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Sequence analysis of the *N*-acetyltransferase 2 gene (NAT2) among Jordanian volunteers

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ABSTRACT

The present study aimed to identify the *NAT2* haplotypes, linkage disequilibrium, and novel *NAT2* genetic variants among Jordanian population. We isolated the genomic DNA from 68 healthy, Arab, unrelated Jordanian volunteers to amplify the protein-coding region of *NAT2* gene by polymerase chain reaction (PCR). Then, the amplified PCR products were sequenced using Applied Biosystems Model (ABI3730x1). It is found that the allele frequencies of known *NAT2* genetic variants 191G>A, 282C>T, 341T>C, 481C>T, 590G>A, and 803A>G were 0.7, 26.5, 48.5, 35.3, 30.9, and 32.4%, respectively. The *NAT2* allele frequencies were generally similar to those of white Europeans but different from those of Asian and African populations. The most common *NAT2* haplotype was *NAT2*5B* with a frequency of 29.3%. According to the *NAT2* haplotype frequencies, 72% (95% confidence interval 61.4–82.7%) of the volunteers were slow encoding *NAT2* haplotype acetylators. The *NAT2*5* represented variants 341T>C and 481C>T were in strong but not complete linkage disequilibrium ($D' = 0.8$, $r^2 = 0.63$). In addition, this study found a novel nonsynonymous *NAT2* 436G>A genetic variant with low frequency (0.7%). However, this novel variant was predicted to be tolerated and not harmful to the NAT2 protein, using *in silico* prediction tools. It is concluded that the frequency of slow encoding *NAT2* haplotype was high among Jordanian volunteers, which may have effects on drug responses and susceptibility to some diseases, such as cancers.

1. Introduction

Human *N*-acetyltransferase 2 (*NAT2*) is a phase II drug-metabolizing enzyme, which is responsible for the acetylation of some drugs such as antituberculosis isoniazid and antimicrobial sulfonamides [1,2].

Acetylation of the drugs in humans is polymorphic. Humans can be divided, according to acetylation phenotype, into slow, intermediate, and fast acetylators [3]. Slow acetylators have a higher plasma drug concentration than rapid acetylators, which may be associated with drug toxicity [4]. This interindividual variation in drugs acetylation capacity is due to several patient factors, such as health status and genetic polymorphisms on the *NAT2* gene [5].

Genetic variations on the *NAT2* gene may significantly influence the acetylating phenotype, thus affecting the drug's efficacy, toxicity, and susceptibility to some diseases. Many studies have reported the association of genetic variants on *NAT2* gene and cancers [6]. In addition, *NAT2* polymorphisms have been linked to developing autoimmune diseases and sulphasalazine-induced systemic lupus erythematosus [7].

The *NAT2* gene is located on chromosome 8, at p22. This gene is an intron-less protein-coding exon of 870-bp open reading DNA sequence encoding 290 amino acids [8–10]. The NAT2 protein receives its entire translated polypeptide sequence from exon 2 [8].

In Jordan, the main *NAT2* genetic alleles, *NAT2*4, *5, *6*, and *7*, were genotyped using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique, and it was found that the frequencies of these variants were different from those in Saudis and Egyptians but closer to those of the Iranian population [11]. However, other *NAT2* genetic alleles have not been investigated, and no study has screened the novel genetic variants, on the *NAT2* gene, among the Jordanian population. Therefore, the current study aimed to identify the structure of the *NAT2* gene in Jordanians, which may have an influence on the interethnic and interindividual variations in drug efficacy, toxicity, and susceptibility to the diseases.

2. Materials and methods

2.1. Chemical compounds

The PCR primers were designed and purchased from Integrated DNA Technologies (Iowa, USA). The Taq polymerase, Taq polymerase buffer, dNTPs, nuclease-free water, DNA ladder, and $MgCl_2$ were obtained

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from Promega (Madison, WI, USA). Agarose gel and the gel electrophoresis buffer Tris-Borate-EDTA (TBE) were purchased from Bio-basic (Markham, Canada). The Redsafe dye was purchased from Intron Biotechnology (Gyeonggi, South Korea).

