Determination of Deleterious SNPs in *NUDT15* Gene Related to Acute Lymphoblastic Leukemia by using Bioinformatics Tools

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Abstract

Aim: In acute lymphoblastic leukemia (ALL), thiopurine group drugs are the most basic drugs and are included in almost all treatment protocols, especially in maintenance treatment. The mechanism of action of thioguanine nucleotides is to enter the DNA structure in cells, disrupt DNA synthesis, and trigger programmed cell death. The impact of deleterious SNPs on nucleotide triphosphate diphosphatase protein regarding ALL is not yet fully understood. In this study, it was aimed to determine the possible deleterious impacts of missense variants in the *NUDT15* gene on protein structure and stabilization that play a significant role in susceptibility to the disease, using modern bioinformatics software.

Method: To access SNPs in the *NUDT15,* it was used National Center for Biotechnology Information (NCBI), Single Nucleotide Polymorphism Database (dbSNP). In bioinformatics tools used in this study included SIFT, PolyPhen-2, PROVEAN, SNAP2, and PANTHER, followed by I-Mutant, HOPE, and STRING.

Results: The results of the analysis showed that in a total of 6663 SNPs in the *NUDT15*, 6 variants have been identified as 'deleterious'. According to the I-Mutant software, 4 deleterious SNPs decreased protein stability while 2 deleterious SNPs increased protein stability. In the HOPE database analysis, E115G, E57G, F52L, and K33N mutant amino acids were found to be smaller and more hydrophobic than wild-type amino acids, while G53R and G145D mutant amino acids were found to be larger. Thus, all variations resulted in alterations in the net charge on the NUDT15 protein.

Conclusion: Data on *NUDT15* variants will contribute to the prediction of the patient's response to thiopurine drugs in future studies, to a better understanding of the patient's susceptibility to drug interactions, and ultimately to obtaining information about the prognosis.

Keywords: Leukemia, single nucleotide polymorphism, thiopurine

Akut Lenfoblastik Lösemi ile İlişkilendirilen NUDT15 Genindeki Zararlı SNP'lerin Biyoinformatik Araçlar Kullanılarak Belirlenmesi

Öz

Amaç: Akut lenfoblastik lösemide (ALL) tiopurin grubu ilaçlar en temel ilaçlardır ve idame tedavisi başta olmak üzere hemen hemen tüm tedavi protokollerinde yer almaktadır. Tiyoguanin nükleotitlerinin etki mekanizması hücrelerde DNA yapısına girmek, DNA sentezini bozmak ve programlı hücre ölümünü tetiklemektir. Zararlı tek nükleotid polimorfizmlerin (SNP'lerin) ALL ile ilgili nükleotid trifosfat difosfataz

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proteini üzerindeki etkisi henüz tam olarak anlaşılmamıştır. Bu çalışmada, *NUDT15* genindeki yanlış anlamlı varyantların hastalığa yatkınlıkta önemli rol oynayan protein yapısı ve stabilizasyonu üzerine olası zararlı etkilerinin modern biyoinformatik yazılımları kullanılarak belirlenmesi amaçlanmıştır.

Yöntem: *NUDT15* genindeki SNP'lere erişmek için Ulusal Biyoteknoloji Bilgi Merkezi (NCBI), Tek Nükleotid Polimorfizm Veritabanı (dbSNP) kullanılmıştır. Bu çalışmada SIFT, PolyPhen-2, PROVEAN, SNAP2, PANTHER, I-Mutant, HOPE ve STRING biyoinformatik araçlarının kullanımı yer almıştır.

Bulgular: Analiz sonuçları, *NUDT15* genindeki toplam 6663 SNP'de 6 varyantın 'zararlı' olarak tanımlandığını göstermiştir. I-Mutant yazılımına göre 4 zararlı SNP protein stabilitesini azaltırken 2 zararlı SNP protein stabilitesini arttırmıştır. HOPE veri tabanı analizinde E115G, E57G, F52L ve K33N mutant amino asitlerinin yabanıl tip amino asitlere göre daha küçük ve hidrofobik olduğu, G53R ve G145D mutant amino asitlerinin ise daha büyük olduğu tespit edilmiştir. Bu sebeple, tüm varyasyonlar NUDT15 proteini üzerindeki net yükte değişiklikle sonuçlanmıştır.

