

Prediction of Structural and Functional Effects of Single Nucleotide Polymorphisms in *NAT2* gene, a Computational Analysis

Amna Elsadig Elsaifi Abodlaa^{1, 2, 3}, Dalia Mursi⁴, Mona Abdelrahman Mohamed Khaier^{1, *},
Mai Abdul Rahman Masri⁵, Nazik Elmalaika Obaid Seid Ahmed Husain⁶, Hind Abdelaziz Elnasri¹

¹Department of Molecular Biology and Bioinformatics, University of Bahri, Khartoum, Sudan

²Haematology Department, Research and Laboratory Unit, Khartoum Teaching Hospital, Sudan

³College of Medical Laboratory, Sudan University of Science and Technology, Sudan

⁴Central Laboratory, Ministry of Higher Education and Scientific Research, Sudan

⁵Department of Zoology, Molecular Biology, University of Khartoum, Khartoum, Sudan

⁶Department of Pathology, Omdurman Islamic University, Omdurman, Sudan

Email address:

amna4342@gmail.com (A. E. E. Abodlaa)

*Corresponding author

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Abstract: N-acetyltransferase 2 (encoded by *NAT2*) is a phase II enzyme that detoxifies and metabolizes xenobiotics and drugs components. It is a critical enzyme in clinical pharmacology. It has remarkable genetic polymorphisms, which is associated with the risk of developing cancer due to the change of normally fast acetylation of substrates to slow acetylation. This study assessed single nucleotide polymorphisms (SNPs) in the coding region and (3'UTR) of *NAT2*. Computational approaches were used in this study for functional and structural effects of *NAT2* gene. SNPs. Were retrieved from NCBI SNPs database. The *NAT2* protein sequence and amino acid change were used as an input to the SIFT, PolyPhen-2, PhD-SNP, SNPs& GO, SNP Analyzer, I-Mutant 3.0 and PMut to determine the deleterious and SNPs conditions. Other software for predication of the structural change were Mutation3D, Chimera and Project HOPE. GeneMANIA software was used to show gene –gene interaction. PolymiRTs was used to investigate the disruption or creation of SNPs of miRNA region. In *Homo sapiens* 182 were nonsynonymous SNPs (nsSNPs), 60 synonymous SNPs, 48 3'UTR SNPs and 19 5'UTR SNPs. A total, 65 of those nsSNPs were predicted to be highly damaging with 3-6 score rates when analyzed with six software. Re-computation of results with I-Mutant 3.0 showed a decrease in the effective stability of the protein due to 55 nsSNPs. Consequent structural changes were shown using Project HOPE and Chimera. *NAT2* is a highly polymorphic gene; the majority of deleterious *NAT2* SNPs are nsSNPs that alter the physiochemical and structural properties of the protein, possibly leading to the loss or distortion of the protein's ability to detoxify and metabolize xenobiotic and aromatic amine compounds. There were three SNPs at the 3'UTR that changed the miRNA binding sites, which might affect the gene regulation.

Keywords: *NAT2* gene, Computational Analysis, nsSNPs, 3'UTR

1. Introduction

Single nucleotide polymorphisms (SNPs) are the genetic basis of many complex human diseases [1]. Nonsynonymous coding SNPs (nsSNPs) were responsible for the variety of

human protein changes and frequently are linked with many diseases [2] These nsSNPs leads to change in amino acid residues in the protein product, potentially resulting in deleterious influences on the formation, function, solubility, or constancy of proteins. Any disease resulting from

alterations in protein structure is often watched at either core or interface residues refereeing protein communications [3]. Protein structure may be destabilized by mutations in amino acids found in the core of the protein, while mutations at interface amino acids can affect protein communication. These nsSNPs mutations at the protein–protein interaction locations are responsible for human disorders [2].

Another important source of variation that can affect protein function is SNPs in miRNAs or microRNA binding sites. miRNAs are small noncoding RNAs that are about 20–25 nucleotides in length. They are responsible for the regulation of gene expression in multiple target genes via sequence-specific hybridization to the 3' untranslated region (3'UTR) of mRNAs and then stop translation or direct degradation of their target.

mRNA [4].

