Abstract: Early stages of individual development are defined by active cell division and differentiation processes. Intercellular signaling molecules as well as proliferation control and DNA stabilizing factors act as basic functional groups. Changes in genome stability maintaining genes functioning can cause the mutation number increase during blastomeric cleavage and differentiation. Therefore the goal of current work was to investigate the frequencies of cell cycle and DNA repair control genes polymorphic variants in women with normally progressing pregnancies and in women with first trimester pregnancy loss. The present case-control study included 151 women with spontaneous abortion and 134 women with normally progressing pregnancies. Polymorphisms Pro72Arg in TP53, APEX1 and DNA repair control genes polymorphic variants in women with normally progressing pregnancies and in women with first trimester pregnancy loss. The present case-control study included 151 women with spontaneous abortion and 134 women with normally progressing pregnancies.

Key Words: TP53, APEX1, Pregnancy Loss, Gene Polymorphism, Gene-Gene Interaction

Introduction: Etiological factors of early pregnancy loss are numerous and diverse. Fetal karyotype abnormalities can be registered in case of development termination of normal pregnancy and also in pregnancies after assisted reproductive technologies. (1-3) However the causes for occurrence of mutations still yet to find out. Some data shows that polymorphic variants of Mad1, Mad2, Bub1, Bub3 genes which are responsible for meiotic chromosome segregation may originate from defects in the checkpoint mechanisms.

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Excision DNA-repair protein ERCC2 encoded by XPD gene is involved in excision repair while despiralizing DNA near damaged region and also is involved in chromosome segregation process being a part of the mitotic spindle complex. Also ERCC2 protein could play a role in aging and skin cancer development.(14–16)

Allele polymorphism of repairation system and cell cycle control genes has been studied extensively in connection with etiology and pathogenesis of different cancer diseases; however implication of these genes to pregnancy pathology development is poorly understood.(17–19)

Functional efficiency of cell cycle and DNA repairation control proteins can affect on successful proceeding of early stages of pregnancy development. Thereby the goal of current work was to investigate the frequencies of cell cycle and DNA repairation control genes polymorphic variants: Pro72Arg in TP53, Asp148Glu in APEX1 and Lys751Gln in ERCC2 (XPD) in women with normally progressing pregnancies and in women with first trimester pregnancy loss.

Materials and Methods
Prior to inclusion in the study, all subjects underwent a standard diagnostic work-up. The women were examined using transvaginal ultrasonography for the absence of uterine abnormalities and polycentric ovary syndrome. Women with previously diagnosed arterial hypertension, diabetes, thyroid diseases, autoimmune pathology and infections during pregnancy were excluded from studied population. Women contacting with exogenous risk factors, such as alcohol, electromagnetic radiation, industrial noise, vibration, chemical pollutants were also excluded.

The study was approved by the Southern Federal University Bioethics Committee. The participants willingly signed the informed consent. After approval by institutional review board, 151 women (mean age 29) with spontaneous abortion in 5-11 week of gestation and 134 women (mean age 30) with normally progressing pregnancies and without any history of spontaneous or missed abortion were studied. Genomic DNA was isolated from the EDTA-anticoagulated peripheral blood using the commercial kit “DNA Express” (GenoTech, Russia). <br>Polymorphisms Pro72Arg in TP53 (MIM*191170, rs1042522), Asp148Glu in APEX1 (MIM*107748, rs1130409) and Lys751Gln in ERCC2 (XPD) (MIM*126340, rs1052559) was detected by allele-specific polymerase chain reaction method using SNP-express reaction kits (Lytech, Russia). The PCR products were analyzed by horizontal 3% agarose gel electrophoresis. Gel images were captured using GelDoc XR system (Bio-Rad, USA).

Hardy-Weinberg equilibrium analysis was performed using Hardy-Weinberg equilibrium calculator in www.eoge.org/software/Hardy-Weinberg,(20) Differences in distribution of allele variants between studied groups were assessed by χ²-analysis. Individuals that carried more than one risk allele or genotype may have a higher risk of pregnancy loss. Therefore gene-gene interactions were explored. We analyzed gene-gene interactions among 3 polymorphisms using the multifactor dimensionality reduction (MDR) method (MDR software, version 2.0) and a P value of less than 0.05 was considered statistically significant.