Sonuç: *NUDT15* varyantlarına ilişkin veriler, gelecekteki çalışmalarda hastanın tiopürin ilaçlarına yanıtının tahmin edilmesine, hastanın ilaç etkileşimlerine duyarlılığının daha iyi anlaşılmasına ve nihayetinde prognoz hakkında bilgi edinilmesine katkı sağlayacaktır.

Anahtar Sözcükler: Lösemi, tek nükleotid polimorfizm, tiyopurin

Introduction

Acute lymphoblastic leukemia (ALL) is the most common blood cancer seen in both children and adults and accounts for approximately 78% of all childhood leukemia diagnoses. The causation of ALL is considered to be a multifactorial disease; it is caused by the interaction of genetic and environmental factors. From a genetic perspective, ALL is defined as the abnormal differentiation and proliferation of cells derived from hematopoietic cancer stem cells. These cancer cells accumulate in the bone marrow and inhibit the growth and differentiation of normal healthy cells[1-3].

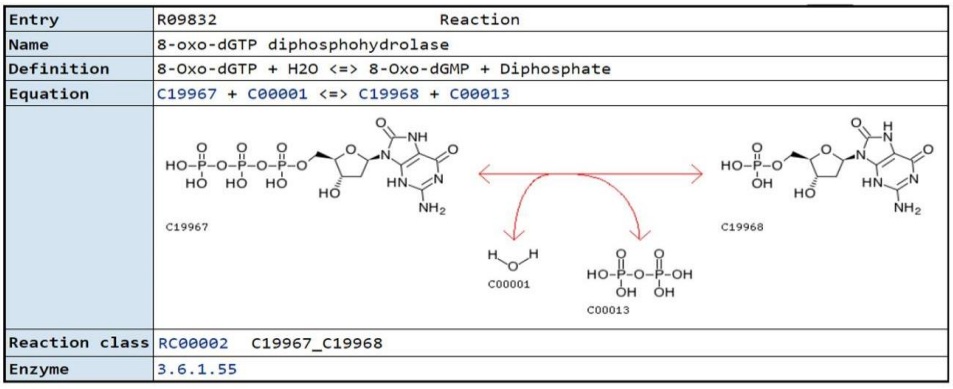
There have been significant improvements in overall survival over the past 50 years; 5-year survival rates for ALL approach 80-90%. This ratio has been achieved by improvements in effective combination chemotherapy, prophylaxis against central nervous system disease, and post-induction therapy. However, despite these significant advances, relapsing ALL remains the leading cause of cancer-related death in children[4].

Today, thiopurine (6-mercaptopurine, azathioprine, and 6-thioguanine) group drugs, which are preferred as anticancer and immunosuppressive agents in the treatment of ALL, are widely used. In ALL, mercaptopurine is an important component of maintenance therapy and indispensable for the treatment of this common malignancy. The thiopurine group drugs, which are designed as purine analogs and are inactive at first, are converted to the active form of thioguanine nucleotides (6-TGN) through enzymatic reactions. The mechanism of action of TGNs is to enter the DNA structure in cells, disrupt DNA synthesis, and trigger programmed cell death[5,6].

Although thiopurines play a very important role in the success of treatment, drug-related complications can be seen and leukopenia is the most serious of these complications. The relationship between thiopurine-induced leukopenia (total white blood cell count < 3000 cells/mm3) and thiopurine methyltransferase (*TPMT*) has been investigated in many populations[7-9]. Despite these studies, thiopurine-related myelosuppression that cannot be explained by *TPMT* genotypes have been observed. In a genome-wide association study (GWAS), the c.415C>T (rs116855232, p.Arg139Cys) variant in the *NUDT15* gene was shown to be strongly associated with thiopurine-associated myelosuppression. According to the study, it has been reported that children with ALL carrying the TT genotype are extremely sensitive to mercaptopurine and can tolerate only 8% of the standard dose[10].

The *NUDT15* (Nudix Hydrolase 15) gene encodes the nucleotide triphosphate diphosphatase NUDT15 enzyme (EC 3.6.1.9) from the nudix hydrolase superfamily, also known as *MTH2*. The *NUDT15* gene (MIM: 615792), localized in the 13q14.2 region of the chromosome, consists of 3 exons. Nudix hydrolase inhibits base mismatches and apoptosis during DNA replication by catalyzing the substrates like 8-oxo-dGTP (Figure 1)[11] and hydrolysis of TGNs, which are active metabolites of thiopurines, thus preventing the cytotoxic effect of thiopurines. Therefore, it acts as a protective mechanism for mammalian cells[10,12,13].

