In silico analysis of coding SNPs and 3′-UTR associated miRNAs in *DCAF17* gene that may affect the regulation and pathogenesis of Woodhouse-Sakati Syndrome

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Abstract:

Background: Woodhouse-Sakati Syndrome refers to a group of inherited disorders characterized by alopecia, hypogonadism, diabetes mellitus, hypothyroidism and progressive extrapyramidal signs. The aim of this study is to identify the pathogenic SNPs in the *DCAF17* gene with their related mciroRNAs and their effect on the structure and function of the protein.
Material and Methods: We used different bioinformatics tools to predict the effect of each SNP on the structure and function of the protein. After that we defined the miRNAs founded in the 3'-UTR region on the *DCAF17* gene and studied the annotations relative to it.
Results: Ten deleterious SNPs out of 339 were found to have a damaging effect on the protein structure and function, with one significant micoRNA in the 3’-UTR region .
Conclusion: This was the first in silico analysis of *DCAF17* gene, in which 10 novel mutations were found using different bioinformatics tools that could be used as a diagnostic markers for Woodhouse-Sakati syndrome, with one relevant microRNA that can regulate the function of the protein.

Keywords: Woodhouse-Sakati Syndrome, DCAF17 gene, SNPs, 3’-UTR ,in silico analysis, diagnostic markers.

1. Introduction:

Woodhouse-Sakati Syndrome (WSS, MIM: 241080) is a rare autosomal-recessive multi systemic disorder which is characterized by a combination of hypogonadism, alopecia, Diabetes Mellitus (DM), mental retardation and extrapyramidal signs.[1-10]

It was originally described in a number of consanguineous Saudi families in the Middle East, but has recently been reported in other ethnicities as well. [11, 12] Since its original description in 1983, approximately 50 cases have been reported until now.[13] Exogenous hormone therapy promoting secondary sex characteristic development and insulin are among the recommended treatments for the disease. [14, 15]

The availability of vast amounts of sequence data, coupled to advances in computational biology in the recent years provides an ideal framework for in silico gene expression analysis. Single Nucleotide Polymorphisms (SNPs) make up about 90% of DNA sequence variations, making it the most common type of genetic variation .The SNPs that are most likely to have a direct impact on the protein product of a gene are those that change the amino acid sequence and variants in gene regulatory regions, which control protein expression levels.(16-18)

The disease was first described in 2008 by al Alazami et al,[4, 11] Mutations in the *DCAF17* gene are the cause of Woodhouse-Sakati syndrome.[3, 19, 20] It is located on chromosome 2q31.1. [4, 21] it encodes a nucleolar protein with poorly understood function, adding to that the pathogenic mechanism underlying WSS is also un known.[13]

The aim of this study was to identify the pathogenic SNPs in *DCAF17* gene located in the coding region and analyzing the microRNA(miRNA) in the 3'- untranslated regions (3'-UTR) using in silico prediction software's that determine the structure, function and regulation of their respective proteins. This is the first *in silico* analysis of it is kind in *DCAF17* gene to address this matter.

2. Material and Methods:

2.1. Retrieving nsSNPs:

SNPs associated with *DCAF17* gene were obtained from the Single Nucleotide Polymorphism database (dbSNP) in the National Center for Biotechnology Information (NCBI). (http://www.ncbi.nlm.nih.gov/snp/).

The sequence and natural variants of DCAF17 protein were obtained from the UniProt database as it considered as the most reliable and unambiguous database for protein sequences. [22] (https://www.uniprot.org/).

A total number of 339 nonsynonymous Single Nucleotide Polymorphisms (ns SNPs) were found from NCBI database, all of them were subjected to in silico analysis using ten different algorithms and softwares; SIFT, PROVEAN, PolyPhen-2, SNPs&GO, PhD-SNP, PMUT, I-mutant, Project Hope, GeneMANIA and Chimera.

2.2. Identifying the most damaging nsSNPs and disease related mutations:

Five different bioinformatics tools were utilized to predict functional effects of nsSNPs obtained from dbSNP database on the protein. These algorithmic include: SIFT, PROVEAN, PolyPhen-2, SNAP2 and PhD-SNP.

The SNPs predicted deleterious by at least four softwares were considered high risk nsSNPs and investigated further.

2.2.1. SIFT Server:

Phenotypic effects of amino acid substitution on protein function were predicted by using Sorting Intolerant From Tolerant (SIFT) server, which is a powerful tool used to fulfill this purpose. A list of nsSNPs (rsIDs) from NCBI's dbSNP database was submitted as a query (original) sequence to SIFT to predict tolerated and deleterious substitutions for every position of a protein sequence. The server divides the results into “Deleterious” and “Tolerated”, nsSNPs with SIFT score ≤ 0.05 were classified as deleterious and are further analyzed to identify the damaging ones, and those > 0.05 were classified as tolerated and are not further analyzed. (Available at: http://sift.bii.a-star.edu.sg/). [23-26]

