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INVESTIGATION OF THE EFFECT OF PLA2R1 POLYMORPHISM IN PATIENTS WITH MEMBRANOUS NEPHROPATHY

MEMBRANÖZ NEFROPATİLİ HASTALARDA PLA2R1 POLİMORFİZMİNİN ETKİSİNİN ARAŞTIRILMASI

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ABSTRACT

Objective: Membranous nephropathy (MN) is the most common cause of nephrotic syndrome in adults. Phospholipase A2 receptor (PLA2R1) is a glycoprotein belonging to the mannose receptor family (~180 kDa). PLA2R1 was found to be associated with idiopathic MN pathogenesis, and the presence of PLA2R1 antibodies was supported by the diseases. In this study, the association of PLA2R1 rs35771982 with membranous nephropathy was investigated.

Material and Methods: The study included 88 patients diagnosed with MN and 101 healthy individuals unrelated to the patients. The PLA2R1 rs35771982 levels of the patient and control groups were examined using the Real Time PCR (Polymerase Chain Reaction) method.

Results: The comparison of the patients with healthy controls in this study showed that the rs35771982 GG genotype was significantly higher in the patients. The genotype of rs35771982 in the Turkish population was found to be common to the CG genotype.

Conclusion: Our findings revealed that the PLA2R1 rs35771982 GG genotype is associated with susceptibility to MN.

Keywords: Membranous nephropathy, PLA2R1, rs35771982, Real-time PCR

ÖZ

Amaç: Membranöz nefropati (MN), erişkinlerde nefrotik sendromun en sık nedenidir. Fosfolipaz A2 reseptörü (PLA2R1), mannoz reseptör ailesine (~180 kDa) ait glikoproteindir. PLA2R1, idiyopatik MN patogenezi ile ilişkili bulunmuş, PLA2R1 antikorlarının varlığının hastalıkla ilişkisi desteklenmiştir. Bu çalışmada PLA2R1 rs35771982'nin Membranöz Nefropati ile ilişkisi araştırıldı.

Gereç ve Yöntemler: Çalışmaya MN tanısı alan 88 hasta ve bu hastalarla ilişkisi olmayan 101 sağlıklı birey dahil edildi. Hasta ve kontrol gruplarının PLA2R1 rs35771982'si Real Time PCR (Polimeraz Zincir Reaksiyonu) yöntemi ile incelendi.

Bulgular: Hastaları sağlıklı kontrollerle karşılaştırdığımızda rs35771982 GG genotipinin hastalarda anlamlı derecede yüksek olduğu görüldü. Türk popülasyonunda rs35771982 CG genotipinin yaygın olduğu bulundu.

Sonuç: PLA2R1 rs35771982 GG genotipinin MN'ye duyarlılıkla ilişkili olduğu bulundu.

Anahtar Kelimeler: Membranöz nefropati, PLA2R1, rs35771982, Gerçek zamanlı PZR

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INTRODUCTION

Membranous nephropathy (MN) is a glomerular pathology that is the most common cause of idiopathic nephrotic syndrome in adults (1). Kidney biopsy is used in the definitive diagnosis of the disease. Considering that biopsy protocols differ in many countries, there are regional differences in the incidence of the disease. The disease is seen twice as often in men compared with the level in women. MN is a chronic disease and spontaneous remission occurs in approximately 30% of the patients. Despite spontaneous remission, 30-40% of MN patients progress to end-stage renal disease (ESRD) within 5 to 15 years (2). Approximately 75% of membranous nephropathy cases are idiopathic (Primary MN-IMN). Secondary MN is associated with autoimmune diseases, infection, and malignancies.

Phospholipase receptor (PLA2R1) is a type I transmembrane receptor that is a member of the mannose receptor family and has been identified as a target podocyte antigen involved in membranous nephropathy. Researchers have shown that the circulating antibodies of the IgG4 subtype against the epitope in PLA2R develop in 70-80% of patients with idiopathic MN (3). Some studies have shown that polymorphisms in the PLA2R1 gene may have a direct effect on the function of the gene (4). The rs35771982 variants are the conversion of the Guanine (G) allele to Cytosine (C) in exon 5 of the gene, which causes the replacement of Histidine with Aspartic acid at position 300 of the PLA2R protein. The G allele of rs35771982 in exon 5 results in a residue change (H300D) at the second of the 8 CTLDs in the protein structure. They reported that this change reduces the collagen-binding activity of cells and may alter antigenicity (5). Several studies have shown that this SNP locus is significantly associated with IMN in Korea, Taiwan, European Caucasians, Beijing, North American Caucasians, and the Indian population (6-10). Two different Chinese cohorts confirmed that the variations in PLA2R1 SNPs (rs35771982, rs4664308, rs3749117) were the risk factors for idiopathic membraneous nephropathy (11, 12).

Researchers studying IMN patients suggested that the G allele of SNP rs35771982 was more selectively expressed in patients than in the controls. The rs35771982 CC genotype is protective, and the rs35771982 GG genotype is a high-risk genotype (7).

