Association between angiotensin II type-1 receptor A1166C polymorphism and the presence of angiographically-defined coronary artery disease in an Iranian population

Article *in* Asian biomedicine · April 2010

DOI: 10.2478/abm-2010-0038

CITATIONS
5

READS
87

14 authors, including:

Akram Assali

7 PUBLICATIONS **22** CITATIONS

SEE PROFILE

Mohsen Moohebati

Mashhad University of Medical Sciences

131 PUBLICATIONS **1,075** CITATIONS

SEE PROFILE

Mitra Hassani

Mashhad University of Medical Sciences

13 PUBLICATIONS **99** CITATIONS

SEE PROFILE

Mohammad Reza Oladi

Mashhad University of Medical Sciences

14 PUBLICATIONS **233** CITATIONS

SEE PROFILE

European Journal of Internal Medicine 22 (2011) 254–261

Contents lists available at ScienceDirect

European Journal of Internal Medicine

journal homepage: www.elsevier.com/locate/ejim

Original article

Association of angiotensin II type 1 receptor gene A1166C polymorphism with the presence of diabetes mellitus and metabolic syndrome in patients with documented coronary artery disease

Akram Assali [a,b,c], Majid Ghayour-Mobarhan [b,d,e], Amirhossein Sahebkar [a,b], Mitra Hassani [a], Jamal Kasaian [a], Farnoosh Tatari [a], Mohsen Moohebati [f], Roghayeh Paydar [b], Mohammadreza Oladi [a], Habib A. Esmaeili [g], Shima Tavallaie [b], Shahireh Omidvar Tehrani [a], Gordon A.A. Ferns [h], Javad Behravan [a,i,*]

[a] Biotechnology Research Center, Avicenna Research Institute, Mashhad University of Medical Sciences (MUMS), Mashhad, Iran
[b] Cardiovascular Research Center, Avicenna Research Institute, MUMS, Mashhad, Iran
[c] Department of Biochemistry, Tehran Payame Noor University, Tehran, Iran
[d] Department of Nutrition and Biochemistry, Faculty of Medicine, MUMS, Mashhad, Iran
[e] Cardiovascular Genetics Research Unit, Henri Poincaré University, 30 rue Lionnois, 54000, Nancy, France
[f] Department of Cardiology, Faculty of Medicine, MUMS, Mashhad, Iran
[g] Department of Statistics, Faculty of Medicine, MUMS, Mashhad, Iran
[h] Institute for Science and Technology in Medicine, University of Keele, Guy Hilton Research Centre, Thornburrow Drive, Stoke on Trent, Staffordshire ST4 7QBy, UK
[i] Department of Pharmaceutical Biotechnology, School of Pharmacy, MUMS, Mashhad, Iran

ARTICLE INFO

Article history:
Received 1 May 2010
Received in revised form 19 November 2010
Accepted 27 November 2010
Available online 23 December 2010

Keywords:
Angiotensin II type 1 receptor
Single nucleotide polymorphism
Diabetes mellitus
Coronary artery disease
Metabolic syndrome

ABSTRACT

Background: There are relatively limited data available on the genetic susceptibility to diabetes mellitus and metabolic syndrome in the Iranian population. We have therefore investigated the association between the angiotensin II type I receptor gene polymorphism (AT_1R/A1166C) and the presence of diabetes mellitus and metabolic syndrome in a well defined group of patients.
Methods: Patients with angiographically defined coronary artery disease (CAD) ($n=309$) were evaluated for the presence of AT_1R/A1166C polymorphism. These patients were classified into subgroups with ($n=164$, M/F: 109/55) and without ($n=145$, M/F: 84/61) diabetes mellitus. The AT_1R polymorphism was assessed using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) based method.
Results: There was a higher frequency of polymorphic genotypes (AC + CC) in the diabetic compared with the non-diabetic group ($p=0.01$). When determined for each gender separately, this difference remained significant in the males ($p=0.04$) but not in females ($p=0.09$). With regard to the allele frequencies, the C allele was significantly higher and the A allele frequency was lower in the diabetic group ($p=0.01$). This remained significant after gender segregation for males ($p=0.01$) but not females. In the binary logistic regression analysis, only serum fasting glucose was found as the independent predictor for the presence of diabetes in the CAD patients ($\beta=1.16$, $p<0.001$ for total population and $\beta=1.29$, $p<0.001$ for male subjects). There was no significant difference in genotype or allele frequencies between subgroups with and without metabolic syndrome, this being unaffected by gender or the definition of metabolic syndrome used apart from a significantly lower frequency of C allele in male subjects with metabolic syndrome defined by the NCEP ATP III criteria ($p=0.04$).
Conclusion: The AT_1R/A1166C polymorphism may be associated with the presence of diabetes mellitus in male subjects with documented CAD.

1. Introduction

Diabetes mellitus is one of the most prevalent endocrine disorders which is now regarded as an important public health concern affecting about 6% of the world's population [1,2]. The prevalence of diabetes is increasing alarmingly and projections on its future burden estimate that the total number of subjects with diabetes will reach about 221 million in 2010 and 300 million in 2025, of whom more than 97% will have type 2 diabetes [3,4].

Several lines of evidence suggest that type 2 diabetes mellitus is a polygenic disease and genetic susceptibility has an important role in the etiology of this disorder [5,6]. With further elucidation of the human genome, identification of multiple genetic polymorphisms

* Corresponding author. Department of Pharmaceutical Biotechnology, School of Pharmacy, Mashhad University of Medical Sciences, P.O. Box 91775-1365 Mashhad, Iran. Fax: +98 511 8823251.
E-mail address: behravanj@mums.ac.ir (J. Behravan).

0953-6205/$ – see front matter
doi:10.1016/j.ejim.2010.11.014

which contribute to the predisposition to type 2 diabetes mellitus is anticipated.

