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ARTICLE

Genetic variants of vascular endothelial growth factor are associated with recurrent implantation failure in Korean women

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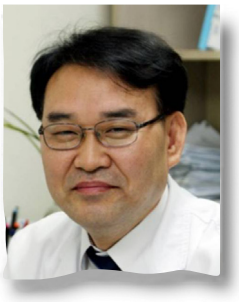

Nam Keun Kim is a full Professor at the Department of Biomedical Science, College of Life Science and Vice-president of the Graduate School at CHA University in Korea. He is the author of more than 120 papers in several international journals. Current research and clinical interests are in the molecular genetics of recurrent pregnancy loss, recurrent implantation failure and primary ovarian insufficiency.

Abstract Vascular endothelial growth factor (VEGF) is involved in embryonic development, decidual vascularization and placenta angiogenesis. This study was performed to determine whether there is an association between genetic polymorphisms in the *VEGF* gene and the development of recurrent implantation failure (RIF) in Korean women. A total of 119 women diagnosed with RIF and 236 control subjects were genotyped for *VEGF* polymorphic sites including rs833061 (-460T>C), rs25648 (-7C>T) and rs3025020 (-583C>T) using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assays and real-time PCR. The *VEGF* rs833061 C allele and rs25648 T allele were significantly associated with increased RIF risk (odds ratio [OR] = 1.813 [1.161–2.831], $P = 0.009$, OR = 2.213 [1.254–3.903], $P = 0.005$). The rs833061/rs25648 TC/CT, TC/CT+TT, and rs833061/rs3025020 TC+CC/TT genotypes were more frequent in the RIF group compared with the control group (OR = 2.130 [1.092–4.156], $P = 0.025$, OR = 2.130 [1.092–4.156], OR = 4.261 [1.163–15.620], $P = 0.028$, respectively). The results of this study suggests that *VEGF* polymorphisms are associated with RIF development. Therefore, we postulate that *VEGF* polymorphisms might be useful markers to predict RIF development. Further studies are warranted to elucidate the role of *VEGF* variants and RIF development.

KEYWORDS: female infertility, genetic association study, recurrent implantation failure, single nucleotide polymorphism, VEGF

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Introduction

Implantation is defined as when the human embryo attaches to the epithelium of the uterus and penetrates through the epithelial lining to embed into the endometrium. In clinical practice, implantation is generally considered successful when the intrauterine gestational sac is identified by ultrasonography.

Recurrent implantation failure (RIF) is defined as repeated failure to achieve a clinical pregnancy following the transfer of adequate quality of embryos (Coughlan et al., 2014). Although there is no consensus definition for RIF among researchers, Polanski et al. proposed defining RIF as the absence of attachment of the embryo to the womb lining after two consecutive cycles of IVF, intracytoplasmic sperm injection (ICSI) or frozen embryo transfer cycles, where the cumulative number of transferred embryos was no less than four for day-2 embryos and no less than two for day-5 embryos (blastocysts), using good quality and developmental stage embryos (Polanski et al., 2014). As with recurrent pregnancy loss, there are several suggested causes associated with RIF, such as gamete/embryo factors, uterine factors, immunological factors and thrombophilic conditions (Choi et al., 2014; Coughlan et al., 2014).

Of those factors, appropriate endometrium is considered to be crucial for successful implantation. After the successful implantation, the fetus is supplied with oxygen and nutrients through adequate placental development. These two processes, implantation and placentation, are required for healthy pregnancy in early stages. Angiogenesis is responsible for these essential processes.

Vascular endothelial growth factor (VEGF) is a key regulator of angiogenesis and vasculogenesis. Single nucleotide polymorphism (SNP), a DNA sequence variation occurring commonly within a population, affects protein expression and function. There are several reports that *VEGF* gene polymorphisms have an association with various obstetrics and gynaecologic disease development and prognosis (Cheng et al., 2013; Lee et al., 2010; Perini et al., 2014). In addition, there are reports that *VEGF* rs2010963 (+405G/C) genotype and *VEGF* rs1570360 (-1154A/A) genotype is associated with RIF (Boudjenah et al., 2012; Goodman et al., 2008).