Based on the conducted studies, we suggest that the rs35771982 genotype in the gene of the transmembrane protein PLA2R1, which causes podocyte damage, may have an effect on protein function. For this purpose, the association of PLA2R1 rs35771982 with membranous nephropathy was investigated.

MATERIAL AND METHOD

Patients and controls

Eighty-eight patients who presented to the Istanbul Faculty of Medicine, Department of Nephrology and were diagnosed with MN under biopsy were included in the study. One tube of blood, 5 cc EDTA (Ethylenediamine tetraacetic acid) was drawn from the patients. The clinicopathological findings of the patients were obtained from the Nephrology Department. The control group included 101 healthy individuals aged over 18 years and younger than 70 years who had no consanguineous relationship with each other and had no diagnosed kidney disease in their family. One tube of blood with 5 cc EDTA was collected from the control groups included in the study. After the ethics committee approval was obtained, the informed consent form was obtained from the volunteers (patients and healthy individuals) included in the study. This study was approved by the Istanbul Medical Faculty Clinical Research Ethics Committee (Date: 28.09.2017, No: 16).

rs35771982 genotyping

Blood samples were centrifuged immediately after being collected. EDTA tubes were used to isolate DNA (EZ1, QİAgen) and stored at 20 °C until the study period. The rs35771982 genotyping was performed with the Real Time Polymerase Chain Reaction (RT-PCR) method using the TaqMan SNP Genotyping (Thermo Scientific, Appliedbiosystems, Carlsbad, CA USA) kit. The probes used in this study were gene- specific FAM labelled MGB (minor Groove binder) probes. TaqMan MGB probes contain an MGB moiety at the 3' end, increasing the probe's melting temperature (Tm) and stabilising probe/target hybrids. TaqMan Universal Master Mix II was used. The results were read using the real-time PCR (Rotor Gene Q) instrument, which can read fluorescence by way of VIC and FAM channels. The sequence used is forward. (CCATCAGACCACTGCCAGCCAGCGT **[C/G]** TTCATCCAGCTGATTGAGGCCCATC)

Statistical analysis

Statistical analysis of the data was performed using the Statistical Package for the Social Sciences version 28.0 (IBM SPSS Corp., Armonk, NY, USA) software programme. Descriptive analysis and descriptive statistical methods were used. In the statistical analysis of the data, continuous data were tested using the Kolmogorov-Smirnov test for normality distribution. The mean ± standard deviations of the data in normal distribution were used. The control and patient groups were in Hardy–Weinberg balance. The chi-square test was used to compare the genotype between the patient and control groups. Chi-square and Fisher's exact tests were used to evaluate the categorical data within the patient group. The chi-square test was used to compare the genotype between the patient and control groups. The chi-square test was used for the odd ratio. One-way ANOVA test was used to measure the P value in table 3. Results were expressed as percentage values. p values<0.05 were considered statistically significant.

RESULTS

The mean age of the patient group was 53.59±13.82 years (age range 25-77y), while the mean age at diagnosis was 46.30±13.53years (age range 19-70y). The patient group consisted of 51 men (58%) and 37 women (42%). In our study, the mean age of 101 healthy individuals was 40.76±12.5 years. 49 men (48.5%) and 52 women (51.5%) were included in the control group. No statistical significance was observed in terms of gender and age in the evaluation of the patient and control groups (Table 1).

Table 1: Demographic and clinical data of the study groups

		Patient group	Control group
		mean±SD	mean±SD
Age		53.59±13.82	40.76±12.5
Age at diagnosis		46.3±13.5	
Gender	Male	51	49
	Female	37	52
BMI (kg/m^2)		78.90±14.7	
Systolic BP (mmHg)		131.42±19.2	
Diastolic BP (mmHg)		81.13±12.5	
Serum albumin (g/dl)		2.80±0.8	
Serum creatinine (mg/dl)		0.9±0.4	
Serum creatinine (mg/dl) follow-up		1.2±1.1	
Haemoglobin (g/dl)		12.93±2.3	
eGFR (ml/min/1.73m^2)		102.21±52.1	
Proteinuria		5677.79±3545.6	
Proteinuria follow-up		2869.82±3003.5	

BMI: Body Mass Index, Systolic BP: Systolic Blood Pressure, Diastolic BP: Diastolic Blood Pressure, eGFR: Glomerular Filtration Rate, *: p<0.05

Table 2: rs35771982 genotype and allele distribution in the patient and control groups

Genotypes	Patient group	Control group	OR	95%CI	p
	n: 88 (%)	n: 101 (%)			
CC	4 (4.5%)	9 (8.9%)	0.644	0.281-1.479	0.265
CG	40 (45.5%)	76 (75.3%)	0.524	0.388-0.708	**0.0001**
GG	44 (50.0%)	16 (15.8%)	2.150	1.618-2.857	**0.0001**
CG + CC vs GG	44 (50%)	85 (84.2%)	0.188	0.095-0.370	**0.0001**
Alleles	n:176 (%)	n:202 (%)			
C	48 (27.3%)	94 (%46.5)	0.623	0.481-0.807	0.828
G	128 (72.7%)	108 (53.5%)			
HWEp	0.171	0.052			

