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Clinical and Genetic Research of Chronic Glomerulonephritis.

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ABSTRACT

The paper presents data on interaction of candidate genes (S311C *PON2*, (-6)A/G *AGT*, (-1166)A/C *AGTR1*, (-592)C/A *IL-10*, VNTR *IL-1Ra*, T113M *IL-9*, K198N *EDN1*, (+46)G/A *ADRB2,* G/A *GNB3 (rs.2301339)*) with oligogenic and continuous characters of chronic glomerulonephritis. The associations of polymorphisms (-6) A/G *AGT* and VNTR *IL-1Ra* with acute nephritic and hematuric syndromes at onset of the disease (OR = 2.62 and OR = 2.04, respectively) were defined. It was found that the molecular genetic markers 311SS *PON2* and (+46)AA *ADRB2* were connected with increased creatinine and haematuria in patients with chronic glomerulonephritis (p = 0.05 and p = 0.03, respectively).

Keywords: chronic glomerulonephritis, candidate genes, genetic polymorphism, clinical syndromes of onset, creatinine level, hematuria level.

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INTRODUCTION

Chronic glomerulonephritis (CGN) is the most severe kidneys disease, which takes the dominant positions among the most common causes of chronic renal failure (Kamyshova et al, 2016). The disease progresses for a long time (during years and decades). It is characterized by a variety of clinical manifestations and, in the end, leads to kidney failure, the morphological substrate of which is contracted kidney. This is one of the most common kidney diseases (Pirkle and Freedman, 2013; Litovkina et al, 2014a). Chronic glomerulonephritis is found in 1% of all autopsies. The disease occurs at any age. Its first symptoms are the most often found between 20 and 40 years (Nickolas et al., 2004). The progression of chronic glomerulonephritis steadily leads to the end-stage of chronic renal failure, and to increase the number of patients, receiving substitutive renal therapy, which requires additional economic expenses for its conducting (Ismail et al., 2016). Therefore, further researches, oriented to identifying the mechanisms of occurrence, development and progression of chronic glomerulonephritis, remains an important problem of modern medicine and nephrology.

In accordance with the above outlined, this work presents the analysis of associations of polymorphic markers candidate genes (S311C *PON2*, (-6)A/G *AGT*, (-1166)A/C *AGTR1*, (-592)C/A *IL-10*, VNTR *IL-1Ra*, T113M *IL-9*, K198N *EDN1*, (+46)G/A *ADRB2*, G/A *GNB3* (rs.2301339)) with oligogenic and continuous characters of chronic glomerulonephritis, as well as with clinically significant syndromes of the disease onset.

MATERIALS AND METHODS

The analysis of candidate genes polymorphisms was conducted among 542 people: 238 patients with chronic glomerulonephritis and 304 individuals from the control group. The samples of patient with chronic glomerulonephritis and the control group included individuals of Russian nationality, who are natives of the Central region of Russia and have no relationships with each other. 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. Clinical and laboratory examinations of patients were carried out on the basis of the Department of Nephrology of the Belgorod Regional Clinical Hospital.

Persons with hypertension, as well as those, who had history of diabetes mellitus or it was identified during the survey, were excluded from the group.

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 standard method of phenol-chloroform extraction (Miller et al., 1988). The analysis of studied loci was carried by the method of polymerase chain reaction of DNA synthesis, using oligonucleotide primers and probes. (Hulkkonen, 2002; Sehouli et al, 2003; Lanfear et al, 2005; Prasad et al, 2006; Jalilian et al, 2008; Ramachandran et al, 2009; Meroufel et al, 2014).

The genotyping of DNA markers was carried out by the following methods: the analysis of polymorphism of restriction fragments length (S311C *PON2*, (-6)A/G *AGT*, (-1166)A/C *AGTR1*, (-592)C/A *IL-10*, VNTR *IL-1Ra*, T113M *IL-9*), the analysis of allelic discrimination by the method of TagMan probes (K198N *EDN1*, (+46)G/A *ADRB2, G/A GNB3 (rs.2301339)*.

