Non-Muscle Myosin Heavy Chain 9 Gene (*MYH9*) Polymorphism (rs4821481) is Associated with Urinary Albumin Excretion in Iranian Diabetic Patients

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Abstract

Background: Myosin heavy chain 9 (*MYH9*) gene polymorphisms have been implicated in different types of renal disease, as well as in nephropathy attributed to type 2 diabetes mellitus.

Objectives: This study sought to analyze the association of *MYH9* gene polymorphism (rs4821481) with diabetic nephropathy (DN), urine albumin excretion value, and glomerular filtration rate (GFR) in an Iranian diabetic population.

Methods: This case-control study included 201 diabetic patients with and without DN, who were referred to the diabetes and metabolic center, Tehran, Iran. The allele and genotype frequencies of rs4821481 were determined using ARMS-polymerase chain reaction (ARMS-PCR). In both groups, blood levels of fasting glucose, HbA1c, urea, creatinine, uric acid, and lipids, as well as urine albumin and creatinine, were measured and GFR was calculated.

Results: Patients who carried the rs4821481 polymorphism had significantly higher urinary excretion of albumin (P < 0.05) and insignificantly lower GFR values (P = 0.08). The frequency of rs4821481 SNP was 22.8% in patients without DN versus 28% in the DN group, which was not statistically significant. Only 2% and 3% of patients without DN and with DN, respectively, had two copies of the C allele. No significant association was found between the rs4821481 polymorphism and DN (OR [95% CI] 1.56 [0.79 - 3.08], P = 0.19).

Conclusions: Although we found an association between *MYH9* gene polymorphism and urinary albumin excretion, the results did not show a significant association between *MYH9* polymorphism (rs4821481) and risk of DN in Iranian diabetic patients.

Keywords: Diabetic Nephropathy, Albuminuria, SNP, rs4821481, *MYH9* Gene

1. Background

Diabetic nephropathy (DN) is one of the most common causes of end-stage renal disease (ESRD). Managing every aspect of chronic kidney disease is one of the greatest challenges that healthcare systems have ever confronted (1-3), and its devastating aftermath may result in earlier morbidities and higher mortality rates. However, this can be monitored and prevented with appropriate insight into the disease, which would lead to less financial pressure on healthcare systems and better care for patients suffering from chronic diseases (4-6). Despite the multifactorial nature of DN, genetic factors have always been considered to be involved in the inheritance and development of disease. Controversial reports of correlations between race and more severe levels of chronic kidney disease have been

published (7-9). A remarkable study by Langefeld et al. (10) demonstrated that glomerular filtration rate (GFR) and urine albumin-to-creatinine ratio (ACR) have strong familial links among Caucasians with type 2 diabetes, signifying that genetic factors have important influences on these parameters. Previous research using genome-wide association study (GWAS) approaches might be useful in finding single nucleotide polymorphisms (SNPs) and variants that may lead to more complex and harmful types of diabetes (11-13). For instance, the GWAS data suggest that polymorphisms of podocyte-expressed *MYH9* gene mutations that encode non-muscle IIA heavy chains are strong candidates to play a major role in the development of nephropathy in diabetic patients (14-16). The Family Investigation of Nephropathy and Diabetes (FIND) study demonstrated an association between *MYH9* and ESRD in non-diabetic

patients (17, 18). On the other hand, several surveys, including an investigation of African Americans, showed a strong correlation between *MYH9* and focal segmental glumerulosclerosis (FSGS), diabetes-induced ESRD, and lupus nephritis (15, 19, 20). Due to recent interest in the *MYH9* gene, several similar studies on Asian, Caucasian, Hispanic American, and African American populations have been carried out (15, 21-23). Given the importance of DN, efforts to identify the genes that influence renal function in diabetic patients have increased in importance and different candidate genes have been studied. Few similar studies have been conducted in Iran, yet extensive studies on the genetics of DN are required.

2. Objectives

This study aimed to examine the effect of rs4821481 of *MYH9* gene polymorphism on urinary albumin excretion and GFR among Iranian diabetic patients and to analyze the association of *MYH9* gene polymorphism (rs4821481) with DN in these individuals.

3. Methods

3.1. Subjects

This was a case-control study conducted at the diabetes and metabolic center (a referral governmental center in Tehran, Iran, affiliated with Tehran University of Medical Sciences,), between February and March 2015. Type 2 diabetes patients aged 30 - 75 years, who had a > 5-year history of diabetes, were included. All patients with increased albumin excretion (urine albumin > 30 mg/24 hours or random urine albumin-to-creatinine ratio [ACR] of > 30 mg/g, confirmed by repeated sampling over 3 - 6 months) and who met the study criteria were included in the DN group. These patients were matched for sex and for duration of diabetes to a group of diabetic patients without nephropathy (ACR < 30 mg/g).

Patients with HbA1c > 9%, urinary tract infection, uncontrolled hypertension, cardiac failure, pregnancy, acute septic infections, hematuria, and recent heavy physical exercise were excluded.

