ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

# Polymorphism of Vascular Homeostasis Genes and Progression of Chronic Kidney Disease in Patients with Chronic Glomerulonephritis.

**Olga Nikolaevna Litovkina*, Elena Vasil'evna Nekipelova, Svetlana Sergeevna Sirotina, Tatiana Igorevna Yakunchenko, Olga Alekseevna Efremova, and Inna Nikolaevna Sorokina.**

Belgorod State National Research University, 85, Pobedy St., Belgorod, 308015, Russia.

**ABSTRACT**

We have presented the results of a study of polymorphic genes' markers of vascular homeostasis (I/D *ACE*, 4a/4b *eNOS*, S311C *PON2*, -6A/G *AGT*, -1166A/C *ATIIR1*, G/A *GNB3* (rs.2301339), G460W *ADD1*, +46G/A *ADRB2*, K198N *ET-1*, +6986G/A *CYP3A5*), associated with progression of chronic glomerulonephritis. Genotype +6986GG *CYP3A5* was identified to be protective factor of progression of chronic glomerulonephritis, and genotypes +6986GA *CYP3A5*, +6986A *CYP3A5 и* 311S *PON2* were found to be risk factors.

**Keywords:** chronic glomerulonephritis, chronic kidney disease, genetic polymorphism, vascular homeostasis genes.

***Corresponding author***

ISSN: 0975-8585

## INTRODUCTION

Chronic glomerulonephritis (CKD) is a chronic immune inflammatory disease, characterized by damage of malpighian tuft. The disease is manifested by arterial hypertension, proteinuria, erythrocyturia, oedemata, creatinine doubling, which leads to chronic kidney disease (CKD) [1]. One of the key factors of CKD progression is activation of pressure systems of vascular homeostasis, like: sympathoadrenal system, rennin-angiotensin-aldosteron system, constricting hormones of endothelium and endothelins [2,3].

In this regard investigators pay growing attention to polymorphic genes' markers of vascular homeostasis in the presence of kidney diseases [4,5], which determine elaboration of specific enzymes (angiotensinogen, angiotensine transforming enzyme, endothelin), relating them to potential genetic risk factors of glomerulopathy. In accordance with the foregoing, in the course of this work the analysis of polymorphic genes' associations of vascular homeostasis was performed (I/D *ACE*, 4a/4b *eNOS*, S311C *PON2*, -6A/G *AGT*, -1166A/C *ATIIR1*, G/A *GNB3* (rs2301339), G460W *ADD1*, +46G/A *ADRB2*, K198N *ET-1*, +6986G/A *CYP3A5*) with progression of chronic kidney disease in patients with chronic glomerulonephritis.

## MATERIALS AND METHODS

Analysis of vasoactive hormones' gene polymorphism was performed with 542 persons: 238 patients with chronic glomerulonephritis and 304 persons from control group. Selections to groups with sick patients and to control groups were performed with individuals of Russian nationality, who were natives of Central region of Russia and who didn't have any family ties. The patients were included into groups only after their diagnoses were determined with the help of clinical and laboratory and instrumental examination techniques. Clinical and laboratory examination was being performed on the base of Nephrology Unit of Belgorod regional clinical hospital.

Exclusionary criteria for the group of patients with CKD were diabetes (in past medical history or revealed in the course of examinations) and hypertensive disease.

Examinations showed that 34 patients had had chronic kidney disease (CKD): creatinine level equaled more than 140 mcmol/l during 6 months of observations.

Test material was presented with venous blood with a volume of 8 to 9 ml, taken from proband's median cubital vein. Genome DNA purification from peripheral blood via standard methods [6].

Analysis of all locuses was performed via polymerase chain reaction (PCR) of DNA synthesis.

Genotyping of DNA markers was performed by means of analysis of amplified fragments lengths polymorphism (AFLP) (Alu polymorphism *ACE*, VNTR *eNOS*), restriction fragment length polymorphism (RFLP) (locuses S311C *PON2*, -6A/G *AGT*, -1166A/C *ATIIR1*) and allele selectivity by means of method Tag Man probes (Tag Man) (locuses *ET-1*, +6986G/A *CYP3A5*, G/A *GNB3* (rs 2301339), G460W *ADD1*, +46G/A *ADRB2*).