Living organisms are always exposed to various substances that may have a carcinogenic and toxic effect [5]. Cancer is among the leading causes of death worldwide [6]. In 2012, about 14.1 million cancer cases were reported and the number is expected to increase to 24 million by the year 2035[7]. Various factors have been found to induce cancer development, including endogenous (genetic) and/or exogenous causes, such as the environment, habits and lifestyle [6]. It has been approximated that about one-third of cancers are attributable to nutrition and lifestyle [7]. Developing databases and recent knowledge about the distribution of highly polymorphic genetic polymorphisms in different populations can aid understanding the variable effects of those mutations and their relation to carcinogenesis and cancer distribution [8, 9]. Among these genes is genes including *NAT2*. Two highly polymorphic isoenzymes of human N-acetyltransferase enzymes, *NAT1* (MIM#108345) and *NAT2* (MIM# 243400) are known. Both genes are located on chromosome 8 and they have different functional roles. *NAT2* is involved in phase II pathway of removal of toxic substances from the human body and metabolism of xenobiotics and arylamine by N- or O-acetylation [10]. The enzyme arylamine N-acetyltransferase type 2 (*NAT2*), encoded by the *NAT2* gene, is a critical enzyme in clinical pharmacology [8]. *NAT2*, also recognized as AAC2; PNAT (NG -012246), is mainly expressed in the liver, but is also expressed in almost all mammalian tissues. The gene is polymorphic, with numerous nucleotide substitutions observed [8, 11]. It is located in chromosome 8, with an open reading frame of 870 bp and consists of 290 amino acids, with variable tissue expression levels and distribution [12, 13]. The occurrence of the acetylator phenotype activity is divided into fast, intermediate, and slow phenotypes and was found to differ through the different ethnic groups [8, 10, 14, 15]. Thus, low or no *NAT2* –enzyme activity have been implicated as a leading cause of increased susceptibility to drug toxicity and many kinds of cancer [16, 17]. About 65 allele variants of *NAT2* have been reported among human populations [13]. These variants have between one to four nucleotide substitutions; the most frequent alleles are the slow acetylator phenotype associated alleles (rs1801280,

rs1799930, rs1799931, and rs1801279) and the fast acetylator phenotype alleles (rs1041983, rs1799929 and rs1208) [13, 14, 17, 18].

The current study is a computational analysis to investigate mutation sin the *NAT2* gene underlying phenotypic variations and predict the importance of the *NAT2* genetic polymorphism as a risk factor in Cancer or human disorders, using an *insilico* approach.

2. Methods

2.1. Data Collection

NAT2 protein structure and function were analyzed using *in silico* translation methods including various databases and software. The related SNP information, protein accession number (GenPept accession number, NP_000006.2) were obtained from the NCBI Protein database during November 2017. *NAT2* SNPs were obtained using the dbSNP database (query, *NAT2*; species, *Homo sapiens* and their dbSNP accession number).

The nucleotide sequence of the *NAT2* gene and amino acid sequence of the *NAT2* protein were obtained from NCBI GenBank (accession number, NG_012246.1).

2.2. The Following Software Were Used During the Study

1. GeneMANIA

The query (*Homo sapiens*; *NAT2*) was submitted to GeneMANIA (<http://www.genemania.org>). GeneMANIA built a complex gene-gene functional interaction network from a gene list containing a greatly adaptive algorithm. The output was given as a diagram and tables illustrating physical interaction co-expression and relation with other genes in a network form [19].

2. Sorting Intolerant From Tolerant SIFT

The functional effects of the deleterious SNPs was predicted using (SIFT) v5.1. SIFT analysis depends on sequence homology and the physical properties of the resulting amino acids in each protein and the possible consequent interrelations and changes in protein function. The FASTA format protein sequence of *NAT2* was obtained from ExPasy/UniprotKB, and amino acids substitution list obtained from dbSNP database (wild type protein/codon position/mutation residue) was entered in to SIFT (Single Protein Tools-SIFT sequence). Positions with regularized possibilities less than 0.05 were predicted to be intolerant (deleterious) and these SNPs were subjected for further analysis. Those with score the same to 0.05 or more were predicted to be tolerated and were not subjected for further analysis [20].

3. PolyPhen-2

The deleterious SNPs in *NAT2* detected by SIFT were subjected to PolyPhen-2 (<http://sift.bii.a-star.edu.sg/>) software. It predicts the constancy and function of human proteins. The *NAT2* protein sequence in FASTA format, amino acid substitutions (AA1 to wild type, AA2 to mutant) and their positions were the input. The results are expressed

as probably or possibly damaging depending on a score range. SNPs that were predicted to be benign were not analyzed further [21].

2.3. Prediction of Disease-associated Variations

2.3.1. SNPs & GO

SNPs & Go is used to find the association between SNPs and diseases. It classifies the SNPs as disease-related or neutral. The NAT2 SNPs that were classified as possibly or probably damaging by PolyPhen-2 were the input for SNPs & GO. The protein sequence and amino acid change (wild type, mutant type and site of the mutation) were submitted into the software. The prediction was displayed either as neutral or disease according to the probability. A probability of more than 0.5 is predicted to be disease-related. Available at: <http://snps.Biofold.org/snps-and-go/snps-and-go.html> [22].

2.3.2. PhD-SNP

PhD-SNP (<http://snps.biofold.org/phd-snp/phd-snp.html>), is a support vector machine(SVM) used to assess whether a given single nsSNP can be classified as disease-related or has a neutral effect, similar to SNPs & GO. The NAT2 FASTA protein sequence and amino acid change (wild type, mutant type and site of the mutation) were submitted into the software. [23]

2.3.3. PMut

Prediction of the pathologic character of a point mutation in a protein using PMut.

It is a server that predicts whether a NAT2 nucleotide substitution is related to pathological mutations or it is neutral. It is used for unusual kinds of sequence information to label single point mutations depending on neural networks to process this information. The input was UniProt ID of the protein (P11245) or protein sequence and mutation to analyze and an output (pathological or not) and a reliability index were given [24] Available at: <http://mmb2pcb.ub.es/PMut/>.

