Medical
Communication

Biosci. Biotech. Res. Comm. 12(3): 584-589 (2019)

BBRC
Bioscience Biotechnology
Research Communications

Role of Genetic Variants in Immunoregulatory and Oxidative Stress Genes with Predisposition to Pre-eclampsia: A possibility for Predicting the High Risks in Synergetic Reaction

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ABSTRACT

The multifactorial basis of pre-eclampsia (PE), also known as pregnancy-induced hypertension, involves a combination of genetic risk factors that cause disease development in women during pregnancy. The prevalence of PE varies in different ethnicities from 8 to 20%. The precise etiology and pathophysiology remain unclear, and immunoregulatory pathway genes Forkhead Box P3 (*FOXP3*) and Cytotoxic T-lymphocyte associated protein 4 (*CTLA4*) and oxidative markers angiotensin converting enzyme (*ACE*) and endothelial nitric oxide synthase (*eNOS*) have been suggested as risk factors for PE development. This review article describes the possible synergic interactions between polymorphic variants in *FOXP3*, *CTLA4*, *eNOS*, and *ACE*, which contribute to immunoregulatory and oxidative stress in women with PE. We screened for studies describing the combinations of all four variants. Previous studies showed that all these genetic markers lower the expression and production of surface molecules, which may enhance the risk and susceptibility to PE. Based on previous studies and meta-analysis, we recommend that genetic screening of a large sample size must be carried out to confirm the roles of these variants in PE.

KEY WORDS: PRE-ECLAMPSIA, FOXP3, CTLA-4, ENOS, ACE, POLYMORPHISM

ARTICLE INFORMATION:

Corresponding Author: saffu.sb1@gmail.com
Received 2nd Aug, 2019
Accepted after revision 20th Sept, 2019
BBRC Print ISSN: 0974-6455
Online ISSN: 2321-4007 CODEN: USA BBRCBA
Thomson Reuters ISI ESC / Clarivate Analytics USA
NAAS Journal Score 2019: 4.31 SJIF: 4.196

Online Contents Available at: http//www.bbrc.in/
DOI: 10.21786/bbrc/12.3/5

INTRODUCTION

Pre-eclampsia (PE) is a pregnancy-specific disorder characterized by hypertension and proteinuria that appears in the 20th week of gestation. This multifactorial pregnancy-related disorder is associated with maternal morbidity and mortality (Steegers et al., 2010, Jahan, et al., 2013, 2014 and Al-Jameil et al., 2014). Globally, PE affects up to 20% of pregnant women. The incidence of PE is associated with genetic and environmental factors. The pathophysiological relationship between genetics and PE remains unknown (Khan et al., 2015 Berhe et al., 2018).

Various factors play important roles in the predisposition to pregnancy-associated conditions including obesity, dyslipidemia, oxidative stress, and possibly maternal immune responses to fetal antigens (Bianco et al., 1998; Hubel, 1998; Zenclussen, 2013). PE, also known as pregnancy-induced hypertension (PIH), disproportionately occurs in the first pregnancy (Kamineni 2015). Genetic evidence from family-based studies revealed that PE is common among the daughters' and sisters of women who had PE (Buurma et al., 2013). PE is typically diagnosed during late pregnancy based on proteinuria, edema, and increased vasoconstriction leading to maternal hypertension and decreased uteroplacental blood flow. Numerous studies confirmed that PE is an immune-mediated disorder. Normal pregnancy involves maternal immune tolerance in the fetus. Immunological factors appear to greatly contribute to the predisposition to PE, particularly in primipara women. Forkhead Box P3 (*FOXP3*) and Cytotoxic T-lymphocyte associated protein 4 (*CTLA4*) are immunoregulatory genes, while angiotensin converting enzyme (*ACE*) and endothelial nitric oxide synthase (*eNOS*) are oxidative stress genes. A relationship between immunoregulatory and oxidative stress genes has been demonstrated in PE, (Bianco et al., 1998; Hubel, 1998; Zenclussen, 2013, Pendeloski et al., 2011,Ye et al., 2017).

