original article

N-acetyltransferase 1 and 2 polymorphisms and risk of diabetes mellitus type 2 in a Saudi population

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BACKGROUND AND OBJECTIVES: There have been inconsistent reports on N-acetyltransferase (*NAT*) gene polymorphism in type 2 diabetes mellitus (T2DM), and data is particularly limited in the Arab population. Therefore, the main objective of this study was to identify whether the genetic polymorphisms of *NAT1* and *NAT2* play a role in susceptibility to T2DM in the Saudi population.

DESIGN AND SETTING: A population-based, prospective genetic association case-control study on a Saudi population.

PATIENTS AND METHODS: Whole blood, anthropometric measurements and biochemistry data were collected from 369 Saudi individuals (186 T2DM patients and 183 healthy controls). DNA was isolated from the blood. Polymorphism of *NAT1* and *NAT2* SNPs [*NAT2**7B, rs1041983(C>T); *NAT2**7, rs1799931(G>A); *NAT2**6A, rs1799930(G>A); *NAT2**5A, rs1799929(C>T); and *NAT1**11A, rs4986988(C>T)] were evaluated by allelic discrimination using real-time PCR.

RESULTS: Subjects with T2DM had a significantly increased body mass index (BMI), waist circumference, systolic and diastolic blood pressure, glucose, triglycerides, and LDL-cholesterol compared with healthy controls ($P<.05$). The rs1799931(G>A) genotype was detected in the control population but not in the T2DM population ($P<.001$). The wild type (G) allele frequency was higher in T2DM than controls ($P=.038$). The mutant allele (A) in rs1799931(G>A) had a protective effect for T2DM (OR 0.32, 95% CI 0.16-0.62; $P=.001$). Regression analysis showed that BMI, systolic BP and triglycerides are potential risk factors for T2DM.

CONCLUSION: The genotypes as well as the individual alleles of rs1799931(G>A) differed significantly between the case and control populations. The variation in the data reported so far suggest that polymorphism of the *NAT* gene may vary among different geographical areas. Environmental or dietary factors may also contribute to disease manifestation.

Diabetes mellitus (DM) is a major health problem. Globally, its prevalence was estimated to increase from 4% in 1995 to 5.4% by the year 2025.[1] The World Health Organization has predicted that most of the burden of this disease will occur in developing countries.[2] In 2004, a national survey indicated that the prevalence of diabetes in Saudi Arabia is 23.7%. A more recent study showed that the prevalence of type 2 DM in Saudi Arabia is 17.7% and 16.4% in men and women, respectively.[3,4] Heredity plays a significant, but variable, role in the etiology of DM. Both type 1 DM (T1DM) and type 2 DM (T2DM) show a familial predisposition, which indicates the involvement of genetic factors in determining susceptibility.

Type 2 diabetes mellitus (T2DM) is known to be influenced by both genetic and environmental factors. Therefore, identification of loci that are associated of T2DM has been investigated.

N-acetyltransferase 1 (*NAT1*) and N-acetyltransferase 2 (*NAT2*) family of enzymes catalyzes the acetylation of many aromatic and hydrazine drugs as well as many aromatic and heterocyclic amine carcinogens.[5,6] This reaction can result in either detoxification (*N-acetylation*) or activation (*O-acetylation*) of

these compounds.[7,8] Both *NAT1* and *NAT2* are products of intronless protein-coding 870 base pair exons.

