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Genes involved in the regulation of vascular homeostasis determine renal survival rate in patients with chronic glomerulonephritis

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ABSTRACT

Chronic glomerulonephritis (CGN) is one of the most severe kidney diseases. Genes of vascular reactivity are thought to play an important role in development and progression of CGN. In this study, we analyzed association of genes of vascular homeostasis with hypertension and renal survival of CGN patients. The study sample included 238 patients with CGN and 304 healthy subjects of population control. Ten polymorphisms of ten genes of vascular homeostasis were genotyped through polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP) analysis and TaqMan assays. Association of the genotypes with renal survival was analyzed by the Kaplan–Meier estimator. Genotypes 311SC and 311SS of the *PON2* gene, (−1166)AC and (−1166)CC of the *AGTR1* gene, (+46)AA of the *ADRB2* gene, and 198KK and 198KN of the *EDN1* gene were associated with decreased rate of renal survival of the patients. Polymorphisms S311C *PON2*, (−1166)A/C *AGTR1*, (+46)G/A *ADRB2*, and K198N *EDN1* were associated with the accelerated decline in kidney function in the CGN patients.

1. Introduction

Chronic glomerulonephritis (CGN) is a genetically determined immune-mediated glomerular disease, which often associated with hypertension, and may result in renal failure and respective increased mortality (Nickolas et al., 2004). Genes of vascular homeostasis play an important role in development and progression of CGN. They regulate renal hemodynamics, mesangial cell proliferation, synthesis and degradation of extracellular matrix, and the rate of development of glomerulosclerosis (Egido, 1996; Jensen and Pedersen, 1997).

CGN often results in development of arterial hypertension (AH). There is a close relationship between hypertension and renal function (Best and Holmes, 2003). Impaired excretion of sodium and water by kidneys is considered as one of the main mechanisms of essential hypertension. In turn, essential hypertension contributes to kidney impairment through vasoconstriction, structural changes in renal arterioles, and parenchymal ischemia (Best and Holmes, 2003). Pathogenesis of arterial hypertension in renal disease is complex. One of the main factors is the activation of pressor hormone systems (sympathoadrenal system, renin–angiotensin–aldosterone system, endothelial constrictor hormones, endothelin). Therefore, genetic markers of these hormones have attracted an increased attention in recent years as possible risk factors for glomerulopathy (Buraczynska et al., 2006).

In this study, we examined polymorphisms of the genes of vascular homeostasis for their possible association with development of hypertension and renal survival in Russian patients suffering from CGN. The polymorphisms were selected on the basis of their possible contribution to pathogenesis of CGN and effect on expression of the genes. In particular, allele D at locus I/D of the *ACE* gene confers higher expression to the enzyme than allele I (Ueda et al., 1996). Allele (−6)A of the *AGT* gene is associated with higher expression of angiotensinogen (Brand et al., 2000). The (−1166)A/C polymorphism of the *AGTR1* gene is known to alter the structure of a *cis*-element within the gene that increases gene expression (Wang et al., 2006). Homozygotes 4a4a of the *NOS3* gene have lower activity of the enzyme as compared to the 4b4b homozygotes (Dosenko et al., 2006). Allele (+6986)A of the *CYP3A5* gene is associated with increased expression of the respective enzyme (Givens et al., 2003). Importantly, the above polymorphisms have been associated with the risk of hypertension in various diseases (Calle et al., 2006; Misono et al., 2009; Tiret et al., 1999).

Abbreviations: CGN, chronic glomerulonephritis; AH, arterial hypertension; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; GFR, glomerular filtration rate; DNA, deoxyribonucleic acid; SNP, single-nucleotide polymorphism; ACE, angiotensin I converting enzyme; NOS3, nitric oxide synthase 3; PON2, paraoxonase-2; AGT, angiotensinogen; AGTR1, angiotensin II receptor, type 1; EDN1, endothelin 1; CYP3A5, Cytochrome P450, Family 3, Subfamily A, Polypeptide 5; GNB3, guanine nucleotide binding protein (G Protein), beta polypeptide 3; ADD1, adducin 1 (alpha); ADRB2, adrenoceptor beta 2; PCR, polymerase chain reaction; HWE, Hardy–Weinberg equilibrium; Ors, odds ratios; CIs, 95% confidence intervals; p_c, Bonferroni correction.

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2. Methods

2.1. Subjects

The study protocol was approved by the Ethics Committee of Belgorod State National Research University. All subjects signed an informed consent before entering the study. In total 542 subjects, including 238 patients with CGN and 304 individuals of population control, were recruited for this study. All study subjects were unrelated Russians from Central Chernozem Region of Russia (Belgorod). Patients were enrolled in the case group only after the clinically confirmed diagnosis of CGN. Clinical and laboratory examination of patients was conducted at the Nephrology Clinic of Belgorod Regional Clinical Hospital.

