ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Genetic Factors Of Renal Survival In Patients With Chronic Kidney Disease.

Elena V. Nekipelova*, Olga N. Novakova, Tatyana I. Yakunchenko, Olga A. Efremova, and Vladimir F. Kulikovskij.

Belgorod State University, 308015, Belgorod, Pobeda Street, 85, Russia.

ABSTRACT

This paper presents the results of the study of associations of polymorphic loci of chemokines (+1931A/T *CCL4* (rs1719153), A/G *CXCL11* (rs4512021), -403A/G *CCL5* (rs2107538), C/G *CCL2* (rs2857657), -801G/A *CXCL12* (rs1801157)) with renal survival in patients with chronic glomerulonephritis. It was established that AA and *CXCL11* AG genotypes (rs4512021) (p=0.05) are the marker of high creatinine level in patients with chronic glomerulonephritis.

Keywords: chronic kidney disease, chemokine genes, genetic polymorphism, creatinine level.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Chronic kidney disease (CKD) occupies a special place among chronic non-infectious diseases, as it is widespread, associated with a sharp deterioration in the quality of life, high mortality, and leads in the terminal stage to the need for expensive replacement therapy – dialysis and kidney transplantation (Sigdel et al., 2012; Lebherz-Eichinger et al., 2014). It should be noted that a significant proportion of patients with CKD are patients with chronic glomerulonephritis (CGN). Chronic glomerulonephritis is characterized by a wide variety of clinical manifestations, but in all cases there is a steady and rapid progression of the disease (Sorokina et al., 2016). Chronic glomerulonephritis is of undulating nature, when the period of remission alternates with exacerbation, when the clinical findings resemble or become similar to acute glomerulonephritis.

At present, the involvement of the genetic component in the development and progression of chronic kidney disease, including CGN, is actively studied [Piotrowski et al., 2010; Litovkina et al., 2014a; Bagci et al., 2015]. According to modern literature, chemokines play an important role in the development of renal pathologies [Anders et al., 2010], which are able to control the migration of various types of leukocytes with the relative receptors from the bloodstream to the tissues, inflammation foci, autoimmune process, participate in activation and differentiation of leukocytes, angiogenesis, fibrogenesis (Lo et al., 2011). However, it is worth noting that the importance of the genetic factors of chemokines in the development of chronic kidney disease has not been sufficiently studied; the available research results are presented mainly in foreign sources [Piotrowski et al., 2010; Litovkina et al., 2014b; Bagci et al., 2015], while in the Russian Federation such works are rare.

Objective of this study was to evaluate the polymorphic loci of chemokines (rs1719153, rs4512021, rs2107538, rs2857657 CCL2, rs1801157 CXCL12) as possible markers of accelerated impairment of the renal function in patients with chronic kidney disease.

MATERIALS AND METHODS

The analysis of polymorphic loci of chemokines was conducted in 700 people: 238 patients with chronic glomerulonephritis (mean age 39.58±14.58 years) and 462 controls (42.12±5.19 years, $p>0.05$). Formation of samples of patients and the control was carried out by a continuous method in the period from 2009 to 2011. The samples included Russian natives of the Central Black Earth region of Russia having no family ties with each other. The patients were included in the study group only after the diagnosis of the disease, confirmed with the help of clinical and laboratory-instrumental methods of examination, by the staff of the Nephrology Unit of St. Joasaph Belgorod Regional Clinical Hospital. The investigated groups of patients with CGN and control are comparable by gender, age characteristics, place of birth and nationality.

As the material for the study we used 8-9 ml of venous blood taken from the cubital vein of a proband. A genomic DNA was isolated from peripheral blood by standard methods (Miller et al., 1988).

Analysis of all loci was carried out by the method of polymerase chain reaction of DNA synthesis with the use of standard primers and probes. Genotyping of DNA markers was performed by the method of analysis of allelic discrimination by the Taq-Man probe method with real-time PCR.

To assess the compliance of the observed distribution of genotypes with the expected one, based on Hardy-Weinberg equilibrium, we used χ^2 test, the observed heterozygosity, the expected heterozygosity, and the Wright fixation index. A comparison of the study groups of patients by the level of creatinine was carried out using the Mann-Whitney test; median (Me) and interquartile range (Q25-Q75) were used for the description. During the study, the heterozygote group was combined with that group of homozygotes that had no differences in median and interquartile interval values from heterozygous carriers or were the least.

