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Combined effect of vascular endothelial growth factor and its receptor polymorphisms in endometriosis: a case-control study

Jéssica Vilarinho Cardoso[a,b], Maurício Simões Abrão[c], Rosane Vianna-Jorge[a,d], Renato Ferrari[e], Plínio Tostes Berardo[f], Daniel Escorsim Machado[b], Jamila Alessandra Perini[a,b,*]

[a] Programa de Pós-Graduação em Saúde Pública e Meio Ambiente, Escola Nacional de Saúde Pública, Fundação Osvaldo Cruz, Rio de Janeiro, RJ, Brazil
[b] Laboratório de Pesquisa de Ciências Farmacêuticas, Unidade de Farmácia, Centro Universitário Estadual da Zona Oeste, Rio de Janeiro, RJ, Brazil
[c] Departamento de Obstetrícia e Ginecologia da Faculdade de Medicina da Universidade de São Paulo, Rio de Janeiro, SP, Brazil
[d] Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil
[e] Instituto de Ginecologia da Universidade Federal do Rio de Janeiro, Hospital Moncorvo Filho, Rio de Janeiro, RJ, Brazil
[f] Serviço de Ginecologia, Hospital Federal dos Servidores do Estado, Rio de Janeiro, RJ, Brazil

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ABSTRACT

Objective: Endometriosis is a multifactorial gynecological disease, whose pathogenesis is crucially dependent on angiogenesis, which is signaled via vascular endothelial growth factor (VEGF) and its receptor (VEGFR2). We hypothesize that single nucleotide polymorphisms (SNPs) in *VEGF* and *VEGFR2* genes may influence the onset and/or the progression of endometriosis. The main aim of this study was to investigate the contribution of *VEGF* and *VEGFR2* SNPs as risk factors for endometriosis, as well as their association with endometriosis symptoms.

Study design: A case-control study was conducted, involving 293 endometriosis patients and 223 controls, who were submitted to laparoscopic or laparotomy surgery at hospitals from the Brazilian public health system. Genotyping of *VEGF* (*−2578C > A, −460T > C, −1154G > A, +405G > C* and *+936C > T*) and *VEGFR2* (*−604T > C, 1192C > T*) SNPs was performed by TaqMan real-time polymerase chain reaction. The association between SNPs and endometriosis, deep infiltrating endometriosis (DIE) or endometriosis symptoms was estimated by odds ratios (OR) with their 95% confidence intervals (CI), which were calculated using multivariate logistic regression models.

Results: VEGF variant alleles *−2578A* and *−1154A* were associated with increased endometriosis risk (OR: 1.39, 95% CI: 1.04–1.87 and OR: 1.63, 95% CI: 1.12–2.37, respectively), whereas *VEGF 405C* and *VEGFR2 1192T* were associated with lower risk of endometriosis (OR: 0.66, 95% CI: 0.43–1.00 and OR: 0.58, 95% CI: 0.40–0.84, respectively). The combination of wild-type genotypes of both *VEGF −2578C > A* and *−1154G > A* with variant genotypes of both *VEGF +405G > C* and *VEGFR2 1192C > T* showed the best protective effect against the development of endometriosis, either considering all cases (OR: 0.33, 95% CI: 0.12–0.89) or only DIE (OR: 0.30, 95% CI: 0.10–0.87). The combination of variant genotypes of *VEGF −2578C > A, −1154G > A, +405G > C* and *VEGFR2 1192C > T* was also protective against DIE (OR: 0.67, 95% CI: 0.46–0.96). *VEGFR2 1192C > T* were associated with reduced cyclical urinary complaints (OR: 0.40, 95% CI: 0.18–0.88).

Conclusions: Our results indicate that *VEGF* SNPs *−2578C > A* and *−1154G > A* increase endometriosis risk, whereas *VEGF +405G > C* and *VEGFR2 1192C > T* are protective against disease development, with *VEGFR2 1192C > T* also reducing cyclical urinary symptoms. The combined analysis of *VEGF–VEGFR2* genotypes suggests a gene–gene interaction in endometriosis susceptibility.

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Introduction

Endometriosis is a complex, heterogeneous and polygenic disease, which may affect various tissues, and present different histological phenotypes [1]. The retrograde menstruation, as proposed in Sampson's theory (1927) [2], is still considered as

* Corresponding author at: Laboratório de Pesquisa de Ciências Farmacêuticas, Unidade de Farmácia, Centro Universitário Estadual da Zona Oeste, Av. Manoel Caldeira de Alvarenga, 1203, Campo Grande, 23070-200 Rio de Janeiro, RJ, Brazil. Fax: +55 21 2332 753.
E-mail address: jamilaperini@yahoo.com.br (J.A. Perini).

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the main mechanism causing the disease development. The survival of ectopic endometrial implants, however, requires the establishment of a new blood supply, and angiogenesis represents a key role during this process [3].

