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EFFECT OF PRO197LEU POLYMORPHISM OF THE GENE GPX 1 ON FUNCTIONAL STATE OF ENDOTHELIUM INDICES OF PATIENTS WITH METABOLIC SYNDROME

Key words: *Pro197Leu polymorphism of GPX 1 gene, the functional state of the endothelium, vascular endothelial growth factor, endotheliocytes, metabolic syndrome.*

Abstract. *Pro197Leu polymorphism of the gene GPX 1 has been studied in 102 patients with MS and 97 healthy individuals. Disorders of genotype frequencies distribution comparing with the control group at the expense of the reduction of Pro/Pro genotype frequency have been found in the main group ($p < 0,05$). Assessment of the relative risk was carried by odds ratio (OR) magnitude. The OR calculation showed the growth of risk of GPX 1 activity disorder in patients with Pro/Leu and Leu/Leu variants of polymorphism comparing with homozygous for the Pro-allele at 4,7 and 6,9 times respectively ($p < 0,05$). Individuals with Leu/Leu genotype had significantly higher level of VEGF and endothelial desquamation intensity as compared with the persons with Pro/Pro genotype ($p < 0,05$). Consequently, Pro-allele has protective properties, it means that presence of these allele in genotype reduses risk of glutathione peroxidase activity decreasing. In Leu-allele carriers, activity of GPX 1 decreases, free radicals accumulate, that cause lipid peroxidation and activation of transcription factors, followed by increasing expression of cytokines, which are also directly affect the endothelium.*

Introduction

Cytoplasmic glutathione peroxidase (GPX 1) is one of the selenoenzymes important for the organism functioning, present in all tissues of the human body, which takes part in detoxication of hydrogen peroxide and products of lipid peroxidation, as catalyzes the interaction of redused glutathione with these substances [4, 5, 9, 10]. It is known that numerous pathologic processes in the organism develop in concequence of disorders in the mechanisms of antioxidant protection. Specially, in patients with insulin resistance accompanied by hyperglycemia and increased production of cytokines there arises oxidative stress. The accumulation of free radicals activates factors of transcription such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kb), which initiate the process of proinflammatory cytokines release [8]. The growth of free radicals results in lipid peroxidation of cellular membranes, causes atherosclerosis and endothelial dysfunction [11].

We studied single nucleotide polymorphism of the gene GPX 1 for going into the question of the dependence of these processes upon the disorders of redox homeostasis. The human gene GPX 1 is localized in 3p21 chromosome and consists of two exons. Several single nucleotide polymorphism variants of this gene are known, but the Pro197Leu polymorphism has been under our study, at which in the position 593 the amino acid cytosine (C) is replaced by thymine (T) (C593T), resulting in substitution of the amino acid proline for leucine in the 197 codon. This mutation refers to missense - functional polymorphisms. Pro-allele is "wild", while Leu- is a "mutant" allele. The presence of Leu-allele causes depression of GPX 1 sensibility to stimulating factors [6].

However, the dependence of functional state of endothelium parameters on Pro197Leu polymorphism of the GPX 1 gene against a background of metabolic syndrome (MS) remains unstudied.

The aim of the study. To investigate the dependence of functional state of endothelium parameters on Pro197Leu polymorphism of the GPX 1 gene against a background of metabolic syndrome.

Material and methods

Pro197Leu polymorphism of the gene GPX 1 have been studied in 102 patients with MS and 97 healthy individuals by isolation of genomic DNA from peripheral blood leukocytes, and then amplification of the polymorphic area in the state of polymerase chain reaction (PCR) was performed on the programmed PCR thermal cyclers "Amply-4L" ("Biocom", Росія) at individual temperature response. Reagents "ДНК-сорб-В" variant 100 (ФГУН ЦНИИЭ, Росія) were used for DNA isolation from

lymphocytes according to instructions. PCR samples were prepared by means of the set "АмплиСенс-200-1" (ФГУН ЦНИИЭ, Росія). Products of PCR were separated using electrophoresis in 3% agarose gel in the presence of tetraborate buffer, concentrated with ethidium bromide. Fragments were visualized by transilyuminator in the presence of a marker of molecular mass 100-1000 bp ("FermentasR", USA).

Pearson's criterion (χ 2) was used to estimate the correspondence of the genotype frequencies under study to theoretically expected distribution at Hardy-Weinberg's equation. Odds ratio (OR) with determination of 95% confidence interval (CI) was calculated with the aim to establish the association of polymorphic variant of the gene with a pathological phenotype.