This review focuses on the combined effects of immune and oxidative components on PE development (Metz et al., 2012). Markers of oxidative stress are pro-oxidant enzymes with endogenously high activity (genetically determined) related to PE. The stimulation and regulation of the immune system is tightly regulated. Additionally, oxidative stress appears to be a central component of both placental and endothelial dysfunction, (Aouache et al., 2018).

Immunoregulatory genes associated with PE

The characteristics of immunopathology for prevalent complex diseases are linked with various immune responses, such as genetic regulation. The initiation, maintenance, and progression of PE have been evaluated by examining combination of genetic factors such as genetic polymorphisms (Mullighan et al., 1999). Numerous genetic polymorphisms and genes were shown to be associated with immunoregulatory pathways; among them, *FOXP3* and *CTLA-4* were shown to be related to PE.

Genetic association with *FOXP3* in women diagnosed with PE

FOXP3 is an immunoregulatory gene that plays an important role in pregnant women and specifically in PE as suggested previously. Regulatory T cells (Tregs) have important functions in the immune response and in genes. *FOXP3* is important in the development of Treg cells (Gholami et al., 2018) and contains 12 exons coding for 431 amino acids with a molecular weight of 47.25 kDa. *FOXP3* is located on chromosome at Xp11.23. In mouse models, inactivation in *FOXP3* results in a lack of Tregs and notable organ-specific autoimmunity (Metz et al., 2012). A few genetic variants (-924A/G (rs2232365); -3279C/A (rs3761548) are associated with PE in the global population.

Recently, Hosseini-Teshnizi et al (2019) performed a meta-analysis of the rs2232365 and rs3761548 polymorphisms in pregnant women with PE and infertile women with recurrent pregnancy loss and concluded as *FOXP3* variants affect pregnant women, including those with PE. The -3279C>A polymorphism is a well-known marker of PE used in many studies in different populations. The CC genotype is associated with low FOXP3 levels, while the AA genotype is associated with overexpression of FOXP3. Thus, genotypes at single-nucleotide polymorphism (SNP) position -3279 may play a decisive role in determining risk or protection for such clinically important conditions like PE given the high maternal/fetal morbidity and mortality of this condition (Jahan et al., 2013).

However, Nourouzian et al (2016) confirmed the negative association between -3279C>A polymorphism and PE risk. The CC genotype (C allele) of the SNP at -3279 has been suggested to play a role in other pregnancy-related complications, such as unexplained recurrent spontaneous abortion (Wu et al., 2011). In individuals with the FOXP3 -3279 CC genotype, due to reduced expression of $FOXP3^{+}$ normal functioning of Treg cells assessed by direct contact with the responder cells showed greatly hampered, as a consequence of this certain degree of inflammatory response of mother against fetal antigens can be expected positively contributing to premature delivery. From this perspective, the characteristics of FOXP3 regulatory molecules are important for identifying high-risk mothers. Chen et al (2015) showed that FOXP3 expression levels in the placental tissue of patients with PE are lower than those in normal pregnant women. This indicates that decreased *FOXP3*

expression decreases immunosuppressive functions and causes a maternal-fetal imbalance to induce PE.

Role of *CTLA-4* in women with PE

CTLA-4 is a member of the immunoglobulin family and transmits inhibitory signals to T cells. *CTLA-4* is a CD28 homologue expressed on the surface of T-lymphocytes. Cytotoxic T-lymphocyte antigen is considered as an important immunoregulatory molecule expressed constitutively in Treg cells. While these regulatory molecules must be expressed in conventional T cells following activation, CTLA affects CD4 T-cell activation (Frauwirth & Thompson, 2002; Rasti & Nasiri, 2016). CTLA-4 dysregulation appears to play a vital role by affecting normal fetal tolerance through exacerbated activation of T-cells on the fetal antigen (Kaufman et al., 1999). Gene mapping analysis revealed that *CTLA*-4 is on chromosome 2q33 and consists of at least 100 polymorphic sites (Karabon et al., 2009). *CTLA-4* plays a role in maintaining pregnancy and fetal-maternal tolerance through its expression in placental fibroblast and decidua cells. Polymorphic variants of *CTLA-4* are associated with reduced expression are likely involved in pregnancy-related disorders (Bonyadi et al. 2017).

