DOI: 10.4149/BLL_2023_028

Bratisl Med J 2023; 124 (3)
175 – 181

CLINICAL STUDY

The effects of angiotensinogen M235T/T174M and angiotensin type 1 receptor A1166C gene polymorphisms on the development of diabetic nephropathy in type 2 diabetes mellitus patients

Fulya YUKCU[1], Tammam SIPAHI[2], Sıbel GULDIKEN[3], Sedat USTUNDAG[4], Necdet SUT[5]

Kütahya Health Sciences University, Faculty of Medicine, Department of Biophysics, Kütahya, Turkey.
fulya.yukcu@ksbu.edu.tr

ABSTRACT
AIMS: Diabetic nephropathy is one of the major complications of Type 2 diabetes mellitus. In this study, we aimed to investigate the effects of angiotensinogen M235T/T174M and angiotensin type 1 receptor A1166C gene polymorphisms on the development of diabetic nephropathy in patients with type 2 diabetes mellitus.
METHODS: This study included 100 type 2 diabetes mellitus patients with diabetic nephropathy patients (patient group) and 99 type 2 diabetes mellitus patients without diabetic nephropathy (control group). Polymerase chain reaction and restriction fragment length polymorphism methods were used to identify polymorphisms in the angiotensinogen M235T/T174M and angiotensin type 1 receptor A1166C genes.
RESULTS: There was no significant difference in genotype frequencies of M235T gene polymorphism between patient and control groups (χ^2 = 4.01, df = 2, p = 0.13). There was no significant difference in genotype frequencies of T174M gene polymorphism between patient and control groups (X^2 = 0.36, df = 2, p = 0.83). There was no significant difference in genotype frequencies of A1166C gene polymorphism between patient and control groups (χ^2 = 0.51, df = 2, p = 0.77).
CONCLUSIONS: The results showed no significant difference in angiotensinogen M235T/T174M and angiotensin type 1 receptor A1166C gene polymorphisms between the patient and control groups. Future studies are needed to validate the results of this study and to explore underlying mechanisms *(Tab. 3, Fig. 3, Ref. 35).* Text in PDF *www.elis.sk*
KEY WORDS: type 2 diabetes mellitus, diabetic nephropathy, angiotensinogen gene polymorphism, angiotensin type 1 receptor, gene polymorphism.

Introduction

Type 2 diabetes mellitus (DM) is a serious public health problem that can lead to both microvascular and macrovascular complications (1). One of the most serious microvascular consequences of diabetes is diabetic nephropathy (DN), which is manifested by proteinuria (1, 2). DN is a clinical syndrome that affects about 20–30 % of diabetic individuals, advances slowly and leaves the patient dependent on renal replacement therapy (3). It is also considered the primary cause of end-stage renal disease (ESRD) in developed countries (4). The pathogenesis of diabetic nephropathy is not fully understood, but present evidence indicates that many factors such as metabolic abnormalities, various growth factors, hemodynamic changes and genetic factors contribute to the pathogenesis of diabetic nephropathy (5).

It is known that the renin-angiotensin system (RAS) plays an important role in renal hemodynamic alterations in DN and that genes involved in the RAS predispose to the development of DN (5, 6). Renin, angiotensinogen (AGT), angiotensin-converting enzyme (ACE), ACE2, angiotensin II type 1 receptor (AT1R), and angiotensin II type 2 receptor (AT2R) are the components of the renin-angiotensin system (RAS) (7). The AT1R, a G-protein-coupled receptor, mediates the impact of angiotensin II on vasoconstriction and cell transport of molecules (8). The AT1R gene is located on chromosome 3q24 and consists of 5 exons and 4 introns (9). Although many polymorphisms have been identified in this gene, the most studied of these is the A1166C variant (10). The A1166C polymorphism in the 3′-untranslated region of the AT1R gene corresponds to adenine (A) to cytosine (C) transversion at position 1166 (11). According to several studies, the A1166C gene polymorphism has been linked to a variety of diseases, including hypertension, left ventricular hypertrophy, coronary heart disease, and DN (12). However, the results obtained from studies

[1]Kütahya Health Sciences University, Faculty of Medicine, Department of Biophysics, Kütahya, Turkey, [2]Trakya University, Faculty of Medicine, Department of Biophysics, Edirne, Turkey, [3]Trakya University, Faculty of Medicine, Department of Endocrinology and Metabolism, Edirne, Turkey, [4]Trakya University, Faculty of Medicine, Department of Nephrology, Edirne, Turkey, and [5]Trakya University, Faculty of Medicine, Department of Biostatistics and Medical Information, Edirne, Turkey