The diagnosis of MS was established according to criteria of the International Diabetes Federation (IDF) [2].

Endothelial function indices were investigated in 102 patients, 20 healthy individuals made control group. The vascular endothelial growth factor (VEGF) level was established by immunoassay method using a set of firm "Vector-Best". The number of circulating desquamated endothelial cells was calculated by Hladovec J. method in Petrishchev N.N. et al. modification [3].

Statistical analysis of the obtained data was carried out using the Student's t-test and Pearson's rank correlation coefficient by means of the software package Statistica 6.0 for Windows. The difference was considered reliable at $p < 0,05$.

Results and discussion

When assessing the distribution of genotype frequencies of the gene GPX 1, it has been found that in the group of patients with MS threre takes place a significant reduction of the frequency of Pro/Pro genotype as compared with the control group (χ^2= 7,0, $p < 0,05$), while a reliable difference between the frequencies of Pro/Leu and Leu/Leu genotypes in the main and control groups ($\chi^2 = 1,9$, $p > 0,05$ and $\chi^2 = 2,6$, $p > 0,05$) has not been found out.

It has been revealed that Pro/Leu and Leu/Leu variants of polymorphism are associated with increased risk of violation of redox system in patients with MS as compared with a group of healthy subjects (table 1). Assessment of the relative risk was carried by odds ratio (OR) magnitude. The OR calculation showed that in patients with Pro/Leu polymorphism the risk of disturbance of GPX 1 activity increases 5,2 times ($p < 0,05$, OR = 1,65, CI = 0,95% 0,94 - 2,90), and in patients with Leu/Leu genotype the risk of such patology is 6,0 times higher than in persons with Pro/Pro genotype ($P < 0,05$, OR = 1,92, CI 0,95% = 0,93 - 3,97).

So, the risk of GPX 1 activity reduction in a dose-dependent way is associated with the presence of "mutant" Leu-allele, while homozygous for the "wild" Pro-allele had significantly lower risk of this disturbance development. Pro allele has protective properties concerning the development of redox system violation.

Table 1

The distribution of genotype frequencies depending on Pro197Leu polymorphism of GPX 1 gene in patients with metabolic syndrome and the control group

Genotypes	*Cases*	*Controls*	χ^2	p	OR	0,95% CI
	102	97				
Genotype frequency *Pro/ Pro*	0,176	0,402	12,91	0,002	0,32	0,17-0,61
Genotype frequency *Pro/Leu*	0,578	0,454			1,65	0,94-2,90
Genotype frequency *Leu /Leu*	0,245	0,144			1,92	0,93-3,97

Note: χ^2 - Pearson criterion, OR - odds ratio, CI - confidence interval.

When studying the dependence of functional state of the endothelium on Pro197Leu polymorphism of GPX 1 gene, a significantly higher level of VEGF in homozygous group for mutant Leu-allele and heterozygous group for this allele in comparison with homozygous ones for wild allele has been received, 45,3% and 62,8% higher respectively ($p<0,05$) (table 2). A credible growth of VEGF in patients with Pro/Pro, Pro/Leu and Leu/Leu genotypes in relation to the group of healthy individuals was found 1,9, 2,8 and 3,2 times higher ($p<0,05$).

VEGF is a cytokine, expression of which increases during hypoxia, hyperglycemia, it is activated in response to proinflammatory cytokines hyperp-

Table 2

Peculitaries of functional state of endothelium indicators in patients with metabolic syndrome according to Pro197Leu polymorphism of the GPX 1 gene

Index	Genotypes *GPX 1* , n=102			Control group, n=20
	Pro/Pro n=18	*Pro/Leu* n=59	*Leu/Leu* n=25	
VEGF, pg / ml	146,3±16,83 */**/***	212,6±24,38 *	238,2±24,73 *	76,6±12,70
Endotheliocytes (10^4/l)	10,22±1,238 */***	13,8±2,214 *	18,25±2,964 *	2,99±0,423

Notes: 1. n - number of observations; 2. * - the probability of changes in relation to control; 3. **- the probability of changes in relation to the group with *Pro/Leu*-genotype; 4. ***- chance changes in relation to group with *Leu/Leu* genotype

roduction [7].

In our investigation VEGF expression increases, probably due to transcription factors activation as a cause of free radicals accumulation against a background of reduced GPX 1 activity. Free radicals start the process of proinflammatory cytokines release by activation of transcription factors such as NF-kb [8].

