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Review article

Vascular endothelial growth factor single nucleotide polymorphisms and haplotypes in pre-eclampsia: A case-control study

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ABSTRACT

An association between vascular endothelial growth factor (*VEGFA*) gene variants and altered VEGF secretion and preeclampsia (PE) were described, often with inconclusive findings. An ethnic contribution to the association of *VEGFA* polymorphisms with PE and its associated features was also suggested. To investigate whether common *VEGFA* single nucleotide polymorphisms (SNP) are linked with PE and associated features in Tunisian women. A case-control study involving 300 women with PE, and 300 age-matched control women. Genotyping of *VEGFA* rs833052, rs699947, rs833061, rs1570360, rs2010963, rs25648, rs833068, rs833070, rs3025020, and rs3025039 SNPs was done by real-time PCR.

Minor allele frequency (MAF) of rs833052, rs699947, rs833061, rs1570360, rs2010963, rs25648, rs833068, rs833070, rs3025020, and rs3025039 *VEGFA* SNP, were not significantly different between PE cases and control women. In addition, there was lack of association of the genotypes of *VEGFA* SNPs with PE, irrespective of the genetic model used. Seven-locus (rs699947, rs833061, rs1570360, rs2010963, rs25648, rs833068 and rs833070) haplotype analysis demonstrated positive association of ATGCCAA, ACAGCAG and CCAGCGG, and negative association of CCAGCAA and ATGCCGG haplotypes with PE, all of which except for ACAGCAG remained associated with PE after correcting for multiple comparisons. Increased and reduced PE severity was associated with ATGCCAA, and with ATGCCGG and CCAGCAA haplotypes, respectively. Furthermore, carriage of CCGGTAG haplotype was associated with reduced risk of PE. Our study suggests that *VEGFA* haplotypes, more so than individual SNPs, play a role in PE pathogenesis in Tunisian women. These findings need confirmation in other ethnic populations.

1. Introduction

The placenta is a critical organ for the transitional maintenance of pregnancy, and for proper embryonic development and fetal growth throughout pregnancy. Establishment of optimal vascularization and placental blood flow is thus crucial for successful pregnancy outcome, and abnormal placentation was suggested as a possible etiologic factor of obstetric complications, including preeclampsia (PE) [1]. This syndrome is a pregnancy-related disorder characterized by hypertension, and occasionally proteinuria, in the second-third week of gestation [1]. PE reportedly affects 5–8% of pregnancies, and is linked with maternal and fetal morbidities, including intrauterine growth restriction (IUGR), and spontaneous preterm birth [1,2]. Animal and clinical studies implicated altered levels of placenta-derived bioactive cytokine and growth factors in the pathogenesis of PE. This includes heightened expression of anti- and pro-angiogenic factors such as soluble endoglin (sEng) and soluble fms-like tyrosine kinase-1 (sFlt-1) [3,4], and an associated decline in the levels of pro-angiogenic factors, including placental growth factor (PlGF) and vascular endothelial growth factor (VEGF) [4,5].

Vascular endothelial growth factor (VEGF) is a 45 kDa heparin-binding homodimeric glycoprotein, which acts as a vasculogenic and pro-angiogenic factor under physiological and pathological conditions, both in paracrine and autocrine fashion [2–4]. VEGF is an important regulator of vascular remodeling and survival of placental trophoblasts [3,4]. Circulating VEGF increases vascular permeability, hence vasodilatation, which are central to many vascular diseases, including hypertension [6].VEGF is secreted by several cell types, including

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endothelial, vascular smooth muscle, as well as corpus luteum, endometrium and placental cells.[7,8]. VEGF is essential for placental trophoblast differentiation and proliferation, the development of embryonic vasculature, and the growth of maternal and fetal blood cells *in utero* [9], and diminished placental trophoblastic VEGF expression was linked with heightened risk of spontaneous miscarriages [10].

The VEGF family includes seven subtypes; VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, (snake venom) VEGF-F, and PlGF. VEGF-A is the most potent member of the VEGF family, and its gene (*VEGFA*) is located on chromosome 6p21.3, and its coding region spans over 14 kb [11]. *VEGFA* gene is organized in eight exons, separated by seven introns, and alternative splicing forms at least nine subtypes with distinct biological activities [11]. Human *VEGFA* gene is highly polymorphic, and 1260 SNPs comprising near-gene (124), 5'-UTR (23), 3'-UTR (125), exonic (209), and intronic (779) variants were reported (www.ncbi.nlm.nih.gov/projects/SNP/snp). An association between *VEGFA* variants and obstetrics and gynecologic diseases [12,13], in particular PE [14–18], were described. *VEGFA* variants were described to directly influence angiogenesis by altering *VEGFA* gene expression, and VEGF secretion [14,15]. However, the associations between *VEGFA* gene variants and PE remain controversial. In this study, we explored the associations between common *VEGFA* polymorphisms and PE among Tunisian women. The association of multi-locus *VEGFA* gene haplotypes PE and its associated features will also be discussed.

