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ORIGINAL RESEARCH

Clinical Significance of NAT2 Genetic Variations in Type II Diabetes Mellitus and Lipid Regulation

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Background: N-acetyltransferase 2 (NAT2) enzyme is a Phase II drug-metabolizing enzyme that metabolizes different compounds. Genetic variations in *NAT2* can influence the enzyme's activity and potentially lead to the development of certain diseases.

Aim: This study aimed to investigate the association of *NAT2* variants with the risk of Type II diabetes mellitus (T2DM) and the lipid profile among Jordanian patients.

Methods: We sequenced the whole protein-coding region in *NAT2* using Sanger's method among a sample of 45 Jordanian T2DM patients and 50 control subjects. Moreover, we analyzed the lipid profiles of the patients and examined any potential associations with *NAT2* variants.

Results: This study revealed that the heterozygous *NAT2*13 C/T* genotype is significantly ($P = 0.03$) more common among T2DM (44%) than non-T2DM subjects (23.5%). Furthermore, the frequency of homozygous *NAT2*13 T/T* genotype was found to be significantly higher ($P = 0.03$) among T2DM patients (26.7%) compared to that of non-T2DM subjects (11%). The heterozygous *NAT2*7 G/A* genotype was exclusively observed in T2DM patients (11.1%) and absent in the control non-T2DM group. Moreover, among T2DM patients, those with a homozygous *NAT2*11* T/T genotype exhibited significantly higher levels of triglycerides (381.50 ± 9.19 ng/dL) with a P value of 0.01 compared to those with heterozygous *NAT2*11* C/T (136.23 ± 51.12 ng/dL) or wild-type *NAT2*11* C/C (193.65 ± 109.89 ng/dL) genotypes. T2DM patients with homozygous *NAT2*12* G/G genotype had a significantly ($P = 0.04$) higher triglyceride levels (275.67 ± 183.42 ng/dL) than the heterozygous *NAT2*12 A/G* (140.02 ± 49.53 ng/dL) and the wild *NAT2*12 A/A* (193.65 ± 109.89 ng/dL).

Conclusion: The finding in this study suggests that the *NAT2* gene is a potential biomarker for the development of T2DM and changes in triglyceride levels among Jordanians. However, it is important to note that our sample size was limited; therefore, further clinical studies with a larger cohort are necessary to validate these findings.

Keywords: type II diabetes mellitus, *N-acetyltransferase 2*, *NAT2*, triglyceride, genetic variants, Jordanian population

Introduction

Type II diabetes mellitus (T2DM) is a metabolic disease that is characterized by a high blood glucose level due to the decrease in insulin release from the pancreas and/or an increase in peripheral cellular resistance to insulin effects. Uncontrolled T2DM can cause harmful complications in the human body.[1] Jordanians, like many other Middle Eastern populations are known to have a high prevalence of T2DM.[2]

T2DM has several risk factors, among which genetics plays a significant role. It was found that specific genes including peroxisome proliferator activator receptor and interleukin-10 genes, increase the risk of T2DM.[3] Recently, it has been reported, in a genome-wide association study, that *N-acetyltransferase 2 (NAT2)* is a candidate gene for the

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development of T2DM. The same study reported that knocking down the *NAT2* gene increased insulin resistance, and hence caused a disruption in lipid metabolism.[4]

NAT2 is a phase II drug metabolizing enzyme that is expressed in several organs of the human body, including the liver. It acetylates certain endogenous and xenobiotic compounds, including drugs and carcinogenic compounds.[5] Moreover, it was found that metabolites of the enzyme can also be toxic to the human body and may induce cancer and immune diseases, such as rheumatoid arthritis.[6–8]

The acetylation capacity of the NAT2 enzyme is influenced by both the health-status and ethnicity of the patient. As previously documented in the literature,[9] individuals with type 2 diabetes exhibit a lower acetylation capacity than those without the condition. Moreover, it has been observed that Caucasians possess a greater proportion of slow-acetylators than Asians.[10] Such variation in acetylation capacity can be attributed predominantly to genetic variants in the *NAT2* gene.[10]

Slow-encoding *NAT2* genotypes are associated with isoniazid-induced hepatotoxicity,[11] rheumatoid arthritis, and other oral diseases.[12] On the other hand, fast-encoding *NAT2* genotypes are at higher risk of developing cancer.[13,14] As for T2DM, the literature has conflicting results for its association with the *NAT2* gene. For example, Irshaid et al did not find a significant association between major *NAT2* genotypes and T2DM among Jordanian patients.[15] Previous studies conducted among Middle Eastern populations, such as those in Saudi Arabia[16] and Turkey,[17] have shown a significant correlation between the *NAT2* genotype and T2DM. However, it is important to note that both of these studies only examined a limited number of *NAT2* genetic variants.