2.2.2. Provean Server:

(Protein Variation Effect Analyzer) is the second software tool used. It also predicts the effect of an amino acid substitution on the biological function of a protein. It predicts the damaging effects of any type of protein sequence variations to not only single amino acid substitutions but also in-frame insertions, deletions, and multiple amino acid substitutions. The results are obtained as either “Deleterious” if the prediction score was ≤ -2.5, while score > -2.5 indicates that the variant is predicted to have a “Neutral” effect. (Available at: http://provean.jcvi.org/index.php). [27, 28]

2.2.3. Polyphen-2 Server:

Polymorphism Phenotyping v2.0 (PolyPhen-2) is another online tool that predicts the possible effects of an amino acid substitution on the structure and function of the protein. The results are classified into "PROBABLY DAMAGING" that is the most disease causing with a score near to 1 (0.7-1), "POSSIBLY DAMAGING" with a less disease causing ability with a score of 0.5-0.8 and "BENIGN" which does not alter protein functions with a score closer to zero; (0-0.4). (Available at: http://genetics.bwh.harvard.edu/pph2/). [22, 29, 30]

2.2.4. SNPs & Go server:

An online web server that used to ensure the disease relationship with the studied single nucleotide polymorphisms SNPs. It gives three different results based on three different analytical algorithms; Panther result, PHD-SNP result, SNPs&GO result. Each one of these results is composed of three parts, the prediction which decides whether the mutation is neutral or disease related, reliability index (RI), and disease probability (if >0.5 mutation is considered as disease causing nsSNP). (Available at: http://snps-and-go.biocomp.unibo. it/snps-and-go/) . [31]

2.2.5. PMUT Server:

PMUT is a powerful web-based tool used for the prediction of pathological variants on proteins. The prediction results are classified as "Neutral" or "Disease". It is available at (http://mmb.irbbarcelona.org/PMut). [32]

2.3. Protein Structural analysis:

I-Mutant:

A online web-tool that is used for the prediction of protein stability changes upon single point mutations, determining whether the mutation increases or decreases the protein stability. (Available at: http://gpcr.biocomp.unibo.it/cgi/predictors/I-Mutant2.0/I-Mutant2.0.cgi). [33]

2.4. Project HOPE:

It is an online web-server used to analyze the structural and functional variations on protein sequence that have been resulted from single amino acid substitution. It searches protein 3D structures by collecting structural information from a series of sources, including calculations on the 3D coordinates of the protein and sequence annotations from the UniProt database. Protein sequences are submitted to project HOPE server then HOPE builds a complete report with text, figures, and animations. It is available at (http://www.cmbi.ru.nl/hope). [34]

2.5. GeneMANIA:

A user-friendly web interface tool approaches to know protein function, analyzing submitted gene lists and prioritizing genes for functional assays. It provides lists with functionally similar

genes that it identifies and recognizes using available genomics and proteomics data. (Available at: (http://www.genemania.org/). [35]

2.6. Modeling nsSNP locations on protein structure:

Chimera:

Chimera 1.8 software is used for visualization and editing of the three dimensional structure of protein. It visualizes the original amino acid with the mutated one to see the impact that can be produced. It is a highly extensible program for interactive visualization and analysis of molecular structures and related data, including density maps, supramolecular assemblies and sequence alignments. High-quality images and animations can be generated. (Availabe at: http://www.cgl.ucsf.edu/chimera). [36]

2.7. 3'-UTR analysis:

2.7.1. Ensemble 95:

RNA sequence were retrieved from ensemble, which is website that makes key genomic data sets available to the entire scientific community without restrictions.[37, 38] (Available at: https://www.ensembl.org/index.html).

2.7.2. RegRNA2.0:

Then the RNA sequences were inserted to RegRNA 2.0 (which is an integrated web server for identifying functional RNA motifs and sites. [39, 40] (Available at: http://regrna2.mbc.nctu.edu.tw/) to find the related microRNA (miRNA).

2.7.3. miRmap22.1:

Then the miRNA sequence were further inserted in to miRmap website to predict the miRNA targets and study the repression strength using thermodynamic, probabilistic, evolutionary and sequence-based approaches.[41] (Available at: https://mirmap.ezlab.org/).

2.7.4. miRBase:

After that we use mirbase website to study its annotations (miRBase website provides a wide-range of information on published microRNAs, including their sequences, their biogenesis precursors, genome coordinates, and community driven annotation).[42-44] (Available at: http://www.mirbase.org/).

Result:

13579 SNPs were retrived from NCBI , 153 were found in coding synominus , 1695… were found in the 3 utr and 640 …. In the 5 utr , 339 were found to be missense . while …. Were found in other regions .

The missense SNPs were submitted to SIFT, Proven, Polyphen-2, SNAP2 respectively. After analysis, 60 mutations out of 339 missense mutations were found to be deleterious in all four server . as found in table (1) .

Furthermore, the above result were submitted to SNP&GO, PHD, and Pmut to study the relation between the SNPs and the disease. Out of 60 SNPs 10 were found to be related to disease. As indicated in table (2) . Then I mutant was used to detect the stability of the protein and the result indicated that all the SNPs except one (rs1279189246 ,H478L) lead to decrease in the stability which lead to unstable amino acid interaction. As found in table (3) .