Further quantitative variations in *CTLA-4* expression affected by genetic variants (rs231775) may be associated with pregnancy-related disorders such as PE. Some studies reported that the +49A>G rs231775 SNP causes recurrent spontaneous abortions. However, Dehaghani et al 2005) suggested that a heterozygous *CTLA-4* A49G allele is a predisposing factor for severe preeclampsia in Iranian women. Zhou et al (2016) found no association with the +49 A/G (rs231775) polymorphism in *CTLA-4* in PE in a Chinese population. Pendeloski et al (Pendeloski et al., 2011) found a similar pattern of a negative association in Brazilian women. Finally, in a Finish population, Jaaskelainen et al (2008) found a significant genetic association with the +49A/G polymorphism in women with PE.

Synergistic effect with combined *FOXP3* and *CTLA-4* variants in identifying genetic risk of PE

Because of the important regulatory function of FOXP3 and requirement for its continuous synthesis in Tregs cells to ensure normal functioning, any SNP either in the promoter or exon regions may greatly impact gene expression. The -3279 variant may alter FOXP3 levels. Reduced expression of this regulatory molecule, as observed in the CC genotype, likely leads to impaired functioning of FOXP3 regulatory molecules, such mutations in combination with CTLA the -4 GG or G allele in heterozygotes, which may confer a high risk of a maternal inflammatory response against the fetus. The G allele in CTLA-4 reduces the regulatory ability of Treg cells because of the significantly decreased CTLA-4 surface levels. A combination of SNPs in these two loci likely to contribute to protection against the mother's response to fetal antigens. This is possible in a *FOXP3* genotype with normal activity which does not affect the normal expression of FOXP3. In combination with CTLA-4 A49G genotype, which results in a normal level of surface expression of CTLA-4 molecule with relatively reduced clonal expansion. This hypothesis is important, as there are no published reports on the synergistic effects of genes involved in predisposition to important pregnancy-associated disorders. However, in recurrent spontaneous abortion, a similar synergistic effect was observed for SNPs in the FOXP3 and CTLA genes which differ from that proposed in the present study (Fan et al., 2018).

Oxidative stress markers: An imbalance between oxidants and antioxidants leads to redox signaling disruption and molecular damage, a condition known as oxidative stress (Aouache et al., 2018). PE is a pregnancy-related disorder considered to have multifactorial origins including the involvement of genetic and environmental factors. Oxidative stress marker (ACE and eNOS) variants have been suggested to significantly affect the development of PE (Hubel, 1998).

Risk of PE in women with *eNOS* genetic markers: Enhanced nitric oxide synthase (eNOS) and PE are both related to hypertension. eNOS is synthesized in endothelial cells by nitric oxide synthase to regulate blood flow and vasomotor tone. eNOS also increases the blood volume to enhance cardiac output and reduce blood pressure. The eNOS gene is on chromosome 7q35-36 in humans. This gene has been widely suggested to be involved in PE development. Three important variants have been reported to increase the risk of PE (-786T/C; 4b/4a; and G894T). A mutation in the promoter region at position 786 causes a T to C substitution. A variable number of 27-base pair tandem repeats in intron 4 (4b/a) and G-T mutation were found at nucleotide position 894 in the eNOS gene. Position 298 is prone to amino acid substitution from Aspirin to glutamine (rs1799983) (Dai, et al. 2013; Ma et al., 2016).

The eNOS enzyme synthesizes NO from L-arginine and functions as a vasodilator molecule crucial for regulating endothelial function and consequently maintaining homeostasis. An SNP in the eNOS gene at G894T results in low NO production, thereby causing endothelial dysfunction and contributing to pregnancy-related hypertension (Rahimi et al. 2013; Zeng et al., 2016). Few reports have also suggested that the polymorphism G894T reduces eNOS activity and decreases the plasma level of NO. Rahimi et al (2013) investigated the influence of eNOS 4a/4b and its synergistic potential with eNOS G894T polymorphisms on affecting PE risk and

confirmed a negative association; the T allele was not associated with PE. However, a concomitant risk was observed for eNOS and T allele did not significantly increase the risk of severe PE. Another study by Zeng et al (2016) confirmed the positive association between the recessive model and PE. Qi et al (2013) found that the inveterate TT genotype is associated with the eNOS G894T polymorphism and an increased risk of PE.