2.2. Samples collection

A total of 68 healthy unrelated Jordanian volunteers of both genders with a mean age of 27 ± 5 years agreed to participate in this study. The ethical committee at AlZaytoonah University of Jordan approved the study (reference number 18/04-2016), and written informed consent was obtained from each participant in accordance with the declaration of Helsinki [12]. From each volunteer, 2 ml of venous blood was collected in EDTA tubes. The volunteers were healthy, as judged by a physical examination, with no chronic or serious diseases.

The sample size was determined according to the allele frequency of the major known *NAT2* genetic variants with the power of the test $(1 - \beta = 0.8)$.

2.3. DNA extraction

DNA was extracted using the Wizard® Genomic DNA purification kit. The genomic DNA was isolated from the blood leukocytes. The extracted DNA concentration was measured using the Nanodrop instrument Quawell DNA/Protein Analyzer (Sunnyvale, CA, USA), and the samples were diluted in nuclease-free water to a final concentration of 100 ng/μl for the PCR reaction.

2.4. Amplification of NAT2 gene

The *NAT2* gene was amplified by PCR using a Bio-Rad T100^{TM} (California, UK) thermal cycler. Two DNA fragments of *NAT2* gene that represent the complete sequence of exon 2 of *NAT2* gene were amplified using specific DNA primers (Table 1). The PCR conditions were as follows: 100 ng of the extracted genomic DNA was amplified in a 50 μl reaction volume containing 0.5 mM MgCl$_2$, green Taq polymerase buffer, 0.2 mM dNTPs, 10 pmoles of each of forward and reverse primers (Table 1), and 1 unit of Taq DNA polymerase. After an initial denaturation at 94°C for 5 min,

30 cycles were performed consisting of denaturation step at 94°C for 1 min, an annealing step at 55°C for 1 min, and an elongation step at 72°C for 1 min, completed with a final cycle of elongation at 72°C for 7 min.

2.5. Gel electrophoresis

The PCR products were separated on 2% agarose gel by applying an electrical current of 125 A for 30 min. The gel was stained by the Redsafe dye (Intron, South Korea). The size of the PCR product was compared with a standard-size 100 bp DNA loading ladder (Promega). The DNA bands were visualized by a benchtop UV transilluminator (Bio Doc-IT™, UK).

2.6. DNA sequencing

The PCR products were purified and then sent to GENEWIZ company (San Diego, USA) for sequencing of the functional exon 2 in the *NAT2* gene using the same PCR forward primer. The novel genetic variant found in this study was confirmed by resequencing of the PCR product using the reverse primer. Sequence analysis was performed using the Applied Biosystems Model (ABI3730x1), which is based on the dye terminator method [13]. The chromatograms of the DNA sequences were visualized using DNA Based v3.5.4 software (Heracle BioSoft, Romania). The alignment of DNA sequences was determined using Multialign software [14]. The NAT2 amino acid sequence of the different species was obtained from GenBank databases [15].

2.7. Linkage disequilibrium and haplotype distribution

The linkage disequilibrium (LD) analysis, estimation of haplotype diversity, and Hardy–Weinberg equilibrium test were carried out using Haploview 4.2 population genetic analysis software. Five *NAT2* variants with minor allele frequencies >5%, 282C>T, 341T>C, 481C>T, 590G>A, and 803 A>G, were applied to Haploview software [16]. The LD for each pair of genetic variants was measured using |D′| and correlation coefficient (r^2) as described previously [17,18].

2.8. In silico prediction of NAT2 protein stability change and molecular modeling

The current study applied three different software programs, PolyPhen 2.0, SIFT, and Mutpred, in order to predict the effect of amino acid substitution, caused by the novel *NAT2* 436G>C nonsynonymous genetic variant on NAT2 protein's stability and function [19,20]. These software programs used numerical scores representing the probability that nonsynonymous substitution is damaging. PolyPhen-2, Mutpred, and SIFT scores use the same

Table 1. Primer names, sequences, and annealing temperatures used in PCR amplification of the *NAT2* gene.