NAT1 and *NAT2* exhibit allelic variations and genetic polymorphisms associated with increased susceptibility toward the toxicity of drug and environmental diseases. Variant *NAT1* and *NAT2* alleles possess various combinations of single nucleotide polymorphisms (SNPs), deletions, and/or insertions.[6] The frequency of *NAT1* and *NAT2* alleles, genotypes, and phenotypes differs markedly among ethnic groups.[5] To date, 28 *NAT1* and 66 *NAT2* alleles have been identified in the human population.[9] The coding region of the genes possess most of the *NAT1* polymorphism, and all of the *NAT2* polymorphisms. The substrate affinity, catalytic activity, protein degradation or stability changes as a result of the polymorphisms.[6,10]

A role for *NAT2* gene polymorphism has been suggested in various types of cancer.[11-13] Interestingly, the association between slow acetylator type of *NAT2* and the risk of bladder cancer differ according different to geographical regions.[14] Reports are inconsistent on *NAT* gene polymorphism in T2DM. Studies form Bosnia and Herzegovina and Turkey demonstrate an association between *NAT2* variation and diabetes in their population.[15,16] However, the results of a Jordanian study are inconsistent with those findings.[17] A study from Saudi Arabia showed an association between the slow acetylator phenotype and T1DM while the other reported an association between the rapid acetylator phenotype and T1DM.[18,19] The data on the association of T2DM and *NAT* genes are inconsistent among different populations so far.

Therefore, we designed the our study with the objective of identifying whether the genetic polymorphisms of *NAT1* and *NAT2* play a role in susceptibility to T2DM in the Saudi population. More specifically, we studied the distribution of selected gene polymorphisms (*NAT1**11, *NAT2**5A, *NAT2**6A, *NAT2**7A/B, *NAT2**14A), which represent a combination of rapid and slow acetylation genotypes, and their association with T2DM patients and normal controls.

PATIENTS AND METHODS

Study subjects

Three-hundred-sixty-nine Saudi individuals (186 T2DM patients and 183 healthy controls) were enrolled in the study. These individuals were part of the Biomarkers Screening in Riyadh Project (Riyadh Cohort), a capital-wide epidemiologic study, taken from over 17 000 consenting Saudis coming from different primary health care centers (PHCCs) during the years 2009-2013. Controls were randomly selected from the master database of this project.".We used a generalized questionnaire to collect demographic information, and a medical history was taken from all subjects. Those with co-morbidities that needed medical attention were excluded from the study. Written informed consent was obtained after orientation for the study. Ethical approval for the study was granted by the Ethics Committee of the College of Science Research Center, King Saud University, Riyadh, Saudi Arabia.

Anthropometry and blood collection

Participating subjects were requested to return to their respective PHCCs after an overnight fast (>10 hours) for anthropometry and blood withdrawal. Anthropometry included height (to the nearest 0.5 cm), weight (to the nearest 0.1 kg), waist and hip circumference using a standardized measuring tape in centimeters, systolic and diastolic blood pressure, and body mass index (BMI, calculated as kg/m^2). Blood was taken from the subjects and placed immediately into a non-heparinized tube for centrifugation. Serum

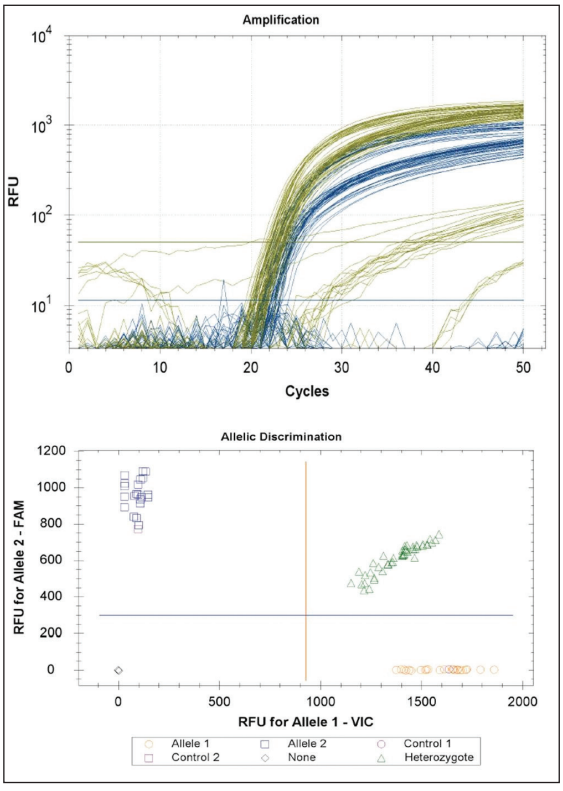

Figure 1. Allelic discrimination plot showing genotype assignment determined by plotting the end point RFU for one fluorophore -VIC (allele 1 on the x-axis) against the RFU for the other fluorophore-FAM (allele 2 on the y-axis).

was separated and transferred to a pre-labeled plain tube, stored in ice, and delivered to the Biomarkers Research Center in King Saud University on the same day.