2.3.4. nsSNP Analyzer

nsSNP Analyzer was used to predict whether NAT2 nsSNP has a phenotypic effect. It works in multistep manner. First the NAT2 protein sequence was to upload, then SNP data (rsSNPs from dbSNP) was uploaded. The output was given 'disease' or 'neutral' [25]. (<http://snpanalyzer.uthsc.edu/help.htm>)

2.4. Impact of nsSNPs on Protein Stability

I-Mutant 3.0

It is a SVM that uses a web tool to study if the protein stability is changed by substitution. The FASTA sequence of the NAT2 protein, in addition to the residues changes, were the input. I-Mutant 3.0 can predict the direction of the free energy change ($\Delta\Delta G$ value (kcal/mol)) and it calculates the reliability index (RI) value. The output determines if the substitution can lead to decrease or increase in the stability of

protein (24).

2.5. Allocation of nsSNPs in MutS Domains Using Mutation3D

Mutation3D (<http://mutation3d.org>), is a useful prediction and imaging server for learning the specific display of residues substitutions on protein forms and structures. It recognizes somatic cancer-causing mutations across several patients, in sequence to see efficient hotspots and increase downstream theories. It is also functional for grouping other types of mutational information, or merely as a tool to rapidly assess the relative sites of residues in proteins. The NAT2 FASTA protein sequence and amino acids substitution were input. The results is shown as a diagram [25].

2.6. Protein Modeling

2.6.1. PROJECT-HOPE

HOPE (<http://www.cmbi.ru.nl/hope/home>), is used to determine the structural effects of a mutation. It collects structural information from various sources. The input was the NAT2 protein sequence in FASTA format or an accession number (GenPeptNP_000006.2). The wild-type residue and the mutant type were specified. HOPE provides a report describing the effect of the amino acid substitution on the protein structure and provides a graphical structure for the protein (if available) [26].

2.6.2. Chimera

Chimera (<http://www.cgl.ucsf.edu/chimera>), is a homology modeling server that investigates the three-dimensional structure of proteins. It was used to visualize and analyze NAT2 protein, molecular constructions and related data, including density maps, supra molecular collectives, sequence arrangements, docking products, trajectories, and conformational ensembles to generate the mutated protein 3D model. The PDB accession number 2PFR was entered and the amino acid substitution through the beta or alpha chain was selected, giving a graphic model depicting the mutant protein.

2.7. Prediction of the Influence of NAT2 SNPs at the 3'UTR

The PolymiRTS

The PolymiRTS (<http://compbio.uthsc.edu/miRSNP>), database was developed to systematically identify SNPs in microRNAs and their target sites. PolymiRTS is used to identify miRNA function, leading to the development of disease and variation in physiological and behavioral phenotypes [27]. It expects miRNA objective sites in the 3'-UTRs of mRNAs with quantitative trait loci (QTLs) for both mRNA appearance traits and higher-order phenotypes [28] SNPs ID of NAT2 were the inputs to the PolymiRTS database, to predict the functional influence of genetic polymorphisms in miRNA seed regions and sequence target sites affecting human diseases and/or biological pathways [29]. The output shows the allele disorders of conserved

microRNA site (D, disrupted) and/or derived allele that generates a new microRNA site (C, created).

respectively.

3. Results

A total of 182 were nsSNPs, 60 were synonymous SNPs, 48 were in the 3'UTR and 19 were in the 5'UTR. General characteristics of *NAT2* collected from different databases are presented in Table 1.

NAT2 SNPs were retrieved from dbSNP and *NAT2* protein sequences was retrieved from ExPasy/UniProtKB.

The relation, co-expression and physical interactions of *NAT2* gene with other genes in the network, produced by GeneMANIA, are presented in Figure 1 and Tables 2 and 3,

Table 1. Descriptive feature for *NAT2* gene.

Character of Nat2	Descriptive
Louse in chromosome (8)	Start from 5001 – 14969
Gene nucleotide NO	16969 bp
No of exon	2exon(1-5001-5101bp, 2-13754-14969bp)
Protein accession number	NP 000006.2
No of amino acid	290aa
Unique stable identifier entry (Expasy UniProtKB)	P11245
Mnemonic identifier of UniProtKB entry	ARY2_HUMAN
PDB ID	2PFR.A