Address for correspondence: Fulya YUKCU, PhD, Kütahya Health Sciences University, Faculty of Medicine, Department of Biophysics, Kütahya, Turkey.
Phone: +905424003137, Fax: +902742652285

conducted so far to investigate the relationship between A1166C polymorphism and nephropathy are not consistent with each other (13, 14). One of the components of RAS is AGT which combines with renin to generate angiotensin I, the precursor of angiotensin II (15). The human AGT gene, which is located on chromosome 1q42–43 and consists of 5 exons and 4 introns, encodes angiotensinogen (16, 17). AGT genetic variations may influence the RAS activity which in turn influences the onset and progression of DN (4). Common polymorphisms of the AGT gene include M235T which encodes threonine instead of methionine at position 235 in exon 2, and T174M which encodes methionine for threonine in exon 2 (3, 12).

Determining whether genetic polymorphisms associated with RAS are associated with diabetic nephropathy may contribute significantly to slowing the disease progression. In this study, we aimed to investigate the effects of AGT M235T/T174M and AT1R A1166C gene polymorphisms on the development of diabetic nephropathy in patients with type 2 diabetes mellitus.

Materials and methods

Study cohort

In our study, the files of the patients who were under the follow-up of Trakya University Faculty of Medicine, Nephrology and Endocrinology Departments were scanned. It was decided to include a total of 199 patients in the study, by taking a total of 100 patients (patient group) and 99 patients without diabetic nephropathy (control group) who were undergoing dialysis due to occult, overt diabetic nephropathy or diabetic nephropathy in two evaluations made at least one month apart. While the mean age of the patient group was 62.14 ± 9.35, the mean age of the control group was calculated as 60.70 ± 9.85. The age range of the patient group was 38–87, and the age range of the control group was 38–81. An informed consent form was given to the individuals in the patient and control groups, and their consent was obtained by informing them about the study.

DNA isolation

Peripheral blood samples from the patient and control groups were taken into vacuum tubes with ethylenediaminetetraacetic acid (EDTA) and stored in the laboratory at + 4 °C. DNA was isolated from the blood samples taken using a DNA isolation kit. DNA purity and quality were evaluated by absorbance values in a spectrophotometer. The isolated DNAs were run in 0.8 % agarose gel electrophoresis and the bands formed were observed under ultraviolet (UV) light.

Determination of genotypes

AGT M235T and T274M, AT1R A1166C gene polymorphisms

The M235T and T274M polymorphisms of the AGT gene, and the A1166C polymorphism of the AT1R gene were determined by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) analysis. Primers sequences for each SNPs, and PCR conditions are listed in Table 1. The products reproduced by PCR were loaded on a 2 % agarose gel and stained with ethidium bromide (EtBr); then, electrophoresis was carried out at 110 volts and observed under UV light. The PCR products were digested with restriction enzymes Tth111, NcoI and HaeIII, respectively, for 1 hour at 37 °C to identify M235T, T74M and A1166C gene polymorphisms. Ethidium bromide staining was used to visualize digested DNA fragment products separated by 2.5 % agarose gel electrophoresis.

Statistical analysis

Statistical analysis of the data obtained in this study was performed using the SPSS (Statistics Package of Social Science) v20 (License No. 10240642) statistical package program. In the evaluation of age, height, weight, body surface area (BSA) and body mass index (BMI) variables, the independent t-test was used to determine whether there was a difference between the two sample groups in terms of means. The chi-square (χ^2) analysis method was used to test whether the relationship between the variables was statistically significant in the comparison of genotype distributions, alcohol,

Tab. 1. Summary of conditions for M235T, T174M and A1166C genetic analyses.

	M235T	T174M	A1166C
Primer sequence (5'3')	F: 5'-CCG TTT GTG CAG GGC CTG GCT CTC T-3' R: 5'-CAG GGT GCT GTC CAC ACT GGA CCC C-3'	F: 5'-TGG CAC CCT GGC CTC TCT CTA TCT-3' R: 5'-CAG CCT GCA TGA ACC TGT CAA TCT-3'	F: 5'-GCA GCA CTT CAC TAC CAA ATG GGC-3' R: 5'-CAG GAC AAA AGC AGG CTA GGG AGA-3'
PCR reaction conditions	95°C for 5 min, 35 cycles of 95°C for 1 min, 68°C for 1 min, 72°C for 1 min, 72°C for 10 min.	95°C for 5 min 38 cycles of 94°C for 15 s, 64°C for 45 s, 72°C for 45 s, 72°C for 10 min.	95°C for 5 min 35 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 1 min, 72°C for 7 min.
PCR product size	165 bp	353 bp	255 bp
Restriction enzyme, incubation conditions	Tth111I 37°C for 1 h	NcoI 37°C for 1 h	HaeIII 37°C for 1 h
Fragment length (bp)	TT: 24 bp - 141 bp TM: 24 bp - 141 bp - 165 bp MM: 165 bp	MM: 155 bp - 198 bp TM: 155 bp - 198 bp - 353 bp TT: 353 bp	CC: 24 bp - 231 bp AC: 24 bp - 231 bp - 255 bp AA: 255 bp

Tab. 2. The clinical characteristics of control and diabetic nephropathy groups.