Primer	Primer sequence (5′–3′)	AT (°C)	Region	Size (bp)
NAT2 1st-F	GTCACACGAGGAAATCAAATGC	57	Exon 2	540
NAT2 1st-R	TCCTCTCTCTTCTGTCAAGCAG			
NAT2 2nd-F	GAATTACATTGTCGATGCTGG	55	Exon 2	610
NAT2 2nd-R	TGAGGGTAGAGAGGATATCTGA			

F, forward primer; R, reverse primer; AT, annealing temperature.

range, 0.0–1.0, but with opposite meanings. A variant with PolyPhen-2 and Mutpred score of 0.0 is predicted to be benign, whereas it is not tolerated in SIFT software.

For molecular modeling of NAT2 protein, visualized (VMD) software was used as described previously [21]. The human NAT2 structure was obtained from the Worldwide Protein Data Bank [22].

2.9. Statistical analysis

The NAT2 allele frequencies were tested for Hardy–Weinberg equilibrium using the chi-square (χ^2) test. Comparison of NAT2 alleles among Jordanians with NAT2 allele frequencies of other ethnic groups was done using the χ^2 test by applying the SPSS statistics program (IBM Analytics, USA). The level $p < 0.05$ was considered as the cut-off value for significance.

3. Results

Two DNA sequences representing exon 2 within NAT2 gene were amplified using the PCR technique. Figure (1)

shows the gel electrophoresis of the two amplified DNA fragments on 2% agarose gel. The sizes of the amplified fragments were 540 and 610 bp.

After DNA sequencing of the amplified NAT2 gene for 68 volunteers, the present study found six major known SNPs: 191G>A, 282C>T, 341T>C, 481C>T, 590 G>A, and 803 A>G. The frequencies of these major NAT2 SNPs are listed in Table 2. This study found that 341T>C was the most frequent genetic variant (48.5%, 95% CI = 40.1–56.9) among healthy Jordanian volunteers.

Table 3 represents NAT2 genotypes among the Jordanian volunteers. All of the NAT2 genotypes were within the Hardy–Weinberg equation. This study found that heterozygote NAT2*5 341T>C (44%) and NAT2*11 481C>T (47.1%) genotypes were the most frequent heterozygote NAT2 genotypes. The homozygote NAT2*5 341T>C (26.4%) genotype was the most common homozygote NAT2 genotype among the Jordanian volunteers in this study.

There is an interethnic variation in the frequencies of NAT2 genetic variants [5]. The present study compared the NAT2 allele frequencies among Jordanians, found in
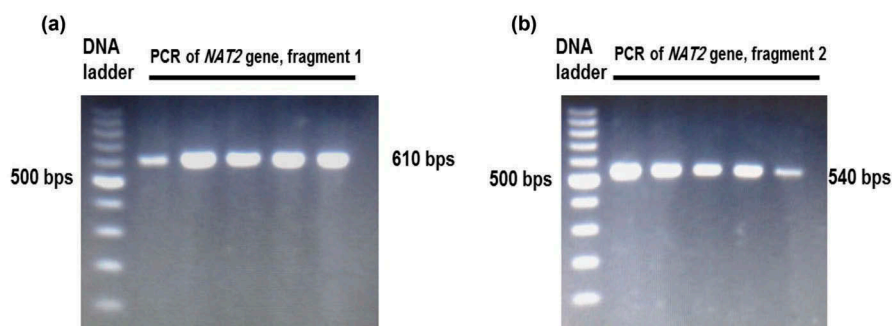

Figure 1. Gel electrophoresis of the PCR products of NAT2 gene. The NAT2 gene sequence was amplified by two PCR reactions represented by the first fragment (A) and second fragment (B).

Table 2. Distribution of NAT2 genetic variants among the healthy Jordanian population ($n = 68$).