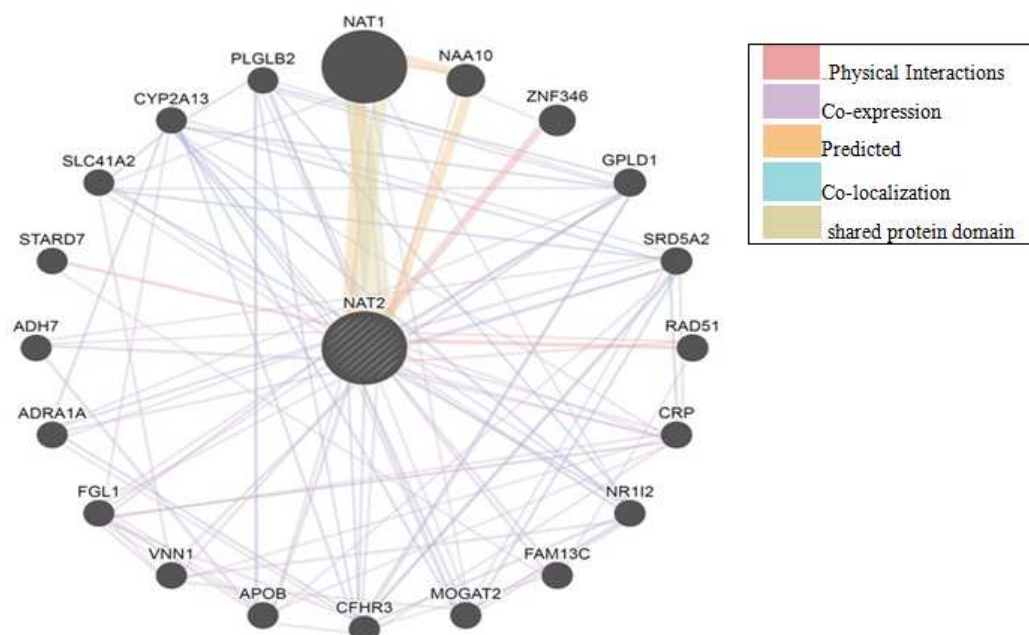


Figure 1. Functional interactions between *NAT2* and related genes using GeneMANIA software.

Table 2. Genes that are co-expressed, have shared domain and/or physical interactions with the *NAT2* gene.

Symbol	Description	Co-expression	Shared domains	Physical interaction
<i>NAT1</i>	N-acetyltransferase 1	YES	YES	YES
<i>NAA10</i>	N(alpha)-acetyltransferase 10, NatAcatalyticsubunit	NO	NO	YES
<i>ZNF346</i>	Zinc Finger Protein 346	NO	NO	YES
<i>GPLD1</i>	Glycosyl phosphatidylinositol Specific Phospholipase D1	YES	NO	NO
<i>SRD5A2</i>	Steroid 5 Alpha-Reductase 2	YES	NO	NO
<i>RAD51</i>	RAD51 recombinase	NO	NO	YES
<i>CRP</i>	C-reactive protein, pentraxin-related	YES	NO	NO
<i>NR112</i>	Nuclear Receptor Subfamily 1 Group 1 Member 2	YES	NO	NO
<i>FAM13C</i>	Family With Sequence Similarity 13 Member C	YES	NO	NO
<i>MOGAT2</i>	Monoacylglycerol O-Acyltransferase 2	YES	NO	NO
<i>CFHR3</i>	Complement Factor H Related 3	YES	NO	NO
<i>APOB</i>	Apolipoprotein B	YES	NO	NO
<i>VNN1</i>	Vanin 1	YES	NO	NO
<i>FGL1</i>	Fibrinogen Like 1	YES	NO	NO
<i>ADRA1A</i>	Adrenoceptor Alpha 1A	YES	NO	NO
<i>ADH7</i>	Alcohol Dehydrogenase 7	YES	NO	NO
<i>STARD7</i>	StAR related lipid transfer domain containing 7	NO	NO	YES
<i>SLC41A2</i>	Solute Carrier Family 41 Member 2	YES	NO	NO
<i>CYP2A13</i>	Cytochrome p450 Family2 Subfamily A Member 13	YES	NO	NO
<i>PLGLB2</i>	Plasminogen-Like B2	YES	NO	NO

Table 3. NAT2 gene relationships and appearance in the network.

Function	FDR	Genes in network	Genes in genome
Cellular Response To Foreign Stimulus	9.63E-07	7	149
Response To Xenobiotic Stimulus	9.63E-07	7	149
Xenobiotic Metabolic Process	9.63E-07	7	148
Drug Catabolic Process	0.243286	2	10
Terpenoid Metabolic Process	0.30869	3	78
Isoprenoid Metabolic Process	0.393928	3	90
Response To Drug	0.628239	3	111
Regulation Of Macrophage Derived Foam Cell Differentiation	0.813281	2	28
Oxidoreductase Action, Acting On The Aldehyde Or Oxo Group Of Donors	0.813281	2	32
Drug Metabolic Process	0.813281	2	30

FDR: False discovery Rate

After analyzing with 6 software; our findings showed that 65 SNPs were highly disordered (lowered enzyme activity SNP), their scores reading range from 3 to 6 in six software, 26 SNPs (out of 65 mutation) were fully alteration (Slow acetylator phenotype), their scores reading 6 in six software.

Using PhD-SNP, 44 SNPs were found to be disease-related and 21 SNPs were found to be neutral. Using SNPs & GO, 31 SNPs were predicted to be disease-related and 24 SNPs were classified as neutral.