The renin–angiotensin system (RAS) comprises a cascade of processes leading to the production of angiotensin II from the angiotensinogen. The activation of this system is considered to play a pivotal role in the pathophysiology of hypertension, coronary artery disease (CAD) and myocardial infarction (MI). This may be mediated by various mechanisms such as an impact on the level of angiotensin II or reducing that of bradykinin [7,8]. There is also evidence that the RAS contributes significantly to the pathogenesis of diabetic cardiovascular disease [9]. Furthermore, the impact of polymorphisms in the RAS genes on the complications of diabetes, particularly diabetic nephropathy, has been widely studied [10–12]. Along with the aforementioned findings, many trials have reported improvement of insulin sensitivity and glucose metabolism and decrease in the risk of type 2 diabetes following the blockade of the RAS by angiotensin converting enzyme inhibitors and some angiotensin II receptor blockers [13–15]. A number of mechanisms that may account for these observations have been proposed such as improvement of blood flow and microcirculation in skeletal muscles. Therefore, based on the recent consistent observations on the reduction in the incidence of type 2 diabetes in hypertensive subjects treated with either of the mentioned drugs, blockade of the RAS by these medications appears to be a promising strategy to prevent the development of type 2 diabetes.

The AT_1 receptor gene (AF245699) is located at 3q21–q25 and extends over a 55 kb segment, consisting of five exons. A single nucleotide polymorphism (SNP) in the 3-prime untranslated region (3′ UTR) of this gene (A1166C, rs5186) has been characterized and investigated in relation to essential hypertension, left ventricular hypertrophy, MI, carotid intimal medial thickening and stroke in a limited number of populations [16–19].

Unlike cardiovascular disease, the association between the AT_1R/A1166C polymorphism and the presence of diabetes and metabolic syndrome – particularly in those with CAD – has not been extensively studied and to our knowledge there is little information about the genetic susceptibility to diabetes mellitus in Iranian population. Therefore, in the present study we aimed to investigate the relationship between A11166C polymorphism of AT_1R gene and the presence of diabetes mellitus in a group of Iranian subjects with angiographically defined CAD. The rationale for this being the improvement of insulin sensitivity and glucose metabolism and decreased risk of type 2 diabetes following the blockade of the RAS system by angiotensin converting enzyme inhibitors and some angiotensin II receptor blockers [13–15]. Because metabolic syndrome increases the risk of both diabetes mellitus and cardiovascular disease, the association between the AT_1R/A1166C SNP and the presence of this syndrome [with either definitions of National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) or International Diabetes Federation (IDF)] was also studied.

2. Methods

2.1. Study population

The study population consisted of a total of 309 subjects with angiographically defined CAD who were recruited from Ghaem Hospital (Mashhad, Iran). These patients were selected from those individuals who underwent coronary angiography from mid 2008 to mid 2009, mainly for stable angina, and were positive for at least one objective test of myocardial ischemia including exercise stress test, Dobutamin stress echocardiography, and Thallium SPECT. Patients who were on oral contraceptives, or hormone replacement therapy as well as pregnant women were excluded from the study. None of the subjects had a prior history of coronary angioplasty or coronary artery bypass graft (CABG). None of the subjects had overt clinical features of infection, or chronic inflammatory disease, and all subjects were negative for HBS antigen, anti-HCV antibody, and anti-HIV antibody. Coronary angiograms were performed using routine procedures. Analysis of the angiograms was performed offline by a Specialist Cardiologist. The presence of one or more stenoses >50% in diameter of at least one major coronary artery (left main, right coronary artery, left anterior descending, and circumflex) was considered as evidence of significant CAD. Subjects who had <50% reduction of coronary artery diameter were excluded from the study. Based on the presence of diabetes mellitus, these subjects were classified into subgroups of diabetic (defined as fasting plasma glucose >7 mmol/L, $n=164$) and non-diabetic ($n=145$). Metabolic syndrome was defined according to each of the NCEP ATP III and IDF definitions [20,21] as described in Table 5. The study protocol was approved by the Mashhad University of Medical Sciences (MUMS) Ethics Committee and written informed consent was obtained from each participant. Clinical characteristics and blood chemistry data were obtained using questionnaires, clinical and laboratory examinations (Table 1).

2.2. Anthropometric and other measurements

Anthropometric parameters including weight, height, BMI, waist circumference, hip circumference and waist/hip ratio as well as systolic and diastolic blood pressures were measured as previously described [22].

2.3. Routine biochemical analysis

A full 12-h fasted lipid profile was determined for each subject. Serum lipid and fasting blood glucose (FBS) concentrations were measured by enzymatic methods.

2.4. Genetic analysis

Whole blood was collected from the study subjects and genomic DNA was isolated from peripheral blood leukocytes using a commercial kit (Biogen). The A1166C variant of AT_1R gene was identified using the polymerase chain reaction (PCR) followed by restriction enzyme digestion of the amplified product as previously described [23,24]. The primers used in the PCR reaction were 5′-GCAC-CATGTTTTGAGTTG-3′ as the forward and 5′-CGACTACTGCTTAG-CATA-3′ as the reverse primer under the conditions described elsewhere [24,25]. The PCR products were digested with the *Dde*I restriction enzyme (MBI Fermentas). Digested products were separated by electrophoresis on a 1.5% (w/v) agarose gel and visualized directly under UV light after staining with ethidium bromide.

2.5. Statistical analysis

Statistical analyses were performed using the SPSS for Windows™, version 14.0 software package (SPSS Inc., Chicago, Illinois, USA). Data were expressed as means ± SD or median and inter-quartile range. The statistical difference in genotype and allele frequencies between the groups was assessed by the χ^2 test. Compliance of genotypes with the Hardy–Weinberg equilibrium in each group was assessed by χ^2 test with one degree of freedom. Demographic characteristics were compared using Student's *t*-test, Mann–Whitney U test or χ^2 test. To identify factors that are independently associated with the presence of diabetes, binary logistic regression analysis (using forward conditional method) was carried out. A two-sided p-value<0.05 was considered statistically significant.