To expand on this knowledge gap, we sequenced the protein-encoding region of the *NAT2* gene among a sample of unrelated healthy Jordanians, and discovered a novel genetic variant in linkage disequilibrium (LD). We also observed that the *NAT2* haplotypes in this population differed slightly from those reported in other ethnic groups.[18]

Given the limited information about the structure of the *NAT2* gene among Jordanian T2DM patients, our study aimed to address this issue by sequencing the protein-coding region of the *NAT2* gene among a sample of T2DM Jordanian patients and comparing the sequence of the *NAT2* gene with that of control non-T2DM subjects.

Materials and Methods

Participants and Ethics of the Study

A total of 45 T2DM patients and 50 non-T2DM subjects of both genders participated in this study. The non-T2DM control subjects were unrelated Jordanians with a glycosylated hemoglobin (HbA1C) value of less than 5.7[19] and without any chronic diseases, such as autoimmunity, liver, kidney, cardiovascular, neurological, or cancer diseases. T2DM patients were diagnosed by endocrinologists at the University of Jordan Hospital between October 2020 and March 2021. The diagnosis of T2DM was in accordance with the guidelines of the American Association of Diabetes.[20] The patients were also unrelated Jordanians.

The protocol for this study was approved by the ethical committee at Al-Zaytoonah University of Jordan and the institutional review board at the University of the Jordan Hospital (Reference number 2022-2021/13-5). Additionally, written informed consent was obtained from each subject before participating in this study. The protocol of this study was done according to the Declaration of Helsinki.

Data Collection

Demographic data, blood lipid and glucose profiles, and total cholesterol, LDL, HDL, TG, and glycated hemoglobin (HbA1c%) data were obtained from records of the University of Jordan Hospital.

DNA Isolation and Genotyping

The genomic DNA was isolated from the whole blood of each subject participating in this study using the Wizard Genomic DNA Purification Kit (Promega, USA). The DNA concentration was measured using a Nanodrop instrument (Quawell DNA/Protein Analyzer, USA). The ratio of 280/260 of DNA samples was 1.8 ± 0.1, which indicates that the DNA samples are free from protein contamination and DNA degradation.[21]

The protein-coding region of the NAT2 gene was amplified for each participant using two PCR reactions carried out in a Bio-Rad thermal cycler (T100TM, UK) as previously described.[18] For each PCR reaction, 200 ng of genomic DNA was added to a 20 μL reaction mixture containing 2X PCR master mix and 10 pmol of each forward and reverse oligonucleotide (Table 1). The PCR conditions were as follows: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 55°C for 50 seconds, and elongation at 72°C for 45 seconds. The final stage of elongation at 72°C for 7 minutes completed the PCR reaction.

Following the PCR reaction, the products were electrophoresed on a 2% agarose gel using a 125 AMP electrical current. The agarose gel was stained with Redsafe dye (Intron, South Korea), which enables the visualization of PCR products at a wavelength of 540 nm, according to the company's instructions. The size of the PCR products was compared with a 100 base pair (bp) DNA loading ladder (New England, USA). Figure 1 shows the gel electrophoresis of PCR products after amplification of the protein coding region of the *NAT2* gene. Lastly, PCR was sequenced by Macrogen company (Seoul, South Korea).[22] The chromatogram of DNA sequences was visualized using DNA-Based v3.5.4 software.[23]

Linkage Disequilibrium and Haplotype Analysis

The analysis of LD, haplotype, and deviation from Hardy–Weinberg equilibrium was done using Haploview 4.2 population genetic analysis software.[24] The LD for each pair of *NAT2* genetic variants was measured using |D′|.[25]

Statistical Analysis

The comparison of NAT2 allele, genotype, and haplotype frequencies between T2DM and non-T2DM subjects was conducted using the chi-square (χ^2) test. The lipid profile continuous data of T2DM patients were compared according to *NAT2* genotype using the analysis of variance (*ANOVA*) test followed by Tukey post-hoc test. All statistical analyses performed in this study were carried out using the Statistical Package for the Social Sciences (SPSS) software (IBM Analytics, USA). A P value below 0.05 was considered significant.

