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Computational Analysis of the Single nucleotide Polymorphisms that Affect Superoxide Dismutase Reveals Important Domain Related to Protein Function

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Abstract **BACKGROUND:** A major focus of neurodegeneration research involves the characterization of how the enzyme Cu, Zn superoxide dismutase is involved in amyotrophic lateral sclerosis (ALS). Despite a wealth of structural and biochemical knowledge, the mechanism by which SOD mutations promote ALS remains controversial. **METHODS:** GENEMANIA software was used to highlight genetic interactions of SOD1. SOD1 was investigated in dbSNP/NCBI database in May 2015. SOD1 had of 2123 SNPs; 622 were identified in human, of which 29 were in the coding region, 8 were non-synonymous SNPs (nsSNPs), 22 were in the 3'un-translated region, and 75 SNPs at 5'un-translated region. Only nsSNPs and 3'UTR SNPs were selected for analysis. Predictions of deleterious nsSNPs was performed by SIFT and Polyphen softwares. The functional impact of the deleterious SNPS was analyzed by project hope. Chimera and project hope softwares were used to highlight the changes occurred as a result of the deleterious SNPs at level of protein 3D structure. The SNPS at 3UTR region were analyzed by Polymirt software. **RESULTS:** Genamania revealed possible role for SoD1 in epidermal cell growth through interaction with *NME2* (MIM 156491) and *NCOA3* (MIM 601937) genes. Two SNPs were found to be deleterious in both SIFT and Polyphen, whereas one SNP was found deleterious in SIFT and not Polyphen. The SNPs predicted to be deleterious in both SIFT and Polyphen are *rs11556621 and rs11556620.* They cause change from the amino acid in position 29 from proline to glutamine, and in amino acid number 87 from asparagine to serine respectively. Both SNPs affect an amino acid at the interpro domain named "Superoxide dismutase, copper/zinc binding domain" (IPR001424) whose function is metal ion binding. The SNP *rs1804450 effect* was found to be a controversial that involves a change in amino acid in position 40 from threonine to isoleucine. Although this residue is part of the "Superoxide dismutase, copper/zinc binding domain" (IPR001424), its site is not conserved. Eight SNPs predicted to induce disruption or creation of mirRNA binding site. **CONCLUSION:** SNPs that affect metal binding domain of superoxide dismutase are the main SNPs that significantly affect enzyme structure and function.

Keywords: *motor neuron disease, SOD1, SNP, computational analysis*

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1. Background

Many point mutations in human Cu-Zn superoxide dismutase (SOD) cause familial amyotrophic lateral sclerosis (FALS); a fatal neurodegenerative disorder in heterozygotes. These mutations cluster in protein regions influencing architectural integrity. Furthermore, crystal structures of SOD wild-type and FALS mutant H43R proteins uncover resulting local framework defects. Mutations of the gene encoding Cu-Zn superoxide dismutase (SOD1) causes 20% of the familial cases of the progressive neurodegenerative disease ALS. [1,2] Despite their widespread distribution, ALS mutations appear positioned to cause structural and misfolding defects. Such defects decrease SOD's affinity for zinc, and loss of zinc from SOD is sufficient to induce apoptosis in motor neurons in vitro. [3] Karch and colleagues analysed aggregation propensities of human/mouse SOD1 chimeras in cell culture and identified two sequence elements in the human enzyme that seem to enhance its aggregation relative to the mouse enzyme. The structural and cell-based data suggest a model in which residues Q42 and Q123 in mouse SOD1 modulate non-native SOD1-SOD1 intermolecular interactions at edge strands in the SOD1

Greek key β-barrel. [4] In fact, in vitro Cu-Zn superoxide dismutase (SOD1) fibrils are transduced into cells and function as seeds to trigger the aggregation of endogenously expressed SOD1. Seeded aggregation of mutant SOD1 will thus play roles in a molecular pathomechanism of SOD1-linked amyotrophic lateral sclerosis [5].