The associations of alleles and genotypes of studied DNA markers with specific clinical syndrome at the onset of the disease were assessed, 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 comparative analysis of continuous characters distribution among different groups of patients with chronic glomerulonephritis (for example, among patients with different genotypes), in order to minimize the errors of the 1st kind, connected with obtaining false positive results, the Bonferroni correction was used (the calculation of adjusted number of comparable pairs, the level of significance p_{cor}).

In the process of study, the interaction of candidate genes polymorphisms with quantitative indicators (the age of onset, the level of creatinine, hematuria and proteinuria, systolic and diastolic blood pressure), the median (Me,), interquartile range (Q25-Q75) and Mann-Whitney test were used in comparative analysis of genotypes, according to these indicators.

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RESULTS

238 patients with chronic glomerulonephritis (middle age 39.58 ± 14.58, ranged from 15 to 76 years), and 304 individuals from the control group (42.20 ± 6.28 years, ranged from 18 to 79 years, p> 0.05) were examined.

During the comparative analysis of allele frequencies and genotypes of studied loci, among patients with chronic glomerulonephritis and the individuals from control group, statistically significant differences were not found (p> 0.05) (Table 1).

Table 1: Summary information about the studied polymorphisms

Polymorphism	Studied groups	Minor allele	MAF (%)	HWE	
				χ2	p
T113M *IL-9*	Case	(-4257)A *IL-13*	17.66	3.67	>0.05
T113M *IL-9*	Control	(-4257)A *IL-13*	18.64	0.22	>0.05
VNTR *IL-1Ra*	Case	*5R IL-1Ra*	4.19	3.57	>0.05
VNTR *IL-1Ra*	Control	5R *IL-1Ra*	0.02	0.01	>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
(+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
(-592)C/A *IL-10*	Case	(-592)A *IL-10*	27.73	3.42	>0.05
(-592)C/A *IL-10*	Control	(-592)A *IL-10*	22.67	0.05	>0.05

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

Then we studied the interrelations between considered loci and quantitative pathogenetically significant signs of chronic glomerulonephritis. These indicators were the following: the age of manifestation, the levels of creatinine, proteinuria and hematuria, systolic blood pressure and diastolic blood pressure. It should be noted, that the distribution of these indicators did not correspond to normal distribution, according to the criterion of Shapiro-Wilk (p <0.01) (Table 2); due to this, in the process of comparative analysis of the genotypes, according to these indicators, the median (Me), interquartile range (Q25-Q75) and Mann-Whitney test were used.

Table 2: The distribution of quantitative indicators in patients with chronic glomerulonephritis

Indicators	N	Me	Q25	Q75	W	p
Age manifestation, years	238	27.50	17.00	41.00	0.97	<0.01
Creatinine level, umol/L	238	112.50	93.90	456.00	0.72	<0.01

Hematuria level, units	238	6.00	2.00	12.00	0.39	<0.01
Proteinuria level, g/day	232	0.50	0.20	1.53	0.50	<0.01
SBP, mm Hg	238	150.00	120.00	170.00	0.96	<0.01
DBP, mm Hg	237	100.00	80.00	100.00	0.89	<0.01

Notes: N, sample size; Me, median; Q25 - Q75, interquartile range - the 25th and 75th percentiles; W, Shapiro-Wilk test. P values were calculated using the Shapiro-Wilk test.

The interaction of polymorphic markers of candidate genes with quantitative pathogenetically significant signs of chronic glomerulonephritis was defined for two loci: paraoxonase-2 (S311C *PON2*), β2-adrenoreceptor ((+46) G/A *ADRB2*) with hematuria and creatinine levels, respectively.

So for the gene S311C *PON2* the following results were obtained: in patients with genetic variant of 311SS, the level of creatinine was significantly higher (median 127.00 mcM/l, Q25-98.30 mcM/l, Q75-537.00 mcM/l) compared with the group of patients with chronic glomerulonephritis, having genotypes 311SC, 311CC *PON2* (median 103.00 mcM/l, Q25-Q75 92.00-336.00 mcM/l, p = 0.05).