For estimation of consistency of observed gene distribution with the expected one based upon Hardy–Weinberg equilibrium, criterion $\chi 2$ was used. Associations of alleles and genotypes of studied DNA markers with creatinine doubling in patients with chronic glomerulonephritis was assessed via analysis of cross tables 2x2 with calculation of criterion $\chi 2$ and with Yates' correction for continuity and odds ratio (OR) with 95% confidence interval (CI).

## RESULTS

238 patients with chronic glomerulonephritis were observed (mean age equaled 39,58±14,58 years, varied from 15 to 76 years old) and 304 persons from control group (42,20±6,28 years, varied from 17 to 79 years old, $p>0,05$).

Comparative analysis of alleles and genotypes' frequency of studied locuses of vascular homeostasis among patients with CKD and patients from control group didn't show any significant differences between these groups (Table 1).

ISSN: 0975-8585

**Table 1: Summary information about the studied polymorphisms.**

| Polymorphism | Studied groups | Minor allele | MAF (%) | HWE | |
|---|---|---|---|---|---|
| | | | | χ2 | p |
| I/D *ACE* | Case | I *ACE* | 45.09 | 0.87 | >0.05 |
| I/D *ACE* | Control | I *ACE* | 48.18 | 0.19 | >0.05 |
| 4a/4b *NOS3* | Case | 4a *NOS3* | 21.37 | 0.26 | >0.05 |
| 4a/4b *NOS3* | Control | 4a *NOS3* | 19.50 | 0.90 | >0.05 |
| S311C *PON2* | Case | 311C *PON2* | 24.58 | 0.17 | >0.05 |
| S311C *PON2* | Control | 311C *PON2* | 28.12 | 0.75 | >0.05 |
| (-6)A/G *AGT* | Case | (-6)G *AGT* | 48.11 | 0.06 | >0.05 |
| (-6)A/G *AGT* | Control | (-6)G *AGT* | 47.69 | 1.38 | >0.05 |
| (-1166)A/C *AGTR1* | Case | (-1166)C *AGTR1* | 26.18 | 1.01 | >0.05 |
| (-1166)A/C *AGTR1* | Control | (-1166)C *AGTR1* | 25.99 | 0.19 | >0.05 |
| G/A *GNB3* | Case | A *GNB3* | 34.18 | 0.24 | >0.05 |
| G/A *GNB3* | Control | A *GNB3* | 31.68 | 0.41 | >0.05 |
| G460W *ADD1* | Case | 460W *ADD1* | 16.31 | 13.55 | <0.001 |
| G460W *ADD1* | Control | 460W *ADD1* | 15.13 | 1.84 | >0.05 |
| (+46)G/A *ADRB2* | Case | (+46)A *ADRB2* | 36.86 | 2.01 | >0.05 |
| (+46)G/A *ADRB2* | Control | (+46)A *ADRB2* | 39.93 | 1.26 | >0.05 |
| K198N *EDN1* | Case | 198N *EDN1* | 17.02 | 0.30 | >0.05 |
| K198N *EDN1* | Control | 198N *EDN1* | 18.54 | 0.38 | >0.05 |
| (+6986)G/A *CYP3A5* | Case | (+6986)A *CYP3A5* | 7.48 | 1.53 | >0.05 |
| (+6986)G/A *CYP3A5* | Control | (+6986)A *CYP3A5* | 5.92 | 0.93 | >0.05 |

Notes: MAF, minor allele frequency; HWE, Hardy–Weinberg equilibrium. P values were calculated using the $\chi^2$ test.

It has been stated that the patients with chronic kidney disease had the concentration of +6986GG *CYP3A5* genotype equaling 72,73% and this concentration was the smallest in comparison with the group of patients without CKD (87,38%, $\chi^2$=2,95, p=0,09) and control group, where this index equaled 88,81% ($\chi^2$=5,55, p=0,019, $p_{cor}$=0,057, OR=0,34, 95%Cl 0,14-0,85). Differences in prevalence of +6986GA *CYP3A5* genotype was determined as well between patients with CKD and control group: among the sick patients the concentration of this marker (27,27%) was 2,5 times higher than that in the control group (10,53%, $\chi^2$=6,32, p=0,013, $p_{cor}$=0,039, OR=3,19, 95%Cl 1,25-7,99).

Similar results were obtained in respect to distribution of alleles of +6986G/A *CYP3A5* locus: higher frequency of +6986A allele was detected (2 times) in the group of patients with CKD (13,64%), comparing to control group (5,92%, $\chi^2$=4,52, p=0,034, OR=2,51, 95%Cl 1,06-5,76).