Results
The distributions of all genotypes and alleles of repairation and cell cycle control system genes polymorphic variants in each group of pregnant women were in Hardy-Weinberg equilibrium. The data on frequencies of polymorphic variants (Asp148Glu in APEX1, Lys751Gln in ERCC2 (XPD) and Pro72Arg in TP53) are shown in Table 1. The distributions of genotype and allele frequency between women with early pregnancy loss and the controls (normally progressing pregnancies) were equal for APEX1, ERCC2 (XPD) and TP53 gene polymorphism.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genotype, allele</th>
<th>Control (n = 134)</th>
<th>EPL (n = 151)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td>Pro72Arg rs1042522</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ProPro</td>
<td>11 (8.2%)</td>
<td>10 (6.6%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ProArg</td>
<td>43 (32.1%)</td>
<td>53 (35.1%)</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>ArgArg</td>
<td>80 (59.7%)</td>
<td>88 (58.3%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>72Arg allele</td>
<td>0.757</td>
<td>0.758</td>
<td></td>
</tr>
<tr>
<td>APEX1</td>
<td>Asp148Glu rs1130409</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AspAsp</td>
<td>28 (20.9%)</td>
<td>35 (23.2%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AspGlu</td>
<td>71 (53.0%)</td>
<td>80 (53.0%)</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>GluGlu</td>
<td>35 (26.1%)</td>
<td>36 (23.8%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>148Glu allele</td>
<td>0.526</td>
<td>0.503</td>
<td>0.59</td>
</tr>
<tr>
<td>ERCC2 (XPD)</td>
<td>Lys751Gln rs1052559</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LysLys</td>
<td>42 (31.3%)</td>
<td>36 (23.8%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LysGln</td>
<td>73 (54.5%)</td>
<td>83 (55.0%)</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>GlnGln</td>
<td>19 (14.2%)</td>
<td>32 (21.2%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>781Gln allele</td>
<td>0.414</td>
<td>0.487</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Gene-gene interactions were analyzed among 3 polymorphisms of these 3 genes using the MDR method. After cross-validation and permutation tests of the gene-gene interactions in relation to the EPL group, the best model included a three-marker model (rs1042522, rs1130409, rs1052559) with accuracy of 51% and a maximum cross-validation (CV) consistency of 10 out of 10. (Table 2).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genotype, allele</th>
<th>Testing accuracy</th>
<th>Cross-validation consistency</th>
<th>p</th>
<th>Testing</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rs1042522, rs1130409, rs1052559</td>
<td>0.51</td>
<td>10/10</td>
<td>0.0044</td>
<td>12.5</td>
<td>6.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(2.1 – 21.3)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Thus we found combination of SNPs APEX1 rs1130409, ERCC2 rs1052559, and TP53 rs1042522 to be the best interaction model for predicting early pregnancy loss.

Discussion:
Reparation processes depend on intracellular signaling cascades which are activated by DNA damage. Signal molecules activate reparation system genes as well as cell cycle arrest and cell cycle stage changes genes. At the same time reparation genes transcription is activated while cyclin genes and replication fork stabilization proteins expression is inhibited. Dysregulation in reactions mentioned above results in fixation of DNA damage and genome changes stabilization in cell populations.

The first trimester of pregnancy is characterized by active development.(14–16) With normally progressing pregnancies and in women with first trimester pregnancy loss.

Table 1: Distribution of TP53, APEX1, ERCC2 genotypes among 151 cases with early pregnancy loss (EPL) and 134 controls

Table 2: MDR analysis of gene-gene interaction in relation to EPL
correction mechanism, occurrence of genome instability and induction of apoptosis in maternal and fetal cells. P53 is involved in apoptosis. Furthermore, p53 protein plays an important role in expression regulation of leukemia inhibitory factor (LIF) which is involved in blastocyst implantation processes.(21) P53 protein polymorphic variant isn’t capable to activate effectively the cytokines of interleukin 6 family, in particular LIF, which is essential for implantation. It is shown that p53 deficient mice have decreased LIF expression levels.(32) There is evidence that Arg72 TP53 has higher transcriptional activity toward a particular subset of p53 target genes, including LIF, than Pro72.(23) P53 protein functional activity change can result in inadequate endometrial decidualization that leads to incomplete or poor cytotrophoblast invasion and suppresses physiological gestation changes in uterine and placental arteries with blood flow decrease.

