Original Article *Iran J Ped Hematol Oncol. 2022, Vol 12, No 4, 246-262*

A comprehensive *in silico* analysis of pathogenic nsSNPs in the *NT5C2* gene involved in relapsed ALL

Reyhane Chamani[1]*, Parnia Sadat Pourhesseini MahmoudAbadi[1], Yasamin Janati[1], Roxana Tajdini[1]

1. Department of Biology, Yazd University, Yazd, Iran
*Corresponding author: Dr Reyhane Chamani, PhD at the Department of Biology, Yazd University, University Blvd, Safayieh, Yazd, Iran, PO Box: 891581841, Tel: 98-3531232652, Email: chamani@yazd.ac.ir ORCID ID: 0000-0002-5160-0159

Received: 09 January 2022 **Accepted:** 02 August 2022

Abstract

Background: About 10-20% of children suffering from acute lymphoblastic leukemia (ALL), experience a relapse, which is a major cause of their death. Purine nucleotide analogs are frequently prescribed to maintain the treatment of ALL. Cytosolic 5´-nucleotidase (NT5C2) catalyzes the 5´ dephosphorylation of purine analogs. Gain-of-function mutations in the *NT5C2* gene result in resistance to the treatment with purine analogs and subsequently in the relapse of the disease.

Materials and Methods: In this descriptive study, bioinformatics tools were used to assess the effect of single nucleotide polymorphisms (SNPs) in the *NT5C2* gene on the function and structure of the protein. So, 352 missense variants were retrieved from the NCBI database and analyzed by SIFT, PROVEAN, PMut, PANTHER, PolyPhen2, SNPs & Go, and PhD-SNP servers. Then, structural evaluations were performed using HOPE, NetSurp-2.0, and PyMOL. Moreover, stability and evolutionary preservation were assessed by I-Mutant2.0 and ConSurf, respectively.

Results: As many as 31 nsSNPs were predicted to be affecting the protein function and stability. Also, the native residues were found to be evolutionarily preserved. The structural evaluation demonstrated that a change of hydrophobicity, flexibility, size, charge, or surface accessibility due to 24 nsSNPs would lead to the change of noncovalent interactions and then the conformation of the protein.

Conclusion: Identification of biomarkers is significant in the prediction of relapses in ALL children. In this study, bioinformatics tools served to identify 24 high-risk deleterious nsSNPs in the *NT5C2* gene. These mutations can be used to predict resistance to chemotherapy and relapse in ALL patients.

Keywords: Bioinformatics tools, *NT5C2* gene, 5´-nucleotidase, Pathogenicity prediction, Relapsed ALL

Introduction

Due to intensified combined chemotherapy regimens, children suffering from Acute Lymphoblastic Leukemia (ALL) can have a survival rate of five years in 90% of the cases. However, 10-20% of patients experience the relapse of the disease and resistance to the treatment, which is a major cause of death in children (1). So, the identification of novel predictive biomarkers is essential for relapsed ALL. Heterozygous mutations in the *NT5C2* gene occur in 10% and 20% of relapsed B-precursor ALL and relapsed T-ALL cases, respectively (2, 3). Recently, Barz et al. (4) identified 110 *NT5C2* mutations in 16.5% of 455 relapses in B-cell precursor ALL.

The *NT5C2* gene, also known as *cN-II*, *GMP*, *NT5B*, *PNT5*, *SPG45*, and *SPG65*, is located on chromosome 10q24.32-q24.33 and has 27 exons. It encodes 5´-nucleotidase, cytosolic II (EC3.1.3.5), a highly conserved enzyme that dephosphorylates 6-hydroxypurine monophosphates, inosine monophosphate (IMP), guanosine monophosphate (GMP), and xanthine monophosphate (XMP) as well as dIMP and dGMP to nucleosides which are subsequently transported out of the cell. This process controls the intracellular purine nucleotide pool (5, 6). Maintenance therapy for ALL is given to sustain remission. The most common chemotherapy regimen includes 6-

[DOI: 10.18502/ijpho.v12i4.10915]

mercaptopurine (6-MP) and 6-thioguanine (TG). These drugs incorporate into the salvage pathway of purine biosynthesis and generate nucleotide monophosphates such as 6-thioinosine monophosphate, 6-thioxanthine monophosphate, and 6-thioguanosine monophosphate that interfere with nucleic acid biosynthesis and activate DNA-mismatch–induced apoptosis (7, 8). The gain-of-function mutations in *NT5C2* intensify the dephosphorylation and clearance of nucleotide monophosphates resulting from thiopurines analogs and, therefore, extremely reduce their cytotoxic effects in patients with the relapsed disease (3, 9, 10). Missense non-synonymous single nucleotide polymorphism (nsSNP) is a point mutation in which a single-base change leads to an amino acid modification in the protein. This substitution may have a neutral or deleterious effect on the protein structure and function. The change in the amino acid may alter the secondary structure, motifs, physicochemical properties (11), dynamics, interacting domain, and the active site of a protein or enzyme, consequently affecting its function. So, damages to missense SNPs are associated with various diseases (12). Besides, some studies have been conducted to find the relationships between SNPs and traits like human diseases. In other words, SNPs serve as genetic markers of diseases in populations (13). Research has shown that SNPs may also impact the response to some drugs. Therefore, the investigation of nsSNPs may help to develop personalized medicine (14). The experimental investigation of how all missense SNPs impact the structure and function of encoded proteins is very difficult, time-consuming, and expensive. Nowadays, bioinformatics and computational tools are valuable for the preliminary screening of deleterious nsSNPs and the determining of candidates for future experimental studies (15). While mutations of cytosolic 5´-nucleotidase play a significant role in the relapsed ALL, their effects on the structure and function of enzymes have not been clarified yet. So, the present study aims to determine high-risk pathogenic nsSNPs in the *NT5C2* gene using various bioinformatics tools.

Materials and Methods

Retrieving nsSNPs

The nsSNP information of the *NT5C2* gene was retrieved from the dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/) and the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar) of the National Center for Biotechnology Information (NCBI).

SIFT

SIFT (Sorting Intolerant From Tolerant, https://sift.bii.a-star.edu.sg/) can predict the tolerance or the effect of amino acid substitution on the protein function using sequence homology and the physical properties of amino acids. A missense SNP is considered to be of effect on protein function when the SIFT score is < 0.05, while a score of ≥ 0.05 indicates that the substitution is tolerated for the protein (16).

PROVEAN

PROVEAN (Protein Variation Effect Analyzer, http://provean.jcvi.org/index.php) at first clusters the aligned sequences with a 75% global sequence identity and then uses the top 30 clusters to predict the effect of amino acid mutations. A cutoff value of -2.5 is also defined. Substitutions with a score more negative than the cutoff value are considered as deleterious, and those with a score more positive than -2.5 are neutral (17).

PMut

Pmut (http://mmb.irbbarcelona.org/PMut/) uses a Random Forest classifier, trained by SwissVar as a manually curated variation database. It calculates a score from 0 to 1 for each mutation. Mutations with a score of 0 to 0.5 are considered neutral, while a

[DOI: 10.18502/ijpho.v12i4.10915]

score between 0.5 and 1 indicates that the substitution is pathogenic (18).