Figure 1. Chemistry of Nudix-catalyzed hydrolysis



The effect of deleterious single nucleotide polymorphisms (SNPs) on nucleotide triphosphate diphosphatase protein regarding ALL is not yet fully understood. Therefore, in this study, it was aimed to investigate the possible deleterious impacts of missense variants in the NUDT15 gene on protein structure and stabilization that play a significant role in susceptibility to the disease, using modern bioinformatics software.

Material and Methods

Retrieval of SNP ID Numbers

SNPs in the NUDT15 (Gene ID: 55270) were obtained from the dbSNP database in August 2022. Missense variations among these SNPs were chosen for further analysis. The sequence and

accession number of the protein (Accession ID: Q9NV35) encoded by the NUDT15 gene were obtained from the NCBI dbSNP and UniProtKB databases.

Bioinformatics Software Analysis

Functional significance of the selected missense mutations in the *NUDT15* gene was evaluated using Sorting Intolerant from Tolerant (SIFT), Polymorphism Phenotyping v2 (PolyPhen-2), Protein Variation Effect Analyzer (PROVEAN), Screening of Non-Acceptable Polymorphism 2 (SNAP2), Protein Analysis Through Evolutionary Relationship (PANTHER) bioinformatics tools. SIFT is software that estimates whether an amino acid replacement impacts protein function and the features of amino acids[14]. PolyPhen-2 software is a tool, that estimates the impacts of an amino acid replacement on the structure and function of its protein using sequence homology and physical features. PROVEAN is used to estimate the possible effect of a changed amino acid on protein structure and biological function[15]. SNAP2 software is a tool that estimates the impact of missense SNPs on protein function based on a neural network classification. PANTHER analyzes the protein sequence based on evolutionary conservation, protein functions, and their interactions with other proteins. The effect of SNPs that are predicted to be deleterious by software tools on the stabilization of protein was determined by I-Mutant 2.0. Modelling of the amino acid substitution effect of the deleterious SNPs on protein structure was performed with the HOPE database analysis[16].

Prediction of Protein-Protein Interactions

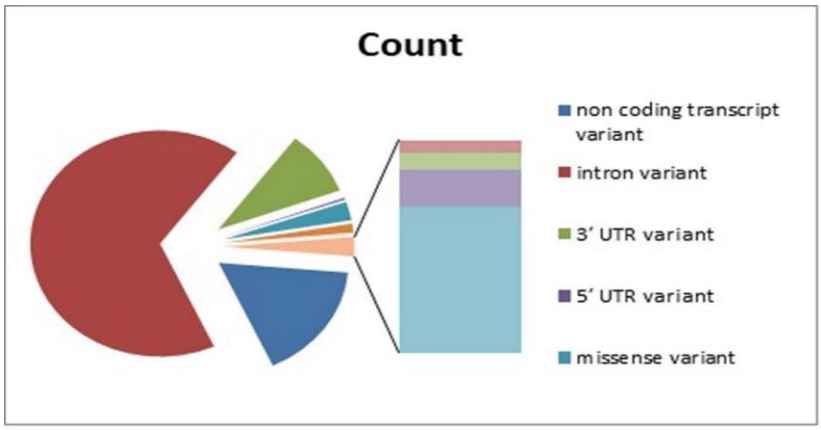
The STRING database provides to merge all known and estimated interactions between proteins, including both functional associations and physical features interactions. For this study, *NUDT15* and Homo sapiens were used as the input[17].

Results

Retrieval of SNPs and Function Prediction

To identify SNPs of NUDT15, NCBI dbSNP and the UniProt were used. A total of 6663 SNPs in the *NUDT15* gene were obtained from NCBI dbSNP: 85 synonymous, 1117 non-coding synonymous, 176 missense, 617 3' Un-translated Region (UTR), 17 5' UTR region, 5 splice acceptor variant, 11 splice donor variant, 14 stop gained, 32 frameshift, 4462 in intronic regions and the remaining 127 SNPs are of other types. A summary of the outcomes is presented in Figure 2.