One significant microRNA was found and investigated as shown in table (4) and (5) .

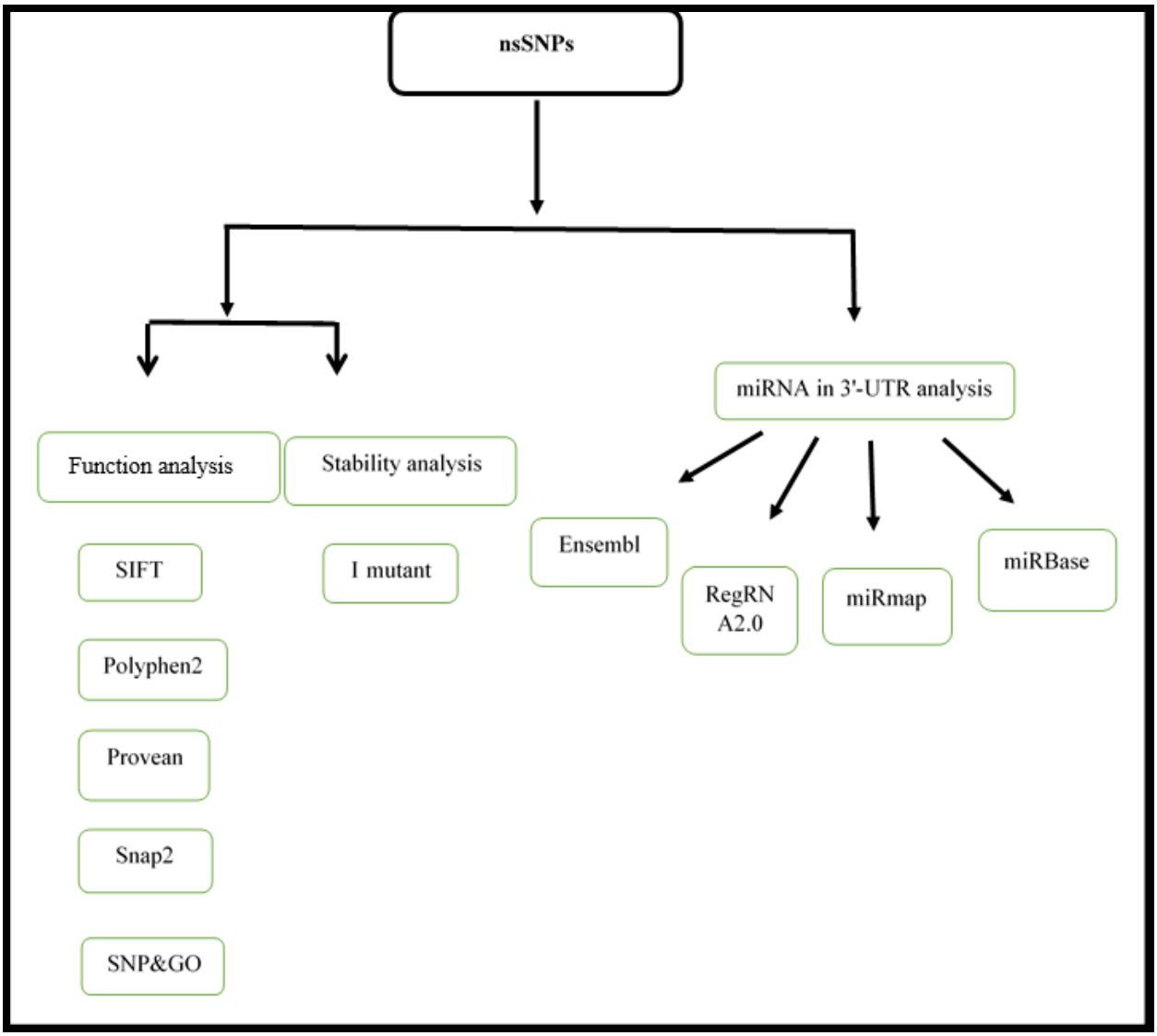

Figure. (1): Diagrammatic representation of in silico work flow for *DCAF17* gene in coding and 3'-UTR regions.

Table. (1): Shows the most deleterious SNPs by four softwares.