Angiogenesis is under the control of numerous inducers and growth factors, including vascular endothelial growth factor (VEGF), which is present in human ectopic and eutopic endometrium [4,5]. VEGF signaling via VEGFR2 is the major transducing pathway in angiogenesis processes [6]. Significantly higher expression of VEGF in glandular epithelium and of VEGFR2 in endometrial blood vessels have been observed in women with endometriosis, as compared with controls [4]. In addition, our group observed that tissue vascularization and the expression of VEGF and VEGFR-2 are significantly higher in ovarian, bladder and rectum sigmoid affected with deeply infiltrating endometriosis (DIE) than compared to controls without endometriosis, suggesting that angiogenesis signaling via VEGF to VEGFR2 is an important event in the development of the disease [5].

VEGF is encoded by *VEGF*, whereas VEGFR2 is encoded by *KDR* (kinase insert domain receptor). Both genes are highly polymorphic, with single nucleotide polymorphisms (SNPs) that may affect the enzyme activity or expression [7–10]. *VEGF* and *KDR* SNPs have been associated with endometriosis risk, although the literature reports show controversial results [9,11–17]. With regards to *VEGF*, our group observed an increased risk of endometriosis in Brazilian women with the variant allele of *VEGF 1154G > A*, and a protective effect for the haplotype *CCGG*, formed by *−2578C > A, −460T > C, −1154G > A* and *+405G > C* [14]. Results from a meta-analysis suggest that *VEGF +936C > T* increase endometriosis risk, whereas *VEGF −2578C > A* and *−1154G > A* might be protective [13]. Another recent meta-analysis explored only *VEGF +405G > C*, and observed that it was not significantly associated with endometriosis risk [15], according to Li et al. [13]. Only two studies investigated the impact of *KDR* SNPs on endometriosis development, with opposite findings regarding the effects of *KDR 1192C > T* [9,17].

No investigation regarding the susceptibility to endometriosis considered the combined effect of *VEGF* and *KDR* SNPs. Thus, the present study aimed to evaluate the role of *VEGF* and *KDR* SNPs as potential risk factors for endometriosis, DIE, as well as for its symptoms, investigating the existence of a possible interaction involving such genetic variations.

Materials and methods

Study design

The study was approved by the Human Research Ethics Committees of *Hospital das Clínicas da Universidade de São Paulo*, *Hospital Federal dos Servidores do Estado* and *Hospital Moncorvo Filho* (Protocol numbers 910/2011, 414/2011 and 1.244.294/2015, respectively). Written informed consent was obtained from all participating individuals. Demographics data, gynecological and obstetrical history, and preoperative symptoms were obtained by interviews during preoperative appointments at three hospitals from the Brazilian public health system, between 2011 and 2015.

Women who were admitted for laparoscopy or laparotomy for gynecological procedures were considered eligible (n = 584). Subjects were considered as cases if they had visible ectopic implants, and histologically confirmed diagnosis of endometriosis (n = 293). The control group (n = 223) consisted of women assigned to laparoscopy or laparotomy for tubal ligation (n = 69) or treatment of benign diseases, such as myoma (n = 54), ovarian cysts (n = 38), hydrosalpinx (n = 11) or others (n = 51), and who had no macroscopic signs of endometriosis. The exclusion criteria were: women who had been diagnosed with adenomyosis, with any previous history or current diagnosis of cancer, rheumatoid arthritis or hypertension-related chronic kidney disease. Peripheral blood samples were obtained from all endometriosis patients and controls during preoperative consultations.

The stage of endometriosis was determined according to the revised American Fertility Society classification. Three types of disease were considered: superficial endometriosis (SUP), ovarian endometrioma (OMA) and DIE. Both superficial peritoneal and ovarian endometrioma may be found in association with deep endometriosis [18], and were considered DIE.

The body mass index (BMI) was calculated as the weight (kg) divided by the square of height (m^2). As suggested in our previous study [19], only severe and incapacitating symptoms of pain were included, which defines non-cyclic chronic pelvic pain and dysmenorrhoea: moderate, if there was noticeable interference with normal daily activities and analgesics were usually required; or severe, if the patient was unable to function normally or had to visit emergency units for pain relief; and deep dyspareunia according to limitation of sexual activity with intercourse painful to the point of interruption. Infertility was defined by the couple not being able to conceive after one year of regular, contraceptive-free intercourse [20]. Cyclical intestinal or urinary symptoms were defined as bowel and/or urinary pain and/or bleeding coinciding with menstrual periods [20].

VEGF and KDR genotyping

Genomic DNA was obtained from blood samples as previously described [14]. Validated TaqMan assays were purchased from Applied Biosystems for detection of *VEGF −2578C > A* (rs699947), *−460T > C* (rs833061), *−1154G > A* (rs1570360), *+405G > C* (rs2010963), *+936C > T* (rs3025039), and *KDR −604T > C* (rs2071559), and *1192C > T* (rs2305948). Table 1 summarizes the sets of probes and primers used for the analysis of each *VEGF* and *KDR* SNP. Reactions were performed on a 7500 Real-Time System, and the genotyping call rate was above 90% for all studied SNPs.