3. Results

3.1. Demographic characteristics

Diabetic subjects were significantly younger than non-diabetic subjects ($p=0.004$). This could be attributed to the fact that diabetics are more prone to cardiovascular disease and develop it earlier than non-diabetics. There was no significant difference between the 2 groups regarding gender and serum low-density lipoprotein cholesterol (LDL-C) concentrations ($p>0.05$). However, the frequency of hypertension ($p<0.001$) together with fasting plasma glucose ($p<0.001$) and serum concentrations of total cholesterol ($p=0.01$), triglycerides ($p<0.001$) and high-density lipoprotein cholesterol (HDL-C) ($p=0.02$) was significantly higher in the diabetic group compared to the non-diabetic group. With respect to the anthropometric parameters, the diabetic group had a higher BMI ($p=0.005$) but no significant difference in waist/hip ratio was observed between the groups ($p>0.05$). Although the difference in the frequency of obesity did not reach statistical significance, the overall rate of overweight and obesity was significantly higher in diabetic compared to the non-diabetic group ($p=0.01$). The prevalence of metabolic syndrome using either the NCEP ATP III ($p<0.001$) or IDF ($p<0.001$) criteria, was also significantly higher in the diabetic group. Demographic characteristics of study subjects are summarized in Table 1.

As for the drug consumption, there was no significant difference in the frequency of subjects consuming anti-hypertensive agents, statins, angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers or aspirin between diabetic and non-diabetic groups (Table 2).

3.2. Association between AT_1R/A1166C polymorphism and the presence of diabetes

In vitro DNA amplification of the AT_1R gene using the specific primers resulted in a 540 bp DNA product. On digestion of the amplified fragment (amplicon) with *Dde*I restriction endonuclease, DNA fragments of 540 (AA), 430 (CC) or 540 and 430 (AC) bp length were observed. Thus each of the samples revealed one of the three different electrophoretic patterns.

Frequencies of the AA, AC and CC genotypes were 90, 42 and 13 in the diabetic, and 124, 31 and 9 in the non-diabetic group, respectively. The genotype distributions were not consistent with the Hardy–Weinberg equilibrium either in the diabetic ($\chi^2=5.4$, $p=0.02$) or in the non-diabetic ($\chi^2=10.7$, $p=0.001$) groups. Frequencies for genotypes and alleles in the study population are presented in Table 3. There was a significant difference in genotype distribution between the 2 groups, with a higher frequency of polymorphic genotypes (AC and CC) and lower frequency of AA genotype in the diabetic group ($p=0.01$). After separate analysis for each gender, the results indicated a significant difference in the males ($p=0.044$) but not in females ($p=0.09$). In regard to the alleles, significantly higher frequencies of the mutated C allele (which was paralleled by a lower frequency of the A allele) were observed in the diabetic group ($p=0.007$). However, after gender segregation, this significant difference was maintained only in males ($p=0.007$) and not in females ($p>0.05$).

Table 1
Demographic characteristics of study subjects.

Variable	CAD	CAD + diabetes	*p*-value
N	164	145	
Age (years)	58.8 ± 11.4	55.4 ± 9.7	0.004
<50	47 (28.7)	49 (33.8)	0.53
50–54	25 (15.2)	25 (17.2)	
55–59	21 (12.8)	20 (13.8)	
≥60	71 (43.3)	51 (35.2)	
Sex			
Women	55 (33.5)	61 (42.1)	0.08
Men	109 (66.5)	84 (57.9)	
Body mass index (kg/m^2)	26.10 (22.65–29.79)	27.38 (24.67–30.07)	0.005
Normal	70 (43.7)	38 (26.3)	0.005
Overweight	53 (32.9)	67 (46.4)	
Obese	37 (23.1)	40 (27.3)	
Waist:hip ratio	0.96 (0.90–1.00)	0.95 (0.92–1.00)	0.91
Hypertension	57 (34.8)	83 (57.2)	<0.001
Metabolic syndrome			
Adult Treatment Panel III	55 (33.4)	76 (53.2)	0.001
International Diabetes Federation	51 (30.7)	78 (53.8)	<0.001
Fasting plasma glucose (mmol/L)	5.11 (4.62–5.77)	8.00 (6.05–10.30)	<0.001
Normal (3.8–5.5)	104 (64.3)	30 (20.8)	<0.001
Impaired glucose tolerance (5.6–6.9)	66 (35.7)	22 (15.1)	
Diagnosis of diabetes (>7)	0 (0)	93 (64.1)	
Systolic blood pressure (mm Hg)	130.00 (120.00–158.00)	132.50 (120.00–170.00)	0.09
<125	68 (41.9)	48 (26.3)	0.05
≥125 to <142	46 (27.7)	67 (46.4)	
≥142	50 (30.5)	40 (27.3)	
LDL-C (mmol/L)	2.56 ± 0.92	2.70 ± 1.01	0.18
<3.02	120 (73.2)	98 (67.6)	0.26
≥3.02 to <3.89	29 (17.7)	25 (17.2)	
≥3.89	15 (9.1)	22 (15.2)	
HDL-C (mmol/L)	1.16 (0.91–1.34)	1.22 (0.98–1.45)	0.02
<0.95	53 (32.3)	30 (20.7)	0.05
≥0.95 to <1.15	32 (19.5)	39 (26.9)	
≥1.15	79 (48.2)	76 (52.4)	
Total cholesterol (mmol/L)	4.41 ± 1.10	4.79 ± 1.49	0.01
Triglyceride (mmol/L)	1.23 (0.97–1.86)	1.74 (1.11–2.42)	<0.001

Values are expressed as mean ± SD, median and inter-quartile range or number (%). Comparisons between CAD patients with and without diabetes were performed using Student's *t*-test, Mann–Whitney U test or χ^2 test.
CAD: coronary artery disease; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol.

Table 2
Medications used by the study population.