Blood samples were taken during the period of the patient's hospitalization. The patients were examined monthly by a nephrologist for the period of 6 months to 1 year. Blood pressure (BP) level was measured daily in the morning, in the upper-sitting position of the patient. At least 3 measurements were made and the average systolic blood pressure (SBP) and diastolic blood pressure (DBP) were calculated. SBP ≥ 140 mm Hg and/or DBP ≥ 90 mm Hg were considered as an indication of AH. BP ≥ 160/100 mm Hg in patients taking antihypertensive drugs was considered as an indicator of severe course of AH. The exclusion criteria for the CGN patients were the history of diabetes mellitus or hypertension.

Renal survival in patients with CGN was assessed in a group of 138 individuals with non-terminal renal failure. Of these, 104 patients had normal renal function (creatinine level <140 μmol/l) and 34 patients had chronic renal failure (creatinine level was 140 μmol/l during 6 months of observation). The progress of chronic renal failure was analyzed from the onset of the disease. The endpoint of the observation was doubling of baseline creatinine. Renal function was assessed through glomerular filtration rate (GFR) which was estimated by Cockroft–Gault's formula (Cockcroft and Gault, 1976).

2.2. DNA isolation

Genomic DNA was isolated from 10 ml of whole blood using a method proposed by Miller et al. (1988).

2.3. Genotyping

The ten DNA polymorphisms were genotyped through the analysis of amplified fragment length polymorphism (I/D polymorphism of the *ACE* gene, VNTR polymorphism of the *NOS3* gene), the analysis of restriction fragment length polymorphisms (S311C of the *PON2* gene (rs7493), −6A/G the *AGT* gene (rs5051), −1166A/C of the *AGTR1* gene (rs5186)) and Tag-Man allele discrimination analysis (K198N of the *EDN1* gene (rs5370), +6986G/A of the *CYP3A5* gene (rs776746), G/A (rs2301339) of the *GNB3* gene (rs2301339), G460W of the *ADD1* gene, +46G/A of the *ADRB2* gene (rs1042713)). The structure of the primers and PCR conditions for genotyping the DNA polymorphisms are described in detail elsewhere (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).

2.4. Statistical analysis

The allele frequencies were checked for departures from the Hardy–Weinberg equilibrium (HWE) using the chi-square test. Association of the DNA markers with CGN in hypertensive patients was assessed through the chi-square test with Yates' correction. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to estimate the association between the polymorphisms and the risk of CGN in the hypertensive patients. The calculations were adjusted for multiple testing by Bonferroni correction (p_c).

Table 1
Characteristics of the subjects from the case and control groups.

Characteristics	Cases	Controls
Total	238	304
Males	127 (53.4%)*	164 (53.9%)
Females	111 (46.6%)*	140 (46.1%)
Age, years	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
Creatinine, μmol/l	337.2 ± 44.1**	130.4 ± 7.8
GFR, ml/min	28.2 ± 1.8	81.6 ± 3.4

* $p > 0.05$.
** $p < 0.001$.

The patients were divided into three groups according to their BP: below 140/90 mm Hg (the first group, 84 patients, 36.2%), from 140/90 to 159/100 mm Hg (the second group, 96 patients, 41.4%), and above 160/110 mm Hg (the third group, 52 patients, 22.4%).

Association of renal survival with the genotypes was analyzed using the Kaplan–Meier test. A software package STATISTICA for Windows v. 6.0 (StatSoft, Inc.) was used for the analyses.

3. Results

The average age of the CGN patients and the population control subjects was similar (39.58 ± 14.58 years and 42.20 ± 6.28 years, respectively, $p > 0.05$). The main characteristics of the study subjects are shown in Table 1. Notably, patients with CGN had higher levels of both systolic (148.4 ± 26.5 mm Hg) and diastolic (92.7 ± 14.0 mm Hg) blood pressures as compared to the control group ($p < 0.001$). As shown in Table 1, creatinine level in patients with CGN was 337.2 ± 44.1 μmol/l, significantly higher than that in the control group ($p < 0.001$). Glomerular filtration rate in CGN patients was 28.2 ± 1.8 ml/min, which was significantly lower than that in the control group ($p < 0.001$).

All studied DNA polymorphisms (except G460W of the *ADD1* gene, $p < 0.05$) showed no deviation from the HWE (Table 2).

No statistically significant differences in allele and genotype frequencies were found between the CGN patients and the controls ($p > 0.05$).

Table 2
Summary information about the studied polymorphisms.

Polymorphism	Studied groups	Minor allele	MAF (%)	HWE	
				χ^2	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 χ^2 test.