Results

Demographic Data of Participants

The average age of control subjects was 45 ± 17 years and of the T2DM was 53 ± 11 years. Thirty participants of the controls were males while 20 were females. In addition, 25 T2DM patients were males while 20 patients were females. The body mass index of the control group was 24 ± 4.3 kg/m^2 and of the diabetic group was 29 ± 2.8 kg/m^2.

Analysis of NAT2 Genotype, Allele, and Haplotype

Table 2 shows the frequency of *NAT2* genotypes among T2DM and non-T2DM subjects. This study shows that the frequencies of *NAT2*13* and *NAT2*7* differ significantly (*P* value = 0.0002–0.03) between T2DM and control subjects. The heterozygous *NAT2*13 C/T* genotype is more significantly (*P* value = 0.03) frequent among T2DM (44%) than that of non-T2DM subjects (23.5%). In addition, the frequency of homozygous *NAT2*13 T/T* genotype is significantly higher (*P* value = 0.03) among T2DM (26.7%) compared to that of non-T2DM subjects (11%).

Table 1 Oligonucleotides Used for the PCRs Targeting the *NAT2* Gene

Oligonucleotide	Sequence (5′-3′)	Annealing Temperature (°C)	Size (bp)
1st-Forwad	*GTCACACGAGGAAATCAAATGC*	55	540
1st-Reverse	*TCCTCTCTCTTCTGTCAAGCAG*		
2nd-Forward	*GAATTACATTGTCGATGCTGG*	55	610
2nd-Reverse	*TGAGGGTAGAGAGGATATCTGA*		

Notes: PCR is the abbreviation of polymerase chain reaction, and *NAT2* is the abbreviation of N-acetyltransferase 2 gene.

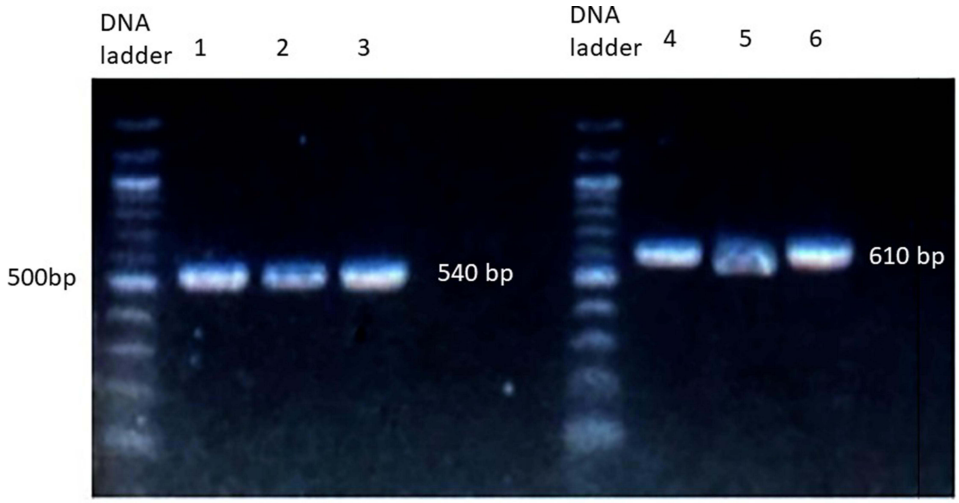

Figure 1 Gel electrophoresis of PCR amplification of the *NAT2* gene. PCR products were run on 2% agarose gel stained with Redsafe stain. Lane 1–3 represents the amplification of the first, while lane 4–6 represents the amplification of the second part of the protein-coding region in *NAT2* gene.

Regarding the *NAT2*7* (*857G>A*) genotype, the homozygous *NAT2*7 A/A* was not found among this sample of T2DM and non-T2DM subjects. The heterozygous *NAT2*7 G/A* genotype was found only in T2DM patients (11.1%), while it was absent in the control non-T2DM group. This difference in the frequency of the *NAT2*7 G/A* genotype between both studied groups is statistically significant (*P* value = 0.002).