Clinical findings of patient and control groups	Diabetic nephropathy group (100)	Control group (99)	p
Male/female (n%)	51 (% 50.5)/ 50 (% 49.5)	33 (% 33.0)/ 67 (% 67.0)	0.012***
Age (year)	62.14±9.35	60.70±9.85	0.290*
Height (cm)	164.09±9.54	161.53± 8.38	0.045*
Weight (kg)	84.85±14.31	81.42±15.50	0.105*
BSA (m^2)	1.93±0.17	1.87±0.19	0.043*
BMI (kg/m^2)	31.70± 5.89	31.27± 5.89	0.599*
SBP (mmHg)	138.55±18.27	123.94±15.67	0.000**
DBP (mmHg)	79.35±13.22	75.00 ±10.52	0.088**
HDL-C (mg/dl)	46.20±13.28	51.95±16.35	0.043**
LDL-C (mg/dl)	112.72±37.43	113.01±34.15	0.911**
HbA1c (%)	7.20±1.35	8.14±7.98	0.908**
Diabetes duration (years)	12.17±7.63	11.02±7.71	0.192**
Microalbuminuria (mg/dl)	27.60±36.29	0.50±0.80	0.000**
Creatine clearance (mg/min)	78.69±57.42	106.34±39.02	0.000**
Total protein (mg/dl)	51.76±76.56	6.70±23.62	0.000**
Total protein result (mg/day)	1202.77±1637.13	85.20±55.33	0.000**
Urine volume (ml)	2649.50±952.22	2412.96±1017.10	0.050**
Drug story	8.77±7.72	7.05±8.85	0.035**
Hypertension Yes/No (n%)	60 (% 90.9)/ 6 (% 9.1)	41 (% 63.1)/ 24 (% 36.9)	0.001***
Smoking Yes/No (n%)	20 (% 19.8)/ 81 (% 80.2)	7 (% 7.0)/ 93 (% 93.0)	0.014***
Family story Yes/No (n%)	66 (%65.3)/ 35 (%34.7)	53 (%53.0)/ 47 (%47.0)	0.075***
Insulin use Yes/No (n%)	47 (% 46.5)/ 54 (% 53.5)	31 (% 31.0)/ 69 (% 69.0)	0.024***

*Student-t test. **Mann-Whitney U test. *** Chi-square (χ^2) test.
BSA: body surface area, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, HDL-C: high-density lipoprotein, LDL-C: low-density lipoprotein, Hb: hemoglobin.

smoking, disease history, hypertension and active ingredients of the drugs used in the patient and control groups. In the evaluation of diabetes age, systolic and diastolic blood pressure, protein in 24-hour urine, total protein in 24-hour urine, microalbumin, urine volume, 24-hour urine creatinine clearance, drug history, HDL-C, LDL-C and HbA1c variables in patient and control groups, the Mann-Whitney U test was used. The found results were expressed as a percentage or mean standard deviation. A value of $p \leq 0.05$ was accepted as the statistical significance limit.

Results

Information on the research population's demographics

Out of 199 studied subjects, 100 cases with DN disease and 99 controls were enrolled in this study. Demographic and clinical findings from the groups participating in the study were examined. The results obtained are shown in Table 2. When the patient and control groups were examined in terms of age, weight, BMI, DBP, LDL-C, HbA1c, diabetes age, family history, no significant difference was found ($p > 0.05$). However, when these two groups are evaluated according to gender, height, BSA, SBP, HDL-C, microalbuminuria, creatinine clearance in urine, total protein, protein in 24-hour urine, urine volume, drug history, hypertension, smoking and insulin use, there is a risk against the disease that could be considered to be factors ($p < 0.05$).

The electrophoretogram of angiotensinogen M235T/T174M and AT1R A1166C gene polymorphisms in the restriction enzyme digestion

Figures 1, 2, and 3 show patient and control truncation products for the AGT M235T/T174M and AT1R A1166C gene polymorphisms, run on 2.5 % agarose gel and viewed under ultraviolet light.