dbSNP rs#	Substitution	sift Prediction	sift Score	Provean Prediction	Provean Score	POLYPHEN2 Prediction	POLYPHEN2 Score	SNAP2 Predicted Effect	SNA Scor
rs1229235381	R5W	AFFECT	0	Deleterious	-2.538	possibly damaging	0.95	Effect	50
rs1330567400	C10R	AFFECT	0	Deleterious	-4.238	probably damaging	0.997	Effect	84
rs1420986481	L13P	AFFECT	0	Deleterious	-3.429	probably damaging	0.99	Effect	63
rs867160323	G19C	AFFECT	0	Deleterious	-4.108	probably damaging	1	Effect	85
rs748586810	N32T	AFFECT	0	Deleterious	-2.666	probably damaging	0.996	Effect	20
	N32I	AFFECT	0	Deleterious	-4.378	probably damaging	0.999	Effect	57
rs1379715347	V40E	AFFECT	0.02	Deleterious	-2.98	possibly damaging	0.828	Effect	35
rs576105333	Y61C	AFFECT	0	Deleterious	-2.61	probably damaging	1	Effect	71
rs1224937342	F68C	AFFECT	0	Deleterious	-2.561	probably damaging	0.992	Effect	31
rs1356908272	D69V	AFFECT	0	Deleterious	-3.745	possibly damaging	0.514	effect	76
rs886055106	R72W	AFFECT	0	Deleterious	-3.542	probably damaging	0.999	effect	85
rs764744666	Y86H	AFFECT	0	Deleterious	-2.834	probably damaging	0.999	effect	68
rs889604735	P89R	AFFECT	0	Deleterious	-3.725	probably damaging	1	effect	19
rs1220794681	E98G	AFFECT	0	Deleterious	-3.449	probably damaging	0.999	effect	50
rs754648178	W129R	AFFECT	0	Deleterious	-5.379	probably damaging	0.999	effect	69
rs1302837695	K167T	AFFECT	0	Deleterious	-3.663	probably damaging	0.994	effect	67
rs377364475	R178W	AFFECT	0	Deleterious	-3.983	possibly damaging	0.95	effect	87
rs1157407921	G181C	AFFECT	0	Deleterious	-2.715	probably damaging	1	effect	57
rs750319630	V186D	AFFECT	0.01	Deleterious	-3.175	probably damaging	0.969	effect	56

rs1181193053	A191E	AFFECT	0	Deleterious	-2.86	possibly damaging	0.948	effect	56
rs778114154	P197L	AFFECT	0	Deleterious	-7.417	probably damaging	1	effect	21
rs778975572	L204P	AFFECT	0	Deleterious	-4.144	possibly damaging	0.772	effect	78
rs769602895	G222R	AFFECT	0	Deleterious	-4.367	probably damaging	0.979	effect	84
rs1422292058	L224P	AFFECT	0	Deleterious	-4.894	probably damaging	0.999	effect	77
rs1474908539	S230P	AFFECT	0	Deleterious	-2.73	possibly damaging	0.718	effect	45
rs748887234	R234I	AFFECT	0	Deleterious	-4.45	probably damaging	0.997	effect	68
rs369174398	Y236C	AFFECT	0	Deleterious	-5.723	possibly damaging	0.927	effect	19
rs1476963715	G253E	AFFECT	0	Deleterious	-3.683	probably damaging	1	effect	76
rs1015369709	C256R	AFFECT	0	Deleterious	-3.356	possibly damaging	0.899	effect	39
	C256G	AFFECT	0	Deleterious	-3.232	possibly damaging	0.718	effect	37
rs778451530	P269L	AFFECT	0	Deleterious	-5.08	probably damaging	1	effect	68
rs1439042141	G271V	AFFECT	0	Deleterious	-4.416	probably damaging	1	effect	60
rs757839826	P282S	AFFECT	0	Deleterious	-5.005	probably damaging	1	effect	50
rs1292011900	P283L	AFFECT	0	Deleterious	-4.623	probably damaging	0.959	effect	38
rs746899310	L285R	AFFECT	0	Deleterious	-4.499	probably damaging	1	effect	42
rs1463889939	W302G	AFFECT	0	Deleterious	-10.032	probably damaging	1	effect	87
rs1412078281	H318R	AFFECT	0	Deleterious	-3.987	probably damaging	0.98	effect	30
rs1319790255	D324G	AFFECT	0	Deleterious	-4.179	probably damaging	1	effect	3
rs78488864	W344R	AFFECT	0	Deleterious	-3.279	probably damaging	0.999	effect	26
rs1439891137	W344C	AFFECT	0	Deleterious	-2.985	probably damaging	1	effect	39
rs752287637	D350H	AFFECT	0	Deleterious	-5.89	probably damaging	1	effect	83
rs764182538	H357Y	AFFECT	0	Deleterious	-2.596	probably damaging	0.998	effect	25
rs890180094	H357P	AFFECT	0	Deleterious	-6.354	probably damaging	1	effect	90
rs764124723	T402I	AFFECT	0	Deleterious	-4.114	probably damaging	1	effect	49