	CAD	CAD + diabetes	*p*-value
Anti-hypertensives	61(49.2)	39(42.4)	0.32
Statins	52(41.9)	27(29)	0.05
ACE inhibitors	36(29.3)	28(30.1)	0.8
ARBs	3(2.4)	0(0)	0.13
Aspirin	74(59.7)	50(53.8)	0.38

	CAD				CAD + diabetes			
	AA	AC	CC	*p*-value	AA	AC	CC	*p*-value
Anti-hypertensives	45(49.5)	10(40)	6(74)	0.225	23(37.7)	10(46.6)	6(60)	0.35
Statins	38(41.8)	9(36)	5(2.5)	0.416	16(25.8)	7(33.3)	4(40)	0.58
ACE inhibitors	29(31.9)	5(20)	2(28.6)	0.51	18(29)	8(38.1)	2(20)	0.56
ARBs	1(1.1)	2(8)	0(0)	0.12	0	0	0	–
Aspirin	56(61.5)	13(52)	8(6.4)	0.68	31(50)	13(61.9)	6(60)	0.58

	Presence of metabolic syndrome (IDF definition)		
	With	Without	*p*-value
Anti-hypertensives	51(44.3)	62(42.2)	0.72
Statins	39(33.6)	49(33.3)	0.96
ACE inhibitors	35(30.4)	38(25.9)	0.41
ARBs	5(4.3)	0	0.011
Aspirin	62(53.4)	85(57.8)	0.47

	Presence of metabolic syndrome (IDF definition)		
	With	Without	*p*-value
Anti-hypertensives	52(49.1)	61(39.1)	0.11
Statins	35(32.7)	53(34)	0.46
ACE inhibitors	34(32.1)	39(25)	0.21
ARBs	5(4.7)	0	0.006

(continued on next page)

To identify factors that are independently associated with the presence of diabetes, binary logistic regression analysis (using forward conditional method) was carried out. The model included the presence of diabetes as dependent variable whilst the presence of polymorphic (AC/CC) genotypes, metabolic syndrome (IDF definition), and hypertension, age, gender, BMI, systolic and diastolic blood pressures, HDL-C, LDL-C, total cholesterol, triglycerides and FBS were inserted as covariates. Besides, the interactions between each of the aforementioned parameters and the presence of polymorphic (AC/CC) genotypes, were inserted as covariate. According to the results of binary logistic regression analysis, only serum FBS was the independent predictor of diabetes in the CAD patients [$\beta = 1.16$, $p<0.001$, odds ratio (95% CI): 3.18 (2.33–4.33)]. The same analysis was also performed among male subjects excluding gender and its interaction with the presence of polymorphic (AC/CC) genotypes. Again, serum FBS was found as the only independent predictor of diabetes in the CAD patients [$\beta = 1.29$, $p<0.001$, odds ratio (95% CI): 3.62 (2.37–5.54)].

3.3. Association between AT_1R/A1166C polymorphism and the presence of metabolic syndrome

Overall, there was a higher prevalence of metabolic syndrome, either defined according to the NCEP ATP III or IDF guidelines, in females compared to males ($p<0.001$, Table 4). In regard to the AT_1R variants, there was no significant difference in genotype or allele frequencies between subgroups with and without metabolic syndrome. This lack of significance remained regardless of gender segregation or metabolic syndrome definition ($p>0.05$, Table 5). The only exception was a significantly lower frequency of the C allele in male subjects with metabolic syndrome based on the NCEP ATP III definition ($p = 0.04$).

Table 3
Genotype and allele frequencies of the AT_1R/A1166C polymorphism in CAD patients with and without diabetes mellitus.

	CAD (164)			CAD + diabetes (145)			*p*-value*
	AA	AC	CC	AA	AC	CC	
Genotype	124 (75.6)	31 (18.9)	9 (5.5)	90 (62.1)	42 (29)	13 (9)	0.01
Women	43 (78.2)	7 (12.7)	5 (9.1)	39 (63.9)	19 (31.1)	3 (4.9)	0.09
Men	81 (74.3)	24 (22)	4 (3.7)	51 (60.7)	23 (27.4)	10 (11.9)	0.04

		CAD	CAD + diabetes	*p*-value
Alleles	A	279 (85.1)	222 (76.6)	0.007
	C	49 (14.9)	68 (23.4)	
Women	A	93 (84.5)	97 (79.5)	0.32
	C	17 (15.5)	25 (20.5)	
Men	A	186 (85.3)	125 (74.4)	0.007
	C	32 (14.7)	43 (25.6)	

Values are expressed as number (%). Comparisons are performed using χ^2 test. *p*-values refer to the comparisons between polymorphic (AC + CC) and non-polymorphic (AA) genotypes; and between alleles. CAD: coronary artery disease.

Table 4
Genotype and allele frequencies of the AT_1R/A1166C polymorphism in patients with and without metabolic syndrome.

Definition			Without metabolic syndrome			With metabolic syndrome		
			AA	AC	CC	AA	AC	CC
IDF	Total	Genotype	117(66.5)	46(26.1)	13(7.4)	84(67.7)	30(24.2)	10(8.1)
		Allele A		280(79.5)			198(79.8)	
		Allele C		72(20.5)			50(20.2)	
	Women	Genotype	25(71.4)	9(25.7)	1(2.9)	53(66.3)	19(23.8)	8(10)
		Allele A		59(84.2)			125(78.1)	
		Allele C		11(15.7)			35(21.9)	
	Men	Genotype	92(65.2)	37(26.2)	12(8.5)	31(70.5)	11(25)	2(4.5)
		Allele A		221(78.4)			73(83)	
		Allele C		61(21.6)			15(17)	
NCEP ATP III	Total	Genotype	114(65.1)	45(25.7)	16(9.1)	87(69.6)	31(24.8)	7(5.6)
		Allele A		273(78.0)			205(82.0)	
		Allele C		77(22.0)			45(18.0)	
	Women	Genotype	29(70.7)	9(22)	3(7.3)	49(66.2)	19(25.7)	6(8.1)
		Allele A		67(81.7)			117(79)	
		Allele C		15(18.3)			31(21)	
	Men	Genotype	85(63.4)	36(26.9)	13(9.7)	38(74.5)	12(23.5)	1(2)
		Allele A		206(76.8)			88(86.3)	
		Allele C		62(23.2)			14(13.7)*	

Values are expressed as number (%). *p*-values refer to the comparisons between polymorphic (AC + CC) and non-polymorphic (AA) genotypes; and between alleles. IDF: International Diabetes Federation; NCEP ATP: National Cholesterol Education Program Adult Treatment Panel. *$p = 0.04$.

