Research Article

Associations of candidate genes with clinical-laboratory parameters in pregnant women with pre-eclampsia

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ABSTRACT

Objectives: The associations of polymorphic variants of folate metabolism genes with clinical and laboratory parameters in pregnant women with pre-eclampsia (PE) were studied, depending on hereditary burden. **Materials and Methods:** The study group included 274 pregnant women, diagnosed with PE. Polymorphisms of the folate cycle genes (+677C>T *MTHFR* [rs1801133], +1298A>C *MTHFR* [rs1801131], −1053C>T *TYMS* [rs699517], IVS6-68C>T *TYMS* [rs1059394], and −1122A>G *TYMS* [rs2790]) were investigated, using the method of polymerase chain reaction (PCR) of DNA synthesis in real-time (real-time-PCR). **Results:** In the group of pregnant women with PE, without a burdened familial history, the genetic variants −1053CT*TYMS* and −1053TT*TYMS* are associated with an increased level of proteinuria. In the group of pregnant women with PE, with a burdened familial history, the polymorphic variants −1053CT*TYMS* and −1053TT*TYMS*, IVS6-68CT*TYMS* and IVS6-68TT*TYMS* are associated with a higher level of glucose in blood, and the genotypes +1298CC *MTHFR* and +1298AC*MTHFR* are associated with the increased activated partial thromboplastin time (APTT). **Conclusions:** Thus, as a result of this study, significant associations of folate metabolism genes with increased levels of proteinuria, glucose, and APTT in groups of pregnant women with PE were established, depending on the burdened familial history.

KEY WORDS: Folate cycle genes, Genetic polymorphism, Pregnancy, Pre-eclampsia

INTRODUCTION

Pre-eclampsia (PE) - is a complication of pregnancy, arising in the period of late pregnancy, and characterizing by the appearance of edemata, proteinuria, arterial hypertension, as well as by deep disorders of the vascular system, hemostasis, immunity, hemodynamics and microcirculation, fetoplacental insufficiency, decreased kidney function, liver, and lungs functions.[1,2] The frequency of PE is 8–20% among all pregnant.[3] During the past decade, PE is one of the main factors of perinatal morbidity in the world and stably ranks №. 3–4 in the structure of the causes of maternal morbidity and mortality.[2,4]

Genetic component of PE development can account for up to 50% of all risk.[5]

An important role in the etiology and pathogenesis of PE belongs to the candidate genes of folate metabolism.[6,7] Mutations in the genes of folate metabolism, causing a decrease in the enzymes activity of methyltetrahydrofolate reductase and methionine synthase reductase, lead to excessive accumulation of homocysteine in the blood and, as a consequence, disruption of methylation processes in cells.[6] Deficiency of folic acid causes the formation of hypertension in pregnant women, the development of total angiopathy, microthrombosis, and the increase of insulin resistance. It should be noted that the role of candidate genes of folate metabolism in the formation of PE is actively investigated, but these studies often give conflicting results in different populations of the world.[1,6,8-11]

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MATERIALS AND METHODS

Object of Study

The study group included 274 pregnant women, diagnosed with PE. 105 of them had hereditary burden for PE and 169 pregnant women had not genetic disposition to PE. The average age of women with PE was 27.19 ± 6.4. All clinical studies were carried out according to the protocols of ethical committee of the

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Russian Federation, with the informed consent of the patients. The present study includes persons of Russian nationality, born in the Central Black Earth region of Russia, who do not have genetic relationships. Clinical and laboratory examination of women from the main and control groups was performed at the delivery time, in the Perinatal Center of the Belgorod Regional Clinical Hospital of St. Joasaph.

Edemata, arterial hypertension, and proteinuria were the base for the diagnosis of PE.[12] Exclusion criteria for the formation of the sample were the following: The presence of uterine pathology (uterine fibroids, internal genital abnormalities), pathology of pregnancy (anomalies of placentation and location of placenta, rhesus-conflict), fetal pathology (congenital malformations), and multifetal pregnancy.

All pregnant women were examined in laboratory conditions including general blood test, coagulogram, biochemical blood test, general urine test (protein, specific gravity, epithelium, leukocytes, and cylinders), Nechiporenko test, and Zimnitsky test (leukocytes and erythrocytes).