PANTHER

PANTHER (http://www.pantherdb.org/tools/csnpScoreForm.jsp) is based on a method called PSEP (position-specific evolutionary preservation). It computes the length of time (in millions of years) to examine the residue in proteins that have been evolutionarily preserved in the ancestry. PANTHER reports the result as a probability of deleterious effects (Pdel). Besides, if the preservation time is more than 450 million years, the substitution may be considered damaging. Also, the mutation would be possibly damaging or benign if the time of preservation is 200-450 or less than 200 million years, respectively (19).

PolyPhen2

Polyphen2 (Polymorphism Phenotyping v2, http://genetics.bwh.harvard.edu/pph2/) uses an interactive greedy algorithm to select some structural and sequence-related features and compare the wild-type and mutant alleles according to these features. It uses multiple sequence alignments, naive Bayes classifiers as well as HumDiv and HumVar datasets to predict the effect of an amino acid mutation on the protein activity. PolyPhen2 defines a score from 0 (neutral) to 1 (damaging). So, 0.00-0.14 is categorized into benign, 0.15-0.84 into possibly damaging, and 0.85-1 into probably damaging (20).

SNPs & Go

SNPs & GO (https://snps-and-go.biocomp.unibo.it/snps-and-go/index.html) are based on a method that uses Support Vector Machines (SVM) and various data derived from the Gene Ontology (GO) annotation, including the data on the sequence, evolution, and function of proteins to predict whether amino acid substitution is related to a disease or not. The method involves calculating the Reliability Index (RI) of prediction, scoring from 0 (unreliable) to 10 (reliable) (21).

PhD-SNP

PhD-SNP (Predictor of human Deleterious Single Nucleotide Polymorphisms, https://snps.biofold.org/phd-snp/phd-snp.html) predicts the disease-related or neutral impact of SNPs on the protein function using Support Vector Machine (SVM) classifiers, sequence and profile information, and BLAST program. It computes a Reliability Index (RI) for each prediction (22).

I-Mutant2.0

I-Mutant 2.0 (https://folding.biofold.org/cgi-bin/i-mutant2.0.cgi) predicts the stability of the protein after an amino acid mutation from its structure (if available) or sequence using the Support Vector Machine and the ProTherm database. As the mutation occurs, the predictor calculates the ΔΔG value (kcal/mol) by subtracting the unfolding Gibbs free energy value of the native protein from the unfolding Gibbs free energy value of the mutated one. The result can be reported in either of the two ways as follows:

a) with the ΔΔG value (a value of < 0 indicates a decrease in the protein stability, while ΔΔG > 0 shows an increase in it)

b) with the ΔΔG sign (+ for increased stability and - for decreased stability) (23).

NetSurfP-2.0

Predicting the Absolute and Relative Surface Accessibility and the secondary structures (helix, strand, and coil) from the sequence of a protein is done by NetSurfP-2.0 (https://services.healthtech.dtu.dk/service.php?NetSurfP-2.0). It also estimates disordered residues and the phi/psi dihedral angles of each amino acid. NetSurfP-2.0 employs a deep neural network architecture for prediction (24).

HOPE

HOPE (https://www3.cmbi.umcn.nl/hope/) is a server that combines the structural information collected from databases and software programs such as WHAT IF web services, UniProt and Reprof to predict the

[DOI: 10.18502/ijpho.v12i4.10915]

effect of amino acid substitution on the structure of a given protein (25).

ConSurf

The ConSurf (https://consurf.tau.ac.il/) server assesses the evolutionary preservation of amino acid sites in a protein. Firstly, ConSurf seeks close homologous sequences using CSI-BLAST, PSI-BLAST or BLAST. Then, a multiple sequence alignment of the homologous sequences is employed to construct a phylogenetic tree through the neighbor-joining algorithm. The empirical Bayesian or ML algorithms are used to calculate the position-specific conservation scores. The continuous conservation scores are shown by nine grades, from the most variable positions (grade 1) to the most conserved positions (grade 9) (26).

Ethical Consideration

The present study was approved at Research Ethics Committees of Yazd University with ID: IR.YAZD.REC.1401.024

Results

Screening the pathogenic nsSNPs

On the purpose of screening, 352 missense variants were retrieved from the dbSNP database. Of them, 69 SNPs were in the intronic regions, and 4 and 2 SNPs were synonymous and stop codon, respectively. So, they were excluded. Seven different *in silico* predicting tools (SIFT, PROVEAN, PMut, PANTHER, PolyPhen2, SNPs & GO, and PhD-SNP) were employed to screen the deleterious *NT5C2* nsSNPs (Figure 4a). Firstly, all the nsSNPs were examined with SIFT, PROVEAN, PMut, and PANTHER. Out of 277 nsSNPs, 176 were predicted as "affect protein function" by SIFT, 134 as "deleterious" by PROVEAN, 122 as "disease" by PMut, and 267 as "probably damaging" by PANTHER (Supplementary Table I).

In addition, 115 common nsSNPs were considered pathogenic by all the four mentioned tools, and then they were explored using PolyPhen2, SNPs & GO, and PhD-SNP. Of them, 69 nsSNPs were predicted as "probably damaging" by PolyPhen2. Also, 61 and 84 nsSNPs were predicted as "disease" by SNPs & GO and PhD-SNP, respectively (Table I). Among them, 31 were considered “pathogenic” by all these three *in silico* tools (Table II). Moreover, the effect of 115 amino acid substitutions on the stability of the protein was explored using I-Mutant2.0 (Table I). As it was found, 98 nsSNPs decreased ($\Delta\Delta G < 0$) but 17 nsSNPs increased ($\Delta\Delta G > 0$) the stability of the protein.

Evaluation of the structural effects of nsSNPs

The effect of 31 screened amino acid substitutions on the secondary structure of the cytosolic 5´-nucleotidase was evaluated using the NetSurfP-2.0 server (Table II). As the results showed, Absolute and Relative Surface Accessibility (i.e., ASA and RSA, respectively) were changed largely by some mutations including S464F, R442C, F441C, F296S, F296V, P293Q, Y225C, L190H, G184R, T183P, Y178C, I142N, and R98G, while the variations in the others were minor.

In addition, the structural effects of mutations were explored using the HOPE server. According to HOPE, in M53T, Y82H, L110S and F296S, the mutated residue was smaller and more hydrophilic than the wild-type residue. This implies that hydrophobic interactions, either in the core of the protein or on the surface, may be lost. In P293Q, the wild-type residue was proline. This residue is known to be very rigid, thus inducing a special backbone conformation that might be required at this position. Mutations can disturb this distinct conformation. In N38S, L109V, Y178C, T183P, Y225C, N250S, and Y461C, mutation leads to a smaller and more hydrophobic residue. This can result in the loss of hydrogen bonds and/or disturb correct folding. In I46M, T56I, L57F, L86W, I142N, L190H, and S464F, the mutant residue is bulkier, which might lead to a bump. It is also more hydrophilic, which means that hydrophobic interactions, either in the core of the protein or on its surface, may be