Other *VEGF* SNP, including rs833061 (-460T>C), rs25648 (-7C>T) and rs3025020 (-583C>T), have been reported to affect VEGF expression and activity (Al-Habboubi et al., 2011; Almawi et al., 2013). However, there are a limited number of studies that evaluate the association between *VEGF* polymorphism and RIF development in Korean subjects.

The objective of this study was to elucidate the association between *VEGF* polymorphisms (rs833061 T > C and rs25648 C > T, and rs3025020 C > T) and RIF in Korean subjects.

Materials and methods

Study participants

This study was a prospective case-control study. Blood samples were collected from 119 subjects with RIF (mean age ± SD, 34.22 ± 3.35 years), and 236 control participants (mean age ± SD, 33.36 ± 5.81). The study population consisted of participants recruited from the Department of Obstetrics and Gynecology of CHA Bundang Medical Center, CHA University (Seongnam-si, Korea) between March 2010 and December 2012. The Institutional Review Board of CHA Bundang Medical Center reviewed and approved the study on 23 February 2010 (reference no. PBC09–120). Informed consent was obtained from all participants.

RIF was defined as failure to achieve pregnancy after two completed fresh IVF–embryo transfer (IVF–ET) cycles with more than 10 cleaved embryos. Serum human chorionic gonadotrophin (HCG) concentrations were less than 5 mIU/ml 14 days after embryo transfer. All transferred embryos were examined by the embryologist before transfer and judged to be of good quality. Both the male and female partner of couples experiencing recurrent implantation failure were evaluated. The following exclusion criteria are commonly adopted to diagnose RIF. Subjects who were diagnosed with RIF due to anatomic, chromosomal, hormonal, infectious, autoimmune or thrombotic causes were excluded from the study group. Anatomical abnormalities were evaluated using several imaging methods, including sonography, hysterosalpingogram, hysteroscopy, computerized tomography and magnetic resonance imaging. Karyotyping was conducted using standard protocols. By measuring the concentrations of prolactin, thyroid-stimulating hormone, free T4, follicle-stimulating hormone, luteinizing hormone and progesterone in peripheral blood, hormonal causes were excluded, including hyperprolactinaemia, luteal insufficiency and thyroid disease. Lupus anticoagulant and anticardiolipin antibodies were examined for autoimmune causes such as lupus and antiphospholipid syndrome. Thrombotic causes were defined as thrombophilia, and were evaluated by protein C and protein S deficiencies and by the presence of anti-α2 glycoprotein antibody. Semen analysis, karyotyping and hormonal assays, including oestradiol, testosterone, FSH and LH, were performed for male partners. Among the initial 152 patients who were enrolled for the study, 33 subjects who had Müllerian anomaly, hypothyroidism, chromosomal abnormality or antiphospholipid syndrome were excluded from the patient group, leaving 119 patients for the study.

Enrolment criteria for the control group included regular menstrual cycles, normal karyotype (46XX), a history of at least one naturally conceived pregnancy and no history of pregnancy loss including abortion history. Data were collected identically for all groups.

Genotyping

Peripheral blood samples were collected for genotyping. Genomic DNA was extracted from anticoagulated peripheral blood using a G-DEX for blood Genomic DNA Extraction Kit (iNtRON Biotechnology, Seongnam, Korea). Nucleotide changes were examined using PCR-restriction fragment length polymorphism (PCR-RFLP) analyses (rs3025020) or real-time PCR (rs833061, rs25648). *VEGF* rs833061T>C, rs25648 C > T and rs3025020C>T were selected using the human genome SNP database (dbSNP: www.ncbi.nlm.nih.gov/snp). The PCR primers used in this study are shown in **Supplementary Table S1**. Restriction enzyme digestion for PCR-RFLP was performed using the following enzyme and conditions: *Msp*I (*VEGF* rs3025020; New England BioLabs, Ipswich, MA) at 37°C for 16 h.