rs751732245	R406W	AFFECT	0	Deleterious	-6.971	probably damaging	1	effect	75
rs1211965571	D416H	AFFECT	0	Deleterious	-2.771	probably damaging	1	effect	25
rs1288356140	D416G	AFFECT	0	Deleterious	-3.396	probably damaging	1	effect	12
	D416V	AFFECT	0	Deleterious	-4.712	probably damaging	1	effect	8
rs139061039	D418G	AFFECT	0	Deleterious	-5.105	probably damaging	1	effect	32
rs759392662	Y429C	AFFECT	0	Deleterious	-7.379	probably damaging	1	effect	40
rs1227872471	D431H	AFFECT	0	Deleterious	-4.105	probably damaging	1	effect	54
rs1256723119	L433S	AFFECT	0	Deleterious	-4.322	probably damaging	1	effect	42
rs766858148	G448R	AFFECT	0	Deleterious	-3.596	probably damaging	1	effect	48
rs1409820369	D473H	AFFECT	0	Deleterious	-4.896	probably damaging	1	effect	52
rs1279189246	H478R	AFFECT	0	Deleterious	-3.431	probably damaging	1	effect	68
	H478L	AFFECT	0	Deleterious	-5.421	probably damaging	1	effect	62
rs1333368709	D483G	AFFECT	0	Deleterious	-5.332	probably damaging	0.995	effect	62
rs753532618	R484G	AFFECT	0	Deleterious	-3.653	probably damaging	0.996	effect	76
rs1387898294	D485E	AFFECT	0	Deleterious	-2.773	probably damaging	0.971	effect	3
rs749237410	F498V	AFFECT	0	Deleterious	-4.135	probably damaging	1	effect	55

Table. (2): Disease effect nsSNPs associated variations predicted by SNPs&GO and PHD-SNP softwares.

dbSNP rs#	SUB	SNP & GO	RI*	Probability	PHD	RI*	Probability	PMUT Score	Prediction
rs1356908272	D69V	Disease	3	0.648	Disease	8	0.893	0.68 (85%)	Disease
rs886055106	R72W	Disease	3	0.625	Disease	7	0.868	0.53 (80%)	Disease
rs750319630	V186D	Disease	0	0.509	Disease	7	0.832	0.64 (84%)	Disease
rs778114154	P197L	Disease	3	0.649	Disease	8	0.921	0.84 (91%)	Disease
rs778975572	L204P	Disease	1	0.533	Disease	7	0.825	0.68 (85%)	Disease
rs1422292058	L224P	Disease	1	0.535	Disease	7	0.836	0.86 (91%)	Disease
rs1256723119	L433S	Disease	1	0.539	Disease	7	0.828	0.86 (91%)	Disease
rs1279189246	H478R	Disease	1	0.564	Disease	8	0.913	0.70 (86%)	Disease
rs1333368709	D483G	Disease	0	0.518	Disease	8	0.885	0.86 (91%)	Disease
rs749237410	F498V	Disease	2	0.62	Disease	8	0.886	0.69 (86%)	Disease

* RI: Reliability Index

Table. (3): Stability analysis predicted by I-Mutant version 3.0**.**

dbSNP rs#	Mutation	i-mutant prediction	RI	Score
rs1356908272	D69V	Decrease	5	-0.6
rs886055106	R72W	Decrease	5	-0.86
rs750319630	V186D	Decrease	7	-1.22
rs778114154	P197L	Decrease	8	-0.84
rs778975572	L204P	Decrease	7	-1.72
rs1422292058	L224P	Decrease	4	-1.5
rs1256723119	L433S	Decrease	9	-2.29
rs1279189246	H478L	Increase	5	0.4
rs1333368709	D483G	Decrease	5	-1.02
rs749237410	F498V	Decrease	9	-1.76

*RI: Reliability Index

Table. (4): miRNAs target identified in 3'- UTR region of *DCAF17* gene by miRmap website.

miRNA	Gene	Position	No. of Nucleotides	Target Sequence	ΔG open	Probability exact	Conservation PhyloP	miRmap score
hsa-miR-4436b-3p	DCAF17	210 ~2124	22	acattaatgtcttcctgcccta	12.32	0.02	0.71	-0.55

* ΔG open: mRNA opening free energy – Accessibility

* PhyloP: evolutionary conservation predictor.

Table. (5): Alignment of Query to mature miRNAs.

Accession	ID	Query start	Query end	Subject start	Subject end	Strand	Score	Value
MIMAT0019941	hsa-miR-4436b-3p	10	21	2	13	-	60	12
MIMAT0025585	mmu-miR-6540-5p	2	22	2	22	-	60	12
MIMAT0027651	hsa-miR-6875-3p	10	21	3	14	+	60	12
MIMAT0017064	mmu-miR-135a-2-3p	4	22	3	21	-	59	15
MIMAT0037309	hsa-miR-135a-2-3p	4	22	4	22	-	59	15
MIMAT0015037	hsa-miR-3163	9	21	10	22	-	56	27
MIMAT0019772	hsa-miR-4685-3p	11	21	6	16	+	55	33
MIMAT0027850	mmu-miR-6974-5p	6	19	5	18	-	52	58
MIMAT0027973	mmu-miR-7034-3p	9	22	4	17	+	52	58
MIMAT0028056	mmu-miR-7075-5p	7	20	2	15	-	52	58
MIMAT0028057	mmu-miR-7075-3p	7	20	6	19	+	52	58
MIMAT0019361	hsa-miR-3976	10	21	6	17	-	51	70
MIMAT0019940	hsa-miR-4436b-5p	10	21	8	19	+	51	70
MIMAT0027746	mmu-miR-6923-5p	10	21	4	15	-	51	70
MIMAT0003339	hsa-miR-421	3	12	7	16	-	50	85
MIMAT0004869	mmu-miR-421-3p	3	12	7	16	-	50	85

Table. (6): The gene co-expressed, share domain and interaction with *DCAF17* gene network.