Using nSNPAnalyzer, it was predicted that 60 SNPs were

disease-related and only five were neutral. Using PMut, almost 54 SNPs were reported as pathogenic, while only 11 SNPs were characterized as neutral. These SNPs were further computed with I-Mutant 3.0, revealing 55 SNPs out of 65 SNPs caused a decrease in the effective stability of the protein.

One domain was found in NAT2; 53 SNP harmful alterations were situated in a protein domain structure (39 covered and 14 clustered), and 3 mutations were located in the inter domain region (Figure 2).

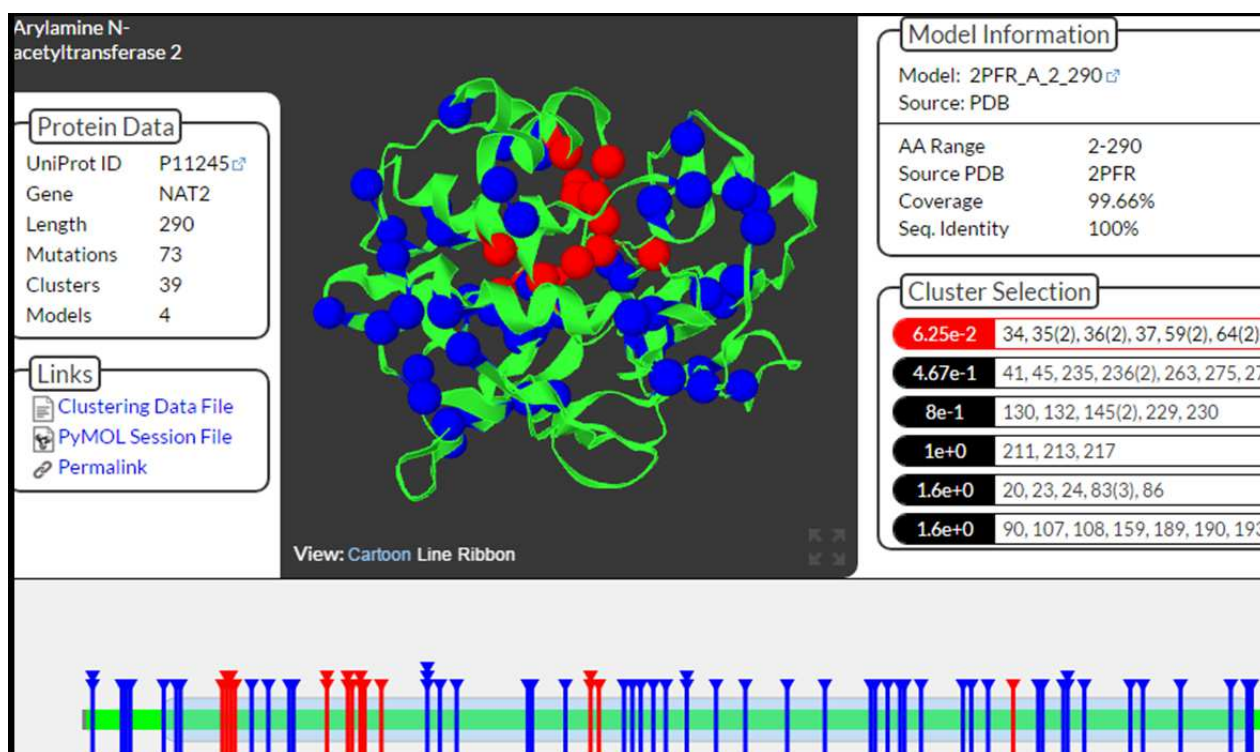


Figure 2. Distribution of extremely damaging mutations SNPs in NAT2 protein domains using Mutations3D.

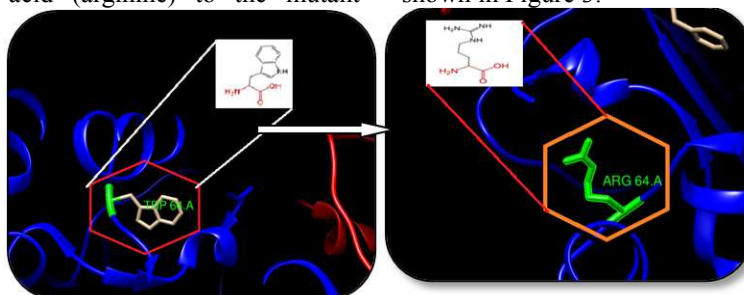
Out of the 72 SNPs reported at the 3'UTR of NAT2, three functional SNPs were predicted to affect miRNA binding Sites when analyzed using PolymiRTS database. The first SNP (rs45539742) was found to have 2 alleles (C and G) contain disrupt three conserved miRSites (D) and one (C) Function class on miRSite. In the second SNP (rs56011639)

analysis showed that the C allele causes a disturbance in a conserved miRSite (class D), while the T allele creates two new miRSites (class C); while the third SNP (rs142360798) had one allele (A) that disturbs three miRSites (class D) (Table 4).