In addition, it is found in this study that the allele frequency of *NAT2*13282 C>T* and *NAT2*7 857A>G* variants is significantly different (*P* value = 0.018–0.043) between T2DM and non-T2DM groups (Table 3). The percentage frequency of *NAT2*13* was 48.8% among T2DM while it was 26.5% among non-T2DM subjects. The *NAT2*7* allele was absent among the healthy non-T2DM group, while it was found at a significant (*P* value = 0.043) percentage frequency (5.6%) among the T2DM group.

Regarding *NAT2* haplotypes, there are some minor *NAT2* haplotypes found only among T2DM patients, including *NAT2*7B, NAT2*12M, NAT2*6B*, and *NAT2*5J* (Table 4). However, the frequency of these NAT2 haplotypes was not significantly different (P value > 0.05) in comparison with the control non-T2DM group. The only significant (P value =

Table 2 The Frequency (Proportion, 95% CI) of NAT2 Genotype Among a Sample of Jordanian T2DM and Non-T2DM Subjects

NAT2 Allele	Wild Genotype			Heterozygote Genotype			Homozygote Genotype:		
	Non-Diabetic	T2DM	*P* value	Non-Diabetic	T2DM	*P* value	Non-Diabetic	T2DM	*P* value
NAT2*13 (282 C>T)	C/C: 33 (0.65)	13 (0.29)	0.0002*	C/T: 12 (0.24)	20 (0.44)	0.0300*	T/T: 5 (0.11)	12 (0.27)	0.0300*
NAT2*5 (T341 T>C)	T/T: 15 (0.29)	12 (0.47)	0.053	T/C: 22 (0.44)	18 (0.40)	0.671	C/C:13 (0.26)	6 (0.13)	0.097
NAT2*11 (481C>T)	C/C: 21 (0.41)	20 (0.44)	0.75	C/T: 24 (0.47)	22 (0.49)	0.832	T/T: 5 (0.11)	3 (0.07)	0.48
NAT2*6 (590 G>A)	G/G: 25 (0.50)	14 (0.31)	0.0470*	G/A: 20 (0.39)	21 (0.47)	0.41	A/A: 5 (0.11)	10 (0.22)	0.115
NAT2*12 (803 A>G)	A/A: 26 (0.53)	20 (0.44)	0.35	A/G: 15 (0.29)	21 (0.467)	0.07	G/G: 9 (0.18)	4 (0.09)	0.23
NAT2*7 (857G>A)	G/G: 50 (1.00)	40 (0.89)	0.0060*	0	5 (0.11)	0.0020*	0	0	1

Notes: "T2DM" is the abbreviation of type II diabetes mellitus, and *NAT2* is the abbreviation of N-acetyltransferase 2 gene. *Indicates a statistically significant *P* value when it is less than 0.05.

Table 3 The Frequency of *NAT2* Genetic Alleles Among a Sample of Jordanian T2DM and Non-T2DM Subjects

NAT2 Allele	Nucleic acid Change[a]	Reference ID	Amino Acid Change	Acetylation Activity	Proportion in Non-T2DM	Proportion in T2DM	*P*-value
NAT2*13	282 C>T	rs1041983	Tyr94Tyr	High	0.27	0.49	0.018*
NAT2*5	T341 T>C	rs1801280	Ile114Thr	Decreased	0.49	0.33	0.095
NAT2*11	481C>T	rs1799929	Leu161Leu	High	0.35	0.31	0.661
NAT2*6	590 G>A	rs1799930	Arg197Glin	Decreased	0.31	0.46	0.582
NAT2*12	803 A>G	rs1208	Arg268Lys	High	0.32	0.32	0.912
NAT2*7	857A>G	rs1799931	Gly286Glu	Decreased	0	0.06	0.043*

Notes: [a]The reference sequence used was GenBank accession No. NC_000008.11, and the position is indicated in respect to the start codon ATG *NAT2* gene; the A in ATG is + 1. "T2DM" is the abbreviation of type II diabetes mellitus, and *NAT2* is the abbreviation of N-acetyltransferase 2 gene. *Indicates a statistically significant *P* value when it is less than 0.05.

0.04) NAT2 haplotype among T2DM subjects is the slow-encoding *NAT2*7B* haplotype, which was not found among non-T2DM subjects. The most frequent *NAT2* haplotype found among T2DM, in this study, was *NAT2*6A* with a percentage frequency of 38.8%, while the most frequent *NAT2* haplotype among non-T2DM subjects was *NAT2*5B* with a percentage frequency of 29.3% (Table 4).