Molecular and Genetic Methods

Typing of polymorphic variants of folate cycle genes of methylene tetrahydrofolate reductase (+677C>T *MTHFR* [rs1801133], +1298A>*CMTHFR* [rs1801131]), thymidylate synthetase (−1053C>T *TYMS* [rs699517], IVS6-68 *C>TTYMS* [rs1059394], and −1122A>G *TYMS* [rs2790]) was carried out for all pregnant women with PE, and pregnant control group, on the basis of the research laboratory "Human molecular genetics" of Belgorod State National Research University. The material for the study was venous blood, obtained in a volume of 8–9 ml from the ulnar vein of pregnant women. All polymorphic variants of folate cycle enzymes were analyzed using the method of polymerase chain reaction (PCR) of DNA synthesis in real-time (real-time-PCR).

Statistical Methods

Formation of the database and statistical calculations was carried out using the program "STATISTICA 6.0." Gene and phenotypic frequencies were calculated using the standard methods. The conformity of the observed distribution of genotypes to the expected one, according to the Hardy-Weinberg equilibrium, was performed using the χ^2 criterion. To minimize the errors of the first kind (false positive results), the Bonferroni correction was used when carrying out multiple comparisons.

The study of the connections of polymorphic variants with pathogenetically significant continuous characters of PE (blood pressure level, fibrinogen level, prothrombin index, etc.) was carried out using non-parametric statistics.[13]

RESULTS

The studied clinical and laboratory parameters (proteinuria, the content of fibrinogen, total protein, urea and creatinine in blood, activated partial thromboplastin time [APTT], and thrombin time) in pregnant women with PE are presented in Table 1.

As a result of the study, it was found that, women with PE, without hereditary burden, with genotypes −1053CT *TYMS* and −1053TT *TYMS,* have higher level of proteinuria (M_e = 0.066 g/l, Q10–Q90 = 0.33–0.163 g/l), than women with the genotype −1053CC *TYMS* for the given locus (M_e = 0.085 g/l, Q10–Q90 = 0.033–0.124 g/l, P = 0.04, respectively).

In pregnant women with PE, having burdened familial history, with genotypes −1053TT *TYMS* and -1053CT *TYMS*, the glucose level in blood (M_e = 4.30 mmol/l, Q10–Q90 = 3.86–4.90 mmol/l) is statistically significantly higher than that of women with the genotype −1053CC *TYMS* (M_e = 3.8 mmol/l, Q10–Q90 = 3.55–4.25 mmol/l, P = 0.004). In women with PE, with hereditary burden, having the genotypes +1298AC *MTHFR* and +1298CC*MTHFR*, the level of APTT (M_e = 35.0 s, Q25–Q75 = 30.0–36.0 s) is higher than that of individuals with genotype +1298AA *MTHFR* (M_e = 30.0 s, Q25–Q75 = 27.50–35.0 s, P = 0.03).

In pregnant women with genotypes IVS6-68CT *TYMS* and IVS6-68TT *TYMS*, the glucose level in blood (M_e = 4.40 g/l, Q25–Q75 = 3.88–4.90 g/l) is higher than in women with genotype IVS6-68CC *TYMS* (M_e = 3.80 g/l, Q25–Q75 = 3.50–4.30 g/l, P = 0.006).

DISCUSSION

As the results of this study indicate, polymorphisms of folate metabolism genes (+1298A>*CMTHFR*, −1053C>T *TYMS*, and IVS6–68 *C>TTYMS*) have an important pathogenetic significance in the formation of clinical and laboratory parameters in pregnant women with PE. Genetic variants −1053CT *TYMS* and −1053TT *TYMS* are associated with the increased level of proteinuria in the group of pregnant women with PE, without a burdened familial history. Polymorphic variants −1053CT *TYMS* and −1053TT *TYMS*, IVS6-68CT *TYMS* and IVS6-68TT *TYMS* are connected with higher level of glucose in blood, and the genotypes +1298CC *MTHFR* and +1298AC *MTHFR* are associated with an increased APTT, in the group of pregnant women with PE, having a burdened familial history.