Ma et al (2016) performed a meta-analysis of 11,700 subjects (4028 cases vs 7672 controls) and observed that the G894T polymorphism in eNOS negatively affected PE/PIH. The samples were selected from 36 case-control studies of African, American, Asian, European, and Latin American populations. In Asians, the T allele was associated with a higher risk of PE compared to the G allele. A dominant model association was observed with PE in Latin American and African populations. However, in both Americans and Europeans, no association was detected. Another meta-analysis study showed that the -786C/T and 4b/a variants greatly contribute to the risk of PE, while the G894T polymorphism showed no association with PE (Dai et al., 2013).

Genetic role of insertion and deletion polymorphism in ACE gene in women with PE

ACE plays a major role in the renin-angiotensin system cascade by converting angiotensin-I to angiotensin-II to affect blood pressure. The 287-base pair Alu sequence affects the presence or absence of the intron 16 sequence in *ACE* (Khan et al 2014). During pregnancy, the circulating and intrarenal renin angiotensin aldosterone system control the salt-water balance to maintain maternal blood pressure and adequate placental perfusion both in the mother and fetus (Cristina et al., 2019).

Gene mapping analysis revealed that the *ACE* gene is located at 17q23 and consists of 26 exons and 25 introns (Khan et al., 2014). Genetic studies have been carried out to evaluate global population and confirmed the significant and non-significant associations of *ACE* I/D gene polymorphisms in women with PE (Cristina et al., 2019; Jahan et al., 2014; Ma et al., 2016; Miao & Gong, 2015; Qi et al., 2013). Four studies conducted in different regions in India revealed negative (Aggarwal, Jain, & Jha, 2010; Aggarwal, Dimri, Tandon, & Agarwal, 2011; Kaur, Jain, Khuller, Gupta, & Sherawat, 2005) and positive associations between the *ACE* gene and PE (Jahan et al., 2014). Meta-analysis studies also showed positive (Zhong, Wang, Zhu, & Zhao, 2012) and negative associations (Shaik, Sultana, Bammidi, Sampathirao, & Jamil, 2011).

Combination of synergistic effect with *ACE* and *eNOS* variants: While ace and eNOS have been evaluated individually, studies are needed to examine their synergistic effects, which may be useful for predicting the risk of PE and gestational hypertension. The synergistic effect may involve a combination of two D alleles with *ACE* I/D polymorphism and low nitric oxide-producing eNOS gene likely enhances the risk of PIH. The ACE enzyme in the renin-angiotensin-aldosterone system converts Ang I to Ang II. Ang II is a central molecule in this system and is responsible for increasing blood pressure via more than one mechanism (Khan et al., 2014) such as by (i) vasoconstriction, (ii) inducing NADPH oxidase, an enzyme involved in generating free radicals of oxygen, and (iii) producing reactive oxygen species which may interact with nitric oxide and converts it into peroxynitrite, thereby reducing the bioavailability of nitric oxide. The synergistic interaction between ACE and eNOS and their effects on PIH require further detailed analysis. Understanding such synergistic interactions conferred by genotypes at two loci in the same patient can enable gynecologists to manage hypertension and associated conditions in mothers with PE and gestational hypertension.

CONCLUSION

A relationship may exist between the genetic polymorphisms documented in immunoregulatory genes and oxidative markers related to PE. Global populations showed genetic associations with *FOXP3*, *CTLA-4*, *eNOS*, and *ACE* polymorphisms in women with PE, which may be related to ethnicity. Thus, similar polymorphisms should be examined in similar populations with larger sample sizes and the results should be compared with those of previous studies in different ethnicities. Genetic screening studies including large sample sizes are needed.

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