The SOD1 gene encodes superoxide dismutase-1 (EC 1.15.1.1); a major cytoplasmic antioxidant enzyme that metabolizes superoxide radicals to molecular oxygen and hydrogen peroxide, thus providing defense against oxygen toxicity. SOD1 is not just a catabolic enzyme, but can also directly regulate NADPH oxidase-dependent production by binding Rac1 and inhibiting its GTPase activity. [6] Soluble cytoplasmic SOD1 is a copper- and zinc-containing enzyme; the SOD1 gene maps to chromosome 21q22. Eukaryotic cells contain two distinct forms of SOD; a mitochondrial manganese-containing enzyme and a cytoplasmic copper/zinc-containing enzyme. The human Cu/Zn SOD-1 is a dimeric protein composed of identical noncovalently linked subunits. SOD2 is a distinct mitochondrial enzyme that contains manganese; the SOD2 gene that maps to 6q25. SOD1 is a homodimer and SOD2 is a tetramer [7].

Sherman et al. isolated clones corresponding to the human SOD1 gene. The deduced 153-residue protein has a calculated molecular mass of approximately 18.5 kD. Two mRNA transcripts of 0.5 and 0.7 kb were detected. Both mRNAs encoded the same protein, which had functional activity in vitro [8].

Human SOD is a tightly associated and unusually stable homodimer of 153 residues per subunit with each chain folding into an eight stranded Greek key β-barrel. The active-site channel on each subunit is formed on the outside of the β-barrel by two long loops (known as loop IV and loop VII). Loop IV contributes His63, the bridging ligand between the copper and zinc sites, and the other three zinc ligands. Loop IV can be divided into a dimer interface sub loop, a disulfide sub loop and a zinc-binding sub loop. The dimer interface sub loop forms 38% of the contact area that builds the dimer. Loop VII, also known as the electrostatic loop, helps attract the anionic superoxide substrate into the active site. Loop VII also contributes Arg143, whose side-chain guanidinium group provides hydrogen bonds to anchor the main chain of loop IV and orient the bound substrate [9].

The copper/zinc superoxide dismutases have been found to have similar physiochemical properties indicating a conservation of structure and function. The properties of this class of enzyme are quite different from those of the manganese or iron enzymes. Sequence analysis has indicated a homology among the manganese and iron class of enzymes but these have no homology with the copper/zinc enzymes. The copper/zinc enzyme therefore has a different ancestor from the manganese or iron enzyme which appear to have a common ancestor.

The cellular location is predominantly cytoplasmic; the pathogenic variants ALS1 Arg-86 and Ala-94 gradually aggregates and accumulates in mitochondria [10].

Single-nucleotide polymorphism (SNPs) most commonly refer to single-base differences in DNA among individuals. Polymorphisms are usually defined as sites where the less common variant has a frequency of at least 1% in the population, but for some purposes rarer variants are important as well. SNPs of various types can change the function or the regulation and expression of a protein. The most obvious type is a non-synonymous SNP, where the alleles differ in the amino acid of the protein product. Some SNPs are polymorphisms at splice sites, and result in variant proteins that differ in the exons they contain [1]. Some SNPs are in promoter regions and are reported to affect the regulation and expression of proteins [11].

A major focus of neurodegeneration research involves the characterization of how the super- oxide-scavenging enzyme Cu- Zn superoxide dismutase (SOD) is involved in amyotrophic lateral sclerosis (ALS) [9]. Despite a wealth of structural and biochemical knowledge, the mechanism by which SOD mutations promote ALS remains controversial. This study explores the effect of non-synonymous SNPs on the function and structure of superoxide dismutase enzyme.

2. Methods

SOD1 gene was investigated in dbSNP/NCBI database in May 2015. SOD1 gene contained a total of 2123 SNPs; 622 were identified in human, of which 29 were in the coding region, 8 were non-synonymous SNPs (nsSNPs), 22 were in the 3'un-translated region, and 75 SNPs at 5'un-translated region. Only nsSNPs and 3'UTR SNPs were selected for computational analysis. Predictions of deleterious nsSNPs was performed by SIFT and Polyphen softwares. The functional impact of the deleterious SNPS was analyzed by project hope. Chimera and project hope softwares were used to highlight the changes occurred as a result of the deleterious SNPs at the molecular level of the protein 3D structure. The FASTA format of the protein was obtained from Uniprot at Expassy database. The 3D structure of a 100% identical protein was retrieved from database by using BLAST/NCBI. The protein used as a template was called “Chain A, Structure of Copper-Zinc Superoxide Dismutase Complexed with Bicarbonate [Homo sapiens]" with ID pdb|4B3E|. The SNPS at the 3UTR region were analyzed by Polymirt software.

