

The effectiveness of glucocorticoid therapy in patients with chronic glomerulonephritis, depending on the polymorphic markers of cytokine genes

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ABSTRACT

Aim: The paper presents the results of a study of interrelationships of polymorphic cytokine loci (rs1800629 *TNFA*, rs909253 *Lta*, rs767455 *TNFR1* and rs1800469 *TGFβ-1*) with the features of glucocorticoid therapy in patients with chronic glomerulonephritis. **Method:** The study of therapy efficiency (medication with glucocorticoids, cytostatics, and angiotensin-converting enzyme inhibitors) was conducted among 169 patients with CGN (79 men and 90 women). The average age of patients was 36.2 ± 8.9 years; the duration of disease was 7.4 ± 5.6 years. In this research, homozygotes by pro-inflammatory and fibroblastic alleles were combined into one group with heterozygotes. **Results and Discussion:** The research materials were processed by statistical methods, using the program Statistica 8.0. The criterion cChi-square was applied with the aim to analyze the compliance of the observed distribution of genotypes with the expected, based on the Hardy–Weinberg equilibrium. In the process of comparative analysis of the frequencies of alleles and genotypes of the studied loci between the control and case groups of patients, the Chi-square criterion was used, with Yates correction for continuity. **Conclusion:** It has been established, that in patients with chronic glomerulonephritis, which have proinflammatory gene alleles of lymphotoxin α (rs909253) and the tumor necrosis factor receptor (rs767455), glucocorticoid therapy is not very effective (only 10-30% of these patients are steroid sensitive). In case of non-inflammatory alleles of these genes, the efficiency of hormone therapy is maximum: steroid-sensitive nephrotic syndrome is observed in 73-90% of patients ($p < 0.001$).

KEY WORDS: Chronic glomerulonephritis, Cytokines, Gene polymorphism, Glucocorticoids

INTRODUCTION

One of the main directions in the development of modern science and medicine is the molecular genetic study of endogenous/genetic factors, which lead to the disease.^[1] Among the significant number of approaches in the study of genetic susceptibility to various pathologies, the search for associations of polymorphic genetic loci with disease is widely used.^[1-7]

Chronic glomerulonephritis (CGN) is an immune complex kidney disease with predominant lesion of the renal glomeruli, leading to progressive death of glomeruli, hypertension, and renal failure.^[8] Majority

of patients with glomerulonephritis have CGN;^[9] their quantity far exceeds the number of people with acute glomerulonephritis. CGN can be either the consequence of acute nephritis or can be primarily chronic. The prevalence of this renal pathology is steadily increasing worldwide.^[10] Therefore, the issue of identifying criteria, which allow to evaluate the prognosis of clinical course and treatment of CGN, is highly relevant.

According to the modern literature sources, cytokines play an important role in the development of renal pathologies, including CGN. They have a leading role in the pathogenesis of immune inflammation.^[11,12] Cytokines are low-molecular peptides, produced by cells. They perform short-distance regulation of intercellular and intersystem interactions, which determine cell survival, stimulation and inhibition of their growth, functional

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activity, and apoptosis of cells.^[13,14] Some sources emphasized the role of tumor necrosis factor (TNF) alpha, TNF receptor, and transforming growth factor β 1 in the pathogenesis of CGN.^[15-17] In this regard, cytokine genes can be considered as candidate genes of CGN.^[18]

It is worth noting that, despite the significant amount of work, aimed at studying the treatment of patients with CGN, this issue remains highly relevant and quite controversial. Special attention should be paid to glucocorticoid therapy, which has a pronounced anti-inflammatory and immunosuppressive effect.^[19] Moreover, the use of these drugs also changes the course of glomerulonephritis.

Thus, the purpose of this study is to evaluate the effectiveness of glucocorticoid therapy in patients with CGN, depending on the polymorphic cytokine loci rs1800629 *TNF α* , rs909253 *Lta*, rs767455 *TNFR1*, and rs1800469 *TGF β -1*.