C: Cytosine, G: Guanin, HWEP: Hardy Weinberg, *: p<0.05

Table 3: Correlation of rs35771982 genotypes with clinical parameters

	CC	CG	GG	p
	mean±SD	mean±SD	mean±SD	
Age(years)	54.8±13.3	53.1±14	45.3±18	0.403
GDF-15	6448±5415	5915±3156	9613±6527	0.303
Proteinuria	5186±2735	6279±4362	5180±1996	0.395
Proteinuria tracing	2878±2615	2989±3440	1380±2183	0.678
Creatinine	0.86±0.35	0.95±0.53	0.81±0.45	0.595
Creatinine tracing	1.1±0.92	1.09±0.77	2.85±4.14	***0.01***
eGFR	95.9±35.5	107.1±63.6	122±77.8	0.457

GDF-15: Growth Differentiation Factor-15, eGFR: Glomerular Filtration Rate, C: Cytosin, G: Guanin, *: p<0.05

Distribution of the rs35771982 genotype in the patient and control groups

The variant homozygous GG genotype (p=0.0001) in the patients and the heterozygous CG genotype (p=0.0001) were found to be significantly higher in the control group. Although the wild CC genotype was numerically higher in the control group, there was no significant difference between the patient and control groups (p=0.05) (Table 2).

Relationship of the genotypes with the clinical parameters

Considering all genotypes of rs35771982, no significance was found between serum GDF-15 level, gender, age, serum creatinine, proteinuria, post-follow-up proteinuria,and eGFR. However, in patients with the CC genotype, the creatine value was found to be significantly higher after follow-up (p=0.01) (Table 3).

DISCUSSION

Membranous nephropathy (MN) is characterised with subepithelial immune complex deposition and thickening of the glomerular basement membrane. MN is typically a disease of adult age and is more common among men compared with the level in women. The progression of the disease is more rapid in older men diagnosed with membraneous nephropathy (13). No significant difference was detected in terms of gender between the patients included in this study. In addition, we found no correlation between disease progression and gender and age. The possible reason may be the difference in the progression of the disease and the smaller number of patients in the advanced stage.

Some studies have shown that polymorphisms in the PLA2R1 gene may have direct effects on the function of the gene. Studies with patients with MN have shown that PLA2R1 SNPs may be associated with the aetiology of the disease. In a study conducted in the Caucasus, researchers have shown that the rs35771982 GG genotype has a strong effect on anti-PLA2R1-positive patients compared with the effect on the negative patients. In the same study, PLA2R1 variants were evaluated in African Americans and no association was detected with the disease (5). In another study, the rs35771982 CC genotype was found to be significantly higher in idiopathic MN patients compared with the level in the control group (6). In a study conducted in China, researchers found that the CC genotype was disease-related and correlated with anti-PLA2R1 positivity (14). In another study, researchers found that the combination of the GG genotype at rs35771982 and AA genotype at rs2715928 was the highest risk of IMN (15). In this study, the evaluation of the rs35771982 genotypes showed that the GG genotype in patients was found to be significant compared with the control group, consistent with the literature. The heterozygous CG genotype was found to be statistically significant in the control group.

The rs35771982 G allele was reported to be a risk marker for the disease, and the C allele is protective in Japanese and Chinese populations (16, 17). A study conducted in the Caucasus found that the G allele was associated with the disease, while a study conducted in China found that the C allele was associated with the disease (5, 14). No significance was found in the allele basis comparison of the patient and control groups included in our study.

No relationship was found between the rs35771982 variants and renal survival and clinical parameters. Disease progression of the CG genotype in the Chinese population in Taiwan was correlated with a low remission rate. No significant difference was found in the gender distribution, age, body mass index, systolic/diastolic blood pressure, serum albumin and creatine levels, haematuria and proteinuria of the rs35771982 variants (7). Thiri et al. stated that the relationship of both alleles of rs35771982 with the disease was independent of the clinical symptoms (16). In our study, although 27 of 39 patients with the CG genotype entered remission, no statistical significance was found. No significant difference was found between the rs35771982 genotypes with gender, age, proteinuria, eGFR and creatine level.

In conclusion; the aim of our study was to determine whether the rs35771982 variant could be used as a serum biomarker that would enable us to diagnose patients with MN. More detailed and larger population research is needed to define the rs35771982 GG genotype as a biomarker that can be used in the diagnosis of the disease. Our study will also be able to eliminate the lack of data regarding the frequencies of rs35771982 variants, which have not been previously studied in the Turkish population.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Istanbul Medical Faculty Clinical Research Ethics Committee (Date: 28.09.2017, No: 16).

Informed Consent: After the ethics committee approval was obtained, the informed consent form was obtained from the volunteers (patients and healthy individuals) included in the study.

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Conflict of Interest: The authors have no conflict of interest to declare.

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