Statistical analysis

Statistical analyses were conducted using Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), version 20.0. The Student's *t* test was used for comparison of quantitative variables, such as age or BMI, with results expressed as means ± standard deviation (SD). Categorical variables, such as age, educational attainment, BMI, menopausal status, family history of endometriosis and painful symptoms, were expressed as percentages and compared between cases and controls with the Chi-square (χ^2) test or the Fisher's exact test, when applicable. Hardy–Weinberg equilibrium analysis was performed to compare the observed and the expected genotype frequencies using the goodness-of-fit χ^2 test. Comparison of allelic or genotypic distributions between cases and controls was performed using the χ^2 test or the Fisher's exact test, when appropriate. The haplotype patterns were inferred using Haploview 4.2 based on the algorithm of expectation and maximization. The associations between SNPs and endometriosis or between SNPs and endometriosis features were estimated by the odds ratio (OR) and their 95% confidence interval (CI), with adjustment for possible confounding factors, using multivariate logistic regression models. The level of significance considered was set as $P < 0.05$. Multiple testing comparisons were adjusted by Bonferroni correction, with the threshold for statistical significance of $P < 0.007$ (0.05/7).

Results

The demographic and clinical variables of endometriosis patients and controls are presented in Table 2. In summary, endometriosis

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Table 1
Characterization of *VEGF* and *KDR* polymorphisms, probes and primers sequences for genotyping by TaqMan real time PCR.

Identified SNP	TaqMan assays	Region	Probe [SNP]	Primer
rs699947	C_8311602_10	PR	GCCAGCTGTAGGCCAGACCCTGGCA**[A/C]** GATCTGGGTGGATAATCAGACTGAC	5′-GGATGGGGCTGACT AGGTAAGC-3′ 5′-AGCCCCCTTTTCCT CCAAC-3′
rs833061	C_1647381_10	PR	GAGTGTGTGCGTGTGGGGTTGAGGG**[C/T]** GTTGGAGCGGGGAGAAGGCCAGGGG	5′-TGTGCGTGTGGGGTTGAGAG-3′ 5′-TACGTGCGGACAGGGCCTGA-3′
rs1570360	C_1647379_10	PR	AGCCCGGGCCCGAGCCGCGTGTGGA**[A/G]** GGGCTGAGGCTCGCCTGTCCCCGCC	5′-TCCTGCTCCCTCCT CGCCAATG-3′ 5′-GGCGGGGACAGGC GAGCATC-3′
rs2010963	C_8311614_10	5′-UTR	CGCGCGGGCGTGCGAGCAGCGAAAG**[C/G]** GACAGGGGCAAAGTGAGTGACCTGC	5′-GCTCCAGAGAGAAGTCGAGGA-3′ 5′-CACCCCCAAAAGCAGGTC-3′
rs3025039	C_16198794_10	3′-UTR	GCATTCCCGGGCGGGTGACCCAGCA**[C/T]**GGTCCCTCTTGGAATTGGATTCGCC	5′-CACACCATCACCATCGACA-3′ 5′-GCTCGGTGATTTAGCAGCA-3′
rs2071559	C___15869271_10	PR	GGTATGGGTTTGTCACTGAGACAGC**[A/T]**TGGCTATAAGAAAGAGATAACAGCG	5′-CCTCCTGTATCCTGAATGAATCT-3′ 5′-GCCTCACATATTATTGTACCATCC-3′
rs2305948	C__22271999_20	Exon 7	AATATTTTGGGAAATAGCGGGAATG**[C/T]**TGGCGAACTGGGCAAGTGCGTTTTC	5′-TGAGGTTAAAAGTTCTGGTGTCCCTGTT-3′ 5′-AAATGTACAATCCTTGGTCACTCCGGGGTA-3′

PR is Promoter Region.

patients were significantly younger than controls (34.9 ± 7.2 versus 37.6 ± 8.4, $P < 0.001$), with lower BMI (24.4 ± 4.6 versus 27.9 ± 5.7, $P < 0.001$), longer educational attainment and higher prevalence of family history of the disease. Endometriosis patients also presented the symptoms of dysmenorrhea, dyspareunia, pelvic pain, urinary and intestinal complaints, and infertility with higher frequency than controls, with the interval between the outset of symptoms and diagnosis of endometriosis being 5.8 ± 6.7 years. Among endometriosis patients, there was a predominance of advanced stages III and IV (n = 172, 61%) and DIE (n = 202, 69%).

The minor allele frequencies of five *VEGF* and two *KDR* SNPs in endometriosis patients and controls are shown in Fig. 1. Significant differences were observed between the two groups with respect to the *VEGF −2578A*, *VEGF −1154A*, *VEGF +405C* and *KDR 1192T*. By contrast, no significant differences were detected in allele (Fig. 1) or genotype (Fig. 2) distribution of *VEGF −460T > C* ($P = 0.77$ and $P = 0.51$, respectively), *VEGF +936C > T* ($P = 0.91$ and $P = 0.69$, respectively) and *KDR 604T > C* ($P = 0.91$ and $P = 0.88$, respectively) SNPs between endometriosis patients and controls. In addition, *KDR 1192C > T* was the only SNP whose allelic distribution was significantly different with regards to symptoms, the variant genotypes *KDR 1192TT* favoring lower occurrence of cyclical urinary symptoms (OR: 0.40, 95% CI: 0.18–0.88). The allelic distribution of *VEGF* and *KDR* SNPs between symptomatic and asymptomatic endometriosis patients is presented in Fig. 3.