The number of circulating desquamated endothelial cells was established to be 7,9% higher in the group with Leu/Leu genotype as compared with the group whith Pro/Pro genotype ($p<0,05$) and respectively 3,4, 4,6 and 6,1 times higher in the groups with Pro/Pro, Pro/Leu and Leu/Leu genotypes in comparison with the control group ($p < 0,05$). Small desquamation of endothelial cells also took place in the control group, which reflected the physiological process of intimal clearance from dead cells [1].

The reason for the increased endothelium desquamation intensity is probably due to lipid peroxidation activation and increased cytokines expression, caused by accumulation of free radicals, which is more pronounced in homozygous for the "mutant" Leu-allele [4, 6, 8].

Thus, our data coincide with the results of other researchers. Bastaki M. et al. have discovered that GPX 1 activity 6 times slows down in homozygous patients for the Leu-allele [4]. Association of endothelial dysfunction with Pro197Leu polymorphism of GPX 1 gene is reflected in the Zelkova T.V. et al. study. They found out that the homozygous for mutant Leu-allele more often suffered from coronary artery disease and myocardial infarction at the age of until 50 years old [12].

Conclusion

1. In patients with metabolic syndrome the risk of reduction of glutathione peroxidase 1 activity is associated in a dose-dependent manner with the presence of "mutant" Leu-allele, while homozygous for the "wild" Pro-allele had a significantly lower risk of this disorder.

2. The presence of Leu-allele in genotype of patients with metabolic syndrome is associated with functional state of the endothelium violation which is probably due to the decreased GPX 1 activity.

Prospects for further research

The survey results indicate the necessity of effective measures for endothelial dysfunction correction development in patients with metabolic syndrome.

Literature. 1.Андреева Н. В. Особенности патогенеза микроангиопатий у больных сахарным диабетом 2 типа разного возраста / Н. В. Андреева // Русский медицинский журнал. - 2006. - Т. 14. № 6. - С. 470 - 471. 2.Метод установления наличия метаболического синдрома у пациентов с артериальной гипертонией и ожирением / Т.Н. Эриванцева, С.П. Олимпиева, И.Е. Чазова [и др.] // Терапевт. арх. - 2006.- № 4.- С. 9 - 15. 3.Петрищев Н.Н. Диагностическая ценность определения десквамированных эндотелиальных клеток крови / Н.Н. Петрищев, О.А. Беркович, Т.Д. Власов [и др.] // Клиническая и лабораторная диагностика. - 2001. - № 1. - С. 50 - 52. 4.Brosnan M.J. One step beyond glutathione peroxidase and endothelial dysfunction/M. Julia Brosnan // Hypertension. - 2008. - №51. - P. 825 - 826. 5.Crawford A. Glutathione peroxidase, superoxide dismutase and catalase genotypes and activities and the progression of chronic kidney disease /A. Crawford, R. Fassett, G. Robert [et al.]. - Nephrology, Dialysis, Transplantation. - 2011. - Vol. 26, № 9. - P. 2806 - 2813. 6. De Oliveira Hiragi C. Superoxide dismutase, catalase, glutathione peroxidase and gluthatione S-transferases M1 and T1 gene polymorphisms in three Brazilian population groups/ C. de Oliveira Hiragi, A. L. Miranda-Vilela, D. M S. Rocha // Genet Mol Biol. - 2011. - Vol. 34, № 1. - P. 11-18. 7. Evidence for a relationship between VEGF and BMI independent of insulin sensitivity by glucose clamp procedure in a homogenous group healthy young men / M. Loebig, J. Klement, A. Schmoller [et al.] // PLoS One. - 2010. - № 5 (9). - Режим доступу до журн.: http://www.ncbi.nlm.nih.gov/pubmed/20830305. 8.Fabre E. E. Gene polymorphisms of oxidative stress enzymes: prediction of elderly renutrition / E. E. Fabre, Agathe Raynaud-Simon, Jean-Louis Golmard [et al.] // Am. J. Clin. Nutr. - 2008. - Vol. 87, № 5. - P. 1504 - 1512. 9.Hu Y. Allelic Loss of the Gene for the GPX1 Selenium-Containing Protein Is a Common Event in Cancer / Y. Hu, R.V. Benya, R.E. Carroll // J. Nutr. - 2005. - Vol. 135, № 12. - P. 3021 - 3024. 10.Miranda-Vilela Ana L. Gene polymorphisms against DNA damage induced by hydrogen peroxide in leukocytes of healthy humans through comet assay: a quasi-experimental study / Ana L. Miranda-Vilela, P. CZ. Alves, A. K. Akimoto //Environmental Health. - 2010.- Vol. 9, № 21. - Режим доступу до журн.: http://www.ehjournal.net/content/9/1/21. 11.Nemoto M. Genetic association of glutathione peroxidase 1 gene with coronary artery calcification in type 2 diabetes: a case control study with multi-slice computed tomography/M. Nemoto, R.