Furthermore, some *NAT2* haplotypes, including *NAT2*5A, *5E, *5K*, and **11A*, were found in this study among the non-T2DM subjects, whereas these *NAT2* haplotypes were not detected among T2DM patients (Table 4). Although, the

Table 4 The Proportion of NAT2 Haplotype Among a Sample of Jordanians T2DM and Non-T2DM Subjects

Haplotype[a]	C282T	T341C	C481T	G590A	A803G	G857A	Proportion		*P*-value	Phenotype
							Non-T2DM	T2DM		
*NAT2*5B*	C	C	T	G	G	G	0.293	0.276	0.91	Slow
*NAT2*6A*	T	T	C	A	A	G	0.235	0.388	0.09	Slow
*NAT2*4*	C	T	C	G	A	G	0.227	0.144	0.24	Rapid
*NAT2*5E*	C	C	C	A	A	G	0.074	0.000	0.07	Slow
*NAT2*5D*	C	C	C	G	A	G	0.052	0.019	0.42	Slow
*NAT2*11A*	C	T	T	G	A	G	0.034	0.000	0.24	Rapid
*NAT2*5C*	C	C	C	G	G	G	0.030	0.011	0.48	Slow
*NAT2*5A*	C	C	T	G	A	G	0.026	0.000	0.24	Slow
*NAT2*13A*	T	T	C	G	A	G	0.019	0.011	0.68	Rapid
*NAT2*5K*	T	C	C	G	A	G	0.010	0.000	0.50	Slow
*NAT2*7B*	T	T	C	G	A	A	0.000	0.056	0.04*	Slow
*NAT2*12M*	T	T	T	G	G	G	0.000	0.012	0.37	Rapid
*NAT2*6B*	C	T	C	A	A	G	0.000	0.038	0.11	Slow
*NAT2*5J*	T	C	C	A	A	G	0.000	0.023	0.21	Slow
*NAT2*12C*	C	T	T	G	G	G	0.000	0.016	0.31	Rapid

Notes: [a]The nomenclature of NAT2 haplotype was obtained from: http://nat.mbg.duth.gr/Human%20NAT2%20alleles_2013.htm. *Indicates statistical significance with *P* value < 0.05. "T2DM" is the abbreviation of type II diabetes mellitus, and *NAT2* is the abbreviation of N-acetyltransferase 2 gene.

Table 5 The Predicted Acetylation Phenotype Among a Sample of Jordanians T2DM and Non-T2DM Subjects

Acetylation Phenotype[a]	Proportion in Non-T2DM	Proportion in T2DM	*P*- value
Fast	0.29	0.18	0.23
Slow	0.71	0.82	0.23

Notes: [a]The acetylation phenotype was predicted depending on the *NAT2* haplotype of participated subjects. "T2DM" is the abbreviation of type II diabetes mellitus, and *NAT2* is the abbreviation of N-acetyltransferase 2 gene.

frequencies of these *NAT2* haplotypes did not reach statistical significance (*P* values > 0.05) when compared between both groups.

Table 5 shows the predicted acetylation phenotype among T2DM and non-T2DM sample groups depending on the analysis of the *NAT2* haplotype. It can be noticed from the represented results that most T2DM and non-T2DM subjects are predicted to be slow acetylators. Although the percentage frequency of the slow-encoding acetylators among T2DM (82%) is slightly higher than in the non-T2DM group (71%), this difference failed to reach statistical significance (P value = 0.23).

LD Analysis

Figure 2 shows the LD of *NAT2* variants among the studied T2DM and non-T2DM groups. It is noticed that *NAT2*7* is not in LD with other *NAT2* variants among T2DM. Additionally, there are some differences in the value of D', which represent the association between *NAT2* variants, between T2DM and non-T2DM groups: *NAT2*13* is in a complete LD (D'=1) with *NAT2*5* and *NAT2*11* among non-T2DM subjects, while the values of D' between *NAT2*13* and *NAT2*5*, and *NAT2*11* are 0.82 and 0.90, respectively. Furthermore, *NAT2*11* and *NAT2*12* are in complete LD (D'=1) among T2DM patients, while the D' value is 0.85 between *NAT2*11* and *NAT2*12* among the control non-T2DM group.