NAT2 allele	Nucleic acid change[a]	Reference ID	Amino acid change	Acetylation activity	Allele frequency 95% CI))
NAT2*4	–	–	Wild	High	0.227 (0.157–0.298)
NAT2*14	191 G>A	rs1801 279	Arg64Gln	Decreased	0.007 (0.000–0.012)
NAT2*13	282 C>T	rs1041983	Tyr94Tyr	High	0.265 (0.191–0.339)
NAT2*5	341 T>C	rs1801280	Ile114Thr	Decreased	0.485 (0.401–0.569)
NAT2*11	481 C>T	rs1799929	Leu161Leu	High	0.353 (0.273–0.433)
NAT2*6	590 G>A	rs1799930	Arg197Glin	Decreased	0.309 (0.231–0.386)
NAT2*12	803 A>G	rs1208	Arg268Lys	High	0.324 (0.245–0.402)

[a]The reference sequence used was GenBank accession No. NC_000008.11. The position is indicated with respect to the start codon ATG NAT2 gene; the A in ATG is +1.

Table 3. Distribution of NAT2 genotype among the healthy Jordanian population ($n = 68$).

NAT2 allele	Wild genotype: frequency (proportion, 95% CI)	Heterozygote genotype: frequency (proportion, 95% CI)	Homozygote genotype: frequency (proportion, 95% CI)
NAT2*14 (191G>A)	G/G: 0	G/A: 0.014 (0.007–0.025)	A/A: 0
NAT2*13 (282C>T)	C/C: 44 (0.647, 0.533–0.761)	C/T: 16 (0.235, 0.134–0.336)	T/T: 8 (0.11, 0.041–0.194)
NAT2*5 (341T>C)	T/T: 20 (0.294, 0.186–0.402)	T/C: 30 (0.44, 0.323–0.559)	C/C:18 (0.264, 0.160–0.370)
NAT2*11 (481C>T)	C/C: 28 (0.411, 0.295–0.529)	C/T: 32 (0.471, 0.352–0.589)	T/T: 8 (0.11, 0.41–0.194)
NAT2*6 (590G>A)	G/G: 34 (0.500, 0.381–0.619)	G/A: 26 (0.382, 0.267–0.498)	A/A: 8 (0.11, 0.41–0.194)
NAT2*12 (803A>G)	A/A: 36 (0.529, 0.411–0.648)	A/G: 20 (0.294, 0.186–0.402)	G/G: 12 (0.176, 0.086–0.267)

CI: Confidence interval.

this study, with the frequencies reported among the major ethnic groups in HapMap data [23], Egyptian [24] and Saudi Arabian [25]. This study found that Chinese Asians had a significant difference (χ^2, $p < 0.05$) in all *NAT2* allele frequencies, in comparison with Jordanians (Table 4). Sub-Saharan Africans had more significant *NAT2*13* 282C>T (52.2%) and less significant *NAT2*5* 341T>C (25%) and *NAT2*11* 481C>T (15%) frequencies, in comparison with Jordanians. The frequency of *NAT2* alleles was similar, with no significant difference, to *NAT2* allele frequencies reported in Northern and Western Europeans, although, the 191G>A variant, which is found only in Africans but not in Caucasians, was detected among the Jordanian volunteers (0.7%). The frequency of 341T>C and 590G>A variants was not statistically different between Jordanians and what was reported among the Middle Eastern Egyptian and Saudi populations (Table 4).

The haplotypes of the *NAT2* gene among Jordanian volunteers were analyzed using Haploview software. It was shown in the current study that the *NAT2*5B* slow acetylator haplotype (29.3%) was the most common frequent *NAT2* haplotype (Table 5). In addition, the slow acetylator *NAT2*6A* (23.5%) and fast acetylator *NAT2*4* (22.7%) haplotype were highly frequent among the Jordanian volunteers. The lowest frequent *NAT2* haplotype among Jordanians, found in this study, was for the *NAT2*5K* haplotype (1%).

It is reported that the acetylation phenotype is strongly affected by *NAT2* genetic variants, and human acetylation capacity can be divided into fast and slow acetylators according to the *NAT2* genetic variations [10,26]. Therefore, the present study predicted the acetylation phenotype among the Jordanian volunteers according to the results of the *NAT2* haplotype (Table 6). Accordingly, the present study found that 28% of the volunteers were fast, while 72% were slow encoding acetylators.