2. Subjects and methods

2.1. Study subjects

Between May 2012 and June 2013, 300 Tunisian women with PE (mean age 31.3 ± 7.0 yr), and 300 control women with normal pregnancy (mean age 30.5 ± 5.8 yr), were recruited into this retrospective case-control study, from outpatient gynecology service of Farhat Hached University Hospital (Sousse, Central Tunisia), Fattouma Bourguiba University Hospital (Monastir, Central Tunisia), Taher Sfar University Hospital (Mahdia, Eastern Tunisia), and Gafsa Hospital (Southern Tunisia). The inclusion criteria were PE during natural pregnancy, defined as gravidic hypertension, and assessed as systolic blood pressure [BP] > 140 mmHg, diastolic BP > 90 mmHg, and/or rise in systolic BP > 30 mm, or diastolic BP > 15 mmHg on at least two measurements 6 h apart, after 20 weeks of gestation, along with significant proteinuria (> 300 mg/24 h) or proteinuria > 2 + (determined by the dipstick method).

Control women were recruited from the same geographical area, with no known personal or family history of PE. While 25.2% of PE cases developed severe early-onset PE form according to these criteria before 34 weeks of gestation, no cases of HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets) were recorded. Local ethics committees approved the study protocol (Poject Number: PI-15-91; November 2014), and both PE cases and control women gave written informed consent for participation in the study. Demographic data of participants and clinical characteristics of patients are shown in Table 2.

2.2. VEGFA genotyping

VEGFA polymorphisms with minor allelic frequency (MAF) of > 5% in Caucasians were selected. Allelic discrimination method (VIC- and FAM-labeled) was used for genotyping the *VEGFA* variants rs833052, rs699947, rs833061, rs1570360, rs2010963, rs25648, rs833068, rs833070, rs3025020, and rs3025039, using assay-on-demand TaqMan assays (Applied Biosystems; Foster City, CA). The reaction was carried out in a 6.3 µl volume according to the instructions of the manufacturer (Applied Biosystems), using both StepOne and StepOne Plus real-time PCR systems (Applied Biosystems). Quality control measures consisted of genotyping replicate blinded specimens to evaluate genotyping reproducibility; concordance exceeded 99%.

2.3. Statistical analysis

Statistical analysis was done using SPSS (version. 22.0, IBM). Continuous variables were presented as means (± SD) for, while categorical variables were expressed as percentages of total. Differences in means were assessed by Student's *t*-test, while inter–group significance was evaluated by Pearson χ^2 test (or Fisher's exact test for small samples). Testing for Hardy–Weinberg equilibrium (HWE) was done by Haploview (www.broad.mit.edu/mpg/haploview). Calculation of study power was done by Genetic Power Calculator (http://pngu.mgh.harvard.edu/~purcell/cgi-bin/cc2k.cgi); the parameters used were 300 women with PE and 300 control women, genotypic relative risk for heterozygote (1/2) and minor allele homozygous (2/2), and the MAF for PE cases and controls for the ten tested SNPs, and assuming a 5.0% population prevalence of PE (unpublished statistics). Assuming these parameters, we calculated the overall power (69.9%) as the average power of the ten tested SNPs. Haploview was used to check linkage disequilibrium (LD) between SNPs, beside their haplotype patterns, which were reconstructed by the expectation maximization method. Taking the control group as reference, regression analysis was used for determination of the odds ratios (OR) and 95% confidence intervals (95%CI) associated with PE risk.

3. Results

3.1. Study subjects

Baseline and clinical characteristics of PE cases and control women are described in Table 1. Women with PE were matched to controls with respect to age at examination, and delivery methods. Women with PE had significantly higher BMI, elevated systolic and diastolic BP, and gestational age at blood sampling. Significant differences between PE cases and control women were also noted with regards to the newborn weight, and to the status of pregnancy. Accordingly BMI, regional origin, newborn weight, gestation age, and pregnancy status were selected as the covariates that were controlled for in subsequent analysis.

3.2. Association of VEGFA SNPs with PE

Ten *VEGFA* SNPs were selected for this study based on their MAF (> 5%) in Tunisians, and likely association with adverse pregnancy complications, including PE. The allele distributions of the tested *VEGFA* SNPs between PE cases and control women are summarized in Table 2. The genotype distributions of the tested *VEGFA* variants did not deviate from Hardy-Weinberg equilibrium. MAF of all tested *VEGFA* SNPs were not significantly different between PE cases and control women, even before correcting for multiple comparisons. Setting homozygous major allele-carrying genotype (codominant and dominant models) or major allele-carrying genotypes (receive model) as reference (OR = 1.00), results from Table 3 demonstrated lack of association of the genotypes of the tested *VEGFA* SNPs with PE under the codominant, dominant, or recessive genetic association models.

3.3. Haploview analysis

Haploview analysis revealed high LD between rs699947, rs833061, rs1570360, rs2010963, rs25648, rs833068 and rs833070 (Block 1), and between rs3025020 and rs3025039 (Block 2) (Fig. 1). From the theoretical 128 possible haplotypes in Block 1, only 10 were found to be common (haplotype frequency > 1.5%), capturing 86.3% of Block 1 haplotypes, while of the 3 of 4 possible haplotypes were common, capturing 99.4% of all haplotypes. Results from Table 4 demonstrated enrichment of ATGCCAA, ACAGCAG, and CCAGCGG haplotypes, and reduced frequency of CCAGCAA and ATGCCGG haplotypes among PE cases than control women, thereby assigning PE susceptibility and protective nature to these haplotypes, respectively. Apart from