4. Discussion

Development of type 2 diabetes mellitus is thought to be the result of complex interactions between genetic and environmental factors. The involvement of a genetic component in the pathophysiology of this disorder is supported by strong evidences such as higher risk of diabetes in close relatives of an affected patient [26], and ethnic differences in the prevalence of the disease [27]. The RAS system is considered to play a pivotal role in the development of macro- and microvascular disease in diabetic subjects [9] and could also be involved in the pathogenesis of diabetes mellitus. This latter hypothesis is supported by the findings that indicate improvement of insulin sensitivity and glucose metabolism and decrease in the risk of type 2 diabetes following the blockade of the RAS system by angiotensin converting enzyme inhibitors and some angiotensin II receptor blockers [13–15]. Whilst the impact of genetic variations in the RAS system on the development of diabetes and its complications is not fully understood, a number of genes have been investigated in this regard [28,29].

In the present study, significantly higher frequencies of the polymorphic genotypes (AC and CC) and C allele were observed at the 1166 position of AT_1 receptor gene in the diabetic compared to the non-diabetic group. However, separate analysis of genotypes and alleles in each gender revealed that this difference is remained significant for males but not females. The lack of significant difference in allele frequencies between diabetic and non-diabetic females could be attributed to the smaller sample of females compared to males, or a gender difference in the genotypes of the AT_1R gene. A number of previous reports have indicated that the activity of RAS is influenced by gender which may be responsible for differences in blood pressure

Table 5
Prevalence of the metabolic syndrome and its components among study subjects.

Metabolic syndrome components	Total	Male	Female	*p*-value
NCEP ATP III criteria (three or more of the following)				
Central obesity: Waist circumference ≥102 cm for men and ≥88 cm for women	87(28.2)	17(19.5)	70(59.3)	<0.001
Triglycerides: ≥1.7 mmol/L	124(40.3)	68(35.6)	56(47.9)	0.03
HDL-C: <1.03 mmol/L in males and <1.29 mmol/L in female	133(43.2)	69(36.1)	64(54.7)	0.002
Fasting plasma glucose: ≥6.1 mmol/L	173(56.5)	97(51.3)	76(65)	0.02
Blood pressure: Systolic BP ≥130 or diastolic BP ≥85 mm Hg	180(49.7)	102(54.8)	87(67.2)	0.03
NCEP ATP III-defined metabolic syndrome	124(41.3)	44(23.8)	80(69.6)	<0.001
IDF criteria (central obesity plus any two of the following four factors)				
Central obesity: Waist circumference ≥94 cm for men and ≥80 cm for women	177(57.5)	75(39.3)	102(87.2)	<0.001
Triglycerides: ≥1.7 mmol/L	124(40.3)	68(35.6)	56(47.9)	0.03
HDL-C: <1.03 mmol/L in males and <1.29 mmol/L in female	133(43.2)	69(36.1)	64(54.7)	0.002
Fasting plasma glucose: ≥5.6 mmol/L	172(55.7)	96(50.3)	76(64.4)	0.01
Blood pressure: Systolic BP ≥130 or diastolic BP ≥85 mm Hg	180(49.7)	102(54.8)	87(67.2)	0.03
IDF-defined metabolic syndrome	125(41.7)	51(27.6)	74(64.3)	<0.001

Values are expressed as number (%). Comparisons are performed using χ^2 test. CAD: coronary artery disease; HDL-C: high-density lipoprotein cholesterol; NCEP ATP: National Cholesterol Education Program Adult Treatment Panel; IDF: International Diabetes Federation.

Table 6
Minor allele (C) frequency of the A1166C polymorphism in Iranian population.

Study	Subjects	Number	Male/female	MAF
Noroozianavval et al. (2007) [50]	Renal transplant recipients	108	66/42	23.6%
Behravan et al. (2007) [25]	Essential hypertension	74	41/33	18.9%
Behravan et al. (2007) [25]	Healthy	91	54/37	13.2%
Alavi-Shahri et al. (2010) [51]	Healthy	101	Only females	18.1%
Alavi-Shahri et al. (2010) [51]	Metabolic syndrome	249	Only females	11.9%
Namazi et al. (2010) [52]	Healthy	70	Only females	25.7%
Namazi et al. (2010) [52]	Breast cancer	70	Only females	21.4%
Assali et al. (2010) [53]	Healthy	135	72/63	7.4%
Assali et al. (present)	CAD	164	109/55	14.9
Assali et al. (present)	CAD + diabetes	145	84/61	23.4

MAF: minor allele frequency.

or perhaps blood glucose between men and women [30,31]. This gender difference in the activity of RAS may be a consequence of basic genetic differences in which AT_1R gene is a candidate. In a previous study by Behravan et al., a significantly higher frequency of the 1166C allele was reported in Iranian hypertensive females but not males which may imply a role for this allele as a predisposing factor for essential hypertension in females [25]. In another study, the 1166A allele of the AT_1R gene was reported to be higher in Tibetan hypertensive males compared to normotensives [32]. In contrast, Reich and colleagues reported that the mutated genotypes (AC and CC) are associated with higher blood pressure in males but not females [31].

