EXPERIMENTAL STUDY

ERVW-1 gene polymorphisms related to preeclampsia

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ABSTRACT

OBJECTIVES: Identification of genetic association between the gene ERVW-1 and preeclampsia.

BACKGROUND: Preeclampsia is a multifactorial disease affecting women during pregnancy and it is one of the main causes of perinatal and maternal morbidity and mortality. The pathophysiology of preeclampsia is very complex and several aspects of the disease have not been elucidated yet. Abnormal placentation frequently occurs during severe preeclampsia. Protein syncytin 1, a product of the ERVW-1 gene, plays a crucial role in the syncytiotrophoblast differentiation and optimal placentation. The syncytin 1 expression is disturbed during preeclampsia. The main focus of this study was the analysis of the ERVW-1 regulatory regions and identification of DNA polymorphisms associated with preeclamptic cases in Slovak population.

METHODS: Regulatory region of gene ERVW-1 was analyzed by sequencing to identify genetic variants.

RESULTS: We identified four DNA variants, namely rs4727276, rs148592540, rs569899772 and rs555416193, in samples of Slovak population.

CONCLUSION: No relation between polymorphisms and preeclampsia was observed, indicating that further investigations with a larger sampling are still required. However, our work represents new original approach in genetic differential diagnosis of preeclampsia with possible useful findings in the future (Tab. 3, Fig. 1, Ref. 34).

KEY WORDS: Preeclampsia, ERVW-1, syncytin 1, DNA polymorphisms, diagnostics.

Introduction

Preeclampsia is a multisystem disorder with heterogeneous pathophysiology which affects women during or immediately after pregnancy. This disease is one of the major causes of maternal morbidity and mortality, preterm birth, perinatal death and intrauterine growth restriction (IUGR) (1). Symptoms, which are easily detected during this disease and can indicate preeclampsia, are hypertension (systolic blood pressure > 140 mmHg, diastolic blood pressure ≥ 90 mmHg) and proteinuria (≥ 0.3 g/24 h) (2). The course of the disease can vary among the women. The root causes of the disease are pathological processes in the placenta and abnormal placental development (3). Many studies have demonstrated that deficient differentiation of trophoblast during preeclampsia leads to insufficient cell fusion and formation of unstable syncytiotrophoblast and also to defective invasion of spiral arteries by trophoblast (2, 4, 5). Disruption of remodeling of spiral arteries results in decreased placental perfusion which is enabled to provide requirements of this tissue (6). This pathophysiological process causes hypoxia and inflammation in the placenta (3). Simultaneously trophoblastic cells are released from unstable syncytiotrophoblast to maternal blood circulation where they can initiate reaction of maternal immune system. The most serious consequences of preeclampsia are eclampsia, HELLP syndrome (hemolysis, elevated liver enzymes, low platelet count) (7), damage of the mother’s internal organs (mostly brain and liver), cardiovascular disorders (8) and restricted growth of the embryo (9). Delivery of the fetus is the only effective treatment for preeclampsia. Only symptomatic treatment is available to address hypertension or convulsive seizures (10). Diagnosis of preeclampsia is difficult and consists of numerous clinical, biochemical and sonographic tests with different validity (5, 11, 12). No accurate non-invasive specific screening test currently exists.

Syncytin 1 is one of the factors, which mediates the fusion of placent al cytotrophoblastic cells to multinucleated syncytiotrophoblast and differentiation of syncytium (13, 14). A high level of the syncytin 1 gene expression is typically exclusive to the placent al tissue under normal physiological conditions (15–17). Syncytin 1 is encoded by the ERVW-1 gene on 7q21.2 (gene ID: 30816), which is a member of the multigene family of human endogenous retroviral elements – HERV-W (18, 19). The ERVW-1 locus consists of a complete provirus and is localized to the hu-
ERVW-1 gene expression is regulated on the transcriptional, post-transcriptional, translational and epigenetic levels (20). Studies have shown that dysregulation of syncytin 1 expression in trophoblast correlates with the development and severity of preeclampsia (4, 19, 21) and that the regulation of syncytin 1 can be affected already at the transcriptional level (22). The well-described regulatory region of ERVW-1 has a bipartite character and consists of a 5′ long terminal repeat (5′ LTR) retroviral element and an upstream regulatory element (URE) which consists of a distal regulatory element (DRE), mammalian apparent LTR retrotransposon (MaLR) and trophoblast specific enhancer (TSE). These elements and their parts can be positive or negative regulators of gene expression depending on the tissue type. There are localized binding sites for transcriptional factors such as GCMa, Sp-1, AP-2, GATA, Oct-1, PPAR-gamma/RXR (23, 24), etc. Interactions of these transcriptional factors are essential for optimal gene expression of ERVW-1. Mutations in the binding sites can alter the expression of ERVW-1 (23). Some studies suggest the existence of an additional potential enhancer in the upstream region (from position -1519 to -437) (24).