[DOI: 10.18502/ijpho.v12i4.10915]

lost. In H486N and F441C, the mutant residue is smaller, and the wild-type and mutant residues differ in terms of hydrophobicity. These differences might lead to a loss of interactions. In L235P, the wild-type residue is located in a region annotated in UniProt to form an α-helix. Proline disrupts an α-helix when not located at one of the first three positions of that helix. So, the helix will be disturbed, which can have severe effects on the structure of the protein. The mutation G184R introduces a charged residue that can cause repulsion with other residues in the protein or ligands. Also, the bigger size of the mutant might lead to bumps. In R98G, D103G, D200G, R442C, and R456H, the wild-type is a charged residue, whereas the mutant is neutral. This can cause a loss of interactions with other molecules or residues. In the case of mutation to G, glycine is very flexible and can disturb the required rigidity of the protein at that position. When an amino acid position is evolutionarily preserved, it powerfully reflects its structural and functional importance in the protein. The evolutionary preservation of the native residues was analyzed by the ConSurf web server. As the results indicated, out of 31 residues, 27 cases were highly preserved with a score of more than 7 (Table II). The others were intermediately preserved with a score of 5. If a residue in the sequence of a protein is evolutionary preserved, its mutation almost certainly affects the protein function. Amino acid side-chains vary in polarity and charge. This variation affects the formation of polar contacts with other residues in the protein, which may alter the 3D conformation and, subsequently, the function of the protein (27). So, the polar contacts in the native and mutant tertiary structure of the protein were analyzed using PyMOL (The PyMOL Molecular Graphics System, Version 2.0 Schrodinger, LLC.). Out of 31 nsSNPs in H486N, H209N, L110S, L109V, L86W, Y82H and I46M, the length and the number of the polar contacts with the other residues did not change, while the alteration in the length and the number of the polar contacts was detected in the others using PyMOL (Figures 1-3). These results support the data obtained by NetSurfP-2.0. So, regardless of these seven mutations, 24 nsSNPs were introduced as high-risk mutations affecting the function of 5´-nucleotidase. The flowchart of the steps for the screening and identification of high-risk deleterious nsSNPS in *NT5C2* is presented in Figure 4A.

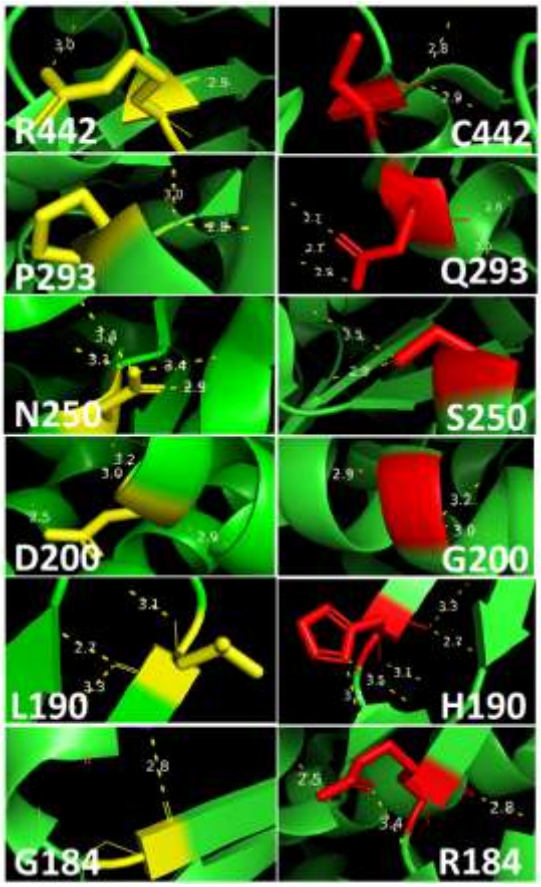

Figure 1. Evaluation of the polar contacts using PyMOL: The 3D structure of 5´-nucleotidase (PDB ID: 2J2C) was illustrated by the PyMOL software (The PyMOL Molecular Graphics System, Version 2.0 Schrodinger, LLC.). Using the mutagenesis wizard of the software, mutations were performed on the structure, and then the lengths (dashed line) as well as the numbers of the polar contacts of the native (yellow) and mutant (red) residues with the others (green) were evaluated (The number on the dashed line indicates the length of the contact). The length and the number of the polar contacts were changed due to R442C, P293Q, N250S, D200G, L190H, and G184R mutations.

[DOI: 10.18502/ijpho.v12i4.10915]

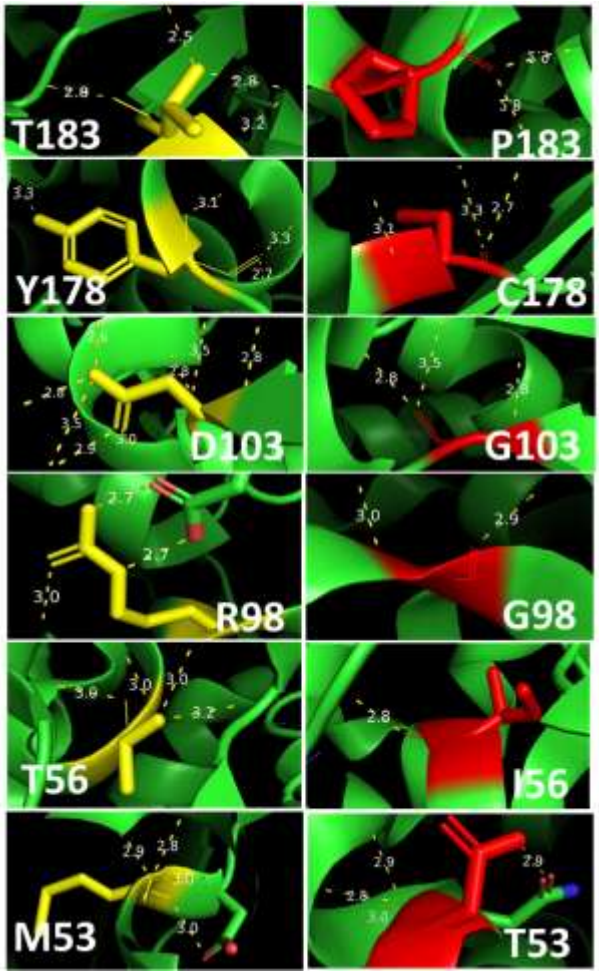

Figure 2. Change of the polar contacts due to mutations: The tertiary structure of the 5´-nucleotidase (PDB ID: 2J2C) was shown by the PyMOL software (The PyMOL Molecular Graphics System, Version 2.0 Schrodinger, LLC.). Using the mutagenesis wizard of the software, mutations were created in the structure, and then the lengths (dashed line) as well as the numbers of the polar contacts of the native (yellow) and mutant (red) residues with the others (green) were compared (The number on the dashed line indicates the length of the contact). The length and the number of the polar contacts were altered after T183P, Y178C, D103G, R98G, T56I, and M53T mutations.

