Vascular endothelial growth factor pathway in endometriosis: genetic variants and plasma biomarkers

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Objective: To study single nucleotide polymorphisms (SNPs) involved in angiogenesis (*VEGF*, *PLGF*, *VEGFR1*, *VEGFR2*, *HIF-1α*) and plasma levels of the corresponding proteins (VEGF, PLGF, sVEGFR1, sVEGFR2) in women with and without endometriosis.

Design: Allele frequencies of vascular endothelial growth factor (VEGF) pathway SNPs and plasma levels of the corresponding proteins were investigated in patients with endometriosis and in controls.

Setting: University hospital.

Patient(s): Samples of DNA from 1,931 Caucasian patients were included (1,109 patients with endometriosis and 822 controls). An additional study group included 973 DNA samples from volunteers, self-reported to be healthy without laparoscopic evaluation.

Intervention(s): Women who underwent a laparoscopy for subfertility and/or pain and healthy volunteers without laparoscopic evaluation.

Main Outcome Measure(s): Functional SNPs of the *VEGF*, *VEGFR1*, *VEGFR2*, *HIF-1α* genes and Hap Map tagging SNPs of the PLGF gene were genotyped by using iPLEX technology on a Sequenom MassArray and TaqMan SNP Genotyping Assay. The VEGF levels were determined in ethylenediaminetetraacetic acid plasma samples by using Bio-Plex Protein Array System. PLGF, sVEGFR1, and sVEGFR2 levels were measured in ethylenediaminetetraacetic acid plasma samples by using ELISA Quantikine kits.

Result(s): A significant association was found between the rs2268613 polymorphism in the *PLGF* gene and PLGF plasma levels. In all study subjects, women with the AA variant of the rs2268613 *PLGF* gene had significantly lower PLGF plasma levels (median [interquartile range] 9.36 [8.19–10.43] pg/mL) than those with the AG variant (12.1 [11.81–20.84] pg/mL; P^a=.0085, P^b=.04), both before and after multiple testing. Plasma levels of VEGF were elevated in endometriosis patients (especially in minimal–mild endometriosis during the menstrual cycle phase) compared with laparoscopic controls but had a moderate diagnostic performance (area under the curve, 0.73) in this discovery dataset. At a cut-off plasma level of VEGF >3.88 pg/mL, minimal–mild stages of endometriosis were diagnosed with a sensitivity of 74% and a specificity of 80% during the menstrual phase of cycle. The associations between the presence of endometriosis and SNPs in *PLGF* (rs2268614), *HIF-1α* (rs11549465), and *VEGFR1* (rs9582036) genes lost statistical significance after multiple testing.

Received July 15, 2015; revised December 7, 2015; accepted December 16, 2015; published online January 7, 2016.

A.V. has nothing to disclose. B.T.Y. has nothing to disclose. C.M.K. has nothing to disclose. A.B. has nothing to disclose. D.S. has nothing to disclose. D.H. has nothing to disclose. C.M. has nothing to disclose. K.P. has nothing to disclose. C.T. has nothing to disclose. X.B. has nothing to disclose. D.L. has nothing to disclose. T.D. has nothing to disclose. A.F. has nothing to disclose.

Supported by a TBM (Toegepast Biomedisch Onderzoek met Primair Maatschappelijke Finaliteit) grant from the Institute for Innovative Science and Technology IWT (Innovatie door Wetenschap en Technologie) in Flanders, Belgium.

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Fertility and Sterility® Vol. 105, No. 4, April 2016 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2015.12.016

Conclusion(s): Genetic variants in the PLGF rs2268613 gene may influence plasma levels of the corresponding protein. Plasma levels of VEGF were elevated in endometriosis patients compared with controls. The associations between the presence of endometriosis and SNPs in PLGF (rs2268614), HIF-1α (rs11549465), and VEGFR1 (rs9582036) genes lost statistical significance after multiple testing. (Fertil Steril® 2016;105:988–96. ©2016 by American Society for Reproductive Medicine.)
Key Words: Endometriosis, plasma biomarkers, single-nucleotide polymorphism, VEGF pathway

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Endometriosis is a complex benign gynecologic disorder, characterized by the growth of endometrial-like tissue outside the uterine cavity and associated with pelvic pain and subfertility. Endometriosis can appear as peritoneal lesions, ovarian endometriotic cysts, and deeply infiltrative endometriosis (1) and can be classified into four stages: minimal, mild, moderate, and severe (2). At present, the only way to conclusively diagnose endometriosis is through laparoscopic inspection, preferably with histologic confirmation.