Gene 1	Gene 2	Weight	Network group
GEMIN2	C18orf25	0.020875	Co-expression
C18orf25	DCAF17	0.01954	Co-expression
POT1	CUL4B	0.015105	Co-expression
POT1	TMEM267	0.010743	Co-expression
SKIV2L2	DCAF17	0.004223	Co-expression
VIPAS39	BBS7	0.006134	Co-expression
POLI	DCAF17	0.01365	Co-expression
GEMIN2	SKIV2L2	0.001957	Co-expression
RAD17	CUL4B	0.009911	Co-expression
RAD17	POT1	0.005801	Co-expression
RAD17	VIPAS39	0.002685	Co-expression
EYA3	POT1	0.02177	Co-expression
RAD17	SKIV2L2	0.012174	Co-expression
TMEM267	DCAF17	0.008714	Co-expression
POT1	TMEM267	0.010902	Co-expression
SKIV2L2	DCAF17	0.010967	Co-expression
SKIV2L2	CUL4B	0.014126	Co-expression
SKIV2L2	POT1	0.015277	Co-expression
BBS7	DCAF17	0.016686	Co-expression
EXOC2	BLMH	0.015184	Co-expression
GEMIN2	CUL4B	0.011145	Co-expression
GEMIN2	INTS7	0.011782	Co-expression
RAD17	SKIV2L2	0.013417	Co-expression
CUL4B	DCAF17	0.009401	Co-expression

TMEM267	DCAF17	0.002878	Co-expression
TMEM267	CUL4B	0.005961	Co-expression
TMEM267	C18orf25	0.003443	Co-expression
SKIV2L2	DCAF17	0.010745	Co-expression
POLI	TMEM267	0.004744	Co-expression
GEMIN2	POT1	0.011051	Co-expression
RAD17	DCAF17	0.010133	Co-expression
RAD17	TMEM267	0.007081	Co-expression
BLMH	SKIV2L2	0.011372	Co-expression
C18orf25	DCAF17	0.026408	Co-expression
TMEM267	DCAF17	0.016632	Co-expression
SKIV2L2	DCAF17	0.01629	Co-expression
NAA15	DCAF17	0.022654	Co-expression
GEMIN2	DCAF17	0.019081	Co-expression
GEMIN2	TMEM267	0.015272	Co-expression
SKIV2L2	POT1	0.003939	Co-expression
BBS7	DCAF17	0.021653	Co-expression
GEMIN2	TMEM267	0.0039	Co-expression
GEMIN2	POT1	0.006586	Co-expression
TMEM267	DCAF17	0.017157	Co-expression
POT1	DCAF17	0.018457	Co-expression
POT1	CUL4B	0.016976	Co-expression
INTS7	PTTG1	0.009717	Co-expression
POLI	DCAF17	0.024595	Co-expression
POLI	POT1	0.015922	Co-expression
GEMIN2	POT1	0.014995	Co-expression

POT1	DCAF17	0.019193	Co-expression
NAA15	SKIV2L2	0.005278	Co-expression
INTS7	SKIV2L2	0.0093	Co-expression
INTS7	NAA15	0.011806	Co-expression
GEMIN2	CUL4B	0.014478	Co-expression
GEMIN2	POT1	0.017858	Co-expression
RAD17	CUL4B	0.018899	Co-expression
RAD17	GEMIN2	0.015564	Co-expression
AGO1	POT1	0.012863	Co-expression
VIPAS39	AGO1	0.006072	Co-expression
EXOC2	INTS7	0.01908	Co-expression
RAD17	POT1	0.027534	Co-expression
RAD17	VIPAS39	0.012628	Co-expression
INTS7	AGO1	0.019896	Co-expression
GEMIN2	PTTG1	0.002871	Co-expression
RAD17	CUL4B	0.013971	Co-expression
RAD17	GEMIN2	0.012185	Co-expression
INTS7	SKIV2L2	0.006518	Co-expression
BLMH	EYA3	0.014397	Co-localization
POT1	DCAF17	0.009539	Co-localization
EYA3	DCAF17	0.02755	Co-localization
EYA3	POT1	0.008808	Co-localization
AGO1	DCAF17	0.026643	Co-localization
AGO1	POT1	0.008327	Co-localization
ARL15	DCAF17	0.025506	Co-localization
ARL15	POT1	0.006414	Co-localization