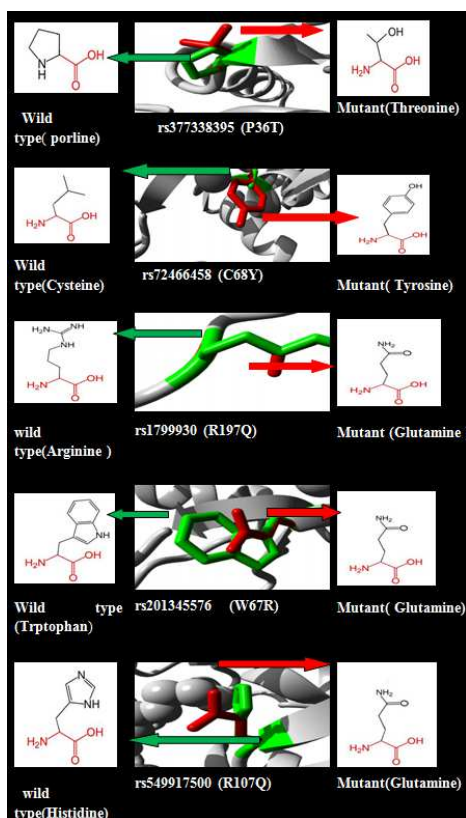
Table 4. *NAT2* SNPs predicted by PolymiRTS to make disturbance of conserved miRNA site or creation of new microRNA binding location D, the resulting allele disorders a conserved binding site; C, the derived allele generates a new binding site.

Location	dbSNP ID	Allele	miR ID	Conservation	Binding Site	Function class	Context +score change
18258614	rs45539742	C	hsa-miR-15a-3p	4	gATGGCCTgtgat	D	-0.246
			hsa-miR-1972	2	gaTGGCCTGtgat	D	-0.278
			hsa-miR-4529-5p	4	GATGGCCtgtgat	D	-0.278
18258647	rs56011639	G	hsa-miR-122-3p	4	gATGGCGTgtgat	C	-0.265
			hsa-miR-1287-3p	4	ttcatGCTAGAAa	D	-0.192
			hsa-miR-4263	3	ttcatgTTAGAAA	C	-0.045
18258715	rs142360798	A	hsa-miR-576-5p	3	ttcatgTTAGAAA	C	-0.058
			hsa-miR-16-2-3p	3	taaagAATATTGt	D	-0.069
			hsa-miR-195-3p	3	taaagAATATTGt	D	-0.051
			hsa-miR-338-5p	2	taaagaATATTGT	D	-0.082

Modelling annotation An amino acid residue substitution from the original amino acid (arginine) to the mutant (tryptophan) at codon 64, produced using Chimera, are shown in Figure 3.

**Figure 3.** Chimera modeling of arginine mutated to tryptophan.

Three-dimensional structure modeling in the wild type of amino acid substitution by the mutant SNP revealed by ProjecHOPE; *NAT2* protein sequence ProjecHOPE server is shown in Figure 4.

**Figure 4.** HOPE Modeling of the 3D structure of the *NAT2* protein with selected SNPs. The protein (grey color) residues changed from wild type (green color) to mutant (red color).

4. Discussion

SNPs are the simplest shape and the most frequent cause of hereditary variation in the human genome [30]. SNP variations are a frequent source of information used in bioinformatics tools and are thus involved in subsequent proteomics studies. A main role of this branch of bioinformatics is to predict the effect of SNPs at the protein level and possible consequences on the function of the protein [9]. SNP mutations in *NAT2* gene have been described as an important risk factor of various cancers (including acute lymphoblastic leukemia, and cancer of the bladder, colon, head and neck, lung and breast), driven by low or no *NAT2* activity [8, 15, 17, 31, 32].

The mutant residue of the R64Q SNP (rs1801279) was predicted to be smaller in size than the wild-type and is neutrally charged while the wild-type is positive. This variation in charge could disturb the ionic interaction bonds made by the native amino acid, while the size difference acetylator phenotype changes the position of the new residue in the protein, preventing the creation of the same hydrogen bonds made by the original wild-type amino acid. Due to the difference in size, the mutation will cause the formation of an empty area in the core of the protein. Other consequences were also reported; for example, the mutant residue of the C68Y (rs72466458) is bigger than the wildtype and is situated close to a highly conserved site of protein that is changed from being buried in the core to being present on the surface, leading to loss of hydrophobicity. This conformational change can be seen in the three-dimensional modeling structure, where the original residue has an interaction with a ligand, while the mutant lacks this interaction, meaning that the protein function must be disturbed. Other SNPs, like P36T (rs377338395), change the hydrophobicity of the amino acid; the original amino acid (proline) is more hydrophobic than the mutant residue. Prolines are recognized to be very stiff and therefore make a specific backbone modification which might be needed at this position; the interruption of this special modulation could cause failure of hydrophobic interactions in the center of the protein. In the 3'UTR, the polymorphisms occurrences were predicted to affect microRNA, because the three SNPs disrupted and/or created new binding sites in the gene, leading to deregulation of gene function.

