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

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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.

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

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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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