The interaction between polymorphic marker of gene β2- adrenoreceptor ((+46) G/A *ADRB2*) with the level of hematuria was defined. In patients who are the carriers of genotype (+46) AA, the median level of hematuria is 11.00 units, interquartile range is 3.0-25.0 units, that is significantly higher than in the group of patients with genetic variants (+46) GG and (+46) GA, according to given polymorphism (Me - 5.0 units, Q25-Q75 2.0-12.0 units, p = 0.03.).

The study of clinical syndromes in onset of chronic glomerulonephritis showed, that in 26.7% of patients the disease begins with acute nephritic syndrome, in 30.4% - with nephritic syndrome, in 24.4% - with urinary syndrome; hematuric syndrome was observed in 18.5% of patients with chronic glomerulonephritis.

The information about statistically significant interaction between two studied polymorphic markers of candidate genes ((-6) A/G *AGT,* VNTR *IL-1Ra*), with predominance of specific clinical syndrome at the onset of the disease, was obtained in the process of the research. Thus, in patients with chronic glomerulonephritis with acute nephritic syndrome, the frequency of genetic variant (-6) AG *AGT* is 67.2%, that is significantly higher than the frequency of the genotype as in the control group (43.9%, $\chi2 = 9.72$, p = 0,003, p_{cor} = 0,009, OR = 2.62, 95% Cl 1.40-4.96), as in the patients with chronic glomerulonephritis with other syndromes in the onset of the disease: urinary (43.4%, $\chi2 = 5.46$, p = 0.020, p_{cor} = 0.060), nephritic (44.6%, $\chi2 = 5.47$, p = 0.019 , p_{cor} = 0.057), hematuric (47.5%, $\chi2 = 3.05$, p = 0.08).

Also, we set the association of genotypes 2R/2R, 2R/4R of the gene VNTR IL-1Ra with the development of hematuric syndrome at the onset of chronic glomerulonephritis. The patients with genotypes 2R/2R, 2R/4R had this syndrome in 2.5 times often, than patients with genotype 4R/4R (28.4% vs. 11.4%; $\chi2 = 8.15$, p = 0.005, p_{cor} = 0.015, OR = 2.04, 95% Cl 1.28-3.87).

DISCUSSION

In our work we revealed the association of polymorphisms (-6) A/G *AGT* and VNTR *IL-1Ra* with acute nephritic and hematuric syndromes at the onset of chronic glomerulonephritis, respectively. Molecular genetic markers 311SS *PON2* and (+46) AA *ADRB2* are connected with increased levels of creatinine and haematuria in patients with chronic glomerulonephritis, respectively.

The interrelation of genotype (+46) AA *ADRB2* with increased level of hematuria can be explained as follows. The renin release occurs with increasing expression of β2-adrenergic receptors of juxtaglomerular

apparatus in the kidneys. As a consequence, there is the activation of the renin-angiotensin-aldosterone system, (Buraczynska et al, 2006), the main component of which is angiotensin II, leading to systemic and local-renal spasm of arterioles with increasing as total peripheral vascular resistance, as renal vascular resistance. It also causes increased reabsorption of sodium, acting directly on the renal tubules. It increases the hypertrophy of renal structures and enhances the proteinuria. All this ultimately weakens the kidney function and may be the cause of hematuria development.

The paper revealed that the genetic marker 311S *PON2* correlated with increased level of creatinine in patients with chronic glomerulonephritis. According to the literature (Sawant et al., 2010), in which in the process of study of oxidative stress and activity of paraoxonase, among 30 hemodialysis-dependent patients, 20 of which had the primary manifestation in the form of chronic glomerulonephritis, it was found an increase, in comparison with the creatinine level control ($p < 0.001$) and decrease of hormone paraoxonase activity by more than 30% ($p < 0.001$), that was seen as the decrease of antioxidant protection. It is known, that the reduction of antioxidant effects of paraoxonase-2 leads to oxidative stress and sclerosis of renal tissue, and, respectively, to decrease in kidneys function (Litovkina et al., 2014b).