This study was approved by the ethics committee of the endocrinology and metabolism research institute (EMRI) affiliated with Tehran University of Medical Sciences (IR.TUMS.EMRI.REC.1394.0018, 2015). The aim of the study and the methodology were explained to all participants, who signed written informed consent.

3.2. Demographic Data Collection and Biochemical Analysis

Demographic information and data regarding past medical history were collected by trained staff via interviews and from medical records available at the diabetes and metabolic center. Height and weight were measured and BMI was calculated (kg/m^2). After resting in the sitting position, each patient's blood pressure was measured twice and the mean of the measurements was used.

After 8 - 12 hours of fasting, venous blood samples (with and without anticoagulant) and early morning urine were collected. The serum samples were separated after centrifugation and stored for analysis. In both groups, the blood levels of glucose, urea, uric acid, triglycerides, cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and creatinine, as well as urine albumin and creatinine, were measured with commercial kits (Pars Azmun, Iran) using an automated chemistry analyzer (Prestige 24i, Tokyo Boeki Medical Systems, Japan). The urine ACR was calculated manually. HbA1c levels were determined with high-performance liquid chromatography (TosohG8, Tosoh Bioscience, Japan). The instruments used in this study were calibrated and maintained according to the manufacturer's instructions. The modification of diet in renal disease (MDRD) calculation was used for GFR estimations.

3.3. Genetic Analysis

Samples were collected in tubes containing EDTA as an anticoagulation reagent, and used for genetic analysis. DNA extraction was performed using a kit from Tiangen Biotech (Beijing, China). Alleles and genotype frequencies were determined with the ARMS-PCR technique. The appropriate primers (Pishgam Co., Iran) were designed using Gene Runner® and Primer Express® software as follows:

Forward wild: TCTCACGACTGGCAAAGAAGAGCTGTCC;

Forward mutant: GGTCTCACGACTGGCAAAGAAGAGC-TATCT;

Reverse common: GCCGTGGATGGGAGAGTGTGGTCAC.

The thermal cycler (Techne LTD, UK) program was as follows: an initial hold for 5 minutes at 95°C, followed by 30 cycles (30 s at 95°C, 30 s at 65°C, and 35 s at 72°C each), then 35 s at 72°C. PCR products were visualized on 1% gel stained with gel red (Biotium, Hayward, CA, USA).

3.4. Statistical Analysis

Considering Z = 1.96, P = 50%, and accuracy=10%, a sample size of 96 for each group was calculated. This study included 100 and 101 diabetic patients in the DN and non-DN groups, respectively.

Data were checked for normal distribution with the Kolmogorov-Smirnov test. Results with normal distribution were presented as mean ± standard deviation (SD)

and others were presented as median with interquartile range (IQR). The natural logarithm (Ln) was calculated for data without a normal distribution. Student's t-test, the Mann-Whitney U test, and χ^2 test were used to compare the results. A logistic regression analysis was conducted to estimate the odds ratio (OR). Statistical analyses were performed using SPSS software version 21 for Windows (SPSS Inc., Chicago, IL, USA). A P < 0.05 was considered statistically significant.

4. Results

The patients' demographic and biochemical parameters are shown in Table 1. In both groups, females comprised 54% of the patients. The DN patients were older and had significantly higher diastolic blood pressure, serum creatinine, and urine ACR, and lower GFR levels (P < 0.05). There were no significant differences between the groups for the other values, such as BMI, systolic blood pressure, lipid profile, uric acid, fasting blood sugar, HbA1c and, surprisingly, urea.

The allele and genotype frequencies of rs4821481 were calculated and data were analyzed for associations between the studied variants and nephropathy (Table 2). The overall frequency of the T allele (wild type) was higher than that of C allele (risk allele) in both groups. The frequency of the C allele (risk allele) in the DN patients was higher than in patients without DN (Table 2), although this difference was not significant (OR [95% CI]: 1.56 [0.79 - 3.08], P = 0.19). The results did not change even after adjustment for sex, age, duration of diabetes, and BMI (OR [95% CI]: 1.24 [0.66 - 2.35], P = 0.49). The Hardy-Weinberg equilibrium point of 0.6 demonstrated a constant stability of genotype frequency in the studied population.

As seen in Table 3, when the results of all participants were grouped together using the regression analysis, a significant association between the C allele and Ln ACR was demonstrated (P < 0.05) in the crude and adjusted models. GFR was lower in the patients with the C allele, but this difference was not significant (P = 0.16) even after adjusting for sex, age, duration of diabetes, and BMI (P = 0.08).

5. Discussion

This report is the first to investigate *MYH9* SNP associations with DN and urinary albumin excretion in Iranians. We found that individuals with at least one copy of the rs4821481 SNP in the *MYH9* gene had significantly higher excretion of albumin in urine. Although GFR values were lower in these subjects, the difference was not significant. The frequency of rs4821481 SNP was 22.8% in diabetic patients without DN versus 28% in the DN group, which was not statistically significant. As our patients were recruited from a referral center in Tehran and were from different parts of the country with different ethnicities, the results can be generalized to the Iranian population.

