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# Genetic Factors of Decreased Kidney Function in Patients with Chronic Glomerulonephritis

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**ABSTRACT**

The article presents the results of studying the interaction of polymorphic variants of vascular homeostasis genes (I/D *ACE*, 4a/4b *eNOS*, S311C *PON2*, (-6) A/G *AGT*, (-1166) A/C *AGTR1*, G/A *GNB3* (rs.2301339), G460W *ADD1*, (+46) G/A *ADRB2*, K198N *EDN1*, (+6986) G/A *CYP3A5*) with the state of renal function at the onset of chronic glomerulonephritis. The factors, reducing the risk of decrease in kidneys function, in patients with chronic glomerulonephritis, at the onset of the disease, were found (311S allele and genotype 311SS *PON2*, OR = 2.14 and 2.60 respectively). The protective factors for renal dysfunction development at the onset of disease are 311C *PON2* allele and genotypes 311SC *PON2,* -1166AC *AGTR1* (OR = 0.47; 0.42 and 0.17 respectively).

**Keywords**: chronic glomerulonephritis, genetic polymorphism, genes of vascular homeostasis, preserved and reduced kidney function.

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## INTRODUCTION

Chronic glomerulonephritis (CGN) – is a group of diseases of different origin and morphological manifestations. Chronic glomerulonephritis is characterized by genetically determined immune-mediated inflammation of the renal glomeruli, which may be accompanied by involvement of all renal structures in pathological process (Litovkina et al, 2014a; Gao et al, 2016), by the development of renal failure, renal scarring, arterial hypertension, as well as it can lead to death from chronic renal failure (Litovkina et al., 2014b).

The leading direction of modern nephrology development is the molecular genetic study of endogenous/genetic factors, which cause the disease. One of such promising approaches, in the study of genetic susceptibility to the disease, is the use of so-called candidate genes (Westland et al, 2015; Westland et al, 2015). As candidate genes, whose expression products can determine the rate of progression of renal disease, first of all are considered the genes of vascular homeostasis, which regulate hemodynamic processes, as well as the processes of synthesis and degradation of extracellular matrix and affect the rate of glomerulosclerosis development (Litovkina et al., 2014a; Gao et al, 2016). It should be noted, that the results of works, dedicated to the study of vascular homeostasis polymorphisms involvement in the formation, development and progression of chronic glomerulonephritis, are inconsistent in different populations of the world, and in the Russian Federation such studies are also few.

## MATERIALS AND METHODS

Two samples were formed for carrying out the study. The control group consisted of 304 persons; the group of patients with chronic glomerulonephritis included 238 individuals. The sample data consisted of Russian citizens of Central Black Earth region of Russia, with no family ties between them. The patients were included in the group of medical cases only after making the disease diagnosis, confirmed by clinical and laboratory instrumental methods of examination on the basis of Nephrology Department of the Belgorod Regional Hospital. The exclusion criteria for the group of patients with chronic glomerulonephritis were diabetes (in history or revealed in the process of examination), hypertension.

In the group of patients with chronic glomerulonephritis and in the control group, it was carried out the genotyping of ten polymorphic markers of vascular homeostasis - angiotensin-converting enzyme (I/D *ACE*), endothelial synthase of nitric oxide (4a/4b *eNOS*), paraoxonase-2 (C311S *PON2*), angiotensinogen ((-6 ) A/G *AGT*), angiotensin-II receptor of the first type ((-1166) A/C *ATGTR1*), β3-subunits of the guanine binding protein (G/A *GNB3* rs.2301339), α-adducin (G460W *ADD1*), β2- adrenoreceptor ((+46) G/A *ADRB2*), endothelin-1 (K198N *EDN1*), cytochrome 3A5 ((+6989) A/G *CYP3A5*).

The venous blood in the amount of 8-9 ml, taken from the cubital vein of proband, was the material for the study. The isolation of genomic DNA from peripheral blood was conducted by the method of phenol-chloroform extraction of Miller S.A. et al. (1988). The molecular genetic analysis of all loci was carried by the method of polymerase chain reaction of DNA synthesis, using oligonucleotide primers and probes. (Agerholm-Larsen et al., 2000; Asai et al., 2001; Jalilian et al., 2008; Lanfear et al., 2005; Picard et al., 2007; Prasad et al., 2006; Yazdanpanah et al., 2007).