The genotype frequencies of *VEGF −2578C > A*, *−1154G > A*, *+405G > C* and of *KDR 1192C > T*, which were significantly different between endometriosis patients and controls, and their association

Table 2
Demographic and clinical characteristics of study population.

Variable	Controls N (%)	Endometriosis N (%)	*P*-value[a]
Age (year)			
<20	5 (2.3)	5 (1.8)	<0.001
21–30	37 (16.8)	70 (24.7)	
31–40	92 (41.8)	150 (53.0)	
41–50	76 (34.5)	55 (19.4)	
>51	10 (4.5)	3 (1.1)	
Educational attainment			
Fundamental education	22 (21.8)	16 (12.3)	<0.001
Secondary school	63 (62.4)	51 (39.2)	
Higher education	15 (14.9)	62 (47.7)	
None	1 (1.0)	1 (0.8)	
BMI			
<18,5	4 (1.8)	18 (6.2)	<0.001
18,5–24,9	59 (26.9)	159 (55.0)	
25–29,9	58 (26.5)	64 (21.5)	
30–40	62 (28.3)	37 (12.8)	
>40	36 (16.4)	13 (4.5)	
Menopausal status			
No	100 (96.2)	138 (95.2)	0.71
Yes	4 (3.8)	7 (4.8)	
Family history of endometriosis			
No	153 (87.9)	107 (75.9)	0.005
Yes	21 (12.1)	34 (24.1)	
Symptom[b]			
Dysmenorrhoea	68 (33.8)	169 (58.7)	<0.001
Non-cyclic chronic pelvic pain	33 (16.4)	89 (30.9)	<0.001
Deep dyspareunia	46 (22.9)	174 (61.3)	<0.001
Cyclical intestinal complaints[c]	18 (10.5)	114 (40.6)	<0.001
Cyclical urinary complaints[c]	7 (4.1)	74 (26.3)	<0.001
Infertility (primary or secondary)	23 (11.4)	132 (45.7)	<0.001

[a] Chi-square test or Fisher's exact test.
[b] A patient can have more than one concomitant symptom.
[c] Pain and bleeding.

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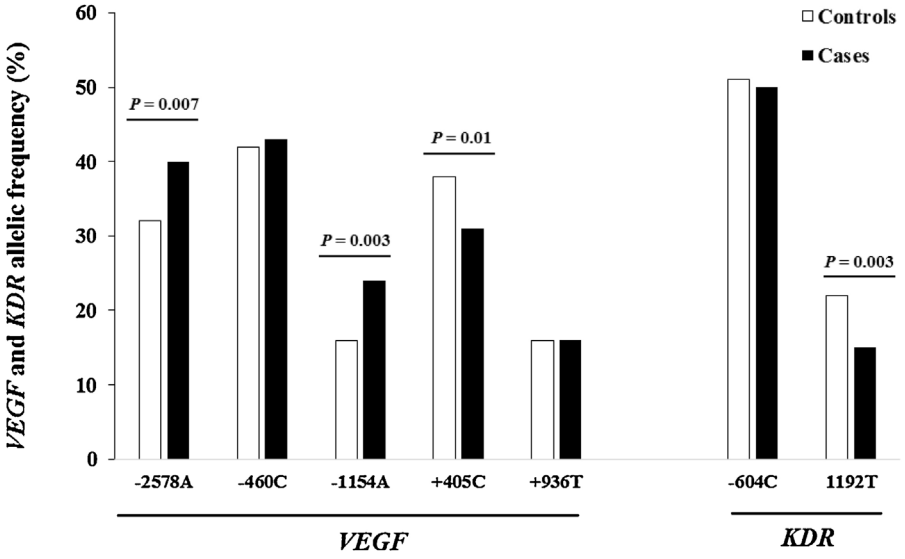

Fig. 1. Minor allelic frequencies of the *VEGF* and *KDR* polymorphisms in study population. *P*-value from Chi-square test (Pearson *P*-value).

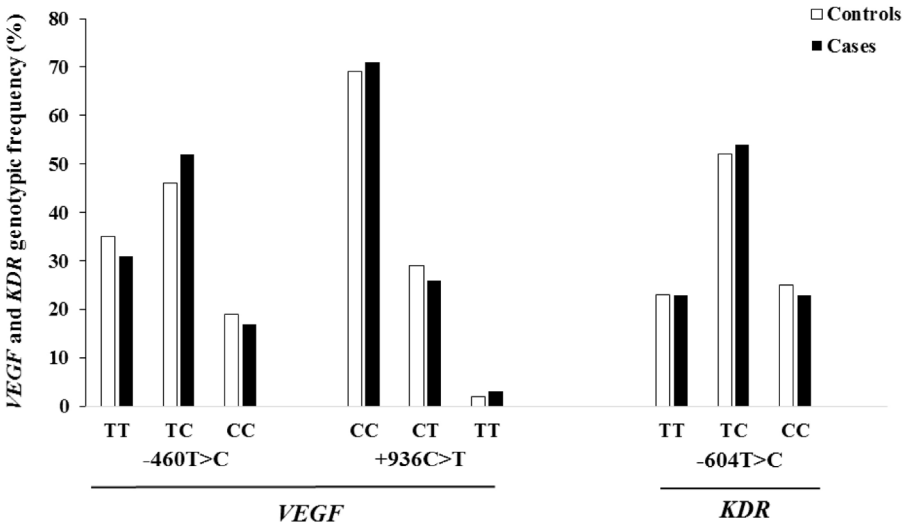

Fig. 2. Genotypic frequencies of the *VEGF* and *KDR* polymorphisms in study population.