Figure 2. Distribution of SNPs found in the *NUDT15* gene



Analysis of Deleterious SNPs

The results showed that there are 6663 SNPs in the *NUDT15* gene, among which 176 SNPs contain missense variants. 7 variants were detected to be deleterious with SIFT software. The results were shown in Table 1.

Table 1. Results of SIFT score and prediction for NUDT15

SNP	Ref Allele	Alt Allele	Amino Acid Change	SIFT Score	SIFT Median	SIFT Prediction
rs76601525	A	G	E115G	0,048	2,66	DELETERIOUS
rs138667875	A	G	E57G	0,01	2,66	DELETERIOUS
s149436418	C	G	F52L	0,012	2,66	DELETERIOUS
rs150241065	G	C	K33N	0,048	2,64	DELETERIOUS
rs199676691	G	C	G53R	0,006	2,66	DELETERIOUS
rs200025929	G	A	G145D	0,01	2,77	DELETERIOUS
rs369372549	C	T	H71Y	0,035	2,66	DELETERIOUS

For Polyphen-2 there were, 6 deleterious SNPs predicted as damaging while one was benign, the deleterious SNPs have an effect on protein structure and function (Table 2).

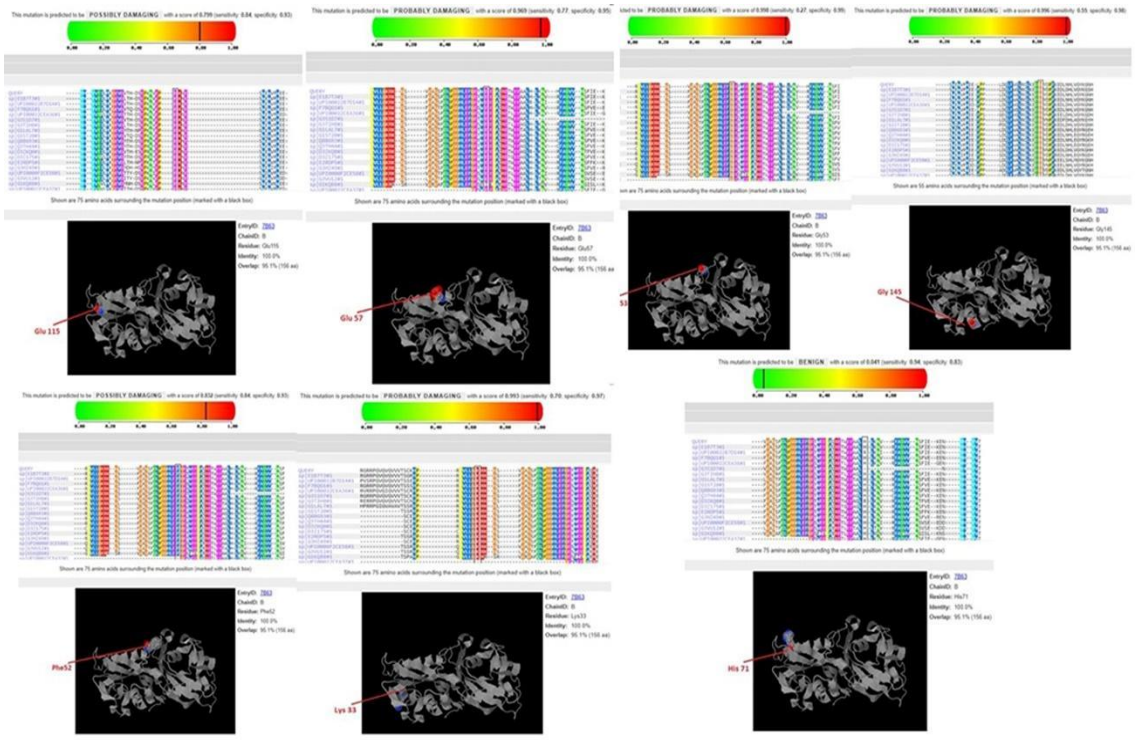
Table 2. Results of Polyphen-2 score and prediction for *NUDT15*