The analyses of alleles and genotypes associations of studied DNA markers with renal function in patients with chronic glomerulonephritis was carried out using the analysis of 2x2 contingency tables, with calculation of criterion $\chi 2$, with Yates correction for continuity and odds ratios (OR) with 95% confidence intervals (CI). In the process of carrying out the multiple comparisons, in order to minimize the errors of the 1st kind, connected with obtaining false positive results, the Bonferroni correction was used ($p_{cor}$).

## RESULTS

238 patients with chronic glomerulonephritis and 304 people from the control group were examined. The main characteristics of the study group of patients with chronic glomerulonephritis and the group of population control are shown in Table 1.

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**Table1. Characteristics of the subjects from the case and control groups**

| Characteristics | Cases | Controls |
|---|---|---|
| Total | 238 | 304 |
| Males | 53.4% | 53.9% |
| Female | 46.6% | 46.1% |
| Age, yrs | 39.58 ±14.58 | 42.20 ± 6.28 |
| Weight, kg | 63.4 ± 2.1 | 67.4 ± 1.7 |
| Height, cm | 165.4 ± 3.4 | 168.6 ± 2.7 |
| SBP, mm Hg | 148.4 ± 26.5 | 128.1 ± 4.4 |
| DBP, mm Hg | 92.7 ± 14.0 | 82.2 ± 2.0 |

The control group is fully comparable with the sample of patients with chronic glomerulonephritis, according to given characteristics ($p > 0.05$).

To assess kidney function at the onset of the disease, the following two groups were formed: the first group consisted of patients with preserved function (n = 107 individuals), the second group consisted of patients with decrease in kidney function, they had excessive level of creatinine (more than 140 mcM/l) (n = 55 individuals).

In the process of the study of polymorphic genetic markers distribution among the patients with chronic glomerulonephritis with preserved and reduced kidney function, as well as in the control group, it was found statistically significant differences in frequencies of genotypes and alleles in locus S311C of paraoxonase-2. It was defined, that among the patients with chronic glomerulonephritis with reduced kidney function, the concentration of genotype 311SS was 72.72% and it was the highest, compared as to the control group, where this index was 50.66% ($\chi2 = 8.27$, $p = 0.005$, taking into account the Bonferroni correction $p_{cor} = 0.015$, OR = 2.60, 95% Cl 1.32-5.15), as to the patients with chronic glomerulonephritis with preserved renal function (52.83%, $\chi2 = 5.16$, $p = 0.02$, $p_{cor} = 0.06$). The differences in the prevalence of genotype 311SC *PON2* between the patients with reduced renal function, and the control group were defined: among the patients, the concentration of the marker was more than 1.7 times lower than in the control group ($\chi2 = 6.12$, $p = 0.01$, $p_{cor} = 0.03$, OR = 0.42, 95% Cl 0.21-0.85).

According to the distribution of alleles of the locus S311C *PON2,* the following results were obtained: higher frequency of allele 311S is in patients with reduced renal function (84.55%), compared as to the group of patients with preserved renal function (72.17%, $\chi2 = 5.48$, $p = 0.02$ ) as to the control group (71.88%, $\chi2 = 7.10$, $p = 0.009$, oR = 2.14, 95% Cl 1.20-3.84).

As for the locus (-1166) A/C *AGTR1,* significant differences in genotypes concentrations among the patients with chronic glomerulonephritis with preserved and reduced kidney function, were also obtained. Thus, in patients with reduced renal function, the frequency of genotype (-1166) AC equaled 28.85%, that was significantly lower (1.7 times) compared to the concentration of given genotype among patients with preserved renal function (50.47%, $\chi2 = 5.81$ $p = 0.017$, $p_{cor} = 0.05$).

## DISCUSSION

Molecular genetic markers, which are the risk factors for decreased renal function in patients at the onset of chronic glomerulonephritis (311S and 311SS *PON2*, OR = 2.14 and 2.60), and protective factors - allele 311C *PON2* and genotypes 311SC *PON2*, (- 1166) AC *AGTR1* (OR = 0.47; 0.42 and 0.17, respectively) were defined in our research.

It should be noted, that in the literature, the information about the interaction of polymorphism S311C *PON2* with renal function in patients with nephropathy is few. Today, the considerable number of facts is collected; they showing that an important pathogenetic link in the development of glomerulonephritis is the imbalance in the functioning of the lipid peroxidation and antioxidant protection system (Sawant et al, 2010; Litovkina et al, 2014b). The damage of the kidney structures may begin with the formation of reactive oxygen

intermediates, stimulated by neutrophils, macrophages and mesangial cells of renal glomeruli. If the antioxidant defense system becomes untenable, it may lead to enchancement of lipid peroxidation and membranes damage of nephrothelial cells. One of the main enzymes, preventing lipid peroxidation, is paraoxonase. The production decrease of this enzyme reduces the reserves of antioxidant defense, facilitates the progression of glomerulosclerosis and renal function decrease (Litovkina et al., 2014b).