The following medical and biological mechanisms can be the base of revealed by us associations of genetic polymorphisms of folate cycle genes with clinical-laboratory parameters of women with PE. The decrease

Table 1: The distribution of clinical and laboratory parameters in the study group of women with pre-eclampsia

Parameters	Lower quartile, (Q25)	Median, (M_e)	Upper quartile, (Q75)	Shapiro-Wilk statistics	Level of significance (*P*)
Proteinuria, g/l	0.03	0.06	0.12	0.48	0.000000
The content of fibrinogen in blood, g/l	3.70	4.20	5.00	0.98	0.00004
APTT, s	29.00	35.00	37.00	0.97	0.000001
TT, s	14.00	15.00	15.00	0.88	0.000000
The total protein content in blood, g/l	61.00	65.15	69.00	0.99	0.01
Urea content in blood, mmol/l	3.57	4.40	7.10	0.89	0.000000
Creatinine level in blood, μmol/l	64.00	72.00	84.00	0.98	0.0001

APTT: Activated partial thromboplastin time, TT: Thrombin time

in the activity of folate cycle enzymes (in individuals with genetic variants +1298CC *MTHFR,* +1298AC *MTHFR*) leads to the disruption in the delivery and metabolism of folic acid, the accumulation of homocysteine in the blood plasma and the development of hyperhomocysteinemia.[14] Herewith, homocysteine begins to show its toxic properties, which primarily concern the vessel wall. According to the literature, a high level of homocysteinemia is a risk factor for the development of both atherosclerotic and thrombogenic vascular diseases. An increase in the concentration of homocysteine increases oxidative stress, stimulates the production of smooth muscle cells, and alters the elastic properties of the vessel wall.[15] The malfunction of microcirculation increases permeability of blood vessels that leads to deterioration of rheological properties of blood, slowing of blood flow in the microvasculature, increasing of peripheral resistance and, consequently, to hypertension; that, in turn, increases the risk of PE and the severity of its manifestations (proteinuria and hypoproteinemia) (Baranova and Bolshakova, 2004).[16]

In various studies on the search for associations of folate cycle genes with clinical-laboratory parameters in pregnant women, conflicting results were obtained. Thus, in the Portuguese population, the genotype +677TT *MTHFR* was associated with a decrease in SBP and DBP, in women with gestational hypertension.[17] Moreover, in the study of Yakut, Buryat, and Russian populations, the associations of polymorphism +677C>T *MTHFR* with blood pressure, proteinuria, and hypoproteinemia were not revealed in pregnant women with PE.[6] In a similar study, conducted on the Russian population of Central Russia, there were also no associations of polymorphic marker of gene *MTHFR* with proteinuria and arterial hypertension, in case of PE.[18]

The inconsistency of the results, obtained in various studies, may be related to the differences in the ethnic and, respectively, genetic background of the studied populations.[19,20] This feature of the Russian gene pool determines the need to take into account the population sample, for which the results are obtained.[21-23]

SUMMARY

Thus, as a result of this study, significant associations of folate metabolism genes with increased levels of proteinuria, glucose, and APTT in pregnant women with PE were established, depending on the burdened familial history.

CONCLUSION

The results of this study broaden the concept of molecular and genetic determinants of PE development. The obtained data can be used in the work of women's consultation clinics and obstetric-gynecologic hospitals, with the purpose to identify the groups with an increased risk of PE development, among women in preconception period and at the early stages of pregnancy.