SNP	Ref Allele	Alt Allele	Amino Acid Change	Polyphen-2 Score	Polyphen-2 Prediction
rs76601525	A	G	E115G	0,799	POSSIBLY DAMAGING
rs138667875	A	G	E57G	0,969	PROBABLY DAMAGING
rs149436418	C	G	F52L	0,832	POSSIBLY DAMAGING
rs150241065	G	C	K33N	0,993	PROBABLY DAMAGING
rs199676691	G	C	G53R	0,998	PROBABLY DAMAGING
rs200025929	G	A	G145D	0,996	PROBABLY DAMAGING
rs369372549	C	T	H71Y	0,041	BENIGN

PolyPhen-2 score ≥0,5 = probably/possibly damaging

NUDT15 deleterious SNPs associated variations predicted by PolyPhen-2 software are shown in Figure 3.

Figure 3. *NUDT15* deleterious SNPs associated variations predicted by PolyPhen-2 software

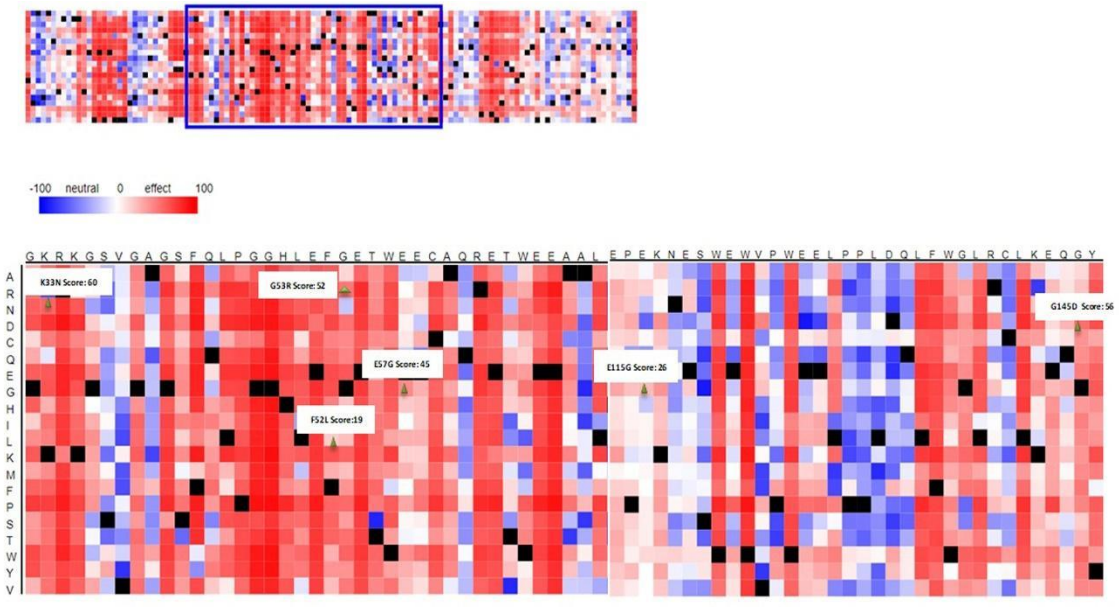


The 6 SNPs that detected both SIFT and PolyPhen-2 software tools to be deleterious were loaded into the online software tools PROVEAN, SNAP2, and PANTHER (Table 3). Figure 4 shows the NUDT15 heatmap.

Table 3. Results of PROVEAN, SNAP2, and PANTHER software for *NUDT15*

SNP	PROVEAN Result	PROVEAN Score	SNAP2 Predicted Effect	SNAP2 Score	SNAP2 Expected Accuracy	PANTHER Result
rs76601525	Deleterious	-4.54	Effect	26	63%	Probably Damaging
rs138667875	Deleterious	-5.77	Effect	45	71%	Possibly Damaging
rs149436418	Deleterious	-4.59	Effect	19	59%	Probably Damaging
rs150241065	Deleterious	-3.49	Effect	60	80%	Probably Damaging
rs199676691	Deleterious	-6.77	Effect	52	75%	Probably Damaging
rs200025929	Deleterious	-3.85	Effect	56	75%	Probably Damaging

Figure 4. SNAP2 prediction scores for the *NUDT15* shown as a heatmap



Prediction of NUDT15 Stability

I-Mutant was used to define the impact of amino acid change on the stabilization of the protein. The result of stability was obtained to be increased/decreased with RI ranging from 0 to 10, shown in Table 4. Using I-Mutant software, 4 deleterious SNPs were decreasing the protein stability while 2 deleterious SNPs were increasing the protein stability.