In relation to the association of AT_1R/A1166C polymorphism with diabetes, a recent study has indicated that the risk of developing diabetes in subjects who are under treatment with angiotensin II receptor blockers is 5 times higher in those with the CC genotype compared to the carriers of A allele [33]. Although not universal [34,35], there are also reports on the association between the 1166C allele of the AT_1R gene and nephropathy in patients with type 1 or type 2 diabetes [36–38]. Moreover, in another study it was found that this polymorphism is associated with diabetic retinal complications and the 1166C allele could be a risk factor for diabetic retinopathy [39]. In another work by Thomas and colleagues among Chinese subjects, the presence of glucose intolerance which was defined as the presence of type 2 diabetes, impaired fasting glucose or impaired glucose tolerance, was not found to be significantly associated with the AT_1R/A1166C polymorphism nor any other aspect of the metabolic syndrome [28].

Since the A1166C polymorphism is in the 3′-untranslated region of the AT_1R gene, it would not influence the amino acid sequence of the receptor. However, the association of this polymorphism with diabetes mellitus and other reported phenotypic effects [13–16] could be due to the presence of linkage disequilibrium with functional variants in the AT_1R gene or other genes in the region. Another possible reason for a putative functional importance of the AT_1R/A1166C polymorphism pertains to its potential impact on the regulation of gene expression by microRNAs (miRNAs). miR155 has been shown to be expressed in the same cell types as AT_1R and interacts directly with the 3′ UTR region of the AT_1R mRNA. Using a receptor silencing reporter assay, Sethupathy and colleagues have reported that the miR155 only downregulates the expression of the AT_1R gene when associated with the 1166A, but not the 1166C allele [40]. Moreover, Martin et al. have reported that the 1166C allele decreases the ability of miR155 to regulate the expression of an AGTR1 reporter gene in transfected Chinese hamster ovary cells [41]. Regarding previous reports on the association between the 1166C allele and phenotype, specifically the presence of hypertension, in many studies, the 1166C allele may have functionality through disturbance in the miR155 related regulation, thereby being associated with elevated AT_1R levels.

We also assessed the relationship between the AT_1R/A1166C SNP and the presence of metabolic syndrome (with either NCEP ATP III or IDF definitions). We found a higher prevalence of the metabolic syndrome in females which is consistent with previous reports in Iranian adult [42–45]. Furthermore, in the present study no significant difference in AT_1R genotypes or alleles was found between subgroups with and without metabolic syndrome. This is consistent with the findings of Milionis et al. who did not find a significant difference in the frequency of AT_1R genotypes and alleles between newly diagnosed untreated hypertensive patients with and without metabolic syndrome [46]. Whilst other studies of this SNP have reported an association with an increased risk of metabolic syndrome, data have been inconsistent [47–49].

There have been few studies that investigated the association between AT_1R/A1166C polymorphism and the presence of diabetes and metabolic syndrome. Moreover, the participants of this study were patients with documented CAD in whom the coexistence of diabetes could seriously predispose to cardiovascular endpoints and end-stage renal disease (ESRD). Our findings indicate that the 1166C allele may be associated with the presence of diabetes in a group of Iranian male patients with angiographically defined CAD and might serve as a predisposing factor and/or predictor for the development of diabetes in these patients. Nevertheless, the aforementioned association was not confirmed by the binary logistic regression analysis. Besides, the present results should be interpreted with some caution. One of the limitations of our study is the fact that we have looked specifically among patients with CAD and hence it may not be possible for these results to be generalized to the whole population. It is worth noting that minor allele (C) frequency of the A1166C polymorphism in Iranian healthy subjects has been reported in 3 previous studies, being 13.2% (54 males, 37 females) [50], 18.1% (101 females) [51] and 25.7 (70 females) [52] (Table 6). Another limitation is the relatively few number of female subjects and individuals with metabolic syndrome that may explain the lack of an association in these subgroups. However, the significantly higher frequency of the C allele which was found in males with NCEP ATP III-defined metabolic syndrome needs to be confirmed in future studies with larger populations. Finally, since diabetes mellitus is a polygenic disease for which multiple genes are involved in genetic susceptibility, different SNPs are probably needed to be assessed simultaneously by linkage studies and haplotype analysis to identify genes that might have significant influence on the risk of diabetes.

Learning points

- The AT_1R/A1166C SNP may be associated with the presence of type 2 diabetes mellitus in Iranian male patients with angiographically-defined CAD.
- The AT_1R/A1166C SNP does not seem to be associated with the presence of metabolic syndrome (based on the IDF definition) in Iranian patients with angiographically-defined CAD.

Acknowledgements

The authors acknowledge the Research Council of the Mashhad University of Medical Sciences (MUMS) for the financial support of the study. We also sincerely thank all of the subjects who participated in the study. The authors have no conflict of interest to declare.