Significant differences in alleles' concentrations between patients with CKD and control patients were observed per S311C of paraoxonase-2 as well: the frequency of 311S *PON2* allele equaled 83,82% among the patients with CKD, which was higher than that in the control group (71,88%, $\chi^2$=3,85, p=0,05, OR=2,03, 95%Cl 1,01-4,20).

## DISCUSSION

As it can be seen from the above, +6986GG genotype of *CYP3A5* locus (OR=0,34)can be considered protective factor for progression of chronicle kidney disease in patients with chronic glomerulonephritis, and risk factors of CKD progression are genetic markers +6986GA *CYP3A5* and +6986A *CYP3A5* (OR=3,19 and OR=2,51, respectively).

According to literature data [12], in the process of genetic polymorphism +6986G/A *CYP3A5*, it is +6986A allele that defines higher level of cytochrome 3A5 production. Moreover, individuals with production of *CYP3A5* in levels that are higher than normal range, more frank medico-biological effects can be expected, one of which increased reabsorption of sodium, stricture of renal tubules [12], which inevitably leads to progression of glomerular affection. Correlation of high-productive allele +6986A *CYP3A5* with increased creatinine level was demonstrated in other studies as well [13]. And it is known that hemodynamic disorders are the ones that lead among non-immune mechanisms of progression of glomerular affection, including decrease of deputinating kidney function, which is marked by increased creatinine level in blood.

At the same time, it has been stated that genetic marker 311S *PON2* is a risk factor of chronic kidney disease's progression (OR=2,03). The mechanism of such associations may be related to decreased antioxidative effects of paraoxonase-2 that lead to oxidative stress and hardening of kidney tissue, and, thus, to depression of renal function. The work of Sawant J. and contributing authors (2010), dedicated to studying of oxidizing stress and paraoxonase in 30 patients on hemodialysis, 20 of which had initial manifestation of CKD, demonstrated increased creatinine level, comparing to control ($p<0,001$) and decreased activity of paraoxonase hormone for more than 30% ($p<0,001$), which considered to be decrease of antioxidative defense [14].

## CONCLUSION

Thus, we can note that +6986GG genotype of *CYP3A5* locus (OR=0,34)can be considered protective factor for progression of chronicle kidney disease in patients with chronic glomerulonephritis, and risk factors of CKD progression are genetic markers +6986GA *CYP3A5* and +6986A *CYP3A5* (OR=3,19 and OR=2,51, respectively).

## REFERENCES

[1] Pirkle JL, Freedman BI. Minerva Urol Nefrol 2013;65(1):37-50.
[2] Litovkina O, Nekipelova E, Dvornyk V, Polonikov A, Efremova O, Zhernakova N, Reshetnikov E, Churnosov M. 2014. Genes involved in the regulation of vascular homeostasis determine renal survival rate in patients with chronic glomerulonephritis.
[3] Sato W, Sato Y. Br J Pharmacol 2014;171(4):879-87.
[4] Shaikh R, Shahid SM, Mansoor Q, Ismail M, Azhar A. J Renin Angiotensin Aldosterone Syst 2014 15(2):124-130.
[5] Shah VN, Cheema BS, Sharma R, Khullar M, Kohli HS, Ahluwalia TS, Mohan V, Bhansali A. Mol Cell Biochem 2013;372(1-2):191-8.
[6] Mathew CC. Human Press 1984; 2:31-34.
[7] Meroufel DN, Médiène-Benchekor S, Dumont J, Benhamamouch S, Amouyel P, Brousseau T. J Renin Angiotensin Aldosterone Syst 2014;15(1):1-6.
[8] Hoffmann IS, Tavares-Mordwinkin R, Castejon AM. J Human Hypert 2005;19:233-240.
[9] Jalilian A, Javadi E, Akrami M. et al. Arch Iranian Med 2008;11(5):544 – 549.
[10] Prasad P, Tiwari AK, Kumar KMP. et al. BMC Med Gen 2006; 7:42.
[11] V Ramachandran, P Ismail, J Stanslas. Cardiovasc. Diabetol 2009;8(11):1-12.
[12] RC Givens, YS Lin, AL.Dowling, et al. J Appl Physiol 2003;95(3):1297-1300.
[13] M Bochud, CB Eap, RC Elston, et al. J Hypertens 2006;24(5): 923-29.
[14] J Sawant, S Nair, N Redkar. The Internet J Nephrol 2010;6(1):122-29.