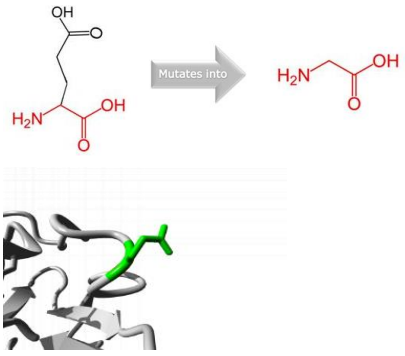
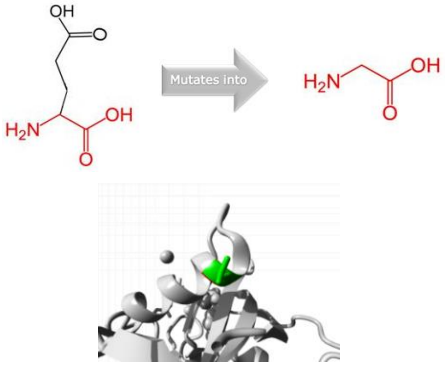
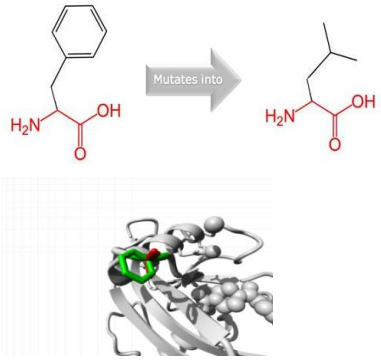
Table 4. Results of I-Mutant result and RI for *NUDT15*

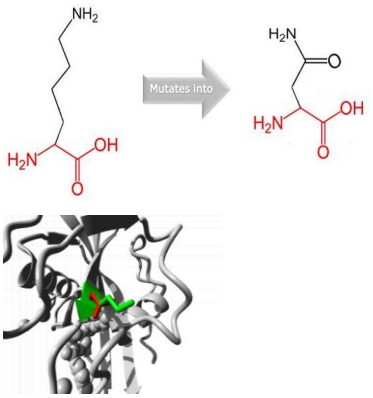
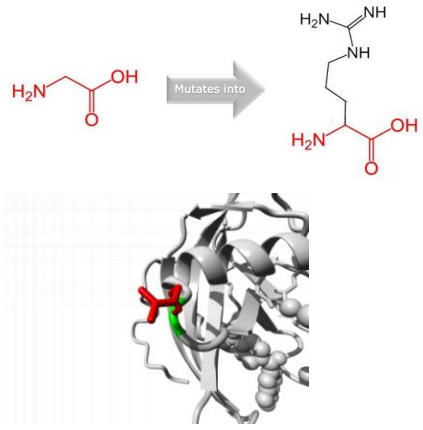
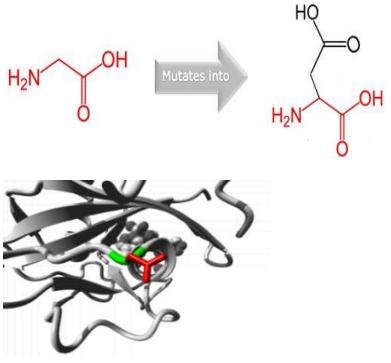
SNP	Amino Acid Change	I-Mutant Result	I-Mutant RI
rs76601525	E115G	Decrease	4
rs138667875	E57G	Decrease	5
rs149436418	F52L	Decrease	3
rs150241065	K33N	Increase	2
rs199676691	G53R	Increase	1
rs200025929	G145D	Decrease	7

Modelling of *NUDT15* and Its Variants

Modeling was done using HOPE software to see the effects of changing amino acids on the three-dimensional structure of the protein. Its results showed that E115G, E57G, F52L, K33N, G53R, and G145D are very conserved. Also, the identified variations are likely to damage the protein structure. According to the results, E115G, E57G, F52L, and K33N were smaller amino acid changes from the wild-type residue, while G53R and G145D were bigger amino acid changes. All variations resulted in a change in the net charge of NUDT15 protein. Schematic structures and three-dimensional models of the wild-type and the mutant amino acid are shown in Table 5. The backbone, which is the same for each amino acid, is colored red. The side chain, unique for each amino acid, is colored black. It is known that protein charge and mass affect spatio-temporal dynamics of protein-protein interaction[18,19]. Thus, these variations may alter the ability of NUDT15 to interact with other proteins.