References

[1] Adeghate E, Schattner P, Dunn E. An update on the etiology and epidemiology of diabetes mellitus. Ann NY Acad Sci 2006;1084:1–29.
[2] Townsend T. A decade of diabetes research and development. Int J Diab Metab 2000;8:88–92.
[3] Amos AF, McCarty, Zimmet P. The rising global burden of diabetes and its complications: estimates and projections to the year 2010. Diab Med 1997;14: S1–S85.
[4] Zimmet P. The burden of type 2 diabetes: are we doing enough? Diab Metab 2003;29:6S9-6S18.
[5] Sacks DB, McDonald JM. The pathogenesis of type II diabetes mellitus: a polygenic disease. Am J Clin Pathol 1996;105:149–56.
[6] Awdeh ZL, Yunis EJ, Audeh MJ, Fici D, Pugliese A, Larsen CE, et al. A genetic explanation for the rising incidence of type 1 diabetes, a polygenic disease. J Autoimmun 2006;27:174–81.
[7] Gardemann A, Nguyen QD, Humme J, Stricker J, Katz N, Tillmanns H, et al. Angiotensin II type 1 receptor A1166C gene polymorphism. Absence of an association with the risk of coronary artery disease and myocardial infarction and of a synergistic effect with angiotensin-converting enzyme gene polymorphism on the risk of these diseases. Eur Heart J 1998;19:1657–65.
[8] Companioni Napoles O, Sautie Castellanos M, Leal L, Casalvilla R, Camacho H, Ferrer A, et al. ACE I/D polymorphism study in a Cuban hypertensive population. Clin Chim Acta 2007;378:112–6.
[9] Hanes DS, Nahar A, Weir MR. The tissue renin–angiotensin–aldosterone system in diabetes mellitus. Curr Hypertens Rep 2004;6:98–105.
[10] Osawa N, Koya D, Araki S, Uzu T, Tsunoda T, Kashiwagi A, et al. Combinational effect of genes for the renin–angiotensin system in conferring susceptibility to diabetic nephropathy. J Hum Genet 2007;52:143–51.
[11] Jacobsen P, Tarnow L, Carstensen B, Hovind P, Poirier O, Parving HH. Genetic variation in the renin–angiotensin system and progression of diabetic nephropathy. J Am Soc Nephrol 2003;14:2843–50.
[12] Padró-Miquel A, Alía-Ramos P, González-Álvarez MT, Navarro-Moreno MA. Survival in type 2 diabetic patients in dialysis and the number of risk alleles in polymorphisms of the renin–angiotensin system genes. Clin Biochem 2009;42: 5–11.
[13] Kotani K, Fujiwara S, Tsuzaki K, Sano Y, Matsuoka Y, Hamada T, et al. An association between angiotensin II type 2 receptor gene A/C3123 polymorphism and glycemic control marker in a general Japanese population. Mol Biol Rep 2009;36:917–20.
[14] Scheen AJ. Prevention of type 2 diabetes mellitus through inhibition of the renin–angiotensin system. Drugs 2004;64:2537–65.
[15] Gillespie EL, White CM, Kardas M, Lindberg M, Coleman CI. The impact of ACE inhibitors or angiotensin II type 1 receptor blockers on the development of new-onset type 2 diabetes. Diab Care 2005;28:2261–6.
[16] Stankovic A, Zivkovic M, Glisic S, Alavantic D. Angiotensin II type 1 receptor gene polymorphism and essential hypertension in Serbian population. Clin Chim Acta 2003;327:181–5.
[17] Chistiakov DA, Kobalova ZD, Tereshchenko SN, Moiseev SV, Nosikov VV. Polymorphism of vascular angiotensin II receptor gene and cardiovascular disorders. Ter Arkh 2000;72:27–30.
[18] Chapman CML, Palmer LJ, McQuillan BM, Hung J, Burley J, Hunt C, et al. Polymorphisms in the angiotensinogen gene are associated with carotid intimal–medial thickening in females from a community-based population. Atherosclerosis 2001;159:209–16.
[19] Takami S, Imai Y, Katsuya T, Ohkubo T, Tsuji I, Nagai K, et al. Gene polymorphism of the renin–angiotensin system associates with risk for lacunar infarction: the Ohasama study. Am J Hypertens 2000;13:121–7.
[20] Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285: 2486–97.
[21] Alberti KG, Zimmet P. IDF Epidemiology Task Force Consensus Group. The metabolic syndrome—a new worldwide definition. Lancet 2005;366:1059–62.
[22] Ghayour-Mobarhan M, Sahebkar A, Parizadeh SMR, Moohebati M, Tavallaie S, Kazemi-Bajestani SMR, et al. Antibody titres to heat shock protein 27 are elevated in patients with acute coronary syndrome. Int J Exp Pathol 2008;89: 209–15.
[23] Lahiri DK, Nurnberger Jr JI. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Res 1991;19:5444.
[24] Reich H, Duncan JA, Weinstein J, Cattran DC, Scholey JW, Miller JA. Interactions between gender and the angiotensin type 1 receptor gene polymorphism. Kidney Int 2003;63:1443–9.
[25] Behravan J, Naghibi M, Mazloomi MA, Hassany M. Polymorphism of angiotensin II type 1 receptor gene in essential hypertension in Iranian population. Daru 2006;14:82–6.
[26] American Diabetes Association. Type 2 diabetes in children and adolescents. Diab Care 2000;23:381–9.
[27] Arslanian S, Suprasongsin C. Differences in the in vivo insulin secretion and sensitivity of healthy black versus white adolescents. J Pediatr 1996;129: 440–3.
[28] Thomas GN, Tomlinson B, Chan JC, Sanderson JE, Cockram CS, Critchley JA. Renin–angiotensin system gene polymorphisms, blood pressure, dyslipidemia, and diabetes in Hong Kong Chinese: a significant association of the ACE insertion/deletion polymorphism with type 2 diabetes. Diab Care 2001;24: 356–61.
[29] Strazzullo P, Iacone R, Iacoviello L, Russo O, Barba G, Russo P, et al. Genetic variation in the renin–angiotensin system and abdominal adiposity in men: the Olivetti Prospective Heart Study. Ann Intern Med 2003;138:17–23.
[30] Miller JA, Anacta LA, Cattran DC. Impact of gender on the renal response to angiotensin II. Kidney Int 1999;55:278–85.