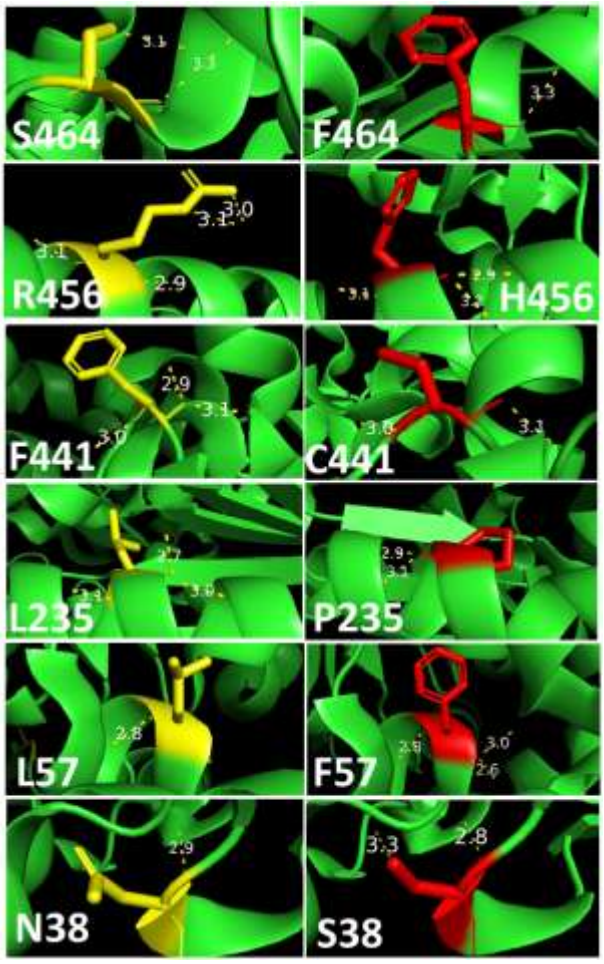

Figure 3. Alteration of polar contacts caused by mutations: The structure of 5´-nucleotidase (PDB ID: 2J2C) was displayed by the PyMOL software (The PyMOL Molecular Graphics System, Version 2.0 Schrodinger, LLC.). Using the mutagenesis wizard of the software, mutations were induced in the structure, and then the lengths (dashed line) as well as the numbers of the polar contacts of the native (yellow) and mutant (red) residues with the others (green) were predicted (The number on the dashed line indicates the length of the contact). The length and the number of the polar contacts were changed due to S464F, R456H, F441C, L235P, L57F, and N38S mutations.

[DOI: 10.18502/ijpho.v12i4.10915]

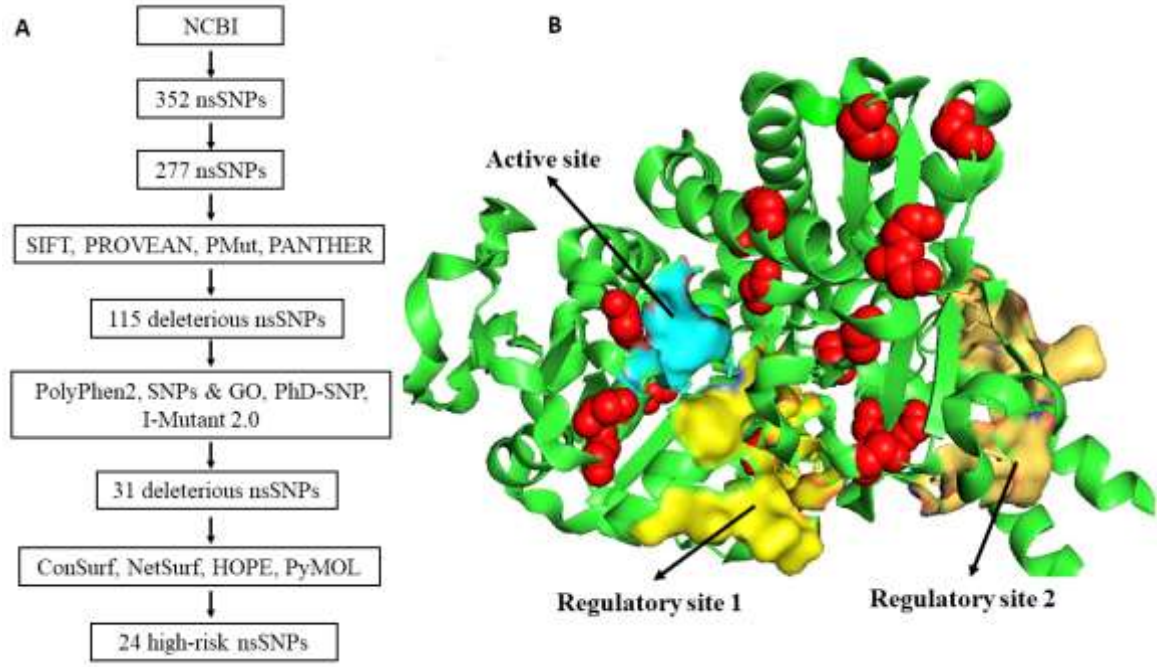

Figure 4. A) The flowchart of the steps to screen and identify the high-risk nsSNPS in *NT5C2* using bioinformatics tools and B) The structure of the 5´-nucleotidase (NT5C2) enzyme illustrated by PyMOL: The active site and regulatory sites 1 and 2 are marked in blue, yellow and orange, respectively. Also, 24 high-risk mutations are demonstrated with red spheres.

Table I: Prediction of deleterious nsSNPs in NT5C2 using PolyPhen-2, SNPs & Go and PHD-SNP as well as stability by I-Mutant2.0

rs ID*	Amino acid change	PolyPhen-2	PolyPhen-2 score	SNPs & Go	SNPs & Go RI #	PHD-SNP	PHD-SNP RI	I-Mutant	I-Mutant RI
rs2066199416	E550K	possibly damaging	0.461	Neutral	7	Neutral	6	Decrease	1
rs745326714	R526W	probably damaging	1.000	Neutral	3	Neutral	4	Decrease	6
rs368467266	H522Y	benign	0.104	Neutral	6	Neutral	5	Decrease	1
rs777429683	R521W	probably damaging	1.000	Neutral	3	Neutral	4	Decrease	5
rs765678401	R507W	probably damaging	1.000	Neutral	2	Neutral	2	Decrease	7
rs1335692779	P503L	possibly damaging	0.921	Neutral	6	Disease	1	Decrease	2
rs1352405866	H486N	probably damaging	1.000	Disease	4	Disease	8	Decrease	1
rs1180354638	N467I	probably damaging	1.000	Disease	5	Disease	9	Increase	5
rs1186701443	N467D	probably damaging	0.982	Disease	0	Disease	6	Decrease	6
rs1266276091	S464F	probably damaging	0.999	Disease	2	Disease	7	Increase	4
rs2066545902	A463V	probably	0.994	Disease	1	Disease	5	Increase	1

[DOI: 10.18502/ijpho.v12i4.10915]