The Association of NAT2 Genotype with the Lipid Profile of T2DM Patients

The results of this study showed only *NAT2*5* genotype affected significantly (*P* value = 0.03) on the plasma levels of HbAC1 among T2DM patients, where carriers of *NAT2*5 C/C* genotype had less significant HbAC1 levels (6.20 ± 0.57 ng/dL) than carriers of the *NAT2*5 T/C* (8.27 ± 1.29 ng/dL) and *NAT2*5 T/T* (7.71 ± 1.66 ng/dL) genotypes. In addition, it is found that *NAT2*11* and **12* genotypes affected significantly (*P* value = 0.01–0.04) on levels of triglyceride in the blood of T2DM patients. T2DM patients with homozygous *NAT2*11 T/T* genotype had significantly (*P* value = 0.01) higher levels of triglycerides (381.50±9.19 ng/dL) than heterozygous *NAT2*11 C/T* (136.23 ± 51.12 ng/dL) and wild *NAT2*11 C/C* (193.65 ± 109.89 ng/dL) genotypes. Furthermore, T2DM patients with homozygous *NAT2*12 G/G* genotype had a significantly (*P* value = 0.04) higher triglyceride levels (275.67 ± 183.42 ng/dL) than the heterozygous *NAT2*12 A/G* (140.02 ± 49.53 ng/dL) and wild *NAT2*12 A/A* (193.65 ± 109.89 ng/dL), as represented in Table 6.

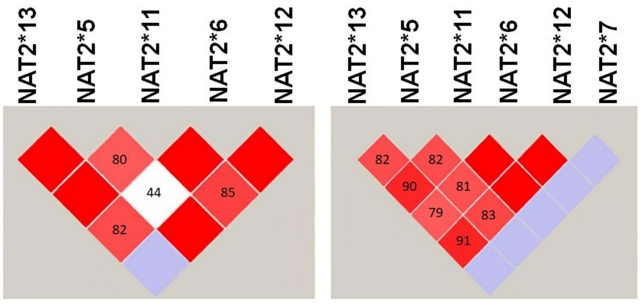

Figure 2 The LD between *NAT2* variants among samples of Jordanians T2DM and non-T2DM. The LD was generated using Haploview software. The red square indicates a strong while the white square indicates a weak LD between *NAT2* variants. The blue square indicates that there is no LD between *NAT2* variants.

Table 6 Effects of *NAT2* Genotype on the Lipid and Glycemic Profile of T2DM Patients

NAT2 Allele	Genotype	Lipid and Glycemic Data				Number of T2DM Patients
		HbAC1 (%)	LDL (ng/dL)	HDL (ng/dL)	TGs (ng/dL)	
NAT2*13	C/C	6.891±1.27	111.63±25.60	46.54±10.02	170.54±114.49	11
	C/T	8.31±1.47	100.842±46.79	42.73±13.01	160.13±75.56	19
	T/T	7.64±1.60	99.92±40.67	44.50±12.54	203.50±118.13	12
	P- value	0.07	0.74	0.71	0.48	–
NAT2*5	T/T	7.71±1.66	104.86±40.37	42.57±9.43	195.14±104.88	21
	T/C	8.27±1.29	98.88±42.57	44.69±14.22	144.09±48.67	16
	C/C	6.20±0.57	111.80±34.16	49.80±14.62	191.40±173.81	5
	P- value	0.03*	0.80	0.48	0.29	–
NAT2*11	C/C	7.84±1.68	100.55±39.65	44.35±11.73	193.65±109.89	20
	C/T	7.79±1.44	103.55±41.95	44.85±12.78	136.23±51.12	20
	T/T	6.40±0.99	130.50±14.85	37.00±7.07	381.50±9.19	2
	P- value	0.46	0.61	0.69	0.01*	–
NAT2*6	G/G	7.50±1.47	106.62±30.82	45.08±11.17	157.58±108.17	13
	G/A	7.93±1.58	108.11±46.53	43.42±12.05	183.37±90.62	19
	A/A	7.72±1.67	90.30±37.32	44.70±13.93	182.80±109.23	10
	P- value	0.75	0.50	0.92	0.75	–
NAT2*12	A/A	7.834±1.68	100.55±39.65	44.35±11.73	193.65±109.89	20
	A/G	7.88±1.41	102.26±42.70	44.63±13.09	140.02±49.53	19
	G/G	6.27±0.74	129.67±10.60	41.00±8.54	275.67±183.42	3
	P- value	0.23	0.50	0.89	0.04*	–
NAT2*7	G/ G	7.77±1.60	102.89±41.44	44.24±12.53	171.89±96.87	37
	G/ A	7.60±1.15	107.20±28.54	44.20±7.60	200.10±122.63	5
	P- value	0.83	0.82	0.99	0.56	–