[31] Reich H, Duncan JA, Weinstein J, Cattran DC, Scholey JW, Miller JA. Interactions between gender and the angiotensin type 1 receptor gene polymorphism. Kidney Int 2003;63:1443–9.
[32] Liu Y, Zhuoma C, Shan G, Cui C, Hou S, Qin W, et al. A1166C polymorphism of the angiotensin II type 1 receptor gene and essential hypertension in Han, Tibetan and Yi populations. Hypertens Res 2002;25:515–21.
[33] Bozkurt Ö, de Boer A, Grobbee DE, Kroon AA, Schiffers P, de Leeuw P, et al. Renin–angiotensin system polymorphisms and the association between use of angiotensin II receptor blockers or angiotensin-converting enzyme inhibitors and the risk of diabetes. J Renin Angiotensin Aldosterone Syst 2009;10:101–8.
[34] Miller JA, Thai K, Scholey JW. Angiotensin II type 1 receptor gene polymorphism and the response to hyperglycemia in early type 1 diabetes. Diabetes 2000;49: 1585–9.
[35] Chang HR, Cheng CH, Shu KH, Chen CH, Lian JD, Wu MY. Study of the polymorphism of angiotensinogen, angiotensin-converting enzyme and angiotensin receptor in type II diabetes with end-stage renal disease in Taiwan. J Chin Med Assoc 2003;66:51–6.
[36] Lin J, Hu FB, Qi L, Curhan GC. Genetic polymorphisms of angiotensin-2 type 1 receptor and angiotensinogen and risk of renal dysfunction and coronary heart disease in type 2 diabetes mellitus. BMC Nephrol 2009;10:9.
[37] Savage DA, Feeney SA, Fogarty DG, Maxwell AP. Risk of developing diabetic nephropathy is not associated with synergism between the angiotensin II (type 1) receptor C1166 allele and poor glycaemic control. Nephrol Dial Transplant 1999;14:891–4.
[38] Chowdhury TA, Dyer PM, Kumar S, Gough SCL, Gibson SP, Rowe BR, et al. Lack if association of angiotensin II type receptor gene polymorphism with diabetic nephropathy in insulin-dependent diabetes mellitus. Diabet Med 1997;14: 837–40.
[39] Chistyakov DA, Chugunova LA, Shamkhalova MS, Shestakova MV, Milen'kaya TM, Dedov II, et al. Polymorphism of angiotensin II receptor gene and microangiopathies in patients with insulin-dependent diabetes mellitus. Russ J Genet 1999;35:1111–5.
[40] Sethupathy P, Borel C, Gagnebin M, Grant GR, Deutsch S, Elton TS, et al. Human microRNA-155 on chromosome 21 differentially interacts with its polymorphic target in the AGTR1 3-prime untranslated region: a mechanism for functional single-nucleotide polymorphisms related to phenotypes. Am J Hum Genet 2007;81:405–13.
[41] Martin MM, Buckenberger JA, Jiang J, Malana GE, Nuovo GJ, Chotani M, et al. The human angiotensin II type 1 receptor +1166A/C polymorphism attenuates microRNA-155 binding. J Biol Chem 2007;282:24262–9.
[42] Hadaegh F, Zabetian A, Tohidi M, Ghasemi A, Sheikholeslami F, Azizi F. Prevalence of metabolic syndrome by the Adult Treatment Panel III, International Diabetes Federation, and World Health Organization definitions and their association with coronary heart disease in an elderly Iranian population. Ann Acad Med Singapore 2009;38:142–9.
[43] Zabetian A, Hadaegh F, Azizi F. Prevalence of metabolic syndrome in Iranian adult population, concordance between the IDF with the ATPIII and the WHO definitions. Diab Res Clin Pract 2007;77:251–7.
[44] Azizi F, Salehi P, Etemadi A, Zahedi-Asl S. Prevalence of metabolic syndrome in an urban population: Tehran lipid and glucose study. Diab Res Clin Pract 2003;61: 29–37.
[45] Azimi Nezhad M, Ghayour-Mobarhan M, Parizadeh SMR, Safarian M, Esmaeili H, Khodaei GH, et al. Metabolic syndrome: its prevalence and relationship to socio-economic parameters in an Iranian population. Nutr Metab Cardiovasc Dis 2008;18:e11–2.
[46] Milionis HJ, Kostapanos MS, Vakalis K, Theodorou I, Bouba I, Kalaitzidis R, et al. Impact of renin–angiotensin–aldosterone system genes on the treatment response of patients with hypertension and metabolic syndrome. J Renin Angiotensin Aldosterone Syst 2007;8:181–9.
[47] Abdollahi MR, Gaunt TR, Syddall HE, Cooper C, Phillips D, Ye S, et al. Angiotensin II type I receptor gene polymorphism: anthropometric and metabolic syndrome traits. J Med Genet 2005;42:396–401.
[48] Goulart AC, Rexrode KM, Cheng S, Rose L, Buring JE, Ridker PM, et al. Association of genetic variants with the metabolic syndrome in 20,806 white women: the women's health genome study. Am Heart J 2009;158:257–62 e1.
[49] Palatini P, Ceolotto G, Dorigatti F, Mos L, Santonastaso M, Bratti P, et al. Angiotensin II type 1 receptor gene polymorphism predicts development of hypertension and metabolic syndrome. Am J Hypertens 2009;22:208–14.

[50] Noroozianavval M, Argani H, Aghaeishahsavari M, Veisi P, Jabbarpourbonyadi M, Hamzeiy H. Renin–angiotensin system polymorphisms and renal graft function in renal transplant recipients in Tabriz Emam Khomeini Hospital. Med J Tabriz Univ Med Sci 2007;29:115–20.
[51] Alavi-Shahri J, Behravan J, Hassany M, Tatari F, Kasaian J, Ganjali R, et al. Association between angiotensin II type 1 receptor gene polymorphism and metabolic syndrome in a young female Iranian population. Arch Med Res 2010;41: 343–9.
[52] Namazi S, Monabati A, Ardeshir-Rouhani-Fard S, Azarpira N. Association of angiotensin I converting enzyme (insertion/deletion) and angiotensin II type 1 receptor (A1166C) polymorphisms with breast cancer prognostic factors in Iranian population. Mol Carcinog 2010, doi:10.1002/mc.20685.
[53] Assali A, Behravan J, Paydar R, Mouhebati M, Hassani M, Kasaeyan J, et al. Association between angiotensin II type 1 receptor A1166C polymorphism and the presence of angiographically-defined coronary artery disease in an Iranian population. Asian Biomed 2010;4:307–14.