In the available literature, there is the evidences on the interaction of polymorphism (-1166) A/C *AGTR1* with various renal pathologies (Jacobsen et al, 2003; Buraczynska et al, 2006). So in the work (Buraczynska et al., 2006) in the process of study of the renin-angiotensin system genes, it was found that the genotype (-1166) CC of angiotensin II receptor type 1 gene, was more frequent in patients with interstitial nephritis. It was found that patients with allele (-1166) C, the average time of the disease development to end-stage of renal failure was significantly less than in individuals with genotype (-1166) AA of *AGTR1* locus. In the study (Jacobsen P. et al., 2003), it found the interaction of allele (-1166C) of *AGTR1* locus with the development of diabetic nephropathy.

The connection of polymorphism (-1166) A/C *AGTR1* with renal function state can be explained in such a way. The polymorphism of *AGTR1* gene, localized in the 3'- non-translating region of the gene, results the substitution in 1166 the position of adenine to cytosine (rs.5186). It has been found, that such replacement modifies the structure of gene's regulatory cis-element, that leads to increase of its expression (Wang et al, 2006; Sethupathy et al, 2007.).

## CONCLUSION

In the course of our study, the polymorphic genetic markers 311S and 311SS *PON2*, which are the risk factors for decreased renal function in patients with chronic glomerulonephritis in the onset of the disease (OR = 2,14 and 2,60 respectively) were defined. The allele 311C *PON2* and genotypes 311SC *PON2,* (-1166) AC *AGTR1* can be considered as protective factors for renal dysfunction development at the onset of the disease (OR = 0,47; 0,42 and 0,17 respectively).

## REFERENCES


[1] Agerholm-Larsen, B., Nordestgaard, B.G., Tybjaerg-Hansen, A., 2000. *ACE gene polymorphisms in cardiovascular disease: metaanalysis of small and large studies in whites.* // Arteriosclerosis, Thrombosis, and Vascular Biology, 20: Pp. 484–492.

[2] Asai, T., Ohkubo, T., Katsuya, T., Higaki, J., Fu, Y., Fukuda, M., Hozawa, A., Matsubara, M., Kitaoka, H., Tsuji, I., Araki, T., Satoh, H., Hisamichi, S., Imai, Y., Ogihara, T., 2001. *Endothelin-1 gene variant associates with blood pressure in obese Japanese subjects. //* Journal of Hypertension, 38: Pp. 1321–1324.

[3] Buraczynska, M., Ksiazek, P., Drop, A., Zaluska, W., Spasiewicz, D., Ksiazek, A., 2006. *Genetic polymorphisms of the renin–angiotensin system in end-stage renal disease.//* Nephrology Dialysis Transplantation, 21: Pp. 979–983.

[4] Gao, J.R., Qin, X.J., Jiang, H, Wang, T., Song, J.M., Xu, S.Z., 2016. *Screening and functional analysis of differentially expressed genes in chronic glomerulonephritis by whole genome microarray.//* Gene, 589(1): Pp. 72-80.

[5] Jacobsen, P., Tarnow, L., Carstensen, B., Hovind, P., Poirier, O., Parving, H.H., 2003. *Genetic variation in the Renin-Angiotensin system and progression of diabetic nephropathy*. // J Am Soc Nephrol., 14(11): Pp. 2843-50.

[6] Jalilian, A., Javadi, E., Akrami, M., Fakhrzadeh, H., Heshmat, R., Rahmani, M., Bandarian, F., 2008. *Association of Cys 311 Ser polymorphism of paraoxonase-2 gene with the risk of coronary artery disease*. // Archives of Iranian Medicine, 11: Pp. 544–549.

[7] Lanfear, D.E., Jones, P.G., Marsh, S., Cresci, S., McLeod, H.L., Spertus, J.A., 2005. *β2-Adrenergic receptor genotype and survival among patients receiving β-blocker therapy after an acute coronary syndrome*. // JAMA. 294: Pp. 1526–1533.