1. GENEMANIA:

GeneMANIA is an online database that helps you predict the function of your favorite genes and gene sets. GeneMANIA finds other genes that are related to a set of input genes, using a very large set of functional association data. Association data include protein and genetic interactions, pathways, co-expression, co-localization and protein domain similarity. You can use GeneMANIA to find new members of a pathway or complex, find additional genes you may have missed in your screen or find new genes with a specific function, such as protein kinases. Your question is defined by the set of genes you input [12].

2. Predicting damaging amino acid substitutions using SIFT (v5.1):

SIFT is a sequence homology-based tool that sorts intolerant from tolerant amino acid substitutions and predicts whether an amino acid substitution in a protein will have a phenotypic effect. SIFT is based on the premise that protein evolution is correlated with protein function. Positions important for function should be conserved in an alignment of the protein family, whereas unimportant positions should appear diverse in an

alignment. SIFT takes a query sequence and uses multiple alignment information to predict tolerated and deleterious substitutions for every position of the query sequence. SIFT is a multistep procedure that searches for similar sequences, then chooses closely related sequences that may share similar function to the query sequence, followed by obtaining the alignment of these chosen sequences, and finally calculates normalized probabilities for all possible substitutions from the alignment. Positions with normalized probabilities less than 0.05 are predicted to be deleterious, those greater than or equal to 0.05 are predicted to be tolerated [13].

3. Prediction of functional modification using Polyphen-2 (Polymorphism Phenotyping v2):

PolyPhen-2 (Polymorphism Phenotyping v2), available as software and via a Web server, predicts the possible impact of amino acid substitutions on the stability and function of human proteins using structural and comparative evolutionary considerations. It performs functional annotation of single-nucleotide polymorphisms (SNPs), maps coding SNPs to gene transcripts, extracts protein sequence annotations and structural attributes, and builds conservation profiles. It then estimates the probability of the missense mutation being damaging based on a combination of all these properties. PolyPhen-2 features include a high-quality multiple protein sequence alignment pipeline and a prediction method employing machine-learning classification. The software also integrates the UCSC Genome Browser's human genome annotations and MultiZ multiple alignments of vertebrate genomes with the human genome. PolyPhen-2 is capable of analyzing large volumes of data produced by next-generation sequencing projects, thanks to built-in support for high-performance computing environments like Grid Engine and Platform LSF [14].

4. Protein Modelling:

a- Chimera 1.8:

Investigating 3D (three-dimensional) structure of proteins is helpful in predicting the effect of SNPs on the structural level and in displaying the degrees of alteration. UCSF Chimera is highly extensible software for interactive visualization and analysis of molecular structures; Chimera (version 1.8) software was used to scan the 3D structure of specific protein and then modifies the original or native amino acid with the candidate to display the impact that can be produced. However some proteins has no readily available 3D structure in database, hence using Chimera would help in predicting the possible 3D structure for proteins under query. It has been innovated by using already available models in database repositories by attaching their protein data bank identity (PDB ID) into chimera for such 3D would be suitable template for innovating a new 3D structure harboring the candidate amino acid. (version1.8) is available at: http://www.cgl.ucsf.edu/chimera [15].

b- Project hope

Project HOPE is an easy-to-use webserver that analyses the structural effects of your mutation of interest. The server allows you to submit a protein sequence and the mutation. Project HOPE will then collect and combine available information from a series of webservers and databases and will produce a mutation report complete with results, figures and animations. Where available, Project HOPE will use the 3D structure of the protein but the server can also build a homology model if necessary. Other information sources include the Uniprot database and a series of DAS prediction servers. Project Hope is available at: http://www.cmbi.ru.nl/hope **[16].**

5. PolymiRTS data base for Polymorphism in microRNA Target Site:

PolymiRTS database was designed specifically for the analysis of non-coding SNPs namely 3'UTR. It aims to identify single-nucleotide polymorphisms (SNPs) that affect miRNA targets in human and mouse. We used this computational server in order to determine 3'UTR SNPs in SOD1 gene that may alter miRNA binding on target sites resulting in diverse functional consequences. All SNPs located in that region were selected and submitted to PolymiRTS (v3.0), available at: http://compbio.uthsc.edu/miRSNP [15,17].