NAA15	DCAF17	0.011778	Co-localization
NAA15	POT1	0.003766	Co-localization
NAA15	ARL15	0.008719	Co-localization
INTS7	DCAF17	0.023841	Co-localization
INTS7	POT1	0.008081	Co-localization
INTS7	NAA15	0.010854	Co-localization
BLMH	DCAF17	0.025363	Co-localization
BLMH	POT1	0.009025	Co-localization
BLMH	EYA3	0.02518	Co-localization
BLMH	NAA15	0.011865	Co-localization
VIPAS39	DCAF17	0.023599	Co-localization
VIPAS39	POT1	0.00637	Co-localization
VIPAS39	ARL15	0.025043	Co-localization
VIPAS39	NAA15	0.008351	Co-localization
EXOC2	DCAF17	0.024342	Co-localization
EXOC2	POT1	0.007643	Co-localization
EXOC2	ARL15	0.024647	Co-localization
EXOC2	VIPAS39	0.028187	Co-localization
GEMIN2	DCAF17	0.012483	Co-localization
GEMIN2	POT1	0.004406	Co-localization
GEMIN2	BLMH	0.01327	Co-localization
RAD17	DCAF17	0.015909	Co-localization
RAD17	POT1	0.005233	Co-localization
RAD17	EYA3	0.015112	Co-localization
PTTG1	DCAF17	0.200927	Genetic Interactions
NAA15	SKIV2L2	0.060909	Physical Interactions

CUL4B	DCAF17	0.115469	Physical Interactions
RAB40B	DCAF17	0.353314	Physical Interactions
ZMAT3	DCAF17	0.300627	Physical Interactions
BLMH	CUL4B	0.065557	Physical Interactions
POLI	CUL4B	0.13731	Predicted

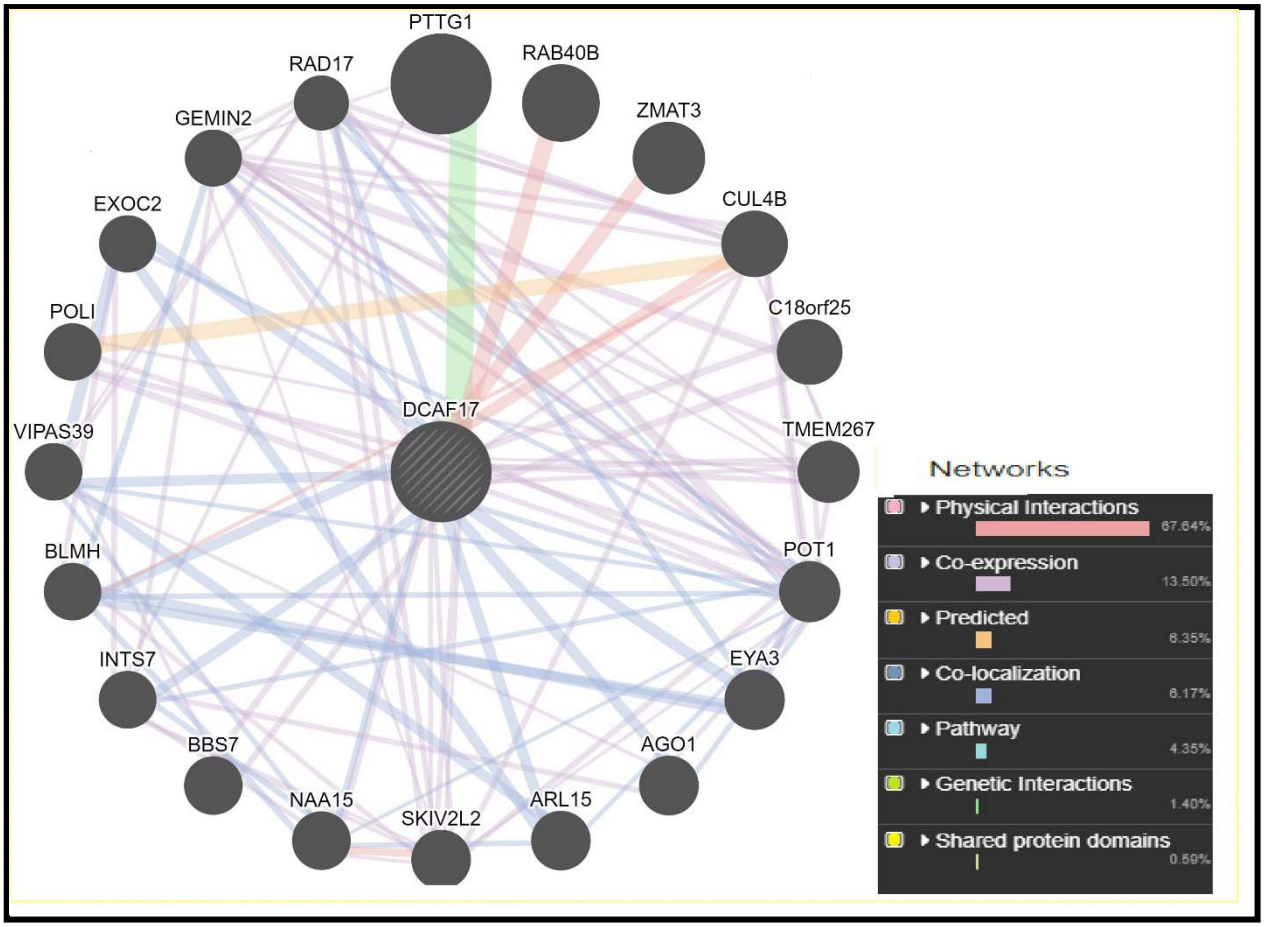

Figure. (2): Interaction between *DCAF17* gene and related genes using GeneMANIA.