Table 5. Schematic structures and 3D models of the original (left) and the mutant (right) amino acid

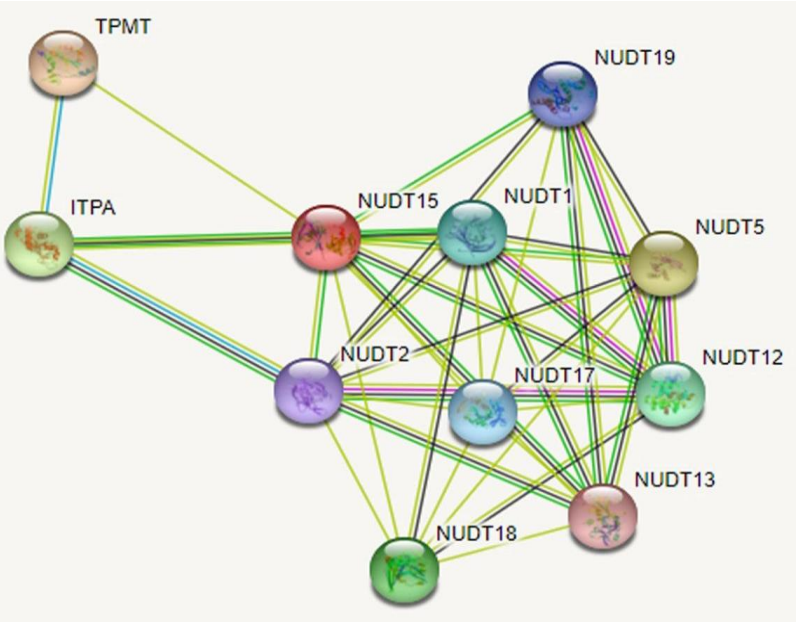
Residue	Structure	Properties
E115G		The mutant residue is smaller than the wild-type reside. The wild-type residue charge was NEGATIVE, the mutant residue charge is NEUTRAL. The mutant residue is more hydrophobic than the wild-type residue. The wild-type residue is very conserved, but a few other residue types have been observed at this position too.
E57G		The mutant residue is smaller than the wild-type residue. The wild-type residue charge was NEGATIVE, the mutant residue charge is NEUTRAL. The mutant residue is more hydrophobic than the wild-type residue. The wild-type residue is very conserved, but a few other residue types have been observed at this position too.
F52L		The mutant residue is smaller than the wild-type residue. The wild-type residue is very conserved, but a few other residue types have been observed at this position too.

K33N		The mutant residue is smaller than the wild-type residue. The wild-type residue charge was POSITIVE, the mutant residue charge is NEUTRAL The wild-type residue is very conserved, but a few other residue types have been observed at this position too.
G53R		The mutant residue is bigger than the wild-type residue. The wild-type residue charge was NEUTRAL, the mutant residue charge is POSITIVE. The wild-type residue is more hydrophobic than the mutant residue. The wild-type residue is very conserved, but a few other residue types have been observed at this position too.
G145D		The mutant residue is bigger than the wild-type residue. The wild-type residue charge was NEUTRAL, the mutant residue charge is NEGATIVE. The wild-type residue is more hydrophobic than the mutant residue. The wild-type residue is very conserved, but a few other residue types have been observed at this position too.

Protein-Protein Interaction of NUDT15

STRING was used to estimate functional interaction between proteins within the cell[20] (Figure 5).

Figure 5. Protein-protein interaction network of *NUDT15* protein shown by STRING



STRING database results estimated the functional interactions with which NUDT15 is associated with TPMT, ITPA, NUDT1, NUDT19, NUDT5, NUDT2, NUDT17, NUDT12, NUDT13 and NUDT18 (Figure 6).