3. Results

Table 1. The genes co-expressed and share a domain with SOD1.(12,18,19)

Gene symbol	*Description*	*Co-expression*	*shared domain*
AK2	adenylate kinase 2 [Acc:362]	no	no
TOMM40L	translocase of outer mitochondrial membrane 40 homolog (yeast)-like [Acc:25756]	no	no
DERL1	derlin 1 [Source:HGNC Symbol;Acc:28454]	no	no
CCS	copper chaperone for superoxide dismutase [Acc:1613]	no	yes
TOMM40	translocase of outer mitochondrial membrane 40 homolog (yeast) [Acc:18001]	yes	no
GSX1	GS homeobox 1 [Acc:20374]	no	no
SOD3	superoxide dismutase 3, extracellular [Acc:11181]	no	yes
HSPA5	heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa) [Acc:5238]	yes	no
DNAJA1	DnaJ (Hsp40) homolog, subfamily A, member 1 [Acc:5229]	yes	no
HSPA1B	heat shock 70kDa protein 1B [Acc:5233]	yes	no
HSPA2	heat shock 70kDa protein 2 [Acc:5235]	no	no
HSPH1	heat shock 105kDa/110kDa protein 1 [Acc:16969]	yes	no
NME2	NME/NM23 nucleoside diphosphate kinase 2 [Acc:7850]	yes	no
HSPA8	heat shock 70kDa protein 8 [Acc:5241]	yes	no
NCOA3	nuclear receptor coactivator 3 [Acc:7670]	no	no
PRDX6	peroxiredoxin 6 [Acc:16753]	yes	no
CAT	catalase [Acc:1516]	yes	no
HSPA4L	heat shock 70kDa protein 4-like [Acc:17041]	yes	no
TMEM68	transmembrane protein 68 [Acc:26510]	no	no
CMPK1	cytidine monophosphate (UMP-CMP) kinase 1, cytosolic [Acc:18170]	yes	no

Genmania revealed that SOD1 gene which codes for superoxide dismutase 1[Acc:11179] has many vital functions. It has a substantial role in antioxidant activity; cellular response to oxidative stress; cellular response to reactive oxygen species; cellular response to superoxide; copper ion binding; epidermal cell differentiation; reactive oxygen species metabolic process; removal of superoxide radicals; response to inorganic substance; response to oxidative stress; response to reactive oxygen species; response to superoxide; and superoxide metabolic process. The genes co-expressed with, share similar protein domain, or participate to achieve similar function are listed in Table 1 below. Table 2 demonstrates non-synonymous SNPs analysed by SIFT and POLYPHEN. The functions accomplished by protein product and its gene-gene interactions are illustrated by using GENEMANIA and shown in Figure 1 and Figure 2 below.

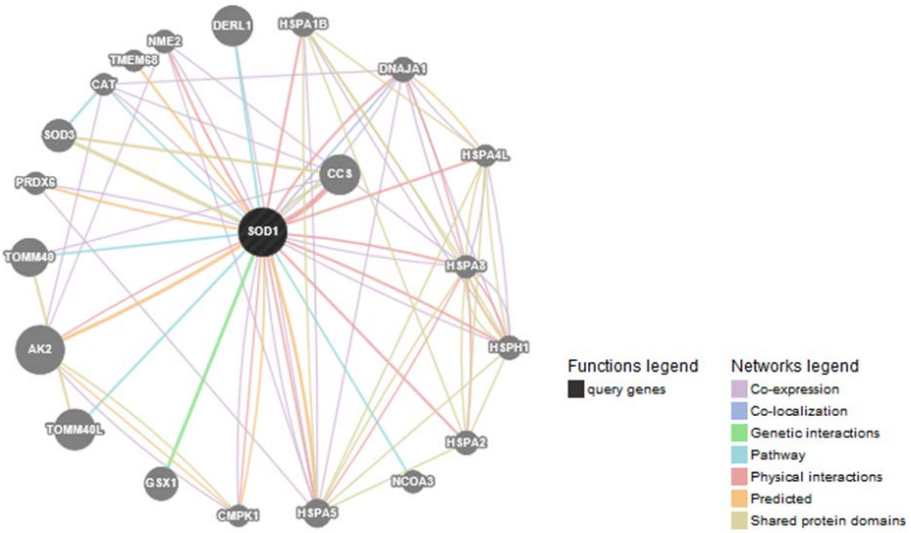

Figure 1. interactions between SOD1 and its related genes [12,18,19]