Notes: *Indicates statistical significance (*P* value < 0.05, *ANOVA*). "T2DM" is the abbreviation of type II diabetes mellitus, and *NAT2* is the abbreviation of N-acetyltransferase 2 gene.

Discussion

The NAT2 enzyme plays a significant role in metabolizing endogenous and exogenous compounds.[5] Genetic variants in the *NAT2* gene can alter the metabolism of chemical compounds and potentially lead to human diseases.[26] The association between *NAT2* genotype and T2DM varies among different ethnic populations.[15,16,27]

In this study, the entire protein-coding region of the *NAT2* gene was sequenced using the Sanger sequencing method and the results were compared to those of non-T2DM control subjects of this study. As a result, we found that there is a significant difference in the frequency of *NAT2*13* and *7* genotypes and alleles between T2DM and non-T2DM subjects. Furthermore, *NAT2*11* and *12* genotypes affected the triglyceride levels among T2DM patients. Collectively, these findings indicate that the *NAT2* gene is associated with the risk of T2DM and triglyceride levels among Jordanian

patients. However, these findings are preliminary, and further clinical studies with larger sample sizes of T2DM patients are needed to confirm our results.

The frequency of *NAT2* alleles, genotypes, and haplotypes among non-T2DM control subjects, found in this study, is similar to what was reported previously[18] among healthy unrelated Jordanians, in which the *NAT2*5* is the most frequent allele and the *NAT2*5B* haplotype is the most frequent *NAT2* haplotype. In this study, it was found that *NAT2*6* and *NAT2*6* are the most frequent allele and haplotype, respectively, among this sample of T2DM patients. However, frequencies of the *NAT2*6* allele, genotype, and haplotype were not statistically (P value > 0.05) higher among T2DM patients, in comparison with the non-T2DM subjects. These results are in line with that of reported results by Yalin et al, in which the *NAT2*6* allele was found at relatively higher frequencies among Turkish T2DM patients.[17] On the contrary, Semiz et al reported that *NAT2*6* is significantly less frequent among T2DM patients from Bosnia and Herzegovina.[28]

The analysis of LD among T2DM patients showed that *NAT2*7* is not in LD with any other *NAT2* variants. However, other *NAT2* variants are in strong LD as represented by the red color of the squares and the high D' values (Figure 2). It can be explained by the low frequency of *NAT2*7* (857 G>A) variant among the diabetic sample[29] or it can be due to other reasons which needs further investigation.

Several studies have been conducted in Jordan to investigate the frequency of major *NAT2* alleles and genotypes among the Jordanian population and their association with diseases using the PCR-restriction enzyme length polymorphism genotyping method.[15,30,31] They examined *NAT2*11* as a representative allele of the loss-of-function *NAT2*5 allele based on previous findings that these two alleles are in complete LD in the Caucasian population. Although Jordanians are considered a Caucasian population, we found in this study that NAT2**11 and NAT2**5 alleles are not in complete LD among non-T2DM and T2DM subjects. This finding confirms what was previously reported by Jarrar et al that both NAT2**11 and NAT2**5 alleles are not in LD among Jordanian healthy volunteers.[18] These findings suggest that carrying the NAT2**11* variant is not a marker for the presence of the *NAT2**5 variant in Jordanians. Accordingly, analyzing only the synonymous *NAT2*11* allele without the *NAT2**5 allele could result in an inaccurate estimation of the functional loss of activity of the *NAT2*5* allele and genotype in Jordanian patients.

We found that only two *NAT2* genetic variants, **13* and **7*, are associated with T2DM among this sample of patients. *NAT2*13 T* and *NAT2*7 G* alleles and their related genotypes were significantly more frequent (P value < 0.05) among T2DM than non-T2DM subjects. *NAT2*13* is a synonymous variant that does not change the amino acid sequence and, hence, the protein structure and activity.[32] However, the *NAT2*13* variant is close to the promoter region of *NAT2* gene and might be in LD with other functional *NAT2* genetic variants that affect the regulation and expression of the *NAT2* gene. Furthermore, several studies found that synonymous variants are associated with T2DM[33] and drug response[34] but the exact mechanisms of how these synonymous variants increase the risk of human diseases are still unclear.