[8] Litovkina, O., Nekipelova, E., Dvornyk, V., Polonikov, A., Efremova, O., Zhernakova,N., Reshetnikov, E., Churnosov, M., 2014a. *Genes involved in the regulation of vascular homeostasis determine renal survival rate in patients with chronic glomerulonephritis.* // Gene, 546(1): Pp. 112-116.

[9] Litovkina, O.N., Nekipelova, E.V., Sirotina,S.S., Yakunchenko, T.I., Efremova, O.A., Sorokina, I.N., 2014b. *Polymorphism of Vascular Homeostasis Genes and Progression of Chronic Kidney Disease in Patients with Chronic Glomerulonephritis. //* Research Journal of Pharmaceutical, Biological and Chemical, 5(5): Pp. 1079-1082.

[10] Miller, S.A., Dykes, D.D., Polesky, H.F., 1988. *A simple salting out procedure for extracting DNA from human nucleated cells.* Nucleic acids research, 16(3): P. 1215.

[11] Picard, N., Djebli, N., Sauvage, F.L.,Marquet, P., 2007. *Metabolism of sirolimus in the presence or absence of cyclosporine by genotyped human liver microsomes and recombinant cytochromes P450 3A4 and 3A5.* Drug Metabolism and Disposition, 35: Pp. 350–355.

[12] Prasad, P., Tiwar, A.K., Kumar, K.M., Ammini, A.C., Gupta, A., Gupta, R., 2006. *Chronic renal insufficiency among Asian Indians with type 2 diabetes: I. Role of RAAS gene polymorphisms*. // BMC Medical Genetics, 7: P. 42.

[13] Sawant, J., Nair, S., Redkar, N., 2010. *Oxidative stress and serum paraoxonase activity in patients on maintenance hemodialysis*. // The Internet Journal of Nephrology, 6(1): Pp. 122-129.

[14] Sethupathy, P., Borel, C., Gagnebin, M. Grant, G.R., Deutsch, S., Elton, T.S., Hatzigeorgiou, A.G., Antonarakis S.E., 2007. *Human microRNA-155 on chromosome 21 differentially interacts with its polymorphic target in the AGTR1 3' untranslated region: a mechanism for functional single-nucleotide polymorphisms related to phenotypes.* Am. J. Hum .Genet., 81(2): Pp. 405-413.

[15] Wang, X., Zhu, H., Dong, Y., Treiber, F.A., Snieder, H., 2006. *Effects of angiotensinogen and angiotensin II type I receptor genes on blood pressure and left ventricular mass trajectories in multiethnic youth*. // Journal of Human Genetics, 9: Pp. 393–402.

[16] Westland, R., Verbitsky, M., Vukojevic, K., Perry, B.J., Fasel, D.A., Zwijnenburg P.J., Bökenkamp, A., Gille, J.J., Saraga-Babic, M., Ghiggeri, G.M., D'Agati, V.D., Schreuder ,M.F., Gharavi, A.G., van Wijk ,J.A., Sanna-Cherchi ,S., 2015. *Copy number variation analysis identifies novel CAKUT candidate genes in children with a solitary functioning kidney.* Kidney Int., 88(6): Pp. 1402-1410.

[17] Yazdanpanah, M., Aulchenk, Y.S., Hofman, A., Janssen Joop, A.M., Sayed-Tabatabaei, F.A., Van Schaik Ron, H N, Klungel Olaf H., Stricker, B.H., Pols Huibertm, A.P., Witteman, J. C., Lamberts, Steven W.J., Oostra,B.A., Van Duijn, C.M., 2007. *Effects of the renin–angiotensin system genes and salt sensitivity genes on blood pressure and atherosclerosis in the total population and patients with type 2 diabetes.* Diabetes, 56: Pp. 1905–1912.

[18] Yoshimura-Furuhata, M., Nishimura-Tadaki, A., Amano, Y., Ehara, T., Hamasaki, Y., Muramatsu, M., Shishido, S., Aikawa ,A., Hamada, R., Ishikura ,K., Hataya, H., Hidaka, Y., Noda, S., Koike, K., Wakui, K., Fukushima ,Y., Matsumoto, N., Awazu, M., Miyake ,N., Kosho, T., 2015. *Renal complications in 6p duplication syndrome: microarray-based investigation of the candidategene(s) for the development of congenital anomalies of the kidney and urinary tract (CAKUT) and focal segmental glomerular sclerosis (FSGS).* Am J Med Genet., 167(3): Pp. 592-601.