Biochemical analysis

Fasting serum samples were stored in a −20°C freezer prior to analysis. Fasting glucose, triglyceride, total and HDL-cholesterol levels were measured by chemistry auto-analyzer (Konelab, Vantaa, Finland) and concentrations of LDL-cholesterol were calculated using Friedwald's formula. The inter- and intra-assay variabilities were 5.3% and 4.6%.

DNA extraction

Whole blood was collected in EDTA-containing tubes and genomic DNA was isolated using the blood genomic prep Mini Spin Kit (GE Healthcare Life Sciences, Piscataway, NJ, USA) according to manufacturer's instructions. Briefly, this method uses a simple genomic DNA purification protocol that uses chemotropic agents to extract DNA from blood cells, denature protein components, and promote the selective binding of DNA to a column-based, silica membrane. The isolated genomic DNA was stored at −20°C until further analysis.

Genotype analysis

Five *NAT1* and *NAT2* SNPs [*NAT2**7B, rs1041983(C>T); *NAT2**7, rs1799931(G>A); *NAT2**6A, rs1799930(G>A); *NAT2**5A, rs1799929(C>T); and *NAT1**11A, rs4986988(C>T)] were evaluated by allelic discrimination real-time polymerase chain reaction (PCR) using pre-designed TaqMan probes from Applied Bio-Systems (Foster City, CA, USA). Amplification reactions were performed in a volume of 10 μL containing 1X TaqMan genotyping Master Mix (Applied Biosystems), 1x mix of unlabeled PCR primers and TaqMan MGB probes, and 50 ng of template DNA. All amplification and detection was conducted on genomic DNA in 96-well PCR plates using a Bio-Rad CFX96 Real-Time PCR Detection System (Bio-Rad, Milan, Italy). Thermal cycling was initiated with a denaturation step of 10 min at 95°C, followed by 50 cycles of 15 s at 95°C and 90 s at 60°C. After PCR was completed, allelic discrimination was analyzed using the Bio-Rad CFX Manager Software (Version 1.6, Bio-Rad). Genotype assignment was determined by plotting the end point relative fluorescent units (RFU) for one fluorophore (allele 1 on the X-axis) against the RFU for the other fluorophore (allele 2 on the Y-axis) on the allelic discrimination plot (**Figure 1**) The genotypes were assigned using the whole data from the study, simultaneously. All PCR reactions were set up in a dedicated PCR area with dedicated PCR pipettes and reagents. For validation, about 10% of the samples were re-genotyped. The results were reproducible with no discrepancies in genotyping.

Statistical analysis

Data are expressed as mean (standard deviation) or number (percentage). A chi-square test or Fisher exact test was used for comparison of categorical variables. A χ^2 test or analysis of variance was used to analyze the difference between continuous variables. Odds ratios (OR), 95% confidence intervals (CI) and corresponding *P* values for risk of T2DM were calculated by using logistic regression analysis. The most common genotype was used as the reference in the model. For the identification of potential risk factors for T2DM, the anthropometric variables that were significantly different between cases and controls were selected as covariates in the logistic regression model. All the statistical analyses were carried out using SPSS statistics software version 17.0 (SPSS Inc, Chicago IL, USA). Chi square with one degree of freedom was used to assess the departure from the Hardy-Weinberg equilibrium. A two-sided *P* value <.05 was considered significant.

The sample size was estimated based on information from previously published studies.

Table 1. Anthropometric and metabolic characterization of study subjects.