The LD of *NAT2* SNPs was also analyzed using Haploview software. As shown in Figure 2, *NAT2* 282C>T was in complete LD ($D' = 1$) with 341T>C and 481C>T genetic variations. The *NAT2* 803A>G was also in complete LD ($D' = 1$) with 590 G>A and 341T>C genetic variations. Furthermore, *NAT2* 481C>T and 590G>A were in complete LD ($D' = 1$, $r^2 = 0.76$). The *NAT2* 341T>C and 481C>T were in strong LD, as represented by a red-colored square on Figure 2, but not in complete LD ($D' = 0.8$, $r^2 = 0.63$).

The present study found a novel genetic variant in Jordanians that was a substitution of guanine to adenine at nucleotide number 436 on the open reading frame of the *NAT2* gene. The current study detected the novel 436G>A variant in only one Jordanian volunteer who carried this variant in heterozygote genotype (Figure 3). To avoid any sequencing errors, this novel variant was confirmed by resequencing the sample using the reverse primer.

Table 4. Comparison of *NAT2* genetic allele frequencies among the Jordanian population with other major ethnic populations.

NAT2 genetic variant	Jordanian allele frequency (this study)	Jordanian allele frequency [11]	Egyptian allele frequency [24]	Saudi Arabian allele frequency [25]	Hapmap project[a]		
					Europe allele frequency	Asian allele frequency	Sub-Saharan African allele frequency
191G>A	0.007	Not determined	Not determined	Not determined	0*	0*	0.08*
282C>T	0.265	Not determined	Not determined	Not determined	0.300	0.365*	0.522*
341T>C	0.485	Not determined	0.497	0.47	0.441	0.023*	0.250*
481C>T	0.353	0.370	Not determined	Not determined	0.401	0.011*	0.150*
590G>A	0.309	0.350	0.26*	0.243*	0.292	0.207*	0.247
803A>G	0.324	Not determined	Not determined	Not determined	0.393	*0.036	0.362

[a]Hapmap data [23]. The Asian, African, and European *NAT2* variant frequencies are for Han Chinese in Beijing, China (CHB), African ancestry of Yoruba in Ibadan, Nigeria (YRI), and Utah residents with Northern and Western European ancestry (CEU), respectively.
*Significant difference (χ^2, $p < 0.05$) in comparison with the proportion of *NAT2* genetic variant among Jordanians found in this study.

Table 5. NAT2 haplotype among the healthy unrelated Jordanian population (n = 68).

Haplotype	C282T	T341C	C481T	G590A	A803G	Allele frequency	Phenotype
NAT2*5B	C	C	T	G	G	0.293	Slow
NAT2*6A	T	T	C	A	A	0.235	Slow
NAT2*4	C	T	C	G	A	0.227	Rapid
NAT2*5E	C	C	C	A	A	0.074	Slow
NAT2*5D	C	C	C	G	A	0.052	Slow
NAT2*11A	C	T	T	G	A	0.034	Rapid
NAT2*5C	C	C	C	G	G	0.030	Slow
NAT2*5A	C	C	T	G	A	0.026	Slow
NAT2*13A	T	T	C	G	A	0.019	Rapid
NAT2*5K	T	C	C	G	A	0.010	Slow

Table 6. Predicted acetylation phenotype among the healthy unrelated Jordanian population (n = 68).

Acetylation phenotype[a]	Frequency (proportion)	95% confidence interval of proportion
Fast	19 (0.28)	0.173–0.386
Slow	49 (0.72)	0.614–0.827

[a]The acetylation phenotype was determined depending on the NAT2 haplotype of the volunteers.
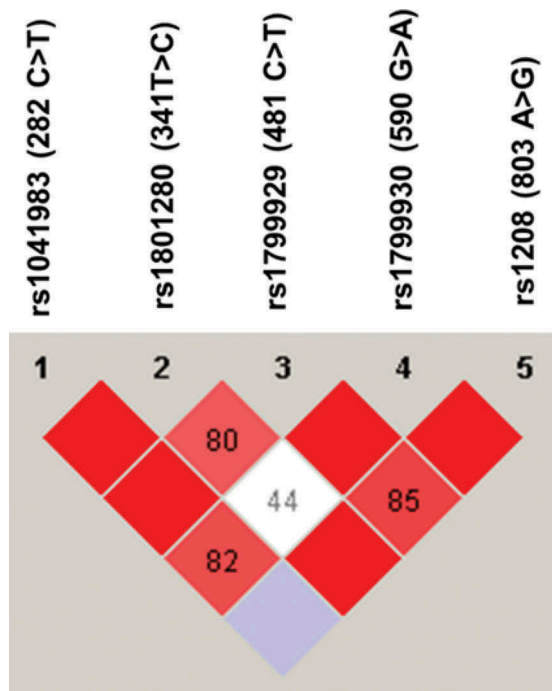
Figure 2. Linkage disequilibrium (LD) of NAT2 genetic variants found among Jordanian volunteers. The LD was carried out using Haploview software. The red squares represent a strong LD, and the white square represents a weak LD. The blue square indicates that there is no LD. Only genetic variants with a frequency higher than 5% were used for identification of LD.