To our knowledge, none of the studies discuss the possible association between the reduction of syncytin 1 gene expression and the existence of genetic variants (polymorphisms) in its regulatory region in preeclamptic cases. Our goal was to analyze the regulatory regions of sequences of the regulatory regions of ERVW-1 (URE, 5′LTR) in the embryonic sample from pregnancies with or without diagnosed preeclampsia, with the aim to identify DNA polymorphisms associated with preeclamptic samples. We assume that mutations in this exact area could lead to changes in gene expression of ERVW-1, which frequently occurs in preeclampsia.

Detection of methylation changes in ERVW-1 promoter has been proposed as a possible diagnostic marker for the early detection of preeclampsia (25, 26). Disadvantages of this marker include the fact that it can only be used during pregnancy because of trophoblast specific expression of syncytin 1 and activation of ERVW-1, for example by hypermethylation, in every other somatic tissue during lifetime. Phosphorylation is another regulatory epigenetic modification of gene expression. It has been proved that phosphorylation status of survivin is different in preeclamptic placentas compared to that in normal placentas. It is worth mentioning that the same regulation mechanism could have a role in the expression of syncytin 1 (27, 28). However, the identification of polymorphisms in DNA associated with PE could provide a diagnostic marker that could be analyzed before pregnancy.

Materials and methods

DNA from 17 aborted embryos (9 preeclamptic and 8 non-preeclamptic) were investigated for the presence of variants in the 5′ LTR and URE regions. Genomic DNA was isolated from microdissected cryostat sections of embryonic tissue specimens by QiAampR Micro Kit (Qiagen Manchester Ltd., Manchester, UK). Regulatory regions of ERVW-1 were amplified separately. Primer sequences for amplification of the LTR and URE regions are: region 1: F: 5′-GCCACGGCATACTATACCC-3′, R: 5′-CCCTCCTCTCCTGTCTGTA-3′, (477-bp amplicon); region 2: F: 5′-AAAAAGAGCAGGGAGCTCCC-3′, R: 5′-GCCCTACCATCCACCC-3′, (438-bp amplicon); region 3: F: 5′-TTGTGGCTGCTGGGTTTAC-3′, R: 5′-GCCACAAATGACTGATGA-3′, (468-bp amplicon); region 4: F: 5′-ACTGAGTCACATGATCTTCACTG-3′, R: 5′-AGAGTGAAATAGCATGAAAACAGCT-3′, (450-bp amplicon); region 5: F: 5′-GCAAAAAGCCTGGAGATACAAC-3′, R: 5′-ATCCAGAGGGATGGGAGATCG-3′, (469-bp amplicon); region 6: F: 5′-TGCAACTCTGACTCTTGTTG-3′, R: 5′-CCACCATGATGTCCTGCGT-3′, (496-bp amplicon).

PCR amplifications were performed in 20-μl reaction volumes containing 150–200 ng of genomic DNA (1 μl), PCR Master Mix (2x) Fermentas (Waltham, Massachusetts USA) (10 μl), 0.5 μM (1 μl) of each primer (Sigma-Genosys, Lambda Life, Slovakia) and nuclease free water to a total volume of 20 μl (29).

After denaturation at 95 °C for 5 minutes, 30 cycles of denaturation at 95 °C for 30 s, annealing for 30 s, and elongation at 72 °C for 30 s were performed, followed by final elongation at 72 °C for 5 minutes. Annealing temperatures were as follows: 59 °C for region 1, 59.7 °C for region 2, 59.5 °C for region 3, 55 °C for region 4, 60 °C for region 5, and 59.3 °C for region 6. Successful amplification of fragments was confirmed by agarose electrophoresis. PCR fragments were purified by ExoSAP-IT® PCR Product Clean Up (Affymetrix, California, USA) as described by the manufacturer and prepared for automated sequencing analysis using BigDye® Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystem, California, USA). Before sequencing by ABI PRISM™ 3100 Genetic Analyzer (Applied Biosystem, California, USA) samples were purified using ExTerminator kit (Ecoli, Bratislava, Slovakia) following the manufacturer’s instructions.