Parameters	T2DM (n=186)	Control (n=183)	*P* value
Male/Female (n, %)	93 (50%)/ 93 (50%)	89 (48.6%)/ 94 (58.4%)	.793
Age (years)	46.1 (5.4)	46.2 (3.5)	.630
BMI (kg/m^2)	31.8 (6.2)	26.5 (4.0)	<.001
Systolic BP (mm Hg)	126.2 (13.3)	117.2 (9.3)	<.001
Diastolic BP (mm Hg)	79.6 (8.7)	76.6 (6.9)	<.001
Cholesterol (mmol/L)	5.4 (1.0)	5.2 (1.1)	.099
HDL (mmol/L)	0.8 (0.3)	0.8 (0.3)	.131
Triglyceride (mmol/L)	2.1 (0.9)	1.6 (0.8)	<.001
Glucose (mmol/L)	9.8 (1.7)	5.1 (0.6)	<.001
LDL-Cholesterol (mmol/L)	4.4 (0.9)	4.1 (1.0)	.031
Waist	96.0 (21.8)	89.4 (15.2)	.003
Hip	103.0 (24.7)	98.9 (16.1)	.082

Values are represented as mean (standard deviation). T2DM, Type 2 diabetes mellitus; BMI, Body mass index; HDL, High density lipoprotein; LDL, Low density lipoprotein; *P* value <.05 was taken as significant.

Table 2. Anthropometric and metabolic parameters by SNP genotype polymorphisms in the overall population (n=369).