For further analysis of the novel 436G>A variant, we found that it is a nonsynonymous genetic variant that substitutes valine to the methionine amino acid at position 146 of the NAT2 protein sequence.

Figure 4 shows the alignment of the NAT2 amino acid sequence from different species: human, chimpanzee, Sumatran orangutan, rhesus monkey, Norway rat, house mouse, and rabbit. It is shown that the amino acid valine (V) at amino acid position 146 was conserved among all the NAT2 amino acid sequences, except in the NAT2 protein of monkeys.
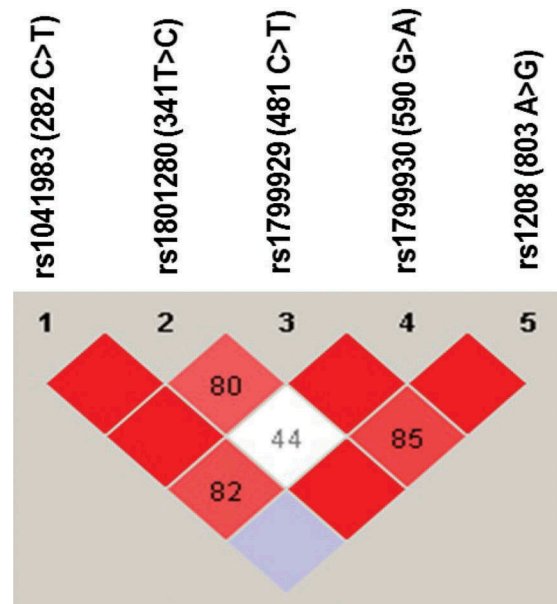
Figure 3. Chromatogram of DNA sequencing of the novel NAT2 436G>A genetic variant. The NAT2 436G>A was identified in only one Jordanian volunteer with a heterozygote genotype.

The effect of a 436G>A novel genetic variant on NAT2 structure and function was predicted using in silico methods. Mutpred, SIFT, and Polyphen2 software programs were used for in silico prediction of the effect of 436G>A variant on NAT2 protein stability. This study found that Mutpred, SIFT and Polyphen2 software programs predicted that 436G>A was not harmful to NAT2 protein function and structure (Table 7). The Mutpred software gave a low score (0.54), SIFT predicted that the effect of the novel variant is tolerated (0.11), and Polyphen2 predicted that the effect of 436G>A is benign (0.04) on NAT2 protein.

The protein structure of wild-type and mutated (V146M) NAT2 was visualized using VMD software. There were no differences in polypeptide folding and structure between the mutant (Figure 5) and the wild-type NAT2 protein reported in the worldwide protein Data Bank [22].

4. Discussion

Genetic variations in NAT2 gene play a major role in the hepatic acetylation capacity, which may have

Figure 4. Alignment of NAT2 polypeptide sequences for different species. The alignment was carried out using Multialign software (Corpet, F. 1998). The NAT2 amino acid sequences of the different species were obtained from the PubMed amino acid sequence bank. Further information is provided in the Materials and methods section.

Table 7. *In silico* prediction of the effect of novel *NAT2* genetic variant by using Polyphen2, SIFT, and MutPred software programs.