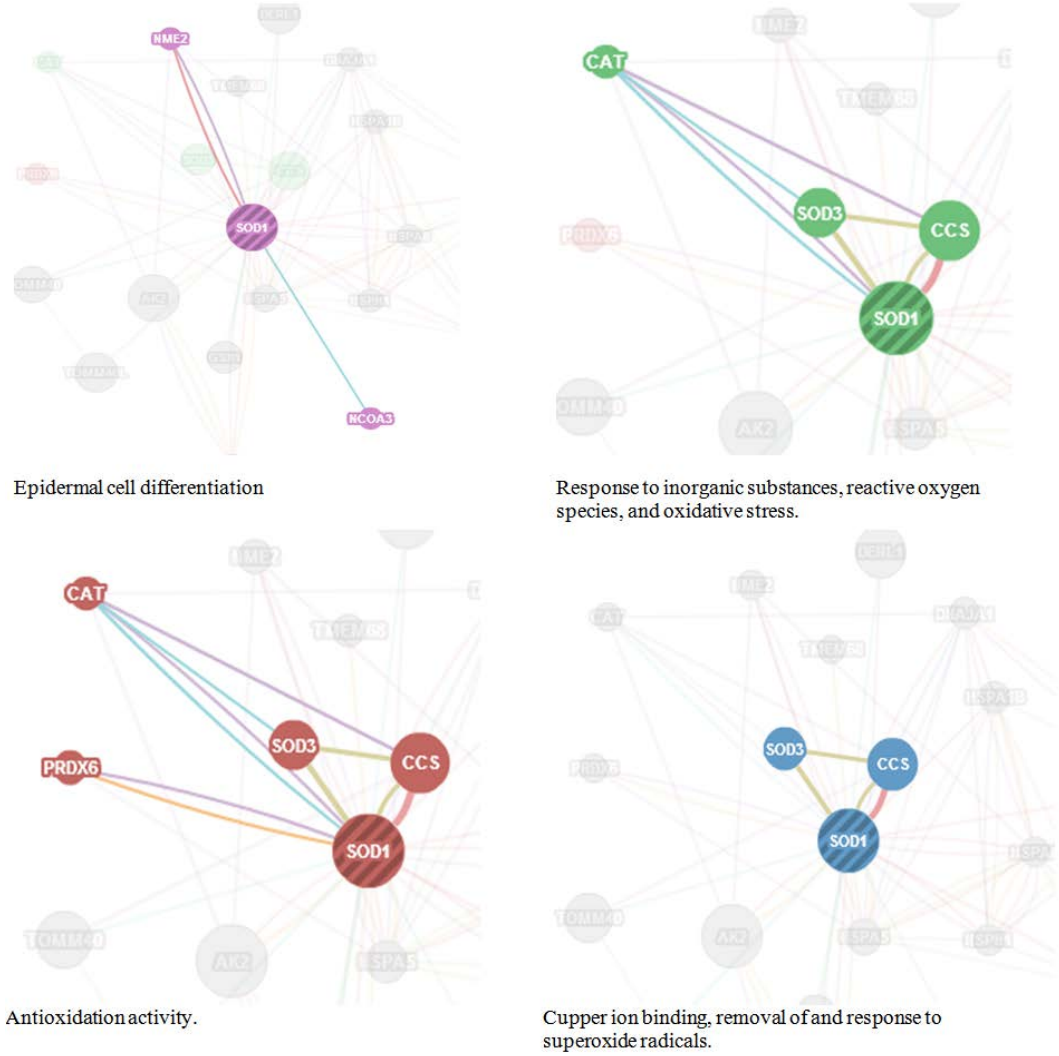

Figure 2. functional interactions between SOD1 and its related genes [12,18,19]

Table 2. list of non-synonymous SNPs with SIFT and POLYPHEN results

SNP	REF ALLELE	ALT ALLELE	AMINO ACID CHANGE	PROTEIN ID	SIFT SCORE	SIFT MEDIAN	NO OF SEQS AT POSITION	SIFT PREDICTION	POLYPHEN PREDICTION
rs199766524	C	T	A5A	ENSP00000374645	0.85	2.93	169	TOLERATED	
rs199766524	C	T	A5A	ENSP00000270142	1	2.7	352	TOLERATED	
rs11556621	C	A	P10Q	ENSP00000374645	0.033	2.71	354	DELETERIOUS	Possibly damaging
rs11556621	C	A	P29Q	ENSP00000270142	0.043	2.73	371	DELETERIOUS	Possibly damaging
rs1804450	C	T	T21I	ENSP00000374645	0.03	2.64	388	DELETERIOUS	Benign
rs1804450	C	T	T40I	ENSP00000270142	0.035	2.7	395	DELETERIOUS	Benign
rs11556620	A	G	N87S	ENSP00000270142	0.001	2.7	398	DELETERIOUS	Probably damaging
rs11556620	A	G	N68S	ENSP00000374645	0.001	2.65	400	DELETERIOUS	Probably damaging
rs202198235	T	C	V95A	ENSP00000270142	0.097	2.7	396	TOLERATED	
rs202198235	T	C	V76A	ENSP00000374645	0.106	2.65	399	TOLERATED	
rs111229903	T	A	D78E	ENSP00000374645	0.573	2.65	397	TOLERATED	
rs111229903	T	A	D97E	ENSP00000270142	0.607	2.7	395	TOLERATED	
rs76731700	NOT FOUND								
rs567432143	NOT FOUND								