Genotype/Allele	Age	BMI (kg/m²)	Systolic BP (mmHg)	Diastolic BP (mmHg)	Cholesterol (mmol/l)	HDL (mmol/l)	Triglyceride (mmol/l)	Glucose (mmol/l)	LDL-Cholesterol (mmol/l)	Waist	Hip
rs1041983											
CC	45.9 (4.6)	28.5 (6.2)	121.0 (11.8)	78.1 (8.4)	5.3 (1.1)	0.8 (0.3)	1.8 (0.9)	7.3 (2.6)	4.2 (1.0)	89.4 (17.5)	100.7 (19.8)
CT	46.3 (4.4)	29.5 (5.3)	121.5 (12.8)	77.3 (7.7)	5.3 (1.1)	0.8 (0.3)	1.9 (0.9)	7.5 (2.7)	4.1 (1.0)	94.9 (19.0)	99.3 (20.3)
TT	46.4 (5.0)	29.6 (6.5)	122.5 (12.4)	79.2 (7.2)	4.9 (0.9)	0.6 (0.3)	1.6 (0.9)	7.0 (2.6)	4.1 (1.0)	92.9 (19.5)	102.9 (20.3)
P value	.729	.253	.778	.386	.161	.029	.142	.556	.795	.117	.682
rs1799929											
CC	45.8 (4.6)	29.8 (5.3)	123.4 (12.4)	78.8 (8.1)	5.1 (0.9)	0.7 (0.3)	1.8 (0.9)	7.4 (2.7)	4.1 (0.9)	93.9 (19.2)	102.5 (21.2
CT	46.6 (4.6)	28.6 (5.5)	120.8 (13.0)	77.4 (7.9)	5.3 (1.1)	0.8 (0.3)	1.8 (0.8)	7.5 (2.7)	4.2 (1.1)	90.4 (19.4)	97.9 (20.6)
TT	45.7 (4.4)	29.2 (7.3)	121.2 (10.6)	78.4 (7.9)	5.3 (0.9)	0.8 (0.3)	1.9 (1.0)	7.3 (2.6)	4.2 (0.9)	92.0 (14.6)	104.2 (14.9)
P value	.240	.298	.227	.350	.128	.246	.563	.752	.621	.363	.027
rs1799930											
AA	46.2 (4.9)	29.4 (6.1)	123.7 (15.3)	80.0 (7.6)	4.9 (1.0)	0.6 (0.3)	1.5 (0.8)	7.4 (2.7)	4.1 (1.1)	96.6 (15.2)	107.3 (14.8)
GA	46.4 (4.4)	29.7 (5.4)	121.5 (11.8)	77.5 (7.4)	5.2 (1.1)	0.8 (0.3)	1.9 (0.9)	7.4 (2.7)	4.1 (1.1)	93.8 (19.4)	93.8 (19.4)
GG	45.9 (4.6)	28.8 (6.3)	121.0 (12.0)	78.0 (8.4)	5.4 (1.0)	0.8 (0.3)	1.8 (0.9)	7.4 (2.6)	4.3 (1.0)	89.4 (17.9)	89.4 (17.9)
P value	.562	.177	.526	.305	.057	.018	.132	.999	.308	.054	.138
rs1799931											
AA	46.1 (5.2)	29.8 (5.9)	120.6 (12.3)	79.3 (10.3)	5.3 (0.7)	0.6 (0.2)	2.1 (1.1)	7.9 (2.6)	4.3 (0.6)	83.0 (24.3)	94.8 (26.2)
GA	46.5 (3.0)	26.5 (4.2)	119.5 (9.0)	79.7 (6.6)	5.2 (1.5)	0.7 (0.2)	1.5 (0.8)	5.0 (0.6)	4.2 (1.4)	88.6 (16.5)	98.4 (17.9)
GG	46.1 (4.7)	29.6 (6.0)	121.9 (12.6)	78.0 (8.1)	5.3 (1.0)	0.8 (0.3)	1.9 (0.9)	7.8 (2.7)	4.2 (1.0)	92.9 (18.4)	101.3 (19.6)
P value	.94	.056	.222	.530	.960	.415	.187	<.001	.886	.114	.438
rs4986988											
CC	46.2 (4.5)	28.9 (6.0)	122.0 (12.7)	78.1 (1.1)	5.3 (1.1)	0.8 (0.3)	1.4 (0.9)	7.5 (2.6)	4.2 (1.0)	91.0 (19.1)	100.1 (19.1)
CT	46.0 (4.7)	29.4 (5.5)	120.5 (11.4)	77.9 (7.7)	5.1 (0.9)	0.7 (0.3)	1.8 (0.8)	7.1 (2.7)	4.0 (0.9)	93.6 (18.2)	100.0 (18.8)
TT	45.7 (4.4)	30.9 (7.7)	120.0 (10.9)	77.4 (7.9)	5.0 (1.2)	1.0 (0.3)	1.5 (0.9)	8.2 (3.0)	3.7 (1.1)	96.9 (16.4)	108.7 (39.4)
P value	.873	.497	.564	.950	.137	.112	.417	.268	.138	.388	.411

Values are represented as mean (standard deviation). BMI, Body mass index; HDL, High density lipoprotein; LDL, Low density lipoprotein; *P* value <.05 was taken as significant.

Table 3. Genotype distribution frequencies of studied *NAT1* and *NAT2* SNPs.

Genotype/Allele	Frequency		*P* value
	T2DM n (%)	Control n (%)	
Genotype rs1041983 (C>T)	180	180	
CC	85 (47.2)	91 (50.6)	.755
CT	78 (43.3)	71 (39.4)	
TT	17 (9.4)	18 (10)	
Allele			
C	248 (68.8)	253 (70.2)	.689
T	112 (31.1)	107 (29.7)	
Genotype rs1799929 (C>T)	186	180	
CT	91 (48.9)	84 (46.7)	.851
CC	53 (28.5)	51 (28.3)	
TT	42 (22.6)	45 (25.0)	
Allele			
C	197 (52.9)	186 (51.6)	.729
T	175 (47.0)	174 (48.3)	
Genotype rs1799930 (G>A)	179	181	
GG	91 (50.8)	94 (51.9)	.919
GA	71 (39.7)	72 (39.8)	
AA	17 (9.5)	15 (8.3)	
Allele			
G	253 (70.6)	260 (71.8)	.729
A	105 (29.3)	102 (28.2)	
Genotype rs1799931 (G>A)	185	154	
GG	176 (95.1)	124 (80.5)	<.001
GA	0 (0)	24 (15.6)	
AA	9 (4.9)	6 (3.9)	
Allele			
G	352 (95.1)	272 88.3))	.038
A	18 (4.8)	36 (11.6)	
Genotype rs4986988 (C>T)	186	181	
CC	140 (75.3)	127 (70.2)	.254
CT	39 (21.0)	50 (27.6)	
TT	7 (3.8)	4 (2.2)	
Allele			
C	319 (85.7)	304 (83.9)	.502
T	53 (14.2)	58 (16.0)	