		damaging							
rs1260681713	Y461C	probably damaging	1.000	Disease	6	Disease	9	Decrease	1
rs948869581	D459E	possibly damaging	0/927	Neutral	0	Disease	7	Increase	1
rs1030219633	R456H	possibly damaging	0/978	Disease	1	Disease	5	Decrease	6
rs1431600760	V454A	possibly damaging	0/996	Neutral	6	Neutral	2	Decrease	5
rs1443548253	F450L	possibly damaging	1	Neutral	0	Disease	9	Decrease	3
rs1222615420	R446W	possibly damaging	0/564	Neutral	0	Neutral	0	Decrease	3
rs2066559867	R446H	possibly damaging	0/669	Neutral	3	Neutral	4	Decrease	7
rs985929069	R442C	possibly damaging	0/999	Disease	4	Disease	9	Decrease	2
rs771233729	F441L	possibly damaging	0/877	Neutral	2	Disease	7	Decrease	4
rs2066562591	F441C	possibly damaging	1	Disease	3	Disease	9	Decrease	5
rs1272078839	S439N	possibly damaging	1	Neutral	2	Disease	4	Increase	3
rs775844720	D431V	possibly damaging	0/999	Disease	7	Disease	8	Decrease	3
rs1433755096	M430R	possibly damaging	0/494	Neutral	2	Disease	6	Decrease	6
rs147120336	I423T	possibly damaging	0/954	Neutral	6	Disease	3	Decrease	8
rs759224877	S418P	possibly damaging	0/821	Neutral	4	Neutral	1	Increase	1
rs2066656074	P414T	benign	0/017	Neutral	8	Neutral	2	Decrease	7
rs2066658373	R413C	possibly damaging	0/716	Neutral	6	Disease	1	Decrease	4
rs1057519868	D407A	possibly damaging	1	Neutral	4	Disease	3	Decrease	7
rs2066664389	H405Y	possibly damaging	0/999	Neutral	5	Neutral	4	Increase	3
rs184419317	L392F	possibly damaging	1	Neutral	4	Neutral	4	Decrease	7
rs775006132	E391G	possibly damaging	1	Neutral	2	Disease	6	Decrease	1
rs774430005	S387L	benign	0/349	Neutral	5	Neutral	6	Increase	0
rs1359768582	G330R	probably damaging	1	Disease	7	Disease	9	Decrease	8
rs1564921342	S328P	probably damaging	0.998	Disease	4	Disease	8	Increase	0
rs1590671523	G324A	benign	0.100	Neutral	3	Disease	4	Increase	0
rs746549118	P320A	possibly damaging	0.553	Neutral	7	Disease	4	Decrease	8
rs776631372	G315A	possibly damaging	0.952	Neutral	7	Disease	0	Decrease	2
rs926878263	I314T	probably damaging	0.988	Neutral	4	Neutral	1	Increase	3
rs1188927869	G310S	benign	0.042	Disease	3	Disease	6	Decrease	6

[DOI: 10.18502/ijpho.v12i4.10915]

rs1415972113	T307S	possibly damaging	0.670	Neutral	6	Neutral	2	Decrease	5
rs1463329393	T307A	possibly damaging	0.504	Neutral	4	Disease	1	Decrease	9
rs780974514	R303H	probably damaging	0.999	Disease	2	Disease	6	Decrease	7
rs745869293	R303C	probably damaging	0.977	Disease	4	Disease	8	Decrease	2
rs1564924220	T300I	benign	0.075	Neutral	4	Neutral	4	Decrease	6
rs2067655015	T300S	possibly damaging	0.723	Neutral	8	Neutral	6	Decrease	6
rs1009620186	F296S	probably damaging	0.980	Disease	8	Disease	10	Decrease	9
rs1234315434	F296V	probably damaging	0.997	Disease	7	Disease	10	Decrease	8
rs2067659333	P293Q	probably damaging	0.972	Disease	6	Disease	8	Decrease	9
rs775316562	R291W	benign	0.042	Neutral	1	Disease	3	Decrease	8
rs1306826286	V288A	probably damaging	0.999	Neutral	2	Disease	6	Decrease	9
rs768829960	S281P	possibly damaging	0.874	Disease	4	Disease	9	Increase	1
rs199588287	Q280P	benign	0.042	Disease	4	Disease	8	Decrease	3
rs2068444135	D257G	possibly damaging	0.732	Disease	2	Disease	6	Decrease	6
rs1344739408	T256R	possibly damaging	0.803	Disease	8	Disease	8	Increase	0
rs767133782	N250S	probably damaging	0.989	Disease	5	Disease	8	Decrease	5
rs753745374	F246L	probably damaging	1	Neutral	1	Disease	8	Decrease	7
rs1226896141	V242L	possibly damaging	0.938	Neutral	7	Neutral	7	Decrease	7
rs1288561387	L235P	probably damaging	1	Disease	3	Disease	8	Decrease	3
rs765117850	P233L	probably damaging	0/998	Neutral	7	Neutral	3	Decrease	6
rs752467649	P233T	probably damaging	1	Neutral	7	Neutral	5	Decrease	5
rs1590754368	D229H	probably damaging	1	Neutral	4	Disease	2	Decrease	7
rs1355588604	V226A	probably damaging	1	Neutral	1	Disease	3	Decrease	9
rs1431496089	Y225C	probably damaging	0/995	Disease	4	Disease	6	Decrease	1
rs774747772	K224N	probably damaging	1	Neutral	2	Disease	6	Increase	1
rs1359934879	E223 K	benign	0/024	Neutral	5	Neutral	4	Decrease	6
rs1453382388	L222 P	probably damaging	0/968	Neutral	8	Neutral	1	Decrease	6
rs2068866642	N221Y	probably damaging	1	Neutral	1	Disease	5	Increase	4
rs1288943666	H209N	probably damaging	1	Disease	7	Disease	8	Decrease	5
rs745543095	W207G	probably	0/997	Neutral	0	Disease	5	Decrease	9

[DOI: 10.18502/ijpho.v12i4.10915]

		damaging							
rs769321277	D206H	probably damaging	1	Disease	2	Disease	4	Decrease	8
rs762580200	D200G	probably damaging	1	Disease	6	Disease	8	Decrease	5
rs952030661	Q199H	possibly damaging	0/918	Disease	1	Disease	2	Decrease	6
rs774448444	R195W	possibly damaging	0/607	Disease	1	Neutral	6	Decrease	7
rs1166034561	L190H	probably damaging	1	Disease	3	Disease	5	Decrease	9
rs1305679810	G184R	probably damaging	1	Disease	4	Disease	7	Decrease	8
rs761543688	T183P	probably damaging	0/997	Disease	3	Disease	3	Decrease	6
rs1425381216	Y178C	probably damaging	1	Disease	3	Disease	8	Decrease	3
rs2069499073	V169A	probably damaging	0/998	Disease	3	Disease	7	Decrease	9
rs1340184014	C167Y	probably damaging	1	Disease	3	Disease	8	Decrease	3
rs200072674	Y163F	probably damaging	0/991	Neutral	3	Neutral	5	Decrease	6
rs1267748680	T162I	probably damaging	0/984	Disease	3	Disease	2	Decrease	2
rs1387152557	P160S	possibly damaging	0/617	Neutral	7	Neutral	0	Decrease	8
rs1369817879	F157L	probably damaging	1	Disease	5	Disease	9	Decrease	7
rs1298943557	T155I	possibly damaging	0/946	Disease	3	Disease	7	Decrease	5
rs587777174	R149G	probably damaging	1	Neutral	7	Disease	4	Decrease	6
rs1195771135	I142N	Probably damaging	1	Disease	3	Disease	8	Decrease	5
rs718137833	N117S	Probably damaging	1	Neutral	5	Neutral	1	Decrease	6
rs768600364	D113N	Probably damaging	1	Disease	5	Disease	8	Decrease	8
rs1307699666	L110S	Probably damaging	1	Disease	6	Disease	6	Decrease	9
rs988179515	L109V	Probably damaging	1	Disease	2	Disease	3	Decrease	7
rs1446916348	G107A	Probably damaging	0/998	Disease	3	Disease	8	Decrease	4
rs761830299	Y106N	Probably damaging	0/999	Disease	3	Disease	7	Decrease	8
rs773870376	D103G	Probably damaging	1	Disease	7	Disease	10	Decrease	8
rs781092106	R98G	Probably damaging	1	Disease	5	Disease	9	Decrease	5
rs377735697	P96L	Probably damaging	1	Neutral	2	Neutral	0	Decrease	5
rs1590866563	L87F	Probably damaging	1	Neutral	1	Neutral	0	Decrease	9
rs2071346838	L86W	Probably damaging	1	Disease	0	Disease	4	Decrease	7

