	Our data	Australian	958	959	19.2 (2)	263.5 (27.5)	673.5 (70.3)	0.70	21.1 (2.2)	233 (24.3)	690.5 (72)	0.98	0.360	0.200	0.730
	All		1210	1240									0.090	0.036	0.890

[a]Association with endometriosis was found in the study.

Table IV. Allelic analyses of genotyped *VEGF* SNPs in endometriosis cases and controls.

SNP name (dbSNP ID)	Study	Sample population	Sample size		Allele counts (%)				Allelic association	Meta-analysis (M-H)		
			Case	Control	Case		Control			OR	95% CI	*P*-value
−460C/T (rs833061)					C	T	C	T	*P*			
	Hsieh *et al*. (2004)[a]	Chinese	122	131	53.9 (22.1)	190.1 (77.9)	83.1 (31.7)	178.9 (68.3)	0.016			
	Kim *et al*. (2005)	Korean	215	219	121.3 (28.2)	103.2 (24)	115.2 (26.3)	322.8 (73.7)	0.533			
	Ikuhashi *et al*. (2007)	Japanese	147	181	82.9 (28.2)	211.1 (71.8)	118 (32.6)	244 (67.4)	0.228			
	Bhanoori *et al*. (2005)	Indian	215	210	206.4 (48)	224 (52.1)	195.7 (46.6)	224.3 (53.4)	0.690			
	Our data	Australian	958	959	954.2 (49.8)	961.8 (50.2)	974.3 (50.8)	943.7 (49.2)	0.540			
	All				1657	1700				0.95	0.82–1.09	0.440
+405C/G (rs2010963)					C	G	C	G				
	Kim *et al*. (2005)[a]	Korean	215	219	189.2 (44)	240.8 (56)	173.9 (39.7)	264.1 (60.3)	0.210			
	Ikuhashi *et al*. (2007)	Japanese	147	181	120.8 (41.1)	173.2 (58.9)	156 (43.1)	206 (56.9)	0.580			
	Bhanoori *et al*. (2005)[a]	Indian	215	210	78.7 (18.3)	351.3 (81.7)	114.7 (27.3)	305.3 (72.7)	0.001			
	Gentilini *et al*. (2008)[a]	Italian	203	140	162.4 (40)	243.6 (60)	86.8 (31)	193.2 (69)	0.018			
	Our data	Australian	958	959	597.6 (31.2)	1318.4 (68.8)	568.7 (29.7)	1349.3 (70.4)	0.300			
	All		1738	1709						1.05	0.91–1.21	0.540
+936T/C (rs3025039)					T	C	T	C				
	Ikuhashi *et al*. (2007)	Japanese	147	181	77.9 (26.5)	216.1 (73.5)	73.1 (20.2)	288.9 (79.8)	0.054			
	Kim *et al*. (2008)	Korean	105	100	41.2 (19.6)	160.8 (80.4)	N/A	N/A	N/A			
	Our data	Australian	958	959	301.6 (15.7)	1614.4 (84.3)	279.1 (14.6)	1638.9 (85.5)	0.304			
	All		1210	1240						1.13	0.92–1.40	0.250

[a]Association with endometriosis was found in the study.

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