with endometriosis are presented in Table 3. The clinical and demographic variables included in multivariate logistic regression models were age, educational attainment, BMI, menopausal status and family history of endometriosis. However, only age and BMI remained as significant co-factors for endometriosis susceptibility. The variant genotypes of *VEGF −2578C>A* and *−1154G>A* were positively associated with endometriosis, suggesting an effect of increased susceptibility. Conversely, the variant genotypes of *VEGF +405G>C* and *KDR 1192C>T* were negatively associated with endometriosis cases, pointing to a lower risk in disease development (Table 3).

A combined analysis of the four SNPs significantly associated with endometriosis (*VEGF −2578C>A*, *−1154G>A*, *+405G>C* and *KDR 1192C>T*) was performed to investigate their interaction on the risk of endometriosis (Table 4). The presence of the variant genotypes of *VEGF +405G>C* and *KDR 1192C>T*, in combination with the wild-type genotypes of *VEGF −2578C>A* and *−1154G>A*, showed a protective effect against the development of endometriosis, either including all cases or only DIE. The concomitant presence of variant genotypes of the four SNPs (*VEGF −2578C>A*, *−1154G>A*, *+405G>C* and *KDR 1192C>T*) was also protective in relation to DIE, but not to all cases of endometriosis.

We used Bonferroni multiple correction to adjust p-values in our study, and *VEGF −2578C>A*, *−1154G>A*, *+405G>C* and *KDR 1192C>T* SNPs remained significant after Bonferroni correction ($P > 0.007$).

Discussion

Endometriosis is a complex gynecological disease, whose molecular mechanisms are not fully elucidated, although angiogenesis via *VEGF-KDR* signaling is recognized as having a central role in the disease development [1]. Accordingly, endometriotic lesions are characterized by dense vascularization, with increased expression VEGF and VEGFR2, as compared to non-affected tissues [4,5,21]. Vodolazkaia et al. studied 11 functional SNPs in genes involved with angiogenesis (*VEGF*, placental growth factor — PLGF, vascular endothelial growth factor receptor 1 — *VEGFR1*, *VEGFR2*, hypoxia inducible factor-1α-HIF-1α) in women with and without endometriosis. They observed that *PLGF*

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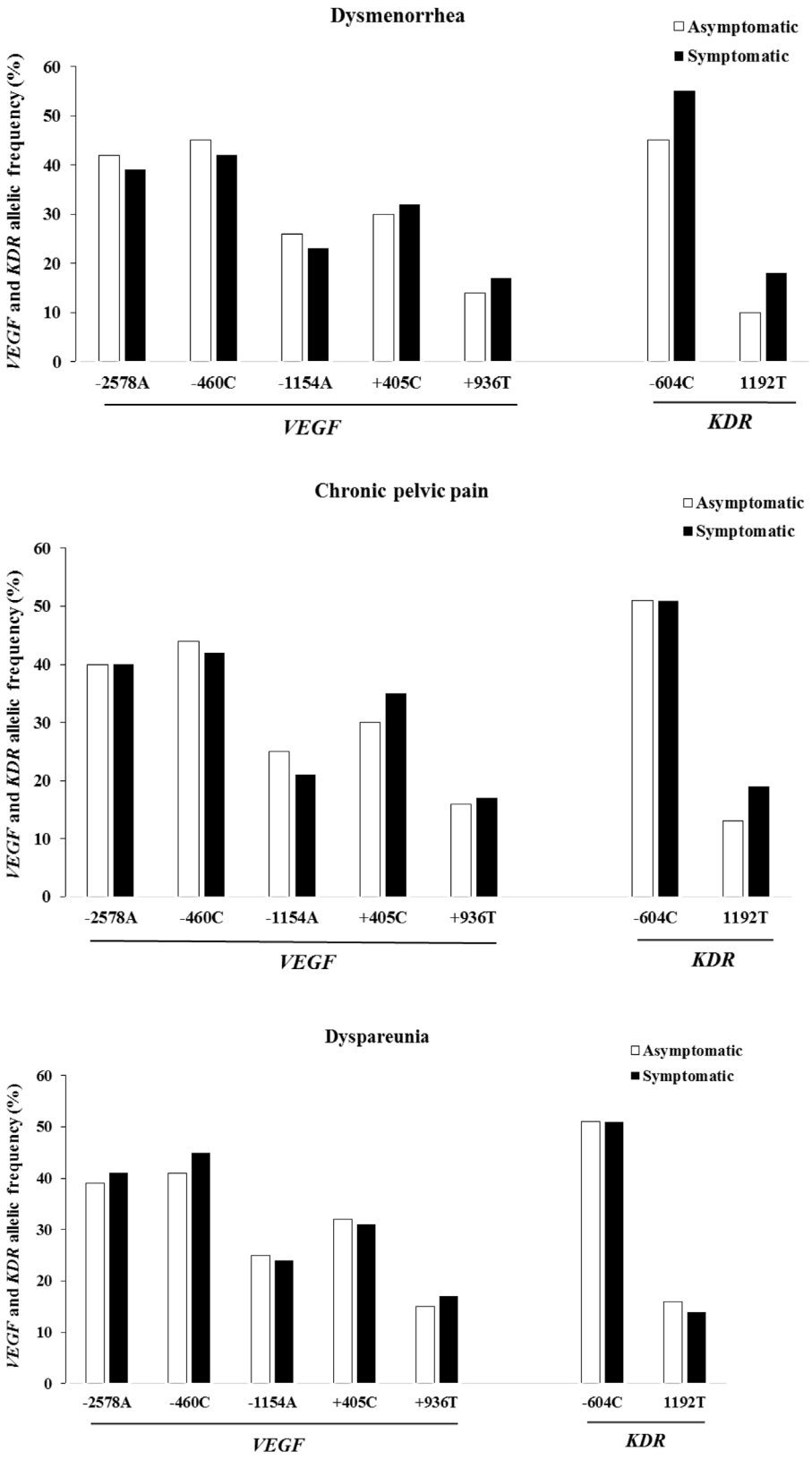