[DOI: 10.18502/ijpho.v12i4.10915]

rs2071348526	Y82H	Probably damaging	1	Disease	5	Disease	9	Decrease	8
rs773823085	R76I	Probably damaging	1	Neutral	0	Neutral	3	Decrease	5
rs773930069	E66G	Probably damaging	1	Disease	1	Disease	5	Decrease	8
rs2071365783	P63L	possibly damaging	0/953	Neutral	2	Disease	1	Decrease	6
rs767057029	K61M	Probably damaging	1	Disease	0	Disease	4	Increase	1
rs2071368159	K61E	Probably damaging	0/972	Neutral	1	Neutral	0	Decrease	5
rs868119072	L57F	Probably damaging	0/998	Disease	4	Disease	5	Decrease	7
rs1400732197	T56I	Probably damaging	1	Disease	7	Disease	9	Decrease	1
rs377751446	M53T	Probably damaging	0/996	Disease	5	Disease	3	Decrease	6
rs766283362	M53V	Probably damaging	0/999	Disease	4	Disease	2	Decrease	8
rs1302349591	I46M	Probably damaging	1	Disease	3	Disease	4	Decrease	8
rs1141098	M43V	Probably damaging	0/998	Disease	0	Neutral	0	Decrease	8
rs1565150621	S40R	Probably damaging	1	Disease	4	Disease	7	Increase	0
rs1393472957	R39L	Probably damaging	1	Disease	7	Disease	9	Decrease	7
rs1330033795	N38S	Probably damaging	0/998	Disease	2	Disease	7	Decrease	7
rs1340377706	F36L	Probably damaging	1	Disease	6	Disease	8	Decrease	5
rs2079961540	F36Y	Probably damaging	1	Neutral	3	Disease	6	Decrease	7

*rs ID: reference SNP cluster identifier, # RI: Reliability Index

[DOI: 10.18502/ijpho.v12i4.10915]

Table II Prediction of structural changes due to mutations by NetSurfP-2.0 and evolutionary conservation by ConSurf

rs ID [a] / (amino acids)	Native / Mutant	RSA[b] (%)	ASA[c] (Å)	SS3[d]	SS8[d]	Surface accessibility	ConSurf score
rs1352405866 (H486N)	H	58	105	coil	coil	buried	9
	N	56	101	coil	coil	buried	
rs1266276091 (S464F)	S	12	14	strand	β-sheet	buried	8
	F	16	32	strand	β -sheet	exposed	
rs1260681713 (Y461C)	Y	3	7	strand	β -sheet	buried	9
	C	3	4	strand	β -sheet	buried	
rs1030219633	R	36	82	helix	α-helix	exposed	9
R456H	H	37	67	helix	α-helix	exposed	
rs985929069	R	21	49	strand	β -sheet	exposed	9
R442C	C	13	18	strand	β -sheet	buried	
rs2066562591	F	9	19	coil	β -sheet	buried	8
(F441C)	C	6	8	strand	β -sheet	buried	
rs1009620186	F	23	46	coil	turn	exposed	9
(F296S)	S	34	40	coil	turn	exposed	
rs1234315434	F	23	46	coil	turn	exposed	9
(F296V)	V	18	28	coil	turn	buried	
rs2067659333	P	17	24	coil	coil	buried	9
(P293Q)	Q	31	56	coil	coil	exposed	
rs767133782	N	18	27	coil	bend	buried	9
(N250S)	S	21	24	coil	bend	buried	
rs1288561387	L	3	5	helix	α-helix	buried	7
(L235P)	P	6	7	helix	α-helix	buried	
rs1431496089	Y	9	18	helix	3_{10} helix	buried	8
(Y225C)	C	5	7	helix	3_{10} helix	buried	
rs1288943666	H	8	15	helix	α-helix	buried	9
(H209N)	N	9	14	helix	α-helix	buried	
rs762580200	D	6	9	helix	α-helix	buried	9
(D200G)	G	4	3	helix	α-helix	buried	
rs1166034561	L	22	41	strand	β-sheet	buried	5
(L190H)	H	40	72	strand	β-sheet	exposed	
rs1305679810	G	17	13	coil	coil	buried	9
(G184R)	R	43	98	coil	coil	exposed	
rs761543688	T	34	47	coil	turn	exposed	5
(T183P)	P	48	68	coil	turn	exposed	

[DOI: 10.18502/ijpho.v12i4.10915]

rs1425381216	Y	18	39	coil	coil	buried	5
(Y178C)	C	13	18	coil	coil	buried	
rs1369817879	F	9	18	coil	turn	buried	9
(F157L)	L	8	14	coil	turn	buried	
rs1195771135	I	10	18	coil	β-sheet	buried	7
(I142N)	N	28	41	strand	coil	exposed	
rs1307699666	L	2	3	strand	β-sheet	buried	8
(L110S)	S	3	3	strand	β-sheet	buried	
rs988179515	L	1	2	strand	β-sheet	buried	8
(L109V)	V	1	1	strand	β-sheet	buried	
rs773870376	D	5	7	strand	β-sheet	buried	9
(D103G)	G	3	3	strand	β-sheet	exposed	
rs781092106	R	19	44	strand	β-sheet	exposed	9
(R98G)	G	9	7	strand	β-sheet	buried	
rs2071346838	L	4	7	helix	3_{10}helix	buried	5
(L86W)	W	5	13	helix	3_{10}helix	buried	
rs2071348526	Y	11	23	coil	coil	buried	9
(Y82H)	H	13	24	coil	coil	buried	
rs868119072	L	3	5	coil	β-sheet	buried	8
(L57F)	F	3	5	coil	β-sheet	buried	
rs1400732197	T	2	2	coil	coil	buried	9
(T56I)	I	2	4	coil	coil	buried	
rs377751446	M	11	22	coil	Turn	buried	9
(M53T)	T	11	16	coil	Turn	buried	
rs1302349591	I	4	7	coil	coil	buried	8
(I46M)	M	4	8	coil	coil	buried	
rs1330033795	N	18	27	coil	coil	buried	9
(N38S)	S	17	20	coil	coil	buried	

[a] rs ID: reference SNP cluster identifier, [b] RSA: Relative Surface Accessibility; [c] ASA: Absolute Surface Accessibility; [d] SS3 and SS8: Secondary Structure using 3- and 8-class definitions predicted by NetSurfP-2.0

Discussion

Cytosolic 5´-nucleotidase is a tetrameric enzyme composed of two dimers of the same type and, like the haloacid dehalogenase (HAD) superfamily, has three conserved motifs. Motif I consists of Asp52, Asp54, Thr56 and Leu57, Motif II of Thr249, and Motif III of Lys292, Asp351 and Asp356. They shape the active site of the enzyme (Figure 4B) and are involved in the binding to the phosphate moiety of the substrate and the catalytic mechanism of the enzyme i.e. dephosphorylation of purine analogs (28). This activity controls the intracellular purine nucleotide pool (5, 6). In this

[DOI: 10.18502/ijpho.v12i4.10915]

tetrameric enzyme, dimers contact the residues of the adjacent dimers at interface A using 19 hydrogen bonds and four salt bridges. The salt bridges are formed between Arg363 and Asp145 and between Arg442 and Glu487 (29). Moreover, cytosolic 5´-nucleotidase is allosterically activated by effectors such as ATP, diadenosine tetraphosphate, and 2,3-BPG (28). Two regulatory sites have been detected in the enzyme (Figure 4B). In regulatory site 1, the activator forms hydrogen bonds with Gln453, Asn154, and several water molecules. In this process, Ile152, Arg144, Phe354, Lys362, Arg456, and Tyr457 are important. In regulatory site 2, the activator forms hydrogen bonds with His428, Met432 and Met436, and residues such as Phe127, Arg129, Arg131, Arg134, Lys140, His428, and Arg446 play a role here. The enzyme has a region of 13 negatively charged residues at the C-terminal. They may interact with the three positively charged regions K(25)KYRR, K(359)SKKRQ and Q(420)RRIKK and participate in the activation and regulation of the cytosolic 5´-nucleotidase (29).