The frequencies of AGT gene M235T and T174M and AT1R gene A1166C genotype and allele among studied groups

Table 3 summarizes the genotype and allele frequencies of the examined AGT gene M235T and T174M and AT1R gene A1166C polymorphisms in DN and control groups. There was no significant difference in genotype frequencies of AGT gene M235T polymorphism between DN and control groups ($\chi^2 = 4.01$, df = 2, p = 0.13). The frequency of TT genotype was very low (27.8 %) and the frequencies of the TM and MM genotypes were higher in the DN group as compared to the control group (52.2 % and 53.3 %,

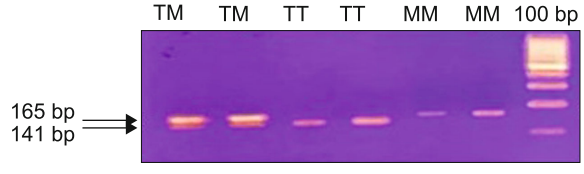

Fig. 1. Gel electrophoresis of PCR amplified product of AGT M235T gene polymorphism.

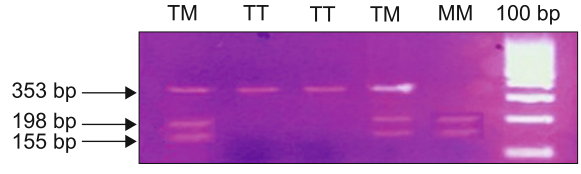

Fig. 2. Gel electrophoresis of PCR amplified product of AGT T174M gene polymorphism.

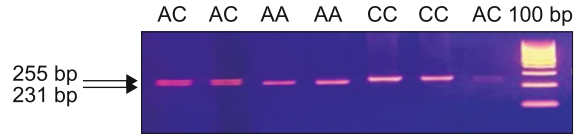

Fig. 3. Gel electrophoresis of PCR amplified product of ATIR A1166C gene polymorphism.

respectively). The risk of developing DN in individuals with the MM genotype is 2.97 times higher than in individuals with the TT genotype (OR = 2.97, 95% CI = 0.91–2.73, p = 0.06). There was no significant difference in allele frequencies of AGT gene M235T polymorphism between DN and control groups (χ^2 = 1.20, df = 1, p = 0.27). The T allele had a very low frequency (47.1 %) while that of the M allele in the DN group was higher (52.7 %) as compared to the control group (52.9 % and 47.3 %, respectively). Individuals with the M allele are 1.25 times more likely to develop DN than those with the T allele (OR = 1.25, 95% CI = 0.84–1.86, p = 0.27) (Tab. 3).

There was no significant difference in genotype frequencies of AGT gene T174M polymorphism between DN and control groups (χ^2 = 0.36, df = 2, p = 0.83). Compared to the control group, the frequency of the TT genotype in the DN group was very low (49.0 %), and the frequencies of the TM and MM genotypes were 53.8 % and 50.0 %, respectively. The risk of developing DN in individuals with the TM genotype is 1.22 times higher than in those with the TT genotype (OR = 1.22, 95% CI = 0.64–2.29, p = 0.54). There was no statistically significant difference in the AGT gene T174M polymorphism allele frequencies between the DN and control groups (χ^2 = 0.28, df = 1, p = 0.59). The T allele had a very low frequency (46.7 %) and the frequency of the M allele in the DN group was higher (53.6 %) as compared to the control group (50.3 % and 46.4 %, respectively). The probability of developing DN in individuals with T allele is 1.14 times higher than in those with M allele (OR = 1.14, 95% CI = 0.65–2.01, p = 0.64) (Tab. 3).

There was no significant difference in genotype frequencies of AT1R gene A1166C polymorphism between DN and control groups (χ^2 = 0.51, df = 2, p = 0.77). Among the DN and control groups, the frequencies of AA genotype were 48.7 % and 21.3 %, respectively, those of AC genotype were 53.7 % and 46.3 %, respectively, while CC genotype frequencies were 46.7 % and 53.3 %, respectively. The risk of developing DN in individuals with the AC genotype is 1.22 times higher than in those with the AA genotype (OR = 1.22, 95% CI = 0.67–2.23, p = 0.51). In addition, the risk of developing DN in individuals with CC genotype is 0.08 (1–0.92) times lower than in those with AA genotype (OR = 0.92, 95% CI = 0.31–2.70, p = 0.88). There was no significant difference in allele frequencies of AT1R gene A1166C polymorphism between the DN and control groups (χ^2 = 0.86, df = 1, p = 0.76). The A allele had a very low frequency (49.8%) and the frequency of the C allele in the DN group was higher (51.5 %) in the DN group than its frequency in the control group (50.2 % and 48.5 %, respectively). Individuals with the A allele are 1.07 times more likely to develop DN than those with the C allele (OR = 1.07, 95% CI = 0.68–1.69, p = 0.76) (Tab. 3).