- **rs11556621:**

This SNP causes a change in the amino acid in position 29 from proline to glutamine as shown in Figure 3.

- **rs11556620:**

This mutation involves a change in amino acid number 87 from asparagine to serine as shown in Figure 4. This mutation matches a previously described variant, with the following description "Amyotrophic lateral sclerosis 1 (ALS1) [MIM: 105400].

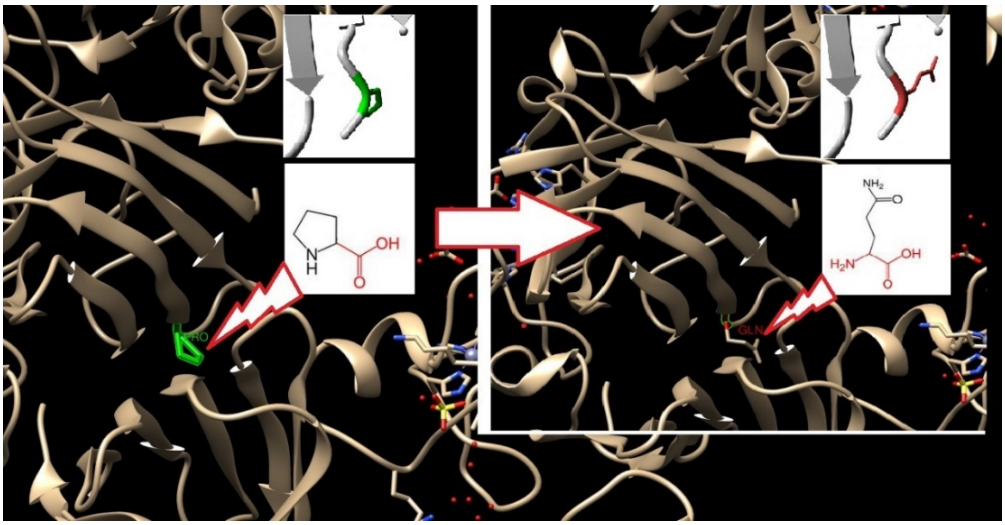

Figure 3. change in the amino acid in position 29 from proline to glutamine

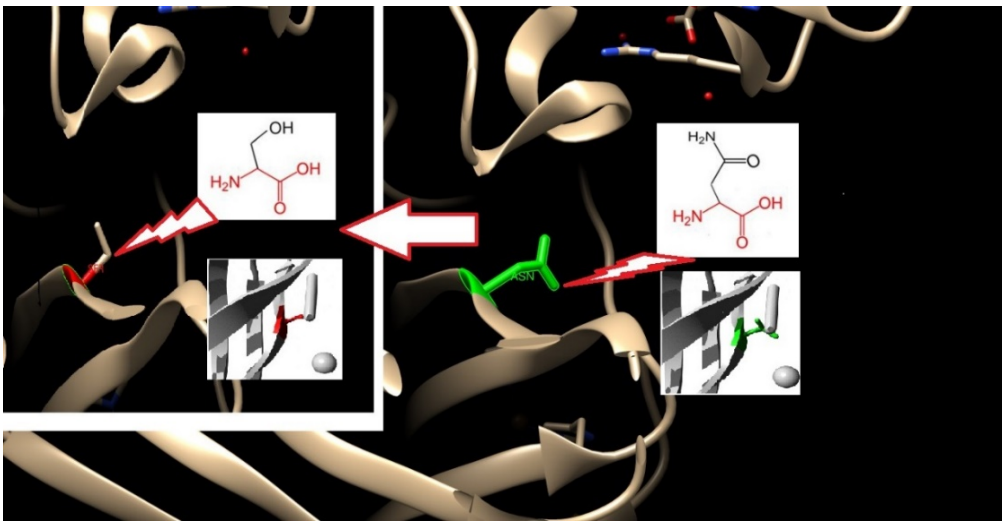

Figure 4. change in amino acid number 87 from asparagine to serine

- **rs1804450**:

This is a mutation that involves a change in amino acid in position 40 from threonine to isoleucine as shown in Figure 5.