MATERIALS AND METHODS

The analysis of polymorphic cytokine loci was carried out among 370 people: 202 patients with CGN (average age 41.08 ± 13.97 years) and 168 people in the control group (average age 42.20 ± 6.28 years, $P > 0.05$). The studied groups consisted of the individuals of Russian nationality, who were natives of the Central Black Earth Region of Russia, and were not related to each other. Patients were included in the certain group only after making the diagnosis, confirmed by clinical and laboratory-instrumental methods of examination, by the staff of the Nephrology Department of St. Joseph Belgorod Regional Clinical Hospital. The studied groups of patients with CGN and control group are comparable by gender, age characteristics, place of birth, and nationality.

Venous blood in a volume of 8–9 ml, taken from the ulnar vein of the proband, was the material for the study. Genomic DNA was extracted from peripheral blood using standard methods.^[20]

Genotyping of DNA markers (rs1800629 *TNF α* , rs909253 *Lta*, rs767455 *TNFR1*, and rs1800469

TGF β -1) was performed by the method of polymorphism analysis of the length of restriction fragments of PCR amplification products of specific genome regions, using appropriate restriction enzymes.

The study of therapy efficiency (medication with glucocorticoids, cytostatics, and angiotensin-converting enzyme inhibitors) was conducted among 169 patients with CGN (79 men and 90 women). The average age of patients was 36.2 ± 8.9 years; the duration of disease was 7.4 ± 5.6 years. In this research, homozygotes by pro-inflammatory and fibroblastic alleles were combined into one group with heterozygotes.

The research materials were processed by statistical methods, using the program Statistica 8.0. The criterion cChi-square was applied with the aim to analyze the compliance of the observed distribution of genotypes with the expected, based on the Hardy–Weinberg equilibrium. In the process of comparative analysis of the frequencies of alleles and genotypes of the studied loci between the control and case groups of patients, the Chi-square criterion was used, with Yates correction for continuity. The calculations were performed in 2×2 contingency tables. Statistical differences were considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

All patients with CGN (202 people) and the individuals of the control group (168 people) received typing of four molecular genetic markers of cytokines: *TNF α* (rs1800629 *TNF α*), lymphotoxin α (rs909253 *Lta*), TNF receptor (rs767455 *TNFR1*), and transforming growth factor β 1 (rs1800469 *TGF β -1*). The inclusion of the above genetic polymorphisms in the analysis is due to the pathogenetic significance of cytokines, determining by them, for CGN.

The investigation of genotype frequencies of polymorphic gene markers showed that for all the considered markers in the control group and for most markers in the group of patients with CGN, the empirical distribution of genotypes corresponds to the theoretically expected at Hardy–Weinberg equilibrium ($P > 0.05$) [Table 1]. However, among patients with

Table 1: Summary information about the studied polymorphisms

SNP	Studied groups	Minor allele	MAF (%)	HWE	
				χ^2	P
rs1800629 <i>TNFα</i>	Case	A	16.25	6.83	>0.05 >0.05
rs1800629 <i>TNFα</i>	Control	A	11.64	0.43	
rs909253 <i>Lta</i>	Case	G	27.92	0.87	>0.05 >0.05
rs909253 <i>Lta</i>	Control	G	30.95	0.15	
rs767455 <i>TNFR1</i>	Case	G	45.71	11.10	<0.01 >0.05
rs767455 <i>TNFR1</i>	Control	G	54.48	0.98	
rs1800469 <i>TGFβ-1</i>	Case	T	42.96	0.89	>0.05 >0.05
rs1800469 <i>TGFβ-1</i>	Control	T	38.34	1.72	

MAF: Minor allele frequency, HWE: Hardy–Weinberg equilibrium. P values were calculated using the Chi-square test

Table 2: Effects of glucocorticoid therapy, depending on polymorphic cytokine loci

SNP	Genotypes	The effectiveness of therapy (%)	
		Steroid-sensitive nephrotic syndrome (n=27)	Steroid-resistant nephrotic syndrome (n=15)
rs1800629 <i>TNFα</i>	GG	62.5	37.5
	GA, AA	70.0	30.0
	χ^2 P		0.81 P>0.05
rs909253 <i>Lta</i>	GG, AG	50.0	50.0
	AA	73.1	26.9
	χ^2 P		10.22 P<0.01
rs767455 <i>TNFR1</i>	AA, AG	53.1	46.9
	GG	90.0	10.0
	χ^2 P		31.80 P<0.001
rs1800469 <i>TGFβ-1</i>	CC	61.5	38.5
	TC, TT	65.5	34.5
	χ^2 P		0.09 P>0.05