Tab. 3. Distribution of genotypes and allele frequencies of AGT M235T and T174M and AT1R A1166C gene polymorphisms in diabetic nephropathy and control groups.

AGT and AT1R Polymorphic sites		Control		Diabetic nephropathy		OR	(95%C)	p
		n=99	%	n=100	%			
	TT	13	72.2	5	27.8	1	–	–
	TM	65	47.8	71	52.2	2.84	0.96–8.40	0.05
	MM	21	46.7	24	53.3	2.97	0.91–2.73	0.06
AGT M235T				χ^2=4.01 df=2 p=0.13				
	T allele	91	52.9	81	47.1	1	–	–
	M allele	107	47.3	119	52.7	1.25	0.84–1.86	0.27
				χ^2=1.20 df=1 p=0.27				
	TT	74	51	71	49	1	–	–
	TM	24	46.2	28	53.8	1.22	0.64–2.29	0.54
	MM	1	50	1	50	1.04	0.06–16.98	0.97
AGT T174M				χ^2=0.36 df=2 p=0.83				
	T allele	172	50.3	170	46.7	1.14	0.65–2.01	0.64
	M allele	26	46.4	30	53.6	1	–	–
				χ^2=0.28 df=1 p=0.59				
	AA	60	51.3	57	48.7	1	–	–
	AC	31	46.3	36	53.7	1.22	0.67–2.23	0.51
	CC	8	53.3	7	46.7	0.92	0.31–2.70	0.88
AT1R A1166C				χ^2=0.51 df=2 p=0.77				
	A allele	151	50.2	150	49.8	1	–	–
	C allele	47	48.5	50	51.5	1.07	0.68–1.69	0.76
				χ^2=0.86 df=1 p=0.76				

Discussion

Diabetes mellitus is a complex syndrome that leads to various metabolic dysfunctions. These metabolic dysfunctions manifest as microvascular complications such as diabetic nephropathy, retinopathy, and neuropathy. DN is an important complication of diabetes, affecting approximately 40 % of diabetic patients. Clinically, it is characterized by proteinuria, decreased rate of glomerular filtration, hypertension, increased cardiovascular morbidity and mortality. An individual with diabetic nephropathy may be predisposed to several risk factors such as hyperglycemia, increased blood pressure, and genetic changes (18). DN pathogenesis has been linked to many molecular and biochemical pathways. The RAS is regarded as a key pathway among these. RAS includes different subsystems that contribute to the development of DN (19). Renin, AGT, ACE,

ACE2, AT1R, and AT2R are the components of RAS (7). Single nucleotide polymorphisms of DN associated genes have been found to have a major impact on the outcome of the disease, but no gene has been identified to be conclusively indicating the presence of DN (20). The angiotensinogen gene, which has five exons and four introns covering 12 kb, is found on human chromosome 1q42–43 (12). M235T and T174M are two frequent polymorphisms of the AGT gene (21).

Chang et al investigated the effect of the AGT M235T gene polymorphism in type 2 DM patients with end-stage renal disease in Taiwan and discovered that the polymorphism had no effect on this condition (22). Chen et al investigated the combined effects of ACE, AGT, AT1R and CYP11B2 gene polymorphisms and arsenic on chronic kidney disease in an arsenic-free zone in their patients, of whom 233 suffered from chronic kidney disease and 449 were included in control groups. In terms of AGT M235T genotype distributions and allele frequencies, they found no statistically significant difference (23). Tang et al conducted a meta-analysis study in Asian whites to determine the susceptibility of type 1 and type 2 DM patients developing DN to the M235T gene polymorphism. They arrived at the conclusion that the M235T polymorphism had no effect on these populations (24). The relationship between end-stage renal disease and AGT M235T gene polymorphism was investigated in an Indian study. It was determined that this polymorphism is a risk factor for the disease (25).