- **SNPS at the 3UTR region**:

The Table 3 below demonstrates the SNPs predicted by Polymirt to induce disruption or formation of mirRNA binding site.

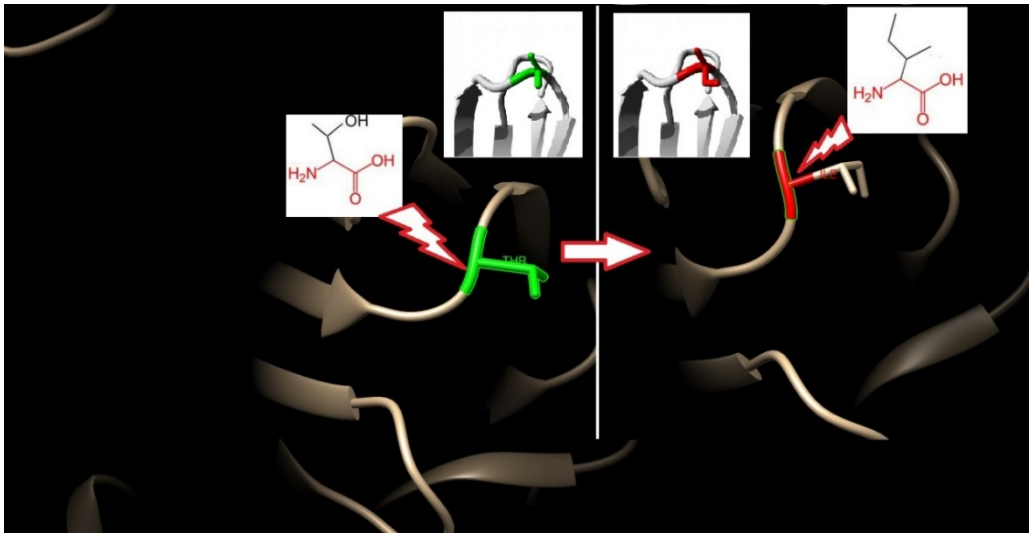

Figure 5. change in amino acid in position 40 from threonine to isoleucine

Table 3. the SNPs predicted by Polymirt to induce disruption or formation of mirRNA binding site

Target sites disrupted by SNPs and INDELs in miRNA seeds								
Location	**miR ID**	**dbSNP ID**	**miR Seed**	**Allele**	**Wobble base pair**	**miRSite**	**Conservation**	**context+ score change**
33041201	hsa-miR-302c-3p	rs199971565	AAG[AAGT/-]GCU	AAGT/-	0	GCACUUA	3	-0.156
33041201	hsa-miR-520d-3p	rs201279305	AAGU[G/C]CU	G/C	0	GCACUUA	3	-0.146
33041131	hsa-miR-8070	rs138552125	U[G/A]UGAUU	G/A	1	AAUCACA	5	-0.188
Target sites created by SNPs and INDELs in miRNA seeds								
Location	**miR ID**	**dbSNP ID**	**miR Seed**	**Allele**	**Wobble base pair**	**miRSite**	**Conservation**	**context+ score change**
33041044	hsa-miR-15b-3p	rs192595529	[G/A]AAUCAU	G/A	1	UGAUUUA	4	-0.027
33041017	hsa-miR-518a-3p	rs200532946	AAAGC[G/A]C	G/A	1	UGCUUUA	8	-0.065
33041017	hsa-miR-518a-3p	rs199969520	AAAGC[G/A]C	G/A	1	UGCUUUA	8	-0.065
33040953	hsa-miR-642b-3p	rs111664333	GA[C/T]ACAU	C/T	0	AUGUAUC	2	-0.226
33041017	hsa-miR-518d-3p	rs73602910	AAAGC[G/A]C	G/A	1	UGCUUUA	8	-0.065

4. Discussion

The deleterious nsSNPs detected cause alteration in the amino acid that usually produced at a specific site. Each amino acid has its own specific size, charge, and hydrophobicity value. The original wild-type residue and newly introduced mutant residue often differ in these properties. This report will evaluate the effect of the mutation on the following features: Contacts made by the mutated residue, structural domains in which the residue is located, modifications on this residue, and known variants for this residue.