The pathogenesis of *MYH9*-related kidney disease is not fully understood. *MYH9*-related disorders change the podocyte cytoskeleton and consequently lead to glomerular filtration barrier damage, proteinuria, and hematuria, and finally to renal failure (24). Many researchers have therefore focused on associations between *MYH9* polymorphism and different types of kidney diseases.

MYH9 polymorphisms have been associated with various non-diabetic kidney diseases in African Americans, such as FSGS nephropathy and HIV-associated nephropathy (19, 25). *MYH9* polymorphisms have also been associated with albuminuria in hypertensive African Americans (23, 26). Effects of *MYH9* polymorphism on renal function in healthy European subjects, but not in diabetic patients, have been suggested (27). Although various reports have displayed associations between *MYH9* polymorphism and non-diabetic ESRD, there are controversial reports about the presence of *MYH9* polymorphism in diabetic ESRD (5, 28-30). Most of the reports have not demonstrated a significant association between *MYH9* polymorphism and diabetic ESRD (18, 28, 29). In the present study, we found no significant association between *MYH9* polymorphism and DN; however, as patients with non-diabetic nephropathy were not included in this research, we cannot confirm an association between *MYH9* polymorphism and non-diabetic nephropathy in Iran.

In research on African Americans, Freedman reported that approximately 16% of patients with type 2 diabetes-associated ESRD had *MYH9* polymorphism, and that several SNPs in the *MYH9* gene, including rs4821481, were associated with DN (OR 1.4, P = 0.047) (15). Another GWAS by Freedman also confirmed this association (30). In a study by Cooke, European Americans with type 2 diabetes and ESRD were compared to type 2 diabetes non-nephropathy controls; it was found that DN was associated with rs4821481 (OR 1.47, P = 0.05) (14). The difference between our study's findings and those of the previous studies may be explained by a lower frequency of the C allele in our patients compared to African Americans (2% - 3% of Iranians versus 60% of African Americans) and by the smaller number of participants in our study. Another source of this difference may be the criteria used for patient recruitment. We included all diabetic patients with increased urinary albumin excretion, not just those who with ESRD.

To the best of our knowledge, this is the first study to investigate the association between *MYH9* gene polymorphisms (rs4821481) and DN in an Iranian population. However, there were a number of limitations; the sample size of

Table 1. Demographic Characteristics and Biochemical Factors of the Diabetic Patients[a]

	Without Nephropathy (n = 101)	With Nephropathy (n = 100)
Age, y	57.5 ± 8.1	61.5 ± 8.3[b]
SBP, mmHg	120 ± 20	125 ± 13
DBP, mmHg	79 ± 7.9	77 ± 8.8[b]
BMI, kg/m²	28.5 ± 4.2	29.0 ± 5.3
GFR, mL/min/1.73m²	76.2 ± 20.6	65.9 ± 20.6[b]
FBS, mg/dL	140.8 ± 34.4	144.0 ± 53.4
HbA1c (%)	7.1 ± 0.7	7.2 ± 0.7
Cr, mg/dL	1.12 ± 0.27	1.27 ± 0.41[b]
Urea, mg/dL	37.1 ± 13.3	42.4 ± 17.4
UA, mg/dL)	5.3 ± 1.4	4.9 ± 1.5
Chol, mg/dL	158.1 ± 34.8	149 ± 30.9
TG, mg/dL	150.9 ± 81.8	141.5 ± 75.6
HDL-C, mg/dL	46.6 ± 10.2	46.9 ± 11.9
LDL-C, mg/dL	76.6 ± 19.9	73.6 ± 18.3
ACR, mg/g	23.0 (20.0 - 26.5)	62.5 (42.2 - 99.7)[b]

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; GFR, glomerular filtration rate; FBS, fasting blood sugar; Cr, creatinine, UA, uric acid; Chol, cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ACR, albumin-to-creatinine ratio.
[a]Values are expressed as mean ± SD or median (interquartile range).
[b]$P < 0.05$.

Table 2. Comparison of Alleles and Genotype Frequency of rs4821481 Variant in Diabetic Patients

	Without nephropathy No. (%)	With nephropathy No. (%)
Genotype		
TT	77 (76.2)	72 (72)
CT	22 (21.8)	25 (25)
CC	2 (2)	3 (3)
Allele		
T	176 (87.1)	169 (84.5)
C	26 (12.9)	31 (15.5)

Table 3. Correlation of GFR and Ln ACR With Allele C in all Subjects[a]

	B	SE	P Value
GFR			
Model 1	-4.7	3.29	0.16
Model 2	-5.92	3.37	0.08
Ln ACR			
Model 1	0.27	0.11	0.015
Model 2	0.35	0.1	0.04

[a]Model 1, crude data; Model 2, adjusted for sex, age, duration of diabetes, and BMI.

5.1. Conclusion

The results of this study showed that rs4821481 SNP is associated with increased urinary albumin excretion. However, we did not find any significant association between rs4821481 *MYH9* polymorphism and risk of DN. Due to the complex role of genetic factors in the development of DN, more comprehensive studies are needed to identify the genetic factors that make diabetic patients prone to ESRD.

our study was smaller than other studies, and the number of diabetic patients with ESRD was limited.

Footnotes

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