Combinations of gene polymorphisms have been investigated in the studies we reviewed above, and inconsistent results have been reported in different populations. In this study, we looked at genotype distributions for the AGT M235T gene polymorphism. When the TT, TM, and MM genotypes in type 2 DM patients and controls were compared, no significant differences were found. We found no statistically significant difference in AGT M235T genotypes between the patient and control groups. Our findings are in contradiction with those of the study conducted by Sarkar et al in India, which concludes that M235T gene polymorphism increases the risk of ESRD. On the other hand, our study is compatible with the views that M235T gene polymorphism in different populations is not associated with the disease (22, 23, 24). The AGT T174M gene polymorphism is a single nucleotide polymorphism characterized by the displacement of threonine and methionine residues at position 174 in AGT exon 2 (26). There were few studies on T174M gene polymorphism and DN in our review of the literature. Tarnow et al were the first to document the association between the AGT T174M polymorphism and DN in a population-based case-control research. Their study included 195 people who had survived DN and 185 people with diabetes who still had normoalbuminuria, and they found that the AGT locus had no discernible effect on the risk of DN. (27). A meta-analysis research that combined 18 case-control studies and involved 8,147 people with coronary artery disease and 5,344 healthy controls was carried out in Russia. The T174M gene polymorphism may increase the risk of coronary artery disease in white people, according to the meta-analysis, while Asians did not show any correlation with the condition. This suggests that the Asian and Caucasian races stem from the differences in their ethnic backgrounds and the environment in which they live (28). Jakubiak et al conducted a meta-analysis study aimed at investigating the RAS gene polymorphism in Canadian Caucasians with heart failure. They discovered a significant increase in the 235TT genotype, T235, and M174 alleles in patients with heart failure as a result of the study (29). Another study conducted on chronic kidney disease patients in an arsenic-free zone found no association between the T174M polymorphism and disease (23). In a study conducted by Freitas et al in Portugal, the effect of AGT T174M gene polymorphism on coronary artery patients was examined and it was determined that the polymorphism had no effect on this condition. However, they reported a significant relationship between the disease and parameters such as gender, smoking, SBP, DBP, HDL-C, HT, DM, and dyslipidemia (30). Agachan et al investigated ACE I/D, AT1R and AGT gene polymorphisms in hypertensive patients in Turkey. They concluded that parameters such as BMI, SBP, DBP, LDL-C between the patient and control groups were statistically significant. While they found the M allele in hypertensive patients to be higher than the control group, they could not find a significant difference between MM genotype and hypertension (31). We found no statistically significant difference in AGT T174M genotypes between the patient and control groups. Since there is limited literature information on T174M gene polymorphism and DN, we had to examine the studies that included the relationship of T174M gene polymorphism with various diseases in our discussion. We discovered a significant relationship between the disease and parameters such as height, BSA, gender, SBP, HDL-C, HT, and smoking in our study, which is consistent with the findings of Freitas et al and Agachan et al In addition, we correlated the differences between the patient and control groups with DN in terms of total protein result, total protein, microalbuminuria, creatinine clearance, urine volume, drug history, and insulin use.

The AT1R gene, which is located on the third chromosome's long arm, is more than 55 kb long and contains 5 exons and 4 introns. The AT1R gene has been linked to a number of polymorphisms (32), of which, the A1166C gene polymorphism has been the most thoroughly examined so far. Various studies have linked the A1166C gene polymorphism to disease processes such as HT, left ventricular hypertrophy, coronary heart disease and DN (12). Although the A1166C gene polymorphism and DN susceptibility have been studied, a consensus perspective has not yet been achieved. Gallego et al found no significant association between AGTR1 A1166C gene polymorphism and DN risk in Caucasian populations (33). In a case-control research carried out in Taiwan, A1166C gene polymorphism genotype distributions were shown to be unrelated to the likelihood of developing chronic renal disease (23). It was shown that the A1166C gene polymorphism's CC genotype was related to the general population, and the C allele was associated with the Asian population in a meta-analysis research that included nine studies on diabetics with diabetic nephropathy (34). In their study of the relationship between the AT1R A1166C gene polymorphism and likelihood of developing diabetic nephropathy, Yin et al discovered that patients with diabetic nephropathy had greater frequencies of the C allele than simple diabetes patients and healthy controls (35).

It is yet unclear how the AGTR1 A1166C gene polymorphism impacts the onset of renal illnesses, particularly that of DN. Inconsistencies in the findings can be attributed to different genetic backgrounds of study participants, different study sample selection criteria, and unequal sample sizes. Our findings are consistent with the literature, particularly in the fact that we discovered more C alleles in the DN group. However, statistical analysis revealed no significant difference in AGT A1166C genotypes between the patient and control groups ($p > 0.05$).

Conclusion

The current study found out that DN patients did not differ from healthy subjects in terms of AGT M235T/T174M and AT1R A1166C gene polymorphisms. More research is needed to be conducted on the Turkish population to understand the role of RAS genes in diabetic nephropathy.

References

1. Aldukhayel A. Prevalence of diabetic nephropathy among Type 2 diabetic patients in some of the Arab countries. Int J Health Sci (Qassim) 2017; 11 (1): 1–4.

2. Atageldiyeva KK, Nemr R, Echtay A et al. Apolipoprotein E genetic polymorphism influence the susceptibility to nephropathy in type 2 diabetes patients. Gene 2019; 715: 144011. https: //doi.org/10.1016/j.gene.2019.144011.