T2DM, Type 2 diabetes mellitus; *P* value <.05 was taken as significant.

RESULTS

For 186 T2DM patients and 183 healthy controls included in the analysis, the median age of all (369) subjects was 47 years (range 31–54 years) and females constituted about 50.7%. The anthropometric, clinical and biochemical features of individuals enrolled in the study are presented in **Table 1**. Subjects with T2DM had significantly increased BMI, waist circumference, systolic and diastolic blood pressure, glucose, triglycerides, and LDL-cholesterol levels compared with the healthy control subjects ($P<.05$). Taking overall study population into consideration, the TT genotype of SNP rs1041983, and AA genotype of rs1799930 was significantly associated with a low HDL count ($P<.05$), whereas the GA genotype was associated with a low glucose level for rs1799931 ($P<.001$; **Table 2**).

The genotype distribution as well as single allele frequencies of rs1041983(C>T), rs1799930(G>A), rs1799931(G>A), rs1799929(C>T) and rs4986988(C>T) variants are summarized in **Table 3**. In general, there was no significant difference in the frequencies of the polymorphisms between the two groups. However, rs1799931(G>A) genotype was not detected in the T2DM population, a difference that was statistically significant as opposed to controls ($P<.001$). Furthermore, the wild type (G) allele frequency was higher in T2DM population than in the non-diabetic population ($P=.038$). Interestingly, the genotype frequencies of rs1041983 (C>T), rs1799929 (C>T), rs4986988 (C>T), rs1799930 (G>A) polymorphisms met the Hardy Weinberg Equilibrium (HWE) in both the control group as well as the T2DM subjects (**Table 4**). Whereas in the case of rs1799931 (G>A), the G and A allele frequencies deviate significantly from the HWE both in the T2DM group ($P<.001$) and controls ($P=.002$).

BMI (OR 1.177, 95% CI 1.09-1.26; $P<.001$), systolic BP (OR 1.085, 95% CI 1.04-1.13; $P<.001$), and triglycerides (OR 1.163, 95% CI 1.18-2.26; $P=.003$) added significantly to the model and were potential risk factors for T2DM. However, diastolic BP, cholesterol, LDL, and BP did not add significantly to the model (**Table 5**). A logistic regression model for the genotype and allele provided a protective effect for mutant allele A in the SNP rs1799931(G>A) (OR 0.32, 95% CI 0.16-0.62; $P=.001$). The allele T in SNP rs1041983(C>T) did produce higher odds for the being the risk factor but the result was not statistically significant. Other genotypes and alleles also did not show any significant effect on T2DM risk (**Table 6**).

Table 4. Genotype, allele frequencies and Hardy-Weinberg chi-square in the *NAT1* and *NAT2* genes in case and control populations.