Maintenance therapy for ALL patients is given to postpone remission. The most common chemotherapy regimen includes 6-mercaptopurine (6-MP) and 6-thioguanine (TG). However, about 15% to 20% of children suffering from acute lymphoblastic leukemia (ALL) experience a relapse, which is a significant cause of death in children. Heterozygous mutations in the *NT5C2* gene occur in 10% and 20% of relapsed B- and T-ALL cases, respectively (2, 3). Gain-of-function mutations in *NT5C2* intensify the dephosphorylation and clearance of nucleotide monophosphates resulting from thiopurines analogs and, therefore, extremely reduce their cytotoxic effects in relapsed patients (3, 9, 10). The change of amino acids due to nsSNPs may alter the secondary and 3D structures, motifs, physicochemical properties (30), flexibility, interacting residues, and the active site of an enzyme, thus affecting its function and causing diseases (12). Moreover, SNPs serve as genetic markers of diseases in populations and may also impact the response to some drugs (14). It is very difficult and expensive to experimentally investigate the impacts of all missense SNPs on the structure and function of encoded proteins (31). Hence, bioinformatics tools are valuable for the preliminary screening of deleterious nsSNPs and determining of candidates for future experimental studies (15). In the present study, 276 nsSNPs were retrieved from the NCBI database. They were screened for deleterious mutations firstly by SIFT, PROVEAN, PMut and PANTHER and then by PolyPhen2, SNPs & GO, and PhD-SNP. However, there are some other prediction tools such as Fathmn, Mutation Assessor, Align-GVGD, CAAD, and MutPred. The servers applied in this study were selected because they are widely used and freely available and can predict pathogenicity based on various criteria and algorithms such as sequence homology, physicochemical properties of amino acids, random forest classifier, evolutionary preservation, interactive greedy algorithm, and support vector machines (SVMs). In the dbSNP database, in addition to single nucleotide variants of *NT5C2*, other types of mutations such as insertion, deletion, frameshift and synonymous have been reported for this gene. Missense mutations were selected for this study because examining all types of mutation is very complicated, time-consuming, and practically impossible. So far, only some missense mutations have been experimentally investigated for their pathogenic effects; most of them have remained unexplored. Furthermore, the effect of nsSNPs on the stability of the protein was predicted with the I-Mutant 2.0 server. In this regard, all the applied predicting tools indicated the effects of 31 nsSNPs on the protein function and stability. Evolutionary conservation analyses with ConSurf also showed that 90% of the 31 native amino acids were

[DOI: 10.18502/ijpho.v12i4.10915]

highly preserved. So, the effects of these mutations on the interactions of amino acids with other residues and on the structure of the protein were explored using the HOPE, PyMOL and NetSurfP-2.0 tools. Based on HOPE, the substitution of a residue with different properties such as hydrophobicity, flexibility, size, and/or charge, compared to a native one, leads to the change of noncovalent interactions including hydrogen bonds, salt bridges, and hydrophobic interactions. It also results in conformational changes in the protein. In addition, according to PyMOL and NetSurfP-2.0, polar contacts and surface accessibility are changed by 24 mutations except for H486N, H209N, L110S, L109V, L86W, Y82H, and I46M substitutions. It is noteworthy that most of these mutations are located within or very close to critical regions including active sites such as I46M, M53T, T56I, L57F, N250S, P293Q, F296V and F296S as well as regulatory sites such as I142N, F157L, F441C, R442C, R456H, Y461C and S464F (Figure 4B). The experimental characterization of the mutant forms of cytosolic 5´-nucleotidase has clarified the mechanisms of some relapse-associated *NT5C2* mutations. Mutations including K359Q and L375F change the conformation of helix A segment into an active α-helix and lead to the amplification of nucleotidase activity in the absence of allosteric activators (7). The NT5C2 enzyme has a self-inactivation mechanism enabled by the arm region and the inter-monomeric pocket. R39Q, R238W/L/G/Q, R367Q, S445F, R446Q, and R478S mutations remove positively charged residues located in the inter-monomeric pocket, or K404N, D407A/Y/E/H, S408R, P414S/A, and D415G mutations alter the arm region. This is how they interfere with the self-inactivation process (7). Therefore, it can be deduced that mutations in the vital regions of the enzyme, including the active and regulatory sites, would alter the conformation and the function of the cytosolic 5´-nucleotidase. Besides their importance in relapsed B-ALL and T-ALL, *NT5C2* mutations play a role in coronary heart disease (CHD) susceptibility (32) and autosomal recessive spastic paraplegia (SPG45) (33). Recently, loss-of-function mutations in *NT5C2* has been reported in patients with spastic paraplegia (34, 35).

Conclusion

In conclusion, with the aid of a variety of bioinformatics tools, 24 high-risk deleterious nsSNPs in the *NT5C2* gene have been found with probable effects on the function of cytosolic 5´-nucleotidase. Therefore, nsSNP can be used as a predictive biomarker for the relapses that occur after maintenance therapy in ALL children.

Conflict of interest

The authors declare no conflict of interest.

References

1. Sayyab S, Lundmark A, Larsson M, Ringnér M, Nystedt S, Marincevic-Zuniga Y, et al. Mutational patterns and clonal evolution from diagnosis to relapse in pediatric acute lymphoblastic leukemia. Sci Rep 2021;11(1):15988-15990.
2. Dieck CL, Ferrando A. Genetics and mechanisms of NT5C2-driven chemotherapy resistance in relapsed ALL. Blood 2019;133(21):2263–2268.
3. Tzoneva G, Dieck CL, Oshima K, Ambesi-Impiombato A, Sánchez-Martín M, Madubata CJ, et al. Clonal evolution mechanisms in NT5C2 mutant-relapsed acute lymphoblastic leukaemia. Nat 2018;553(7689):511–514.
4. Barz MJ, Hof J, Groeneveld-Krentz S, Loh JW, Szymansky A, Astrahantseff K, et al. Subclonal NT5C2 mutations are associated with poor outcomes after relapse of pediatric acute lymphoblastic leukemia. Blood 2020;135(12):921–933.
5. Hunsucker SA, Mitchell BS, Spychala J. The 5′-nucleotidases as