Statistical analysis of results was carried out with the Fisher’s exact test using IBM SPSS Statistics software ver.20. p < 0.05 were considered statistically significant.

Results

We analyzed DNA from microdissected cryostat tissue sections of 9 embryos from preeclamptic pregnancies and 8 embryos from non-preeclamptic pregnancies. We amplified regulation regions (URE and 5′ LTR) localized upstream from the coding portion of the ERVW-1 gene. We analyzed regulatory elements for the presence of DNA variants which could be associated with preeclampsia. We identified 4 variants in the 2139 bp long region at positions -1340, -1046, -246, -30 (Figs 1a–d). A summary of variant frequencies is in Table 1.

<table>
<thead>
<tr>
<th>Variant Position</th>
<th>G/T Polymorphism Frequency</th>
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<tbody>
<tr>
<td>-1340 G&gt;T</td>
<td>7:g.92107752C&gt;G</td>
</tr>
<tr>
<td>-1046 G&gt;T</td>
<td>7:g.92108846G&gt;T</td>
</tr>
<tr>
<td>-246 G&gt;T</td>
<td>rs4727276</td>
</tr>
<tr>
<td>-30 G&gt;T</td>
<td>rs148592540</td>
</tr>
</tbody>
</table>

Allelic frequencies of -1340 G>T and -246 G>T polymorphisms in Slovak samples are 0.118 and 0.147, respectively. Variants at positions -1046 (0.059) and -30 (0.029) were polymorphisms rs555416193.
(7:g.92108552G>A) and rs569899772 (7:g.92107536G>A), respectively (1000 Genomes Project Phase 3) (Tab. 2).

No DNA variants were identified in the rest four preeclamptic and one nonpreeclamptic samples. The statistical analysis of the results was carried out using Fisher’s exact test. The analysis confirmed no statistically significant association between polymorphisms and preeclamptic cases; p for -1340G>T, -1046G>A, -246C>G and -30G>A polymorphisms was 0.294, 1.0, 0.294 and 0.471, respectively.

Discussion

The etiology of preeclampsia, a multifactorial disease with a variety of symptoms, has not yet been fully understood. There are a number of hypotheses about the likely cause of preeclampsia and underlying pathological processes leading to this maternal disorder (30), but evidence suggests that the origin of the disease is in the placenta (3) and involvement of genetics. Abnormal placentation manifests with a disruption of the maternal spiral artery remodeling which leads to insufficient placental perfusion (31). In our study we assumed that the placental abnormalities during preeclampsia are a consequence of a poor trophoblastic differentiation which is controlled by the product of endogenous retroviral element ERVW-1, syncytin 1. According to our knowledge none of the studies focus on ERVW-1 polymorphisms regarding to preeclampsia.

Analysis of the ERVW-1 regulatory regions confirms known high homogeneity of this region (4). 5’LTR shows the highest rate of sequence conservation as all 34 sequences were strictly identical. All identified mutations appeared in the region upstream from ERVW-1 and were in heterozygous form. Mutations at positions -246C>G (7:g.92107752C>G) and -1340G>T (7:g.92108846G>T) are known common polymorphisms (rs4727276 and rs148592540, respectively). Allele frequencies of polymorphisms between the Slovak population and other populations (1000 Genome database) are compared in Table 3. Allelic frequency of the -1340G>T polymorphisms is 0.118 in the Slovak population. This allelic frequency varies slightly from the worldwide average (0.018). When comparing European populations (Finnish, British, Iberian, Tuscan) and population with European ancestry, allelic frequencies
Recent studies show that several subtypes of preeclampsia can be classified (early or late PE, PE with or without fetal growth restriction, with regard to severity, etc.). It is possible that these subtypes have a different genetic background and different contribution of environmental factors which together cause pathophysiology of this disease (34). Severe preeclampsia appears to have the greatest genetic component and higher heritability than the less severe subtypes of PE. Also a decrease in the gene expression of syncytin 1 correlates with the severity of preeclampsia (21) which confirms the important role of syncytin 1 in etiology of preeclampsia and the possibility of its use as a potential marker for the diagnosis of PE. These findings highlight the importance of categorizing the cases of women with preeclampsia into several subtypes during basic research.