In the current study, a computational examination of the reported *NAT2* SNPs using various publicly available software and databases was conducted to understand their possible influence on the protein structure and function, and hence their ability to cause or enhance advance cancer development. The analysis revealed that the *NAT2* gene is highly polymorphic and can contribute to disease formation following changes in its amino acids. The results of this analysis agrees with several studies [15, 18, 33]. It also shows that the *NAT2* had strong mutagenic and carcinogenic effects, due to changes in protein structure and function

produced by deleterious SNPs [8, 31].

Several wet lab studies highlighted the role of a number of *NAT2* SNPs in carcinogenesis [5, 12, 17, 34]. Of these, two SNPs (rs1801279 (R64Q) and rs1799930 (R197Q)) were revealed to be highly damaging and could decrease the stability of protein according to six software and the I-Mutant 3.0 computational analysis tool in your study. In wet lab studies, these SNPs were defined as causing a significant decrease in acetylation capacity and are associated with a slow acetylator phenotype, leading to many diseases (e.g. lung cancer or chronic myelocytic leukemia) [3, 13, 33]. Two other SNPs (rs1799929 and rs1208) were synonymous, showing tolerated (using SIFT) and benign (using PolyPhen-2) effects by computational analysis software (i.e. they do not alter the phenotype), and they have been identified as slow acetylator phenotype in a wet lab setting (these findings agree with another study) [10]. Another SNP (rs1799931 (G386E)) was classified as slow-acetylator in wet lab and the produced protein was considered as slower acetylator in the aforementioned study [5, 12, 17]. However, it was observed as tolerated and benign in this study; this different expression of acetylator phenotype may be due to genetic variations or interactions that differs between individuals.

The coding and non-coding *NAT2*-SNPs might cause protein structure or expression alterations, leading to a wide range of variation at the population level and disease characteristics. Such proteomics information, are in general, closely joined to current (post-genomic) drug discovery studies [9] and individualized medicine.

5. Conclusions

NAT2 is highly polymorphic. A 65-SNP panel infers *NAT2* acetylator phenotype with high accuracy, were predicted by diverse software tools mostly damaging mutations shifting physiochemical properties of the protein; dimension, charge and hydrophobicity essential to a lack or disorder of the protein function and their configuration. Three *NAT2* SNPs at the 3'UTR caused a change in the microRNA binding position, which might result in deregulation of gene function. Thus, low or no *NAT2* activity can be connected to higher susceptibility to unfavorable risk factors and various types of cancer.

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Appendix

Table A1. Prediction of functional modification of NAT2 protein using six software SNPs showing fully damaging NAT2 SNPs.

SNP ID	Amino Acid Change	Sift	TI	Predication	PolyphenPSIC Score	Phd-SNP Predication
rs72466456	I10S	Deleterious	0.01	Probably damaging	0.99	Disease
rs747536177	G11V	Deleterious	0.00	Probably damaging	1	Disease
rs377338395	P36T	Deleterious	0.00	Probably damaging	1	Disease
rs377338395	P36A	Deleterious	0.00	Probably damaging	1	Disease
rs753310036	F37S	Deleterious	0.00	Probably damaging	1	Disease
rs773371959	I59T	Deleterious	0.01	Probably damaging	1	Disease
rs1805158	R64W	Deleterious	0.00	Probably damaging	1	Disease
rs1801279	R64Q	Deleterious	0.00	Probably damaging	1	Disease
rs1019316375	G65V	Deleterious	0.00	Probably damaging	1	Disease
rs201345576	W67R	Deleterious	0.00	Probably damaging	1	Disease
rs72466458	C68Y	Deleterious	0.00	Probably damaging	1	Disease
rs746734312	G83C	Deleterious	0.00	Probably damaging	1	Disease
rs561124342	G83A	Deleterious	0.00	Possibly damaging	0.67	Disease
rs561124342	G83V	Deleterious	0.00	Probably damaging	1	Disease
rs759840221	G90R	Deleterious	0.01	Probably damaging	1	Disease
rs549917500	H107Q	Deleterious	0.00	Probably damaging	1	Disease
rs751243960	L108R	Deleterious	0.03	Probably damaging	1	Disease
rs4986996	D122Y	Deleterious	0.00	Probably damaging	1	Disease
rs559660817	D122G	Deleterious	0.00	Probably damaging	1	Disease
rs764591879	G124V	Deleterious	0.01	Probably damaging	1	Disease
rs72554616	Q145P	Deleterious	0.03	Probably damaging	1	Disease
rs374177543	L152W	Deleterious	0.01	Probably damaging	1	Disease
rs374761885	Y190C	Deleterious	0.00	Probably damaging	1	Disease
rs749948990	G230V	Deleterious	0.01	Probably damaging	1	Disease
rs778253810	G236R	Deleterious	0.00	Probably damaging	1	Disease
rs777549905	L279R	Deleterious	0.00	Probably damaging	1	Disease

Table A1. Continued.