Nishimura1, T. Sasaki1 [et al.] // Cardiovascular Diabetology. - 2007. - Vol. 6, № 23. - Режим доступу до журн.: http://www.cardiab.com/content/6/1/23). 12.Zeikova T.V. The glutathione peroxidase 1 (GPX1) single nucleotide polymorphism Pro197Leu: association with the span and coronary artery disease / Mol. Biol. (Msk.). - 2012. - Vol. 46, № 3.- P.481 - 486.

ВЛИЯНИЕ PRO197LEU ПОЛИМОРФИЗМА ГЕНА GPX 1 НА ПОКАЗАТЕЛИ ФУНКЦИОНАЛЬНОГО СОСТОЯНИЯ ЭНДОТЕЛИЯ У БОЛЬНЫХ С МЕТАБОЛИЧЕСКИМ СИНДРОМОМ

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Резюме. Нами исследовано Pro197Leu полиморфизм гена GPX 1 у 102 пациентов с метаболическим синдромом и 97 практически здоровых лиц. В основной группе выявлены нарушения распределения частот генотипов по сравнению с группой контроля за счет снижения частоты Pro/Pro генотипа ($p<0,05$). При анализе полученных данных мы обнаружили увеличение риска нарушения активности GPX 1 у лиц с Pro/Leu и Leu/Leu вариантами полиморфизма по сравнению с гомозиготами по "дикому" аллелю в 4,7 и 6,9 раза соответственно ($p<0,05$). У пациентов с Leu/Leu генотипом достоверно возрастала экспрессия сосудистого эндотелиального фактора роста и интенсивность десквамации эндотелиоцитов по сравнению с гомозиготами по "дикиму" Pro-аллелю.

Итак, Pro-аллель обладает протекторными свойствами, препятствуя снижению активности глутатионпероксидазы. У носителей Leu-аллеля, на фоне снижения активности GPX 1, происходит накопление свободных радикалов, являющихся причиной перекисного окисления липидов и активации факторов транскрипции с последующим ростом экспрессии цитокинов, которые также непосредственно поражают эндотелий.

Ключевые слова: Pro197Leu полиморфизм гена GPX 1, функциональное состояние эндотелия, сосудистый эндотелиальный фактор роста, эндотелиоциты, метаболический синдром.

ВПЛИВ PRO197LEU ПОЛІМОРФІЗМУ ГЕНА GPX 1 НА ПОКАЗНИКИ ФУНКЦІОНАЛЬНОГО СТАНУ ЕНДОТЕЛІЮ У ХВОРИХ ІЗ МЕТАБОЛІЧНИМ СИНДРОМОМ

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Резюме. Нами досліджено Pro197Leu поліморфізм гену GPX 1 у 102 хворих із метаболічним синдромом та 97 практично здорових осіб. В основній групі виявлено порушення розподілу частот генотипів порівняно із групою контролю за рахунок зниження частоти Pro/Pro генотипу ($p<0,05$). При аналізі отриманих даних ми виявили зростання ризику порушення активності GPX 1 у осіб із Pro/Leu та Leu/Leu варіантами поліморфізму порівняно із гомозиготами за "диким" алелем у 4,7 та 6,9 раза ($p<0,05$). У осіб із Leu/Leu генотипом вірогідно зростала експресія судинного ендотеліального фактору росту та інтенсивність десквамації ендотеліоцитів порівняно із гомозиготами за "диким" Pro-алелем.

Отже, Pro-алель володіє протекторними властивостями щодо зниження активності глутатіонпероксидази. У носіїв Leu-алелю, на тлі зниження активності GPX 1, відбувається накопичення вільних радикалів, що є причиною пероксидного окиснення ліпідів та активації факторів транскрипції із наступним зростанням експресії цитокінів, які також безпосередньо вражають ендотелій.

Ключові слова: Pro197Leu поліморфізм гена GPX 1, функціональний стан ендотелію, судинний ендотеліальний фактор росту, ендотеліоцити, метаболічний синдром.

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