Changes in the level of ERVW-1 expression and the amount of syncytin 1 in cells depend on several factors including different regulatory mechanisms of gene expression. Disruption of ERVW-1 regulatory region can be one of many causes of decreased gene expression and we hypothesize that it could play a part in triggering the transition from mild preeclampsia to a severe one. To our knowledge, none of the studies focus on genetic association between ERVW-1 gene and preeclampsia. Identification of genetic variants in the ERVW-1 regulatory regions could be used as a diagnostic marker for prediction of preeclampsia development.

References

3. Roberts JM, Bell MJ. If we know so much about preeclampsia, why haven’t we cured the disease? J Reprod Immunol 2013; 99: 1–9.

Tab. 3. Comparison of allelic frequencies of polymorphisms rs4727276, rs148592540, rs569899772 and rs555416193 among populations (source: 1000 Genome Browser).

<table>
<thead>
<tr>
<th>Populations</th>
<th>Frequency of polymorphisms</th>
</tr>
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<tbody>
<tr>
<td>World average</td>
<td>G = 0.217 T = 0.018 A = 0.0 A = 0.0</td>
</tr>
<tr>
<td>African</td>
<td>G = 0.282 T = 0.004 A = 0.0 A = 0.0</td>
</tr>
<tr>
<td>American</td>
<td>G = 0.184 T = 0.036 A = 0.0 A = 0.0</td>
</tr>
<tr>
<td>East Asian</td>
<td>G = 0.323 T = 0.0 A = 0.0 A = 0.0</td>
</tr>
<tr>
<td>South Asian</td>
<td>G = 0.115 T = 0.08 A = 0.001 A = 0.0</td>
</tr>
<tr>
<td>Gujarati Indians</td>
<td>G = 0.107 T = 0.015 A = 0.005 A = 0.0</td>
</tr>
<tr>
<td>European</td>
<td>G = 0.145 T = 0.054 A = 0.0 A = 0.001</td>
</tr>
<tr>
<td>Finnish in Finland</td>
<td>G = 0.146 T = 0.061 A = 0.0 A = 0.005</td>
</tr>
<tr>
<td>Slovak</td>
<td>G = 0.147 T = 0.118 A = 0.029 A = 0.059</td>
</tr>
</tbody>
</table>

There are more similar. The same tendency was observed in cases of -246C>G polymorphism with the allelic frequency in the Slovak population equaled to 0.147 and the worldwide average equaled to 0.217. Modest discrepancies between the Slovak and European populations could be explained by the small number of analyzed samples or can be specific to the Slovak population.

Variants at the positions -1046G>A (7:g.92108552G>A) and -30G>A (7:g.92107536G>A) were only identified by 1000 Genome project as rs555416193 and rs569899772, respectively. Rs569899772 was identified in South Asian population (Gujarati Indians) with the frequency of 0.005. Rs555416193 was identified in European population (Finnish) also with the frequency of 0.005. Frequencies of rs569899772 and rs555416193 are significantly higher than average frequencies of polymorphisms identified by 1000 Genome project in mentioned populations. It is not possible to determine if these variants are rare mutations or common benign polymorphisms in the Slovak population because of the small number of tested samples.

Variants -1340G>T, -1046G>A and -246C>G do not disrupt the known binding sites for transcriptional factors. -30G>A is localized in the binding site of the c-Myb oncogene. A recent study revealed that point mutations in the 3’LTR allows binding of c-Myb to DNA and enhances syncytin 1 promoter activity and expression (32). However, it is not possible to predict the effect of the variants found in this study or on the gene expression without additional experiments.

ERVW-1 has yet to be associated with pathological processes such as preeclampsia, HELLP and IUGR (33) which affect placental function. To our knowledge, polymorphisms in ERVW-1 have not been analyzed for association with preeclampsia despite known aberrant expression of ERVWE-1 during this disease. Our study confirmed no association of DNA polymorphisms in ERVW-1 with PE likely because the number of available samples was relatively small (all archive preeclamptic materials) but it is important to consider the size of the Slovak population.

ERVW-1 regulatory region can be one of many causes of decreased gene expression and we hypothesize that it could play a part in triggering the transition from mild preeclampsia to a severe one. To our knowledge, none of the studies focus on genetic association between ERVW-1 gene and preeclampsia. Identification of genetic variants in the ERVW-1 regulatory regions could be used as a diagnostic marker for prediction of preeclampsia development.

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