CGN, there was a deviation from Hardy–Weinberg equilibrium for the locus *TNFR1* (rs767455), due to a decrease in actual heterozygosity, compared to theoretical ($\chi^2 = 11.10$, $P < 0.01$), as evidenced by negative values of fixation index ($D = -0.23$).

Comparative analysis of the frequencies of alleles and genotypes of the studied loci among patients with CGN and the control group did not reveal statistically significant differences ($P > 0.05$).

At the next stage of work, the investigation was conducted on the effectiveness of glucocorticoid therapy in patients with CGN ($n = 42$), depending on the polymorphic cytokine loci [Table 2]. It has been established that among patients with CGN, with non-inflammatory allele A (AA genotype) of lymphotoxin α gene (rs909253) and allele G of the TNF receptor gene (rs767455) (GG genotype), the proportion of patients with steroid-sensitive nephrotic syndrome is significantly higher, compared to individuals with pro-inflammatory alleles of these genes: For lymphotoxin α gene – 73.1% and 50.0%, respectively, ($P < 0.01$) and for the TNF receptor gene – 90.0% and 53.1%, respectively ($P < 0.001$) [Table 2]. As for other analyzed genes (rs1800629 *TNF α* and rs1800469 *TGF β -1*), there were no significant differences in the proportion of steroid-sensitive patients, among people with pro-inflammatory and non-inflammatory genotypes. In case of combination of non-inflammatory alleles of lymphotoxin α genes and the TNF receptor in the genotype, the proportion of steroid-sensitive patients is 88.2%, that is, almost 3 times higher than the similar indicator for patients with pro-inflammatory alleles of these genes – 33.3% ($P < 0.001$).

According to literature data,^[21] individuals with “wild” (non-inflammatory) alleles of cytokine genes have reduced levels of cytokines. In patients with CGN,

they are effectively suppressed by the administration of glucocorticoids. Medicinal preparations, belonging to this group, and used for the treatment of CGN, have a pronounced immunosuppressive and anti-inflammatory effect.^[19] However, in case of elevated concentrations of cytokines observed according to Karplus *et al.*, 2002,^[22] in individuals with mutant (pro-inflammatory) alleles of cytokine genes, monotherapy for the arresting of CGN exacerbation is insufficient, and simultaneous administration of glucocorticoids and cytostatics is required (combined therapy). In this case, the joint pathogenetic action of drugs, belonging to these groups, is more pronounced – they produce anti-inflammatory and immunosuppressive effects, and that ultimately provides a positive therapeutic outcome in the process of CGN treatment.

It should be noted that the data obtained are important for practical nephrology because molecular genetic typing of loci rs1800629 *TNF α* , rs909253 *Lta*, rs767455 *TNFR1*, and rs1800469 *TGF β -1* in patients with CGN will allow not only to identify groups of patients with a poor prognosis of CGN (early development of chronic renal failure, end stage of chronic renal failure, etc.) but also to determine a more optimal strategy for CGN treatment.

CONCLUSION

Thus, in the framework of this work, the investigation of the effectiveness of glucocorticoid therapy was conducted. It was performed depending on the polymorphic cytokine loci (rs1800629 *TNF α* , rs909253 *Lta*, rs767455 *TNFR1*, and rs1800469 *TGF β -1*), in patients with CGN, who are the residents of the Central Black Earth Region of Russia. It has been established that in patients with CGN, which have pro-inflammatory gene alleles of lymphotoxin α (rs909253 *Lta*) and the TNF receptor (rs767455

TNFR1); glucocorticoid therapy is not very effective (only 10–30% of these patients are steroid sensitive). In case of non-inflammatory alleles of these genes, the efficiency of hormone therapy is maximum: Steroid-sensitive nephrotic syndrome is observed in 73–90% of patients.

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