Analysis of associations of renal survival with polymorphic genetic markers was carried out using the Kaplan-Mayer multiplier method.

ISSN: 0975-8585

RESULTS AND DISCUSSION

All patients with CGN and individuals in the control group underwent typing of five molecular-genetic markers of chemokines: regulator of normal T-cell expression activity (rs2107538), β-cell precursor growth factor (rs1801157), monocyte chemoattractant protein 1 (rs2857657), interferon-inducible α T-cell chemoattractant (rs4512021), and macrophage inflammatory protein 1β (rs1719153).

The study of the distribution of the genotypes of the studied polymorphic loci of chemokines showed that for all the genetic polymorphisms examined in the control sample and in the group of CGN patients, the empirical distribution of the genotypes corresponds to the theoretically expected at Hardy-Weinberg equilibrium ($p>0.05$). Comparative analysis of the frequencies of alleles and genotypes of the investigated loci among patients with CGN and control group revealed no statistically significant differences ($p>0.05$).

Next, the following quantitative pathogenetically significant indices in CGN patients were examined: the age of the manifestation of the disease, the level of creatinine, hematuria, and proteinuria. Since the distribution of the analyzed indicators, estimated using the Shapiro-Wilk test, does not correspond to the law of normal distribution ($p<0.05$) (Table 1), the median (Me) and interquartile range (Q25-Q75) were used to describe the quantitative indices in question, and for comparison of individuals with different genotypes by these parameters a nonparametric method - the Mann-Whitney test - was used.

Table 1: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, µmol/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

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 study of the links of the chemokine loci in question with these pathogenetically significant quantitative indicators revealed significant associations only for *CXCL11* A/G genetic polymorphism (rs 4512021) with the creatinine level in CGN patients (Table 2). CGN patients with *CXCL11* AA and AG genotypes have significantly higher creatinine levels (median 119.0 µmol/l lower quartile 94.4 µmol/l, upper quartile 541.0 µmol/l compared to individuals with the GG genotype (median 101.0 µmol/l, interquartile interval - 87.0-181.0 µmol/l, p=0.05, respectively.) Thus, the data obtained may indicate that the *CXCL11* A/G locus (rs4512021) is associated with a renal function.

The evaluation of renal survival in patients with CGN, depending on the genetic polymorphisms of chemokines, conducted using the Kaplan-Meyer multiplier method, which allows determining the influence of any factors, including genetic ones, on a time before the onset of the studied outcome (renal function impairment) presented the following results: rs1719153, p=0.58; rs4512021, p=0.78; rs2107538, p=0.46; rs2857657; rs1801157, p=0.32. We should note the absence of statistically significant associations of polymorphic loci under investigation with the rate of progression of chronic glomerulonephritis ($p>0.05$).

ISSN: 0975-8585

Table 2: The level of creatinine in patients with chronic glomerulonephritis, depending on the genetic polymorphisms of chemokines

SNP	Genotype	Level of creatinine, µmol/L		
		N	Me	Q25-Q75
rs1719153	+1931AA	114	110.5	92.5-313.0
	+1931AT, +1931TT	111	119.0	93.2-685.0
	p	>0.05		
rs2107538	-403AA	4	180.0	135.5-416.5
	-403GG, -403GA	180	110.0	91.65-528.5
	p	>0.05		
rs 2857657	GG	10	95.0	71.00-54.0
	CC, CG	218	112.5	94.0-401.0
	p	>0.05		
rs1801157	-801AA	8	102.0	98.0-329.0
	-801GG, -801GA	218	112.0	92.5-401.0
	p	>0.05		
rs4512021	AA, AG	218	119.0	94.4-541.0
	GG	8	101.0	87.0-181.0
	p	**0.05**		