Variants	Altered amino acid	Polyphen2 (probability score)[a]	SIFT (probability score)[b]	MutPred (probability score)[a]	
				Probability score	Molecular mechanism disrupted (*p*-value)
436G>A	V146M	Benign (0.04)	Tolerated (0.89)	0.542	Gain of ubiquitination at K141 ($p = 0.07$)

[a]Probability of being pathogenic, 0 = lowest; 1 = highest. [b]Probability of being pathogenic; 0 = highest; 1 = lowest.
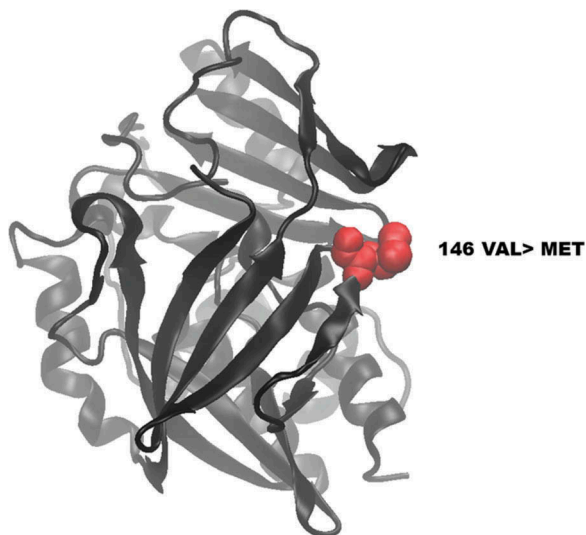
Figure 5. Visualization of wild-type and mutated NAT2 proteins. (A) shows the location of the novel mutated residue VAL146MET in the predicted model structure of NAT2 protein.

effects on drug metabolism and susceptibility to certain diseases. In this study, the protein-coding exon of *NAT2* gene was completely sequenced among a sample of Arab Jordanian volunteers. To the best of our knowledge, it is the first study in Jordan to have identified the *NAT2* haplotype and the LD of *NAT2* SNPs. In addition, the present study could identify the novel *NAT2* genetic variant (436G>A) among Jordanians with *in silico* prediction. The findings of this study may increase our understanding of the interindividual and interethnic variations in the acetylation capacity, which may affect drug response, toxicity, and susceptibility to diseases.

The size of the sample was relatively small, and the statistical power of the test ($\beta = 80\%$) indicated that the sample size of 68 volunteers was sufficient to study the high frequency of NAT2 variants among Caucasian populations. In addition, several studies have investigated the genetic variants among other populations, such as Koreans [27], and have used a sample size of 48, smaller than that in this study. Therefore, the sample size of the current study was statistically sufficient to investigate NAT2 variants among the Jordanian population.

In this study, the *NAT2* gene was genotyped by the DNA-sequencing technique. Previous studies on *NAT2* gene among Jordanians genotyped major *NAT2* genetic polymorphisms using the PCR-RFLP method [11,28]. It has been reported that DNA sequencing is the golden method for genotyping [29], while the PCR-RFLP technique has large errors with a higher probability of false-positive results [30]. Some studies have found that the PCR-RFLP technique is less accurate than other genotyping assays, such as direct DNA sequencing and pyrosequencing [31–33]. These may indicate that the results of *NAT2* genotyping obtained from this study were more accurate than the previous reports on *NAT2* genotyping among Jordanians.

The *NAT2*5 allele is represented mainly by the presence of the 341T>C variant. Many studies have analyzed the 481C>T genetic variant as a representative variant of *NAT2*5 allele [11,34]. These studies, based on the 341T>C and 481C>T variants, are in complete LD. The current study found that 341T>C and 481C>T variants were not in complete LD among Jordanian volunteers. Some volunteers were found to carry only the 481C>T but not the 341T>C variant on the *NAT2* gene, which indicates that those volunteers

were considered genetically as fast acetylators. Therefore, detection of 481C>T variant as a representative SNP of *NAT2*5* allele among Jordanians may give a false-slow encoding *NAT2* genotype and can overestimate the prevalence of slow acetylator phenotypes among Jordanians [35]. Therefore, genotyping the slow allele *NAT2*5* using the 481C>T variant among Jordanian population is not recommended.