Population	Genotype frequency			Allelic frequency		HWE	
	CC	CT	TT	Wild type (C)	Mutant (T)	HW χ^2	HW *P*
rs1041983 C>T							
T2DM	85 (47.2)	78 (43.3)	17 (9.4)	248 (68.8)	112 (31.1)	0.026	.883
Control	91 (50.6)	71 (39.4)	18 (10)	253 (70.2)	107 (29.7)	0.561	.454
rs1799929 C>T							
T2DM	53 (28.5)	91 (48.9)	42 (22.6)	197 (52.9)	175 (47.0)	0.061	.805
Control	51 (28.3)	84 (46.7)	45 (25.0)	186 (51.6)	174 (48.3)	0.775	.379
rs4986988 C>T							
T2DM	140 (75.3)	39 (21.0)	7 (3.8)	319 (85.7)	53 (14.2)	3.745	.053
Control	127 (70.2)	50 (27.6)	4 (2.2)	304 (83.9)	58 (16.0)	0.128	.721
	GG	GA	AA	Wild type (G)	Mutant (A)	HW χ^2	HW *P*
rs1799930 G>A							
T2DM	91 (50.8)	71 (39.7)	17 (9.5)	253 (70.6)	105 (29.3)	0.333	.563
Control	94 (51.9)	72 (39.8)	15 (8.3)	260 (71.8)	102 (28.1)	0.054	.817
rs1799931 G>A							
T2DM	176 (95.1)	0 (0)	9 (4.9)	352 (95.1)	18 (4.8)	185	<.001
Control	124 (80.5)	24 (15.6)	6 (3.9)	272 88.3))	36 (11.6)	9.25	.002

HWE, Hardy-Weinberg equation; T2DM, Type 2 diabetes mellitus; HW P, Hardy–Weinberg *P* value; All the values are represented as n (%); d.f.=1 for all tests. *P* value <.05 was taken as significant.

DISCUSSION

In this case-control study, we investigated whether the polymorphism of *NAT1* and *NAT2* genes plays role in the development of T2DM. Drug-metabolizing enzyme activity is altered in diseases such as cancers, infectious and inflammatory disease, and immune system disorders.[16] The relevance of *NAT* genes in drug metabolism and disease susceptibility has been a central theme of pharmacogenetic research mainly because of the genetic variability among different human populations.

The protein product of the *NAT2* gene is capable of N-acetylation and O-acetylation which is implicated in the metabolism and detoxification of naturally occurring xenobiotics, including carcinogens and drugs.[20] The acetylator phenotype is determined by studying the acetylation of a variety drugs such as isoniazid, sulfadimidine, dapsone or caffeine. Therefore, the acetylation capacity in humans has been linked to *NAT2* gene polymorphisms, which alters susceptibility to cancer and other diseases including adverse drug reactions.[21,22]

Studies describing the association of *NAT* alleles with the T2DM are few and have been inconsistent in different populations and geographical regions. Only one of the SNPs of the *NAT* gene polymorphism (rs1799931 G>A) that we studied was significantly different between the cases and controls. The frequency of wild type genotypes was higher in the T2DM patients than the control group. Also, the individual allelic frequency of major alleles differed significantly in both groups with a protecting effect for the mutant allele (OR 0.32). However, another study showed no signifi-

Table 5. Multiple logistic regression analysis of anthropometric and metabolic variables on T2DM.

Parameters	Odds ratio	95% Confidence interval	*P* value
Body mass index	1.177	1.09-1.26	<.001
Systolic blood pressure	1.085	1.04-1.13	<.001
Diastolic blood pressure	0.949	0.89-1.00	.067
Cholesterol	1.102	0.44-2.74	.835
Triglyceride	1.634	1.18-2.26	.003
LDL-Cholesterol	0.943	0.37-2.42	.902
Waist	1.007	0.99-1.03	.466

LDL, low density lipoprotein, *P* value <.05 was taken as significant.

Table 6. Logistic regression analysis of different genotypes and alleles on T2DM.