Fig. 3. Allelic distribution of *VEGF* and *KDR* polymorphisms among endometriosis patients symptomatic and asymptomatic. *P*-value from Chi-square test (Pearson *P*-value).

rs2268613 influences the plasma levels of the corresponding protein; however the SNPs rs2268614 (*PLGF*), rs11549465 (*HIF-1α*) and rs9582036 (*VEGFR1*) showed no statistically significant associations with endometriosis after multiple testing [9]. Recently, a genome-wide association study (GWAS) describing a new endometriosis susceptibility locus on 4q12, upstream of the *KDR* gene, corroborating the importance of the VEGF pathway in the pathogenesis of the endometriosis [22].

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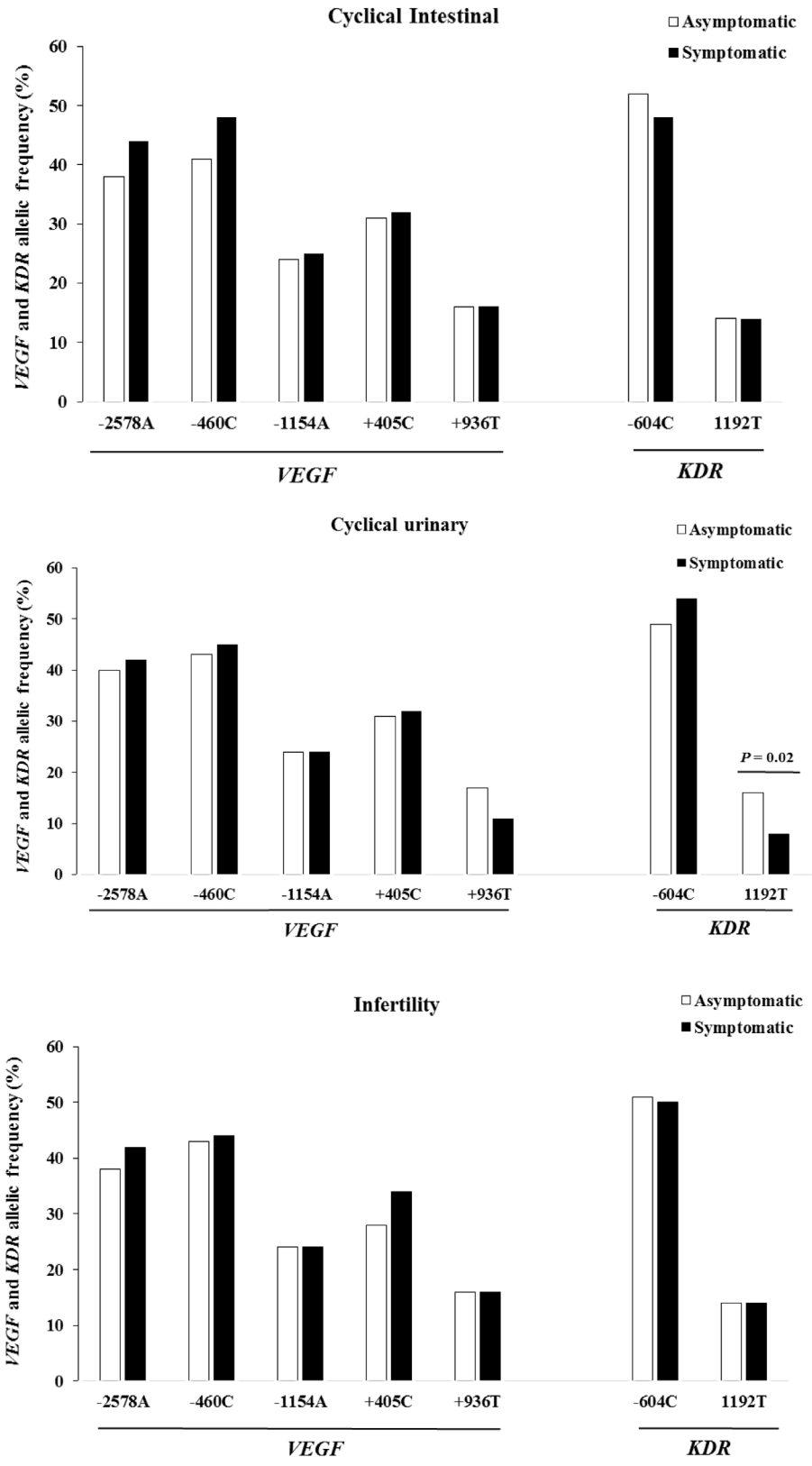