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

According to current literature, a β-cell precursor growth stimulating factor (stromal cell factor - CXCL12) and interferon-inducible T-cell alpha chemoattractant (CXCL11) play a key role in providing an immune response in the chemokine family belonging to the α-chemokine family (Lo et al., 2011). CXCL11 is an important chemotactic factor for T-lymphocytes; it activates Th1CD4 T-cells, NK cells, monocytes in the inflammatory focus, which ultimately can play an important role in maintaining inflammatory processes in the kidney, leading to a decrease in its function. Interferons regulate the production of CXCL11 (Lazzeri et.al., 2002). Interferon γ in combination with tumor necrosis factors and bacterial liposaccharides stimulates the production of interferon-inducible α T-cell chemoattractant by neutrophils. Increased production of CXCL11 also results from the combined effect of interferons γ and IL-1 (Vielhauer et al., 2009; Anders et al., 2010).

CONCLUSION

Thus, the study of the links of polymorphic loci of chemokines (rs1719153, rs4512021, rs2107538, rs2857657, rs1801157) with renal function in patients with chronic glomerulonephritis revealed that the CXCL11 AA and AG genotypes (rs4512021) (p=0.05) are the marker of high creatinine level. No statistically significant associations of investigated genetic polymorphisms were detected with the rate of progression of chronic glomerulonephritis (p>0.05).

REFERENCES

[1] Anders, H.J., Sayyed, S. A., Vielhauer, V., 2010. Questions about chemokine and chemokine receptor antagonism in renal inflammation. Nephron. Exp. Nephrol., 114(2):33-38.

[2] Bagci, B., Bagci, G., Candan, F., Ozdemir, O., Sezgin, I., 2015. The protective effect of MCP-1 -2518 A>G promoter polymorphism in Turkish chronic renal failure patients requiring long-term hemodialysis. Int Urol Nephrol. ,47(3): 551-556.

[3] Lazzeri, E., Lasagni, L., Serio, M., Romagnani, S., Romagnani, P., 2002. Cytokines and chemokines in nephropathies and renal transplant. G Ital Nefrol., 19(6): 641-9.

[4] Lebherz-Eichinger, D., Klaus, D.A., Reiter, T., Horl, WH, Haas M, Ankersmit H.J., 2014. Increased chemokine excretion in patients suffering from chronic kidney disease. Transl Res., 164(6): 433-43.

[5] 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): 112-116.

[6] 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): 1079-1082.

[7] Lo, D.J., Weaver, T.A., Kleiner, D.E., Mannon, R.B., 2011. Chemokines and their receptors in human renal allotransplantation. Transplantation, 15(91): 70–77.

[8] 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): 1215.

[9] Nekipelova, E.V., Novakova, O.N., Yakunchenko, T.I., Krikun, E.N., Zhernakova, N.I., Efremova, O.A., 2016. Clinical and Genetic Research of Chronic Glomerulonephritis. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 7(6): 3222-3227.

[10] Piotrowski, P., Lianeri, M., Gasik, R., Roszak, A., Olesinska, M., Jagodziński, P.P., 2010. Monocyte chemoattractant protein-1 -2518 A/G single nucleotide polymorphism might be associated with renal disease and thrombocytopenia of SLE. J Biomed Biotechnol., 2010:130265.

[11] Sigdel, T.K., Li, L., Tran, T.Q., Khatri, P., Naesens, M., Sansanwal, P., Dai, H., Hsieh, S.C., Sarwal, M.M., 2012, Non-HLA antibodies to immunogenic epitopes predict the evolution of chronic renal allograft injury. J Am Soc Nephrol., 23(4): 750-763.

[12] Sorokina, I., Nekipelova, E., Yakunchenko, T., Novakova, O., Krikun, E., Zhernakova, N., Polonikov, A., 2016. Genetic Factors of Decreased Kidney Function in Patients with Chronic Glomerulonephritis. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 7(6): 3228-3232.

[13] Vielhauer, V., Allam, R., Lindenmeyer, M. T., Cohen, C.D., Draganovici, D., Mandelbaum, J., Eltrich, N., Nelson, P.J., Anders, H.J., Pruenster, M., Rot, A., Schlöndorff, D.,Segerer, S., 2009. Efficient renal recruitment of macrophages and T cells in mice lacking the Duffy antigen/receptor for chemokines. Am. J. Pathol., 175(1): 119-131.