In comparison with previous reports on *NAT2* allele frequencies in Jordan, the frequencies of 481C>T and 590 G>A were similar to those in the report by Jarrar et al. [11]. However, Jarrar et al. detected the 857A>G variant among Jordanians with a low frequency of 1%. The present study could not find the 857A>G variant among the studied Jordanian sample. This may be due to the larger sample size used by Jarrar et al. [11] (n = 150) than that used in the current study (n = 68).

Jordanians are considered a mixture of different ethnic populations, with a majority of Arabs. The present study collected the blood samples from only Arab volunteers and did not include volunteers from minor ethnic groups, such as Kurds and Circassians. Comparison of *NAT2* allele frequencies with other ethnic groups showed that Jordanian *NAT2* allele frequencies were in line with those of Europeans and different from the *NAT2* allele frequencies in Chinese Asians and Africans. These results are in line with those reported previously [11], in which the *NAT2* allele frequencies were similar to those of the European and Middle Eastern populations but different from the Asian and African populations. However, the 191G>A variant, which was reported only among Africans and Middle Eastern Turkish [36] but was absent among European Caucasians, was also found among Jordanian volunteers. Some studies found that Arabs have some genetic variants found in African but not in Caucasian populations [37]. This may indicate a genetic admixture between Caucasian Arabs in the Arabic peninsula and their African neighbors that makes the distribution of genetic polymorphisms among Arabs unique in comparison with other Caucasian populations, such as white Europeans.

The *NAT2*5B* and *NAT2*6A* haplotypes were reported to be the most common haplotypes among different Caucasian populations [38]. The present study found that *NAT2*5B* and *NAT2*6A* haplotypes were the most common slow haplotypes among Jordanians with frequencies of 29.3% and 23.5%, respectively. However, the frequency of the *NAT2*5B* haplotype among Jordanians (27%) was significantly lower (×2, p < 0.05) than the *NAT2*5B* frequency among white Europeans (41.1%) [38].

Depending on the *NAT2* haplotype among Jordanians, it was found in this study that 72% of the volunteers had the *NAT2* slow acetylation encoding haplotype. This high frequency of *NAT2* slow

encoding genotype was also reported previously among Jordanians, which could explain the high proportion of slow acetylation phenotype found in Jordanians [39].

The NAT2 191G>A, 341T>C, and 590G>A clinically decrease the metabolism of isoniazid, sulphasalazine, and hydralazine associated with drug toxicity, such as hepatic, neuro-toxicity, and systemic lupus erythematosus [2,7,8,10]. As Jordanian populations have a high frequency of slow-encoding NAT2 haplotype, the risk of toxicity induced by drugs metabolized by the NAT2 enzyme may be high among Jordanians. Therefore, physicians should consider genotyping NAT2 191G>A, 341T>C, and 590G>A in treatment with drugs metabolized by NAT2.

The present study found and confirmed the presence of the novel *NAT2*436G>A genetic variant among the volunteers. The frequency of this novel variant was very low (0.7%). The novel 436G>A variant is a nonsynonymous variant that causes a substitution of the valine peptide at position 146, in the amino acid sequence, with methionine. The alignment of the human NAT2 protein sequence with the NAT2 protein of other species showed that V146 is conserved in most of the species, which might indicate that V146 plays a role in the structural stability and function of NAT2. In addition, it is reprted that substitution of Q145, which is close to the novel V146M substitution, changed the formation of hydrogen bonds in the NAT2 enzyme [40]. However, *in silico* prediction in all the software programs (Polyphen2, Mutpred, and SIFT) used predicted that V146M is tolerable and probably is not harmful to NAT2 protein.

Collectively, these *in silico* data did not encourage us to proceed further through the functional *in vitro* and *in vivo* functional genetic analysis. Although *in silico* tools predict the probability of a harmful effect in genetic variations in protein function and structure, it is reported that *in silico* software may show inaccurate prediction in comparison with *in vitro* and clinical data [41].

5. Conclusions

In conclusion, the present study identified the haplotype and LD of *NAT2* genetic polymorphisms and found the novel *NAT2* genetic variant 436G>A with *in silico* prediction among Jordanian volunteers. The findings of this study may increase our understanding of the interindividual and ethnic variation in the acetylation of drugs and toxic compounds.

Disclosure statement

Funding

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