Fig. 3. (Continued)

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Table 3
Association analyses of *VEGF* and *KDR* SNPs in endometriosis patients compared with controls.

Polymorphisms	Controls N (%)	Endometriosis N (%)	*P* value[a]	$OR_{adjusted}$ (95% CI)[b]
VEGF-2578 C > A				
CC	101 (46.3)	98 (34.0)		1[c]
CA	95 (43.6)	149 (51.7)	0.01	1.72 (1.12–2.65)
AA	22 (10.1)	41 (14.3)	0.12	1.68 (0.87–3.26)
CA + AA	117 (53.7)	190 (66.0)	0.01	1.71 (1.14–2.58)
A	139 (31.9)	231 (40.1)	0.03	1.39 (1.04–1.87)
VEGF-1154 G > A				
GG	144 (69.6)	157 (58.8)		1[c]
GA	59 (28.5)	92 (34.5)	0.07	1.50 (0.96–2.36)
AA	4 (1.9)	18 (6.7)	0.045	3.73 (1.03–13.6)
GA + AA	63 (30.4)	110 (41.2)	0.03	1.64 (1.06–2.54)
A	67 (16.2)	128 (24.0)	0.01	1.63 (1.12–2.37)
VEGF +405 G > C				
GG	76 (35.2)	140 (48.1)		1[c]
GC	113 (52.3)	121 (41.6)	0.04	0.64 (0.41–0.98)
CC	27 (12.5)	30 (10.3)	0.26	0.69 (0.36–1.32)
GC + CC	139 (64.8)	152 (51.9)	0.04	0.65 (0.43–0.97)
C	167 (38.6)	181 (31.1)	0.05	0.66 (0.43–1.00)
KDR 1192 C > T				
CC	130 (60.2)	211 (74.6)		1[c]
CT	77 (35.6)	61 (21.6)	0.003	0.50 (0.31–0.79)
TT	9 (4.2)	11 (3.9)	0.31	0.60 (0.22–1.62)
CT + TT	86 (39.8)	72 (25.4)	0.002	0.51 (0.33–0.79)
T	95 (22.0)	83 (14.7)	0.004	0.58 (0.40–0.84)

Differences in sample sizes are due to available data from PCR amplification for each SNP. OR: odds ratio; CI: confidence interval.
[a] P-value is P from Chi-square test (Pearson P-value) or Fisher's exact test.
[b] Adjusted by age and BMI.
[c] Reference group.

The present study is the first to focus on the combined effect of *KDR* and *VEGF* SNPs in the susceptibility to endometriosis, as well as in its histological and clinical presentation. Cases and controls were surgically evaluated, with the diagnosis of endometriosis being histologically confirmed. Therefore, asymptomatic endometriosis patients could be appropriately included as cases. The main limitation of this approach is that controls included women with other gynecological diseases, which might be also affected by the studied genetic variants.

Our results indicate increased endometriosis risk in the presence of *VEGF* variant alleles *−2578A* and *−1154A*, whereas *VEGF +405C* and *KDR 1192T* variant alleles appear to be protective. In a previous study from our group, involving 182 cases and 112 controls, we have already reported increased risk of endometriosis for *VEGF −1154A*, whereas the other four *VEGF* SNPs showed no significant associations with the disease [14]. Despite the lack of significant association with endometriosis for *VEGF −2578A* and *+405C* in that previous study, the results already pointed to higher proportion of genotypes containing *VEGF −2578A* and lower proportion of genotypes containing *VEGF 405C* among cases, as compared to controls [14]. A relatively recent meta-analysis, comprehending 3313 endometriosis patients and 3393 controls, from 14 case-control studies, also suggested that *VEGF −2578C > A* increases the risk of endometriosis [13]. However, in contrast to our results, the meta-analysis pointed to a protective effect for the variant genotypes of *VEGF −1154G > A* [13], and showed no significant effect of *VEGF +405G > C* as a risk factor for endometriosis [13], such as [9,15,16,23]. Our results suggest no significant effect of the *VEGF −460T < C* and *+936C < T* on the susceptibility to endometriosis, which is in accordance with previous reports [11,23].