REFERENCES

1. Williams PJ, Pipkin FB. The genetics of preeclampsia and other hypertensive disorders of pregnancy. Best Pract Res Clin Obstet Gynaecol 2011;25:405-17.
2. Sidorova IS. Preeclampsia. Meditsinskoe Information Noeagenstvo. Moscow: Publishing House; 2016.
3. Suhih GT, Murashko LE. Preeclampsia. Moscow: Publishing House, GEOTAR-media; 2010.
4. Ajlamazyan EK, Mozgovaya EV. Gestational Toxicosis: Theory and Practice. Moscow: Publishing House, MEDpress-Inform; 2008.
5. Baranov VS. Genetic Passport is the Basis of Individual and Predictive Medicine. Saint Petersburg: Publishing House N-L; 2008.
6. Vorozhishcheva AY. Genetic factors for development of preeclampsia in populations of different ethnic origins: Extended abstract of Cand. Sci. (Medicine) Dissertation. Tomsk; 2014. p. 24.
7. Zhou L, Cheng L, He Y, Gu Y, Wang Y, Wang C. Association of gene polymorphisms of FV, FII, MTHFR, SERPINE1, CTLA4, IL10, and TNFalpha with preeclampsia in Chinese women. Inflamm Res 2016;65:717-24.
8. Obolenska MI, Rodrihes RR, Martseniuk OP. Folate-relatedprocesses in humanplacenta: Gene expression, aminothiols, proliferation and apoptosis. Ukr Biokhim Zh 2011;83:5-17.
9. Pavlova KK, Trifonova EA, Gotovceva LV, Maksimova NR, Nogovicyna AN, Stepanov VA. The role of polymorphisms of genes eNOS, ACE and MTHFR in the development of gestational toxicosisin the Yakut population. Yakut Med J 2010;3:28-31.
10. Valenzuela FJ, Pérez-Sepúlveda A, Torres MJ, Correa P, Repetto GM, Illanes SE, *et al.* Pathogenesis of preeclampsia:

The genetic component. J Pregnancy 2012;2012:632732.
11. Reilly R, McNulty H, Pentieva K, Strain JJ, Ward M. MTHFR 677TT genotypeanddisease risk: Is there a modulating role for B-vitamins? Proc Nutr Soc 2014;73:47-56.
12. Turner JA. Diagnosis and management of preeclampsia: An update. Int J Womens Health 2010;2:327-37.
13. Rebrova OY. Statistical analysis of medical data. Application of Program Package STATISTISA. Moscow: Publishing House Media Sfera; 2006.
14. Suhovolskaya MA, Subbotina TN, Olhovskiy IA, Bazarin KP. Mutations in the genes of methylenetetrahydrofolatereductase (MTHFR) and methionine synthase (MTR), and risk factors for hyperhomocysteinemiain athletes. Clin Lab Diagn 2012;9:65.
15. Zajnulina MS, Glotov AS, Kornyushina ML. Thrombophilia in obstetric practice. Saint Petersburg: Publishing House N-L; 2009.
16. Baranova EI, Bolshakova OO. Clinical relevance of homocysteinemia (literature review). Arterial Hypertens 2004;1:12-5.
17. Matos A, da Silva AP, Maia H, Ferreira J, Clode N, Areias MJ, *et al.* PP004. The polymorphism C677T of methylenetetrahydrofolate reductase (MTHFR), may increase risk for future higher blood pressure in women with previous hypertension in pregnancy. Pregnancy Hypertens 2013;3:69.
18. Halford-Knyazeva IP. Genetic Markers for the Prediction of preeclampsia: Extended Abstract of Cand Sci (Medicine) Dissertation. Moscow; 2013. p. 16.
19. Churnosov MI, Pesik VY, Rudyh NA. Materials on the study of gene pool structure of the Russian population in Central Russia. Med Gen 2005;6:289a.
20. Sorokina IN, Churnosov MI, Balanovskaya EV. Genetic resources of the population of Belgorod region II."Familial portraits" in groups of regions with different levels of division, and the role of migrations in their formation. Genetics 2007;8:1120-8.
21. Bailey LB, Gregory JF 3rd. Polymorphisms of methylenetetrahydrofolate reductase and other enzymes: Metabolic significance, risks and impact on folate requirement. J Nutr 1999;129:919-22.
22. Friedman G, Goldschmidt N, Friedlander Y, Ben-Yehuda A, Selhub J, Babaey S, *et al.* A common mutation A1298C in human methylenetetrahydrofolate reductase gene: Association with plasma total homocysteine and folate concentrations. J Nutr 1999;129:1656-61.
23. Strizhakov AN, Makacariya AD, Timohina EV, Bajmuradova SM, Kozlova UA. Clinical significance of acquired and hereditary forms of thrombophilia in the pathogenesis of intrauterine growth restriction syndrome. Obstet Perinatol 2009;8:16-21.