It was found that the genotype (-6) AG *AGT* is a risk marker for the development of acute nephritic syndrome at the onset of the disease (OR = 2.62). In the literature, there is the evidence that urinary excretion of angiotensinogen, positively correlates with systolic and diastolic blood pressure, as well as with the ratio of urine albumin/creatinine. On the basis of these data, it was concluded that the increase of angiotensinogen concentration in urine occurs simultaneously with an increase of glomerular endothelial dysfunction (Kobori et al., 2009).

CONCLUSION

Thus, as a result of the study, it was found, that genotype (-6) AG of the gene *AGT* (OR = 2.62) should be considered as the marker of acute nephritic syndrome at the onset of chronic glomerulonephritis, and genotypes 2R/2R, 2R/4R of the gene VNTR *IL-1Ra* are the markers of hematuric syndrome formation (OR = 2.04). The genotype 311SS of the gene *PON2* is connected with increased level of creatinine in patients with chronic glomerulonephritis and the genotype (+46) AA *ADRB2* is associated with increased levels of hematuria in this group of patients ($p = 0.05$ and $p = 0.03$ respectively).

REFERENCES

[1] 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.

[2] Hulkkonen, J., 2002. *Inflammotory Cytokines and Cytokine Gene Polymorphisms in Chronic Lymphocytic Leukaemia, in Primary Sjogren's Syndrome and Haelthy Subjects*. // Tampere, 81p.

[3] Ismail, M.I., Lakouz, K., Abdelbary, E., 2016. *Clinicopathological correlations of renal pathology: A single center experience.* // Saudi J Kidney Dis Transpl., 27(3): Pp. 557-62.

[4] 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.

[5] Kamyshova, E.S., Shvetsov, M.Y., Kutyrina, I.M., Burdennyi, A.M., Chzhen, A., Nosikov, V.V., Bobkova, I.N., 2016. *Clinical value of TNF, IL-6, and IL-10 gene polymorphic markers in chronic glomerulonephritis.//* Ter Arkh., 88(6): Pp. 45-50.

[6] Kobori, H., Alper, B., Shenava, R., Katsurada, A., Saito, T., Ohashi, N., Urushihara, M., Miyata, K., Satou, R., Hamm, L.L., Navar ,L.G., 2009. *Urinary angiotensinogen as a novel biomarker of the intrarenal rennin-angiotensin system status in hypertensive patients. //* Hypertension, 53(2): Pp. 344-350.

[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] Meroufel, D.N., Mediene-Benchekor, S., Dumont, J., Benhamamouch, S., Amouyel, P., Brousseau, T., 2014. *A study on the polymorphisms of the renin-angiotensin system pathway genes for their effect on blood pressure levels in males from Algeria.* // J Renin Angiotensin Aldosterone Syst., 15(1): Pp. 1-6.

[11] 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.

[12] Nickolas, T.L., Frisch, G.D., Opotowsky, A.R., Arons, R., Radhakrishnan, J., 2004. *Awareness of kidney disease in the US population: findings from the National Health and Nutrition Examination Survey (NHANES) 1999 to 2000.* // American Journal of Kidney Diseases, 44: Pp. 185–197.

[13] Pirkle, J.L., Freedman, B.I., 2013. *Hypertension and chronic kidney disease: controversies in pathogenesis and treatment*. // Minerva Urol Nefrol., 65(1): Pp. 37-50.

[14] 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.

[15] Ramachandran, V., Ismail, P., Stanslas, J., 2009. *Analysis of renin-angiotensin-aldosterone system gene polymorphisms in malaysian essential hypertensive and type 2 diabetic subjects*. Cardiovasc. Diabetol., 8(11): Pp. 1-12.

[16] Sawant, J., Nair, S., Redkar, N., 2010. *Oxidative stress and serum paraoxonase activity in patients on maintenance hemodialysis. //* The Journal of Nephrology, 6(1): Pp. 122-29.

[17] Sehouli ,J., Mustea ,A., Koensgen, D., 2003. *Interleukin-1 receptor antagonist gene polymorphism is associated with increased risk of epithelial ovarian cancer.* // Annals of Oncology, 14: Pp. 1501-1504.