Two studies investigated the association between *KDR 1192C > T* and endometriosis [9,17]. Vodolazkaia et al. found no significant association, but Kang et al. suggested a favorable effect in the endometriosis susceptibility, which is in accordance with our results. Wang et al. [10] showed that *KDR 1192C > T* leads to a decreased efficiency of VEGF binding to VEGFR-2, which could reduce angiogenesis signaling, thereby reducing the development of endometriosis. Concerning *KDR −604T > C*, our data suggested no significant effect on endometriosis pathogenesis. There are no previous reports regarding *KDR 604T > C* and endometriosis.

Table 4
Combined genotype frequencies of *VEGF* and *KDR* SNP between controls and cases (all patients or DIE) and their association with endometriosis risk.

Characteristic	Controls N (%)	Endometriosis N (%)	*P*-value[a]	$OR_{adjusted}$ (95% CI)[b]	DIE[c] N (%)	*P*-value[a]	$OR_{adjusted}$ (95% CI)[b]
VEGF −2578C > A, −1154G > A, +405G > C and *KDR 1192C > T*							
WT/WT/WT/WT	8 (4.0)	18 (6.9)		1[d]	13 (7.4)		1[d]
WT/WT/VAR/VAR	80 (39.8)	72 (27.6)	0.03	0.33 (0.12–0.89)	50 (28.6)	0.03	0.30 (0.10–0.87)
VAR/VAR/WT/WT	33 (16.4)	77 (29.5)	0.98	1.00 (0.61–1.66)	51 (29.1)	0.70	0.90 (0.52–1.55)
VAR/VAR/VAR/VAR	80 (39.8)	94 (36.0)	0.11	0.76 (0.55–1.06)	61 (34.9)	0.03	0.67 (0.46–0.96)

OR: odds ratio; CI: confidence interval; DIE: deep infiltrating endometriosis; WT/WT/WT/WT = *CC/GG/GG/CC*; WT/WT/VAR/VAR = *CC/GG/GC/CT* or *CC/GG/GC/TT* or *CC/GG/CC/CT* or *CC/GG/CC/TT* or *CC/GG/GG/CT* or *CC/GG/GG/TT* or *CC/GG/GC/CC* or *CC/GG/CC/CC*; VAR/VAR/WT/WT = *CA/GA/GG/CC* or *CA/AA/GG/CC* or *AA/GA/GG/CC* or *AA/AA/GG/CC* or *CC/GA/GG/CC* or *CC/AA/GG/CC* or *CA/GG/GG/CC* or *AA/GG/GG/CC*; VAR/VAR/VAR/VAR = *AA/AA/CC/TT*.
[a] P-value is P from Chi-square test (Pearson P-value) or Fisher's exact test.
[b] Adjusted by age and BMI.
[c] Controls vs DIE.
[d] Reference group.

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SNPs in the *VEGF* or *KDR* genes also were associated with gynecological disorder, such as, breast cancer [24,25], ovarian cancer [26], cervical cancer [27], ovarian hyperstimulation syndrome [28] and recurrent pregnancy loss [29]. Recently, we evaluated the impact of 5 common *VEGF* SNPs on breast cancer features and prognosis, and concluded that *VEGF −2578C > A* genotyping may add to prognostic evaluation of breast cancer [24]. Moreover, Su et al. observed that SNPs in *VEGF* and *KDR* jointly contributes to consecutive spontaneous miscarriages [29].

When *VEGF* and *KDR* SNPs were analyzed together, the combination of wild-type genotypes of both *VEGF −2578C > A* and *−1154G > A* with variant genotypes of both *VEGF +405G > C* and *KDR 1192C > T* showed the best protective effect against the development of endometriosis, either considering all cases or only DIE. These findings strongly suggest a gene–gene interaction between *VEGF* and *KDR* regarding the development of endometriosis, and point to the importance of their combined analysis in further epidemiological studies to establish their potential as disease biomarkers.

The present study also evaluated the impact of *VEGF* and *KDR* SNPs in endometriosis symptoms. Only *KDR 1192C > T* showed a significant effect, with the variant allele (*T*) being protective against cyclical urinary symptoms (pain and bleeding). The mechanisms involved in the onset of endometriosis symptoms are not known, and not all patients experience the same symptoms [30]. However, several evidences indicate that macrophages are recruited to endometriotic lesions, where they release pro-inflammatory and pro-angiogenic mediators [31,32], including VEGF [21], which might contribute to the onset of pain and bleeding, as observed in cyclical urinary complaints. Indeed, in addition to its role in angiogenesis, which is essential for the maintenance and growth of endometriotic lesions, VEGF-KDR signaling also appears to modulate nociception and to be involved in other pathologies associated with chronic pain [33,34]. Because *KDR 1192C > T* leads to decreased VEGF binding to VEGFR-2 [10], it appears that VEGF-KDR signaling is also essential for the cyclic pain symptoms associated with endometriotic activity.

In summary, the combined analysis of *VEGF-KDR* genotypes suggests a gene–gene interaction in endometriosis susceptibility, possibly as result of altered VEGF-VEGFR2 signaling. The functional understanding of genetic variants in the pathogenesis of endometriosis appears to fundamental, in view of its hereditary susceptibility, and may contribute for the characterization of new biomarkers as well as potential molecular targets.

Conflict of interest

All the authors declare that there is no conflict of interest.

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