[DOI: 10.18502/ijpho.v12i4.10915]

regulators of nucleotide and drug metabolism. Pharmacol Ther 2005;107(1):1–30.
6. Brouwer C, Vogels-Mentink TM, Keizer-Garritsen JJ, Trijbels FJM, Bökkerink JPM, Hoogerbrugge PM, et al. Role of 5′-nucleotidase in thiopurine metabolism: Enzyme kinetic profile and association with thio-GMP levels in patients with acute lymphoblastic leukemia during 6-mercaptopurine treatment. Clin Chim Acta 2005;361(1):95–103.
7. Dieck CL, Tzoneva G, Forouhar F, Carpenter Z, Ambesi-Impiombato A, Sánchez-Martín M, et al. Structure and Mechanisms of NT5C2 Mutations Driving Thiopurine Resistance in Relapsed Lymphoblastic Leukemia. Cancer Cell 2018;34(1):136-147.
8. Moriyama T, Liu S, Li J, Meyer J, Zhao X, Yang W, et al. Mechanisms of NT5C2-mediated thiopurine resistance in acute lymphoblastic leukemia. Mol Cancer Ther 2019;18(10):1887–95.
9. Tulstrup M, Grosjean M, Nielsen SN, Grell K, Wolthers BO, Wegener PS, et al. NT5C2 germline variants alter thiopurine metabolism and are associated with acquired NT5C2 relapse mutations in childhood acute lymphoblastic leukaemia. Leukemia 2018;32(12):2527–35.
10. Meyer JA, Wang J, Hogan LE, Yang JJ, Dandekar S, Patel JP, et al. Relapse-specific mutations in NT5C2 in childhood acute lymphoblastic leukemia. Nat Genet 2013;45(3):290–294.
11. Arshad M, Bhatti A, John P. Identification and in silico analysis of functional SNPs of human TAGAP protein: A comprehensive study. PLoS One 2018;13(1):1-9.
12. Hossain MS, Roy AS, Islam MS. In silico analysis predicting effects of deleterious SNPs of human RASSF5 gene on its structure and functions. Sci Reports 2020;10(1):1–14.
13. Bhatnager R, Dang AS. Comprehensive in-silico prediction of damage associated SNPs in Human Prolidase gene. Sci Rep 2018;8(1):1-9.
14. Elkhattabi L, Morjane I, Charoute H, Amghar S, Bouafi H, Elkarhat Z, et al. In silico analysis of coding/noncoding SNPs of human RETN gene and characterization of their impact on resistin stability and structure. J Diabetes Res 2019;2019:1–9.
15. Zhang M, Huang C, Wang Z, Lv H, Li X. In silico analysis of non-synonymous single nucleotide polymorphisms (nsSNPs) in the human GJA3 gene associated with congenital cataract. BMC Mol Cell Biol 2020;21(1):1–13.
16. Sim NL, Kumar P, Hu J, Henikoff S, Schneider G, Ng PC. SIFT web server: predicting effects of amino acid substitutions on proteins. Nucleic Acids Res 2012;40(W1):W452–457.
17. Choi Y, Sims GE, Murphy S, Miller JR, Chan AP. Predicting the Functional Effect of Amino Acid Substitutions and Indels. PLoS One 2012;7(10):e46688-e46690.
18. López-Ferrando V, Gazzo A, De La Cruz X, Orozco M, Gelpí JL. PMut: A web-based tool for the annotation of pathological variants on proteins, 2017 update. Nucleic Acids Res 2017;45(W1):W222–W228.
19. Mi H, Ebert D, Muruganujan A, Mills C, Albou LP, Mushayamaha T, et al. PANTHER version 16: a revised family classification, tree-based classification tool, enhancer regions and extensive API. Nucleic Acids Res 2021;49(D1):D394–403.
20. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. Nat Methods 2010;7(4):248–249.
21. Calabrese R, Capriotti E, Fariselli P, Martelli PL, Casadio R. Functional annotations improve the predictive score of human disease-related mutations in proteins. Hum Mutat 2009;30(8):1237–1244.

[DOI: 10.18502/ijpho.v12i4.10915]

22. Capriotti E, Fariselli P. PhD-SNPg: A webserver and lightweight tool for scoring single nucleotide variants. Nucleic Acids Res 2017;45(W1):W247–252.
23. Capriotti E, Fariselli P, Casadio R. I-Mutant2.0: Predicting stability changes upon mutation from the protein sequence or structure. Nucleic Acids Res 2005;33(SUPPL. 2):W306.
24. Klausen MS, Jespersen MC, Nielsen H, Jensen KK, Jurtz VI, Sønderby CK, et al. NetSurfP-2.0: Improved prediction of protein structural features by integrated deep learning. Proteins Struct Funct Bioinforma 2019;87(6):520–527.
25. Venselaar H, te Beek TAH, Kuipers RKP, Hekkelman ML, Vriend G. Protein structure analysis of mutations causing inheritable diseases. An e-Science approach with life scientist friendly interfaces. BMC Bioinformatics 2010;11(1):1–10.
26. Ashkenazy H, Abadi S, Martz E, Chay O, Mayrose I, Pupko T, et al. ConSurf 2016: an improved methodology to estimate and visualize evolutionary conservation in macromolecules. Nucleic Acids Res 2016;44:W344-349.
27. Berg Jeremy M, Tymoczko John L, Gatto Jr Gregory J, Stryer L. Biochemistry. 9th eddition. Macmillan; 2019;1-9.
28. Bretonnet AS, Jordheim LP, Dumontet C, Lancelin JM. Regulation and activity of cytosolic 5′-nucleotidase II: A bifunctional allosteric enzyme of the Haloacid Dehalogenase superfamily involved in cellular metabolism. FEBS Lett 2005;579(16):3363–3368.
29. Walldén K, Stenmark P, Nyman T, Flodin S, Gräslund S, Loppnau P, et al. Crystal structure of human cytosolic 5′-nucleotidase II: Insights into allosteric regulation and substrate recognition. J Biol Chem 2007;282(24):17828–17836.
30. Arifuzzaman M, Mitra S, Das R, Hamza A, Absar N, Dash R. In silico analysis of nonsynonymous single-nucleotide polymorphisms (nsSNPs) of the SMPX gene. Ann Hum Genet 2020;84(1):54–71.
31. Ghasemi F, Khatami M, Heidari MM, Chamani R, Zare-Zardini H. In-silico study to identify the pathogenic single nucleotide polymorphisms in the coding region of CDKN2A gene. Iran J Pediatr Hematol Oncol 2021;11(2):114–133.
32. Chen X, Zhang Z, Wang X, Chen Y, Wang C. NT5C2 Gene Polymorphisms and the Risk of Coronary Heart Disease. Public Health Genomics 2020;23(3–4):90–99.
33. Straussberg R, Onoufriadis A, Konen O, Zouabi Y, Cohen L, Lee JYW, et al. Novel homozygous missense mutation in NT5C2 underlying hereditary spastic paraplegia SPG45. Am J Med Genet Part A 2017;173(11):3109–3113.
34. Naseer MI, Abdulkareem AA, Pushparaj PN, Bibi F, Chaudhary AG. Exome Analysis Identified Novel Homozygous Splice Site Donor Alteration in NT5C2 Gene in a Saudi Family Associated With Spastic Diplegia Cerebral Palsy, Developmental Delay, and Intellectual Disability. Front in Gen 2020;11:1–6.
35. Elsaid MF, Ibrahim K, Chalhoub N, Elsotouhy A, El Mudehki N, Abdel Aleem A. NT5C2 novel splicing variant expands the phenotypic spectrum of Spastic Paraplegia (SPG45): Case report of a new member of thin corpus callosum SPG-Subgroup. BMC Med Gen 2017;18:1–7.

[DOI: 10.18502/ijpho.v12i4.10915]