Figure 6. The co-expressed and shared domain with the *NUDT15* gene by STRING

TPMT	*Thiopurine S-methyltransferase; Catalyzes the S-methylation of thiopurine drugs such as 6-mercaptopurine; Belongs to the cla...*
NUDT5	*ADP-sugar pyrophosphatase; Enzyme that can either act as an ADP-sugar pyrophosphatase in absence of diphosphate or cata...*
ITPA	*Inosine triphosphate pyrophosphatase; Pyrophosphatase that hydrolyzes the non-canonical purine nucleotides inosine triphos...*
NUDT18	*8-oxo-dGDP phosphatase NUDT18; Mediates the hydrolyzis of oxidized nucleoside diphosphate derivatives. Hydrolyzes 8-oxo-...*
NUDT12	*Peroxisomal NADH pyrophosphatase NUDT12; Hydrolyzes NAD(P)H to NMNH and AMP (2',5'-ADP), and diadenosine diphosph...*
NUDT1	*7,8-dihydro-8-oxoguanine triphosphatase; Antimutagenic. Acts as a sanitizing enzyme for oxidized nucleotide pools, thus supp...*
NUDT17	*Nucleoside diphosphate-linked moiety X motif 17; Probably mediates the hydrolysis of some nucleoside diphosphate derivativ...*
NUDT19	*Nucleoside diphosphate-linked moiety X motif 19; Coenzyme A diphosphatase that mediates the hydrolysis of a wide range of ...*
NUDT2	*Bis(5'-nucleosyl)-tetraphosphatase [asymmetrical]; Asymmetrically hydrolyzes Ap4A to yield AMP and ATP. Plays a major role i...*
NUDT13	*Nucleoside diphosphate-linked moiety X motif 13; Nudix hydrolase family*

Variants of genes encoding NUDT15 and inosine triphosphatase (ITPA) also affect the metabolism of thiopurine, especially in populations with low frequency of TPMT variants. Hence, NUDT15 and ITPA are estimated to degrade toxic 6-mercaptopurine metabolites during thiopurine metabolism[21-23].

Discussion

Variations in the *NUDT15* gene may be associated with disruption of thiopurine metabolism and thiopurine-induced leukopenia. Studies in different populations have reported that *NUDT15* variants have a strong effect on early myelosuppression of mercaptopurine, and appropriate dose

adjustment is required in patients with both heterozygous and homozygous variants[24-26]. In a study evaluating the relationship between *NUDT15* and *TPMT* variants and leukopenia, the association of NUDT15 rs116855232 (p.R139C) and rs554405994 (p.V18_V19insGV) and TPMT rs1142345 (p.Y240C) variants with mercaptopurine-induced leukopenia was found to be statistically significant (p values, respectively, 5.1×10^{-12}, 1.5×10^{-4}, 0.005)[27]. Another study investigated the relationship between myelosuppression at the 2., 4. and 6. months after initiation of mercaptopurine during maintenance therapy in children with ALL with the NUDT15 c.415C > T genotype. According to the study, cases with the NUDT15 CT or TT genotype showed an importantly increased risk of neutropenia with an OR of 7.4 (95% CI 1.3-42.6) at month 2, and an OR of 14.5 (95% CI 3.4-61.6) at month four and six and 14.4 (95% CI 2.9-72.3) times higher risks[25].

Before thiopurine treatment in patients with ALL, TPMT enzyme activity is examined to prevent serious side effects that may arise from these drugs. The low TPMT enzyme activity causes an increase in toxicity along with drug efficacy. TPMT enzyme activity level differs between individuals depending on the polymorphisms in the *TPMT* gene. Since individuals with low TPMT activity cannot catabolize thiopurines, TGNs accumulate at a very high rate, causing very serious and even fatal hematotoxicity[28]. In order to prevent these complications, each patient's TPMT level is measured before starting treatment. Similarly, determining the *NUDT15* genotypes of each patient before receiving thiopurine treatment at the beginning will reduce the side effects that will occur with the adjustment of the thiopurine dose to be taken by the patient.

Conclusion

In conclusion, E115G, E57G, F52L, K33N, G53R, and G145D SNPs were determined to be deleterious or damaging by SIFT, PolyPhen-2, SNAP2, PROVEAN, PANTHER, I-Mutant and HOPE software tools. The data on *NUDT15* variants obtained as a result of this study will contribute to the predetermination of the response of patients to thiopurine drugs in future studies, to a better understanding of the patient's sensitivity to drug interactions, and ultimately to obtaining information about the prognosis.

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