Several studies have shown the association between Pro72Arg polymorphism in TP53 gene with in vitro fertilization failure, infertility and endometriosis.(24–28) Literature data on the association of TP53 gene polymorphism with pregnancy loss are poor and incomplete.(29–31) Fraga and colleagues (32) stated that TP53 Arg/Arg (rs1042522) genotype frequency in group of women with recurrent pregnancy loss is equal to control group. Therefore the combination of current genotype with MDM TT (rs2279744) genotype results in pregnancy loss risk increase (OR was 2.58). (32)

Polymorphic site in APEX1 gene that causes amino acid substitution in 148 position of protein molecule is located in fifth exon. It encodes C-terminal domain with β-structure. Such amino acid substitution doesn’t affect enzyme endonuclease activity. APEXI is included into big group of proteins capable for regulation of DNA repair processes (33), DNA recombination, DNA demethylation (34), cellular redox homeostasis, DNA-dependent transcription regulation, mRNA stability regulation (35). It is shown that 148Glu allele is associated with mitotic block after ionizing radiation. (36) Amino acid substitution in APEXI molecule caused by allele gene variant doesn’t affect on protein’s enzyme activity but it can cause different effects on the cooperation between other components of gene regulatory network. This can result in common changes in reparation regulation efficiency, transcriptional factors activity and mRNA stability. (37)

Polymorphic site of ERCC2 (XPD) gene located in 23th exon and not in the helicase/ATPase domain. But current amino acid residue (Lys751Gln) is located in highly evolutionary conserved sequence. (38) There is the association between the presence of XPD gene polymorphic variant and decrease of effectiveness and activity of DNA repair among group of older persons. (39,40) There is the data that mutations in C-terminal domain and in region responsible for XPD helicase activity can be associated with placental insufficiency and with higher risk of preeclampsia development. The reason may be found in changes of binding efficiency between XPD, CDK-activating kinase and p44 subunit of IHH transcriptional factor. Mutations in XPD gene cause the disruption in protein-protein interactions of transcription factor with its targets. (18,37)

Down regulation of the ability to interact with other molecular components of the system can lead to decrease of repair processes efficiency. Mosleh R, with colleagues (17) showed the association between repair system genes (XRCC1, XPD6, XPD23) allele variants and high DNA fragmentation index.

The MDR method was introduced to identify gene-gene interactions that are associated with early pregnancy loss. The MDR method reduces high-dimensional genetic data into a single dimension. This permits gene-gene interactions to be detected in relatively small sample sizes. Several loci usually contribute to the phenotypes expressed in complex diseases, including pregnancy loss. Therefore, it is important to identify gene–gene interactions, because they may more accurately predict the risk of pathology than single genes. In the present study, an MDR analysis was used to predict potential gene–gene interactions that may partly determine the early pregnancy loss. We found that rs1042522, rs1130409, and rs1052559 were associated with EPL.

The presence of more than one polymorphism in DNA damage repair genes can cause the remarkable decrease in repair system efficiency. Thus it may influence on pregnancy pathology development by disrupting the processes of cell cycle control and apoptosis, inducing genome instability in maternal and fetal tissues. All changes mentioned above may result in implantation defects and shallow trophoblast invasion of the decidua leading to pregnancy loss.

**Conclusion**

The combination of SNPs APEX1 rs1130409, ERCC2 rs1052559, and TP53 rs1042522 is the best interaction model for predicting early pregnancy loss. According to model of gene–gene interaction gene polymorphism combination of polymorphic variants of repair system and cell cycle control genes increases the relative risk of pregnancy loss.

**Acknowledgement**

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**Conflict of Interest:** None.

**References**