3. Aggarwal N, Kare PK, Varshney P et al. Role of Angiotensin Converting Enzyme and Angiotensinogen Gene Polymorphisms in Angiotensin Converting Enzyme İnhibitor-Mediated Antiproteinuric Action in Type 2 Diabetic Nephropathy Patients. World J Diabet 2017; 8 (3): 112–119. https: //doi.org/10.4239/wjd.v8.i3.112.

4. Zhou B, Wen M, Mi L et al. Associations between angiotensinogen M235T polymorphisms and the risk of diabetic nephropathy: A meta-analysis. Diabet Res Clin Pract 2018; 142: 26–36. https: //doi.org/10.1016/j.diabres.2018.05.015.

5. Ha SK. ACE insertion/deletion polymorphism and diabetic nephropathy: clinical implications of genetic information. J Diabet Res 2014; 2014: 846068. https: //doi.org/10.1155/2014/846068.

6. Zhuang Y, Niu F, Liu D et al. Association between AGTR1 A1166C polymorphism and the susceptibility to diabetic nephropathy: Evidence from a meta-analysis. Medicine (Baltimore) 2018; 97 (41): e07689. https: //doi.org/10.1097/MD.0000000000007689.

7. Qiao YC, Wang M, Pan YH et al. The relationship between ACE/AGT gene polymorphisms and the risk of diabetic retinopathy in Chinese patients with type 2 diabetes. J Renin Angiotensin Aldosterone Syst 2018; 19 (1): 1470320317752955. https: //doi.org/10.1177/1470320317752955.

8. Ali Z, Kusrini I, Shahab A et al. Association between A1166C Polymorphism of the Angiotensin II Type-1 Receptor Gene and Type-2 Diabetic Nephropathy in an Indonesian Malay Population. Acta Med Indones 2018; 50 (4): 314–319.

9. Abouelfath R, Habbal R, Aqli ELM et al. Does signal nucleotide polymorphism of RGS2 and ATIR, individually or in combination modulate the response to antihypertensive drugs in resistant hypertensive subjects? Gene Reports 2019 (18); 100575. https: //doi.org/10.1016/j.genrep.2019.100575.

10. El-banawy H, Bedair R, Mohammed A. Angiotensin II type 1 receptor (A1166C) gene polymorphism in Egyptian adult hemodialysis patients. Alexandria J Med 2015; 51 (4): 339–345. https: //doi.org/10.1016/j.ajme.2014.12.003.

11. Baudin B. Polymorphism in angiotensin II receptor genes and hypertension. Exp Physiol 2005; 90 (3): 277–282. https: //doi.org/10.1113/expphysiol.2004.028456

12. Wang JG, Staessen JA. Genetic polymorphisms in the renin-angiotensin system: relevance for susceptibility to cardiovascular disease. Eur J Pharmacol 2000; 410 (2–3): 289–302. https: //doi.org/10.1016/s0014-2999 (00)00822-0.

13. Shah VN, Cheema BS, Sharma R et al. ACACβ gene (rs2268388) and AGTR1 gene (rs5186) polymorphism and the risk of nephropathy in Asian Indian patients with type 2 diabetes. Mol Cell Biochem 2013; 372 (1–2): 191–198. https: //doi.org/10.1007/s11010-012-1460-2.

14. Moradi M, Rahimi Z, Amiri S et al. AT1R A1166C variants in patients with type 2 diabetes mellitus and diabetic nephropathy. J Nephropathol 2015; 4 (3): 69–76. https: //doi.org/10.12860/jnp.2015.14.

15. Wang B, Guo Q, Peng Y et al. Association of AGT M235T and ACE I/D polymorphisms with the risk of ischemic stroke: meta-analysis in Han Chinese population. J Neurol Sci 2012; 320 (1–2): 79–84. https: //doi.org/10.1016/j.jns.2012.06.022.

16. Borai İH, Hassan NS, Shaker OG et al. Synergistic effect of ACE and AGT genes in coronary artery disease. Beni-Suef Univ J Basic Appl Sci 2018; 7 (1): 111–117.

17. Zhai C, Cong H, Zhang H et al. M235T polymorphism in the angiotensinogen gene and cardiovascular disease: An updated meta-analysis of 39 case-control comparisons. Anatolian J Cardiol 2019; 21 (4): 222–232. https: //doi.org/10.14744/AnatolJCardiol.2019.75282.

18. Bowden DW. Genetics of diabetes complications. Curr Diabet Reports 2002; 2 (2): 191–200. https: //doi.org/10.1007/s11892-002-0080-8.

19. Zain M, Awan FR. Renin Angiotensin Aldosterone System (RAAS): its biology and drug targets for treating diabetic nephropathy. Pakistan J Pharm Scie 2014; 27 (5): 1379–1391.