There is abundant evidence that genetic factors influence the susceptibility to endometriosis (3). Indeed, the prevalence of endometriosis is six to nine times greater in the first-degree relatives of affected women compared with the general population (4). A number of single-nucleotide polymorphisms (SNPs) in candidate genes have been associated with endometriosis (reviewed by Falconer et al [5]); however less than half of the reported associations with endometriosis have been investigated in a separate population, and many associations are not replicated in subsequent studies (reviewed by Montgomery et al [6]), which indicates the need of validation studies in independent data sets.

Although the pathogenesis of endometriosis remains unclear, there is compelling evidence that angiogenesis plays a key role in the ectopic implantation of endometrial tissue and its development into endometriotic lesions (7–9). Vascular endothelial growth factor (VEGF), vascular endothelial growth factor receptor 1 (VEGFR1), vascular endothelial growth factor receptor 2 (VEGFR2), placental growth factor (PGF or PLGF), and hypoxia inducible factor-1α (HIF-1α) are part of the biological system that plays a key role during angiogenesis (10–18). Placental growth factor is involved in angiogenesis and vasculogenesis. The role of PLGF in endometriosis is unknown. However, because it is involved in angiogenesis it must have some implication for endometriosis.

Circulating VEGF levels have been reported to be either increased (19–22) or to be similar (8, 23, 24) in women with endometriosis when compared with controls (Supplemental Table 1, available online), probably owing to differences in study design and methodology (e.g., serum and plasma collection, time of sample collection and processing).

Studies regarding VEGF genetic polymorphisms in endometriosis have been performed in different ethnic groups and led to different conclusions (25–33). Conflicting results of the genetic association studies may be partially explained by common methodologic problems like low sample size, different ethnic backgrounds of the study subjects, variable disease definitions and inclusion/exclusion criteria (34), and the lack of a control group with laparoscopically excluded endometriosis (5).

Therefore, the aim of this study was to test the hypothesis that SNPs in genes involved in angiogenesis (*VEGF*, *PLGF*, *VEGFR1*, *VEGFR2*, *HIF-1α*) are associated with endometriosis and that plasma levels of the corresponding proteins (VEGF, PLGF, sVEGFR1, sVEGFR2) are affected by these polymorphisms.

MATERIALS AND METHODS

DNA Samples

Samples of DNA from 1,931 Caucasian patients of Leuven University Fertility Center (LUFC) were included in our study (1,109 patients with endometriosis and 822 controls) from women who had undergone laparoscopy for subfertility and/or pain in 1998–2010. The cases (women with endometriosis) had either minimal–mild (n = 608) or moderate–severe (n = 484) endometriosis, staged according to the revised classification system of the American Society of Reproductive Medicine (2). The reference control group had laparoscopically confirmed absence of endometriosis (laparoscopic control group). Additionally, DNA samples were obtained from an additional group of female blood donors (n = 973), self-reported to be healthy but without information regarding fertility or pelvic pain status and without laparoscopic evaluation (self-reported healthy volunteers group). Clinical characteristics of the study and control groups are shown in Supplemental Table 2. The study was approved by the Commission for Medical Ethics of the Leuven University Hospital Belgium.

DNA Extraction

Samples of DNA were extracted from peripheral blood (n = 1,712) or peritoneal biopsy (n = 219). Deoxyribonucleic acid purified from ethylenediaminetetraacetic acid (EDTA)-stabilized whole peripheral blood was collected for routine molecular diagnostic tests at the Center for Medical Genetics of University Hospitals, Leuven, Belgium. The following methods were used: Chemagic DNA blood special kit (Chemagen), based on the specific binding of DNA to paramagnetic