The wild-type residue of rs11556620 SNP is much conserved, but a few other residue types was observed at this position too. The mutant residue was among the residues observed at this position in other sequences. This means that homologous proteins exist with the same residue type as the mutant at this position and this mutation is possibly not damaging to the protein. However, the impact on domain structure and hydrophobicity is far more important. Firstly, this residue is part of an interpro domain named "Superoxide dismutase, copper/zinc binding domain" (IPR001424). This domain is annotated with the following Gene-Ontology (GO) terms to indicate its function: metal ion binding (GO: 0046872). More broadly speaking, these GO annotations indicate the domain has a function in ion binding (GO: 0043167). The mutated residue is located on the surface of a domain that is important for binding of other molecules. The differences between the wild-type and mutant residue might disturb the interaction with these other molecules. Secondly, the hydrophobicity of the wild-type and mutant residue differs. The wild type residue forms a hydrogen bond with the Aspartic acid on position 125. The mutant residue is smaller than the wild-type residue. The size difference between wild-type and mutant residue means that the new residue is not in the correct position to make the same hydrogen bond as the original wild-type residue did. The difference in hydrophobicity will affect hydrogen bond formation. This will cause a possible loss of external interactions. According to the PISA-database, the mutated residue is involved in a multimer contact. The mutation introduces a smaller residue at this position. The new residue might be too small to make multimer contact. A more hydrophobic residue is introduced here. Any hydrogen bonds that could be made by the wild-type

residue to other monomers will be lost now and affect the multimeric contacts. All these effects raised the possibility of interfering with the normal protein structure and function to almost 100% [16].

Regarding rs11556621 SNP, Polyphen showed that the wild-type residue is much conserved, but a few other residue types was observed at this position too. The mutant residue was among the residues observed at this position in other sequences. This means that homologous proteins exist with the same residue type as the mutant at this position and this mutation is possibly not damaging to the protein. Nevertheless, Prolines are known to be very rigid and therefore induce a special backbone conformation which might be required at this position. The mutation can disturb this special conformation. In addition, this residue is part of an interpro domain named "Superoxide dismutase, copper/zinc binding domain" (IPR001424). More broadly speaking, these GO annotations indicate the domain has a function in ion binding (GO: 0043167). The mutated residue is located on the surface of this domain that is important for binding of other molecules, and the mutant residue is bigger than the wild-type residue. The mutation might also cause loss of hydrophobic interactions with other molecules on the surface of the protein. These differences between the wild-type and mutant residue might disturb the interaction with these other molecules. Thus this SNP is most likely to affect protein structure and function [16].

The wild-type residue of rs1804450 SNP is not conserved at this position, which may indicate that the SNP may not be pathological. Yet, the mutant residue was not among the residue types observed at this position in other homologous sequences which might indicate that the mutation is possibly damaging to the protein. On the other hand, the mutant residue is bigger than the wild-type residue. The residue is located on the surface of the protein, mutation of this residue can disturb interactions with other molecules or other parts of the protein. Furthermore, the hydrophobicity of the wild-type and mutant residue differs. The difference in hydrophobicity will affect hydrogen bond formation. The wild-type residue forms a hydrogen bond with the Histidine on position 44. The size difference between wild-type and mutant residue makes the new residue not in a correct position to make the same hydrogen bond as the original wild-type residue did. It should be noticed that this residue is also part of an interpro domain named "Superoxide dismutase, copper/zinc binding domain" (IPR001424) annotated above. Hence, the mutated residue is located on the surface of a domain that is important for binding of other molecules. The differences between the wild-type and mutant residue might disturb the interaction with these other molecules [16].

SOD1 is well known for its antioxidation function, yet Genamania revealed a possible role for SOD1 in epidermal cell growth through interaction with *NME2* (MIM 156491) and *NCOA3* (MIM 601937) genes. More interestingly, when five human melanoma cell lines were investigated for their antioxidant activities, results showed that both activity and amount of SOD1 immunoreactive protein is correlated with number of chromosome 21, suggesting a gene dosage effect. This strengthens the probability of finding links between SOD1 and epidermal cell growth, which is an interesting era for research [20].

In short, the copper/zinc binding domain seems to be very sensitive to single nucleotide polymorphisms, and very important for optimal superoxide dismutase function.

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