20. Rizvi S, Syed TR, Farzana M. Association of genetic variants with diabetic nephropathy. World J Diabet 2014; 5 (6): 809–816. https: //doi.org/10.4239/wjd.v5.i6.809.

21. Niu T, Yang J, Wang B et al. Angiotensinogen gene polymorphisms M235T/T174M: no excess transmission to hypertensive Chinese. Hypertension 1999; 33 (2): 698–702. https: //doi.org/10.1161/01.hyp.33.2.698.

22. Chang HR, Cheng CH, Shu KH et al. Study of the polymorphism of angiotensinogen, anigiotensin-converting enzyme and angiotensin receptor in type II diabetes with end-stage renal disease in Taiwan. J Chinese Med Ass JCMA 2003; 66 (1): 51–56.

23. Chen WJ, Huang YL, Shiue HS et al. Renin-angiotensin-aldosterone system related gene polymorphisms and urinary total arsenic is related to chronic kidney disease. ToxicolAppl Pharmacol 2014; 279 (2): 95–102. https: //doi.org/10.1016/j.taap.2014.05.011.

24. Tang W, Zhou TB, Jiang Z. RETRACTED: Association of the angiotensinogen M235T gene polymorphism with risk of diabetes mellitus developing into diabetic nephropathy. J Renin-Angiotensin-Aldosterone System: JRAAS 2015; 16–14. https: //doi.org/10.1177/1470320314563426.

25. Sarkar S, Gupta V, Kumar A et al. M235T polymorphism in the AGT gene and A/G (I8-83) substitution in the REN gene correlate with

end-stage renal disease. Nephron 2015; 129 (2): 104–108. https: //doi.org/10.1159/000370074.

26. Liao X, Yang Z, Peng D et al. Association of T174M polymorphismof angiotensinogen gene with essential hypertension: A meta-analysis. Genet Mol Biol 2014; 37 (3): 473–479. https: //doi.org/10.1590/s1415-47572014000400001.

27. Tarnow L, Cambien F, Rossing P et al. Angiotensinogen gene polymorphisms in IDDM patients with diabetic nephropathy. Diabetes 1996; 45: 367–369. https: //doi.org/10.2337/diab.45.3.367.

28. Wang WZ. Association between T174M polymorphism in the angiotensinogen gene andrisk of coronary artery disease: a meta-analysis. J Geriatric Cardiol 2013; 10 (1): 59−65. https: //doi.org/10.3969/j.issn.1671-5411.2013.01.010.

29. Jakubiak M, Denus S, Dube S et al. Ten renin-angiotensin system-related gene polymorphisms in maximally treated Canadian Caucasian patients with heart failure. Brit J Clin Pharmacol 2008; 65 (5): 742–751. https: //doi.org/10.1111/j.1365-2125.2007.03091.x.

30. Freitas AI, Mendonça I, Brión M et al. RAS gene polymorphisms, risk factors and the advent of coronary artery disease in the Portuguese population. BMC Cardiovasc Dis 2008; 8: 15. https: //doi.org/10.1186/1471-2261-8-15.

31. Agachan B, Isbir T, Yilmaz H et al. AngiotensinconvertingenzymeI/D, angiotensinogen T174M/M235T and angiotensin II type 1 receptor A1166C gene polymorphisms in Turkish hypertensive patients. Exp Mol Med 2003; 35 (6): 545–549. https: //doi.org/10.1038/emm.2003.71.

32. Duncan JA, Scholey JW, Miller JA. Angiotensin II type 1 receptor gene polymorphisms in humans: physiology and pathophysiology of the genotypes. Curr Opinion Nephrol Hypertension 2001; 10 (1): 111–116. https: //doi.org/10.1097/00041552-200101000-00017.

33. Gallego PH, Shephard N, Bulsara MK et al. Angiotensinogen gene T235 variant: a marker for the development of persistent microalbuminuria in children and adolescents with type 1 diabetes mellitus. J Diabet Compl 2008; 22 (3): 191–198. https: //doi.org/10.1016/j.jdiacomp.2007.03.003.

34. Yang C, Zhou T. RETRACTED: Relationship between the angiotensinogen A1166C gene polymorphism and the risk of diabetes mellitus developing into diabetic nephropathy. J Renin-Angiotensin-Aldosterone System 2015; 16–14. https: //doi.org/10.1177/1470320314566221.

35. Yin X, Li H, Xuan J et al. AGTR1 A1166C polymorphism is associated with risk of diabetic nephropathy. J Zhejiang Univ Med Sci 2013; 42 (1): 45–51. https: //doi.org/10.3785/j.issn.1008-9292.2013.01.008.

Received Septeember 26, 2022.
Accepted October 11, 2022.