The *NAT2*7* is a non-synonymous variant that change the amino acid glycine to glutamate, which alters NAT2's affinity to metabolize certain chemical compounds such as sulfamethazine.[35] It was also previously reported that the *NAT2*7* allele is more frequent among T2DM than non-T2DM in the Saudi population.[16] However, more clinical studies are needed to confirm that *NAT2*7* variant is more frequent among T2DM patients.[36,37]

We found in this study that some *NAT2* haplotypes are detected only among T2DM patients, but not in non-T2DM subjects, such as *NAT2*7B, NAT2*6B, NAT2*5J*, and *NAT2*12M*. However, the only statistically significant *NAT2* haplotype found among T2DM patients is the *NAT2*7*B haplotype, which is characterized by the presence of two *NAT2* genetic variants: 282 C>T (*NAT2*13*) and 857 G>A (*NAT2*7*). This may indicate that the inheritance of these two *NAT2* variants together increases the risk of developing T2DM in Jordanians.

Slow acetylators are more common in T2DM patients.[38] In this study, the prediction of the acetylation phenotype depending on the *NAT2* gene structure shows that most of the sample of T2DM patients were slow acetylators (82%). However, this excess of slow acetylators among T2DM patients was not statistically (P value > 0.05) different when we compared the frequency of individuals encoding the slow-acetylation gene between the control of non-T2DM group (72%). It can be concluded that not only the loss of function *NAT2* variants explain the excess of slow-acetylation phenotype among T2DM patients. There are also another pathological factors could decrease the acetylation capacity in the liver. It was reported that the mRNA expression of mouse *nat2* gene decreased significantly in the liver after

induction of DM using streptozocin and was correlated with increased expression of the inflammatory enzyme cyclo-oxygenase 2 in the liver.[39]

We also found that the *NAT2*5* genotype is associated with the glycemic level HbA1c; where carriers of homozygous *NAT2*5/*5* had significant lower HbA1c values than patients carried heterozygous *NAT2*4/*5* and wild genotypes. *NAT2*5/*5* genotype encodes the slow acetylation phenotype.[35] It was reported by Iskakova et al that the *NAT2*13* variant determined the effectiveness of the hypoglycemic drugs dipeptidyl peptidase-4 inhibitors.[40] Accordingly, it can be suggested that the *NAT2*5* variant decreases the metabolism of certain hypoglycemic drugs which hence increases the plasma levels of these drugs and decreases HbA1c levels among T2DM patients. This finding needs further molecular and clinical investigations.

Several studies revealed that genetic variants influence the lipid profile of T2DM patients.[41,42] We found that the *NAT2* genotype is associated with only the level of triglycerides but not the level of cholesterol. The two *NAT2* genotypes, *NAT2*11* and **12*, were associated with triglyceride levels, where T2DM patients with homozygous *NAT2*11/*11 (T/T)* and *NAT2*12/*12 (G/G)* had significantly higher levels of triglycerides than heterozygous and wild genotypes. The role of the NAT2 enzyme and *NAT2* genetic variants in triglyceride metabolism is still unclear. However, it was shown previously that knocking-down the mouse *NAT2* gene increases lipolysis and hence increases the amount of free fatty acids used in the synthesis of triglycerides.[43]

Although the aims of this study were achieved, there are some limitations that should be addressed. First, the sample size of T2DM patients is small. Therefore, this study can be considered a pilot study, and further studies with a larger sample size are needed to confirm the findings of this study. Second, the T2DM patients were on the antihyperlipidemic drug atorvastatin, which influenced the cholesterol levels, and hence the association between the *NAT2* genotype and the lipid profile. Lastly, some lipid parameters including total cholesterol, free fatty acids were not studied in association with *NAT2* variants.

Conclusion

In conclusion, this study found a significant association between the *NAT2* genotype and the risk of T2DM and triglyceride levels among Jordanian patients. However, further clinical studies with larger sample sizes of T2DM patients are needed to confirm these findings.

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Disclosure

The authors declare that there is no conflict of interest in this work.

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