SNP ID	RI	Probability	Snps & GOPredication	RI	Probability	SNPsAnalyze	PMut	Score
rs72466456	8	0.92	Disease	7	0.866	Disease	Pathological	6
rs747536177	6	0.791	Disease	2	0.583	Disease	Pathological	6
rs377338395	7	0.835	Disease	6	0.778	Disease	Pathological	6
rs377338395	4	0.689	Disease	3	0.665	Disease	Pathological	6
rs753310036	8	0.891	Disease	6	0.796	Disease	Pathological	6
rs773371959	5	0.742	Disease	5	0.742	Disease	Pathological	6
rs1805158	9	0.96	Disease	7	0.857	Disease	Pathological	6
rs1801279	9	0.936	Disease	7	0.837	Disease	Pathological	6
rs1019316375	9	0.951	Disease	8	0.878	Disease	Pathological	6
rs201345576	4	0.72	Disease	5	0.757	Disease	Pathological	6
rs72466458	9	0.973	Disease	9	0.925	Disease	Pathological	6
rs746734312	9	0.928	Disease	7	0.854	Disease	Pathological	6
rs561124342	5	0.806	Disease	6	0.758	Disease	Pathological	6
rs561124342	8	0.902	Disease	7	0.851	Disease	Pathological	6
rs759840221	5	0.75	Disease	0	0.518	Disease	Pathological	6
rs549917500	8	0.889	Disease	7	0.865	Disease	Pathological	6
rs751243960	7	0.843	Disease	1	0.569	Disease	Pathological	6
rs4986996	8	0.908	Disease	7	0.832	Disease	Pathological	6
rs559660817	8	0.839	Disease	7	0.755	Disease	Pathological	6
rs764591879	7	0.848	Disease	6	0.798	Disease	Pathological	6
rs72554616	3	0.646	Disease	0	0.509	Disease	Pathological	6
rs374177543	4	0.714	Disease	0	0.52	Disease	Pathological	6
rs374761885	4	0.678	Disease	2	0.607	Disease	Pathological	6
rs749948990	6	0.791	Disease	4	0.723	Disease	Pathological	6
rs778253810	7	0.861	Disease	5	0.728	Disease	Pathological	6
rs777549905	6	0.816	Disease	2	0.582	Disease	Pathological	6

SIFT Tolerance Index: variety from 0 to 1. The amino SNPs is predicted deleterious if the score is <0.05 , and tolerated if the score is >0.05 . PolyPhen-2 outcome: Probably damaging (high prediction) / Possibly damaging (lower prediction), PSIC SD: Position- Specific Independent Counts server if the score is ≥ 0.5 , Key score (deleterious=1, probably damaging =1, possibly damaging =0.5, Disease =1, pathological =1 Neutral)

Table A2. Prediction of NAT2 protein stability using I-Mutant.

I-Mutant Predication	RI	DDG	I-Mutant Predication	RI	DDG
Decrease	7	-0.86	Decrease	4	-0.66
Decrease	3	-0.55	Decrease	3	-0.13
Decrease	4	-0.22	Decrease	8	-2.34
Increase	6	0.05	Decrease	9	-0.67
Increase	2	-0.32	Decrease	9	-2.61
Increase	5	-0.09	Decrease	5	-0.54
Decrease	1	0.51	Decrease	9	-2.37
Decrease	4	-0.52	Increase	1	-0.68
Increase	4	-0.07	Decrease	8	-0.85
Increase	2	0.47	Decrease	8	-0.49
Decrease	6	-0.97	Decrease	7	-1.02
Decrease	0	-0.41	Decrease	5	-0.78
Decrease	8	-0.93	Decrease	8	-1.29
Decrease	9	-1.52	Decrease	1	-0.44
Increase	1	-0.22	Decrease	6	-1.25
Decrease	7	-1.47	Decrease	6	-0.78
Decrease	7	0.45	Decrease	7	-0.58
Increase	1	0.42	Decrease	5	-0.59
Increase	5	-0.49	Decrease	2	-1.18
Decrease	4	-0.11	Increase	5	0.65
Increase	1	0.45	Decrease	6	-1.26
Decrease	3	-0.05	Decrease	8	-1.82
Decrease	5	-0.01	Decrease	8	-1.74
Decrease	3	-0.11	Decrease	1	-0.31
Increase	2	0.17	Decrease	6	-1.14
Decrease	5	-0.65	Decrease	5	-0.77
Decrease	6	-0.89	Decrease	8	-1.78
Decrease	7	-1.16	Decrease	8	-2.31
Decrease	9	-0.97	Decrease	7	-1.33
Decrease	8	-0.61	Decrease	7	-1.57
Decrease	7	-1.15	Decrease	1	-0.22
Decrease	7	-1.56	Increase	3	-0.42
Decrease	9	-1.33	Increase	4	-0.54
Increase	0	0.27	Decrease	4	-1.02
Decrease	5	-1.17	Decrease	7	-0.78
Increase	1	0.05			

$\Delta\Delta$ sign DDG Value: DG(Original Protein)-DG(Wild Type) in kcal/mole, NAT2 rate: DDG<0: decrease stability, DDG>0: increase stability, I-Mutant-SNPRI(Reliability Index): 0=10, where 0 is the lowest reliability and 10 is the highest reliability.

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