Genotype/ Allele	OR	95% CI	*P* value
rs1041983 (C>T)			
CC	Reference		
CT	2.09	0.46-9.45	.338
TT	0.94	0.96-9.09	.955
C	Reference		
T	1.89	0.74-4.82	.182
rs1799929 (C>T)			
CT	Reference		
CC	1.02	0.52-1.97	.964
TT	0.80	0.40-1.59	.525
C	Reference		
T	0.93	0.68-1.28	.654
rs1799930 (G>A)			
GG	Reference		
GA	0.39	0.08-1.79	.225
AA	0.83	0.08-7.99	.869
G	Reference		
A	0.58	0.23-1.50	.262
rs1799931 (G>A)			
GG	Reference		
GA	0.00	--	.998
AA	1.11	0.25-0.49	.889
G	Reference		
A	0.32	0.16-0.62	.001
rs4986988 (C>T)			
CC	Reference		
CT	0.67	0.39-1.16	.148
TT	1.07	0.28-4.03	.924
C	Reference		
T	0.83	0.53-1.29	.417

OR, Odds ratio; CI, Confidence interval; *P* value <.05 was taken as significant.

cant difference between the two groups for this SNP.[15] A study from Japan also reported no significant increase in the prevalence of the slow acetylator phenotype in patients with diabetic nephropathy, suggesting that *NAT* gene polymorphism might not be a risk factor for diabetic nephropathy in diabetic patients.[23]

A previous study from Saudi Arabia showed significant differences in the proportion of rapid acetylators between T1DM and T2DM (*P*=.0436), and between the control and overall diabetic population (*P*=.024) or those with T1DM (*P*=.0028). There was also a significant association between rapid acetylator and T1DM.[18] Mrozikiewicz et al found no relation between the fast acetylator genotypes (homozygous and heterozygous) and T1DM.[24] Another study also found no relationship between *NAT2* polymorphism and T2DM or its complications such as nephropathy and neuropathy.[25] Furthermore, Hegele et al showed no difference in the genotype and allele frequencies of the *NAT2* gene and T2DM.[26] However, Yalin et al[15] concluded that the *NAT2* slow acetylator genotypes could be an important factor that determines DM in a Turkish population. The investigators, in a case-control study, reported that the *NAT2* slow allele (especially *6A) was found to confer a 5-fold increased risk of T2DM.[15] An increased risk for diabetes have also been shown in the *NAT2*5A* mutant genotype and the *NAT2*14A* heterozygous genotype, while the polymorphism in the *NAT2*7A/B* have not been associated with an increased risk.

Another study in the population of Bosnia and Herzegovina reported that *NAT2*5* polymorphism is significantly associated with a 2.4-fold increased risk for developing T2DM, while *NAT2*6* polymorphism significantly decreases the risk of T2DM by 5-fold.[16] In a more recent study, Irdshaid et al explored the association between *NAT2* genotypes and T1MD and T2DM in the Jordanian population.[17] They concluded that there is an excess of genotypes encoding intermediate acetylation in T2DM and an excess of slow acetylator genotypes in T1DM. They further showed that the *NAT2*4/6* genotype is more prevalent in T2DM.[17] Our study does not support the involvement of *NAT2*5A* or *NAT2*6* in an increased risk of T2DM, and also does not indicate a lack of association of *NAT2*7A/B* with the disease phenotype.

The departure from the Hardy Weinberg (HW) equilibrium is evident in the case of SNP rs1799931 (G>A) in the present study. Other studies have reported the frequencies of the wild type and mutant alleles of these SNPs are under the HW equilibrium.[27] Deviation from HW equilibrium can also be caused by selection bias, which may have occurred in our study due to the small sample size , but is unlikely because of random sampling.

Several studies have shown that anthropometric measures including estimates of body composition and BMI are significantly and positively associated with T2DM risk in both men and women independent of age and other individual characteristics.[16,28,29]

CONCLUSION

Our study has demonstrated that genotypes as well as the individual allele of SNP rs1799931(G>A) significantly differs between the case and control popula-

tions. Further, allele A provided a protective effect from diabetes. The BMI, systolic BP and triglycerides are the potential risk factors for T2DM. The variation in the data reported so far suggests that polymorphisms of the *NAT* gene may vary among different geographical areas. It is also possible that some environmental or dietary factors may also contribute to disease manifestation. Therefore, further studies with larger sample sizes on these lines are warranted.

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Conflict of interest

The authors declare that they have no conflict of interest.

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