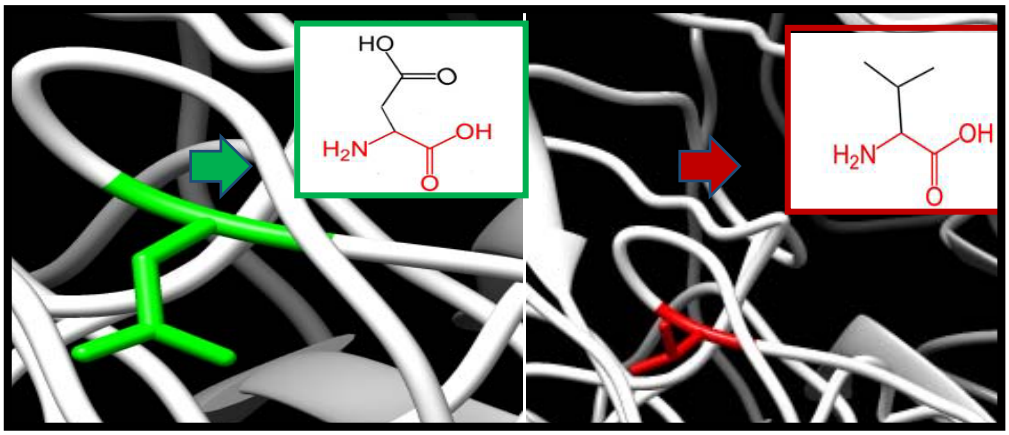

Figure. (3): D69V: amino acid change from Aspartic acid to Valine at position 69.

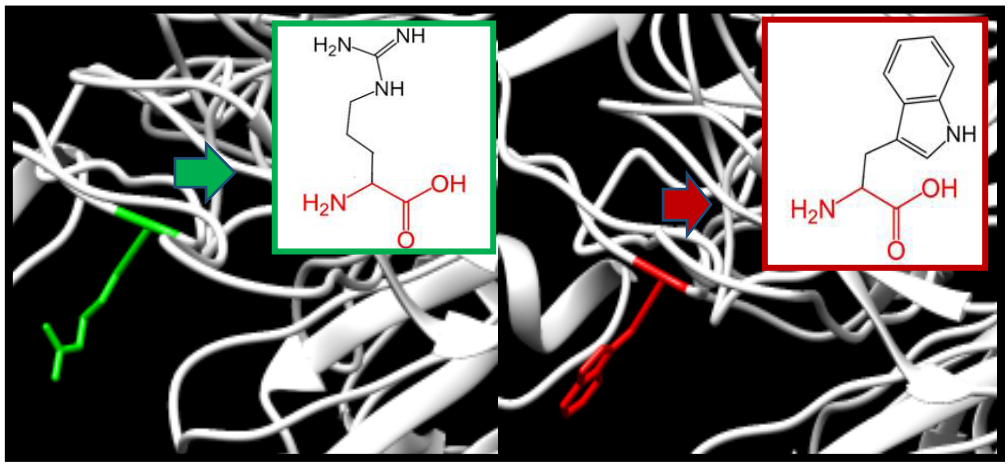

Figure. (4): R72W: amino acid change from Arginine to Tryptophan at position 72.

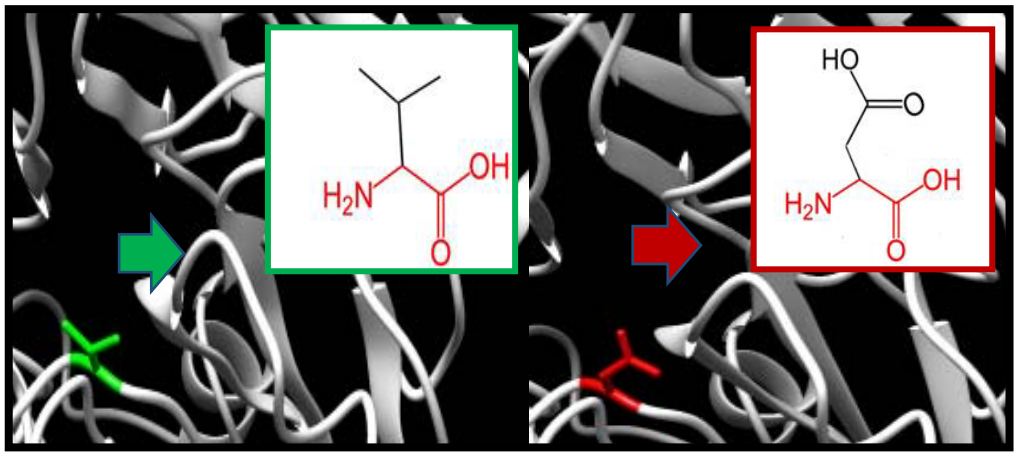

Figure. (5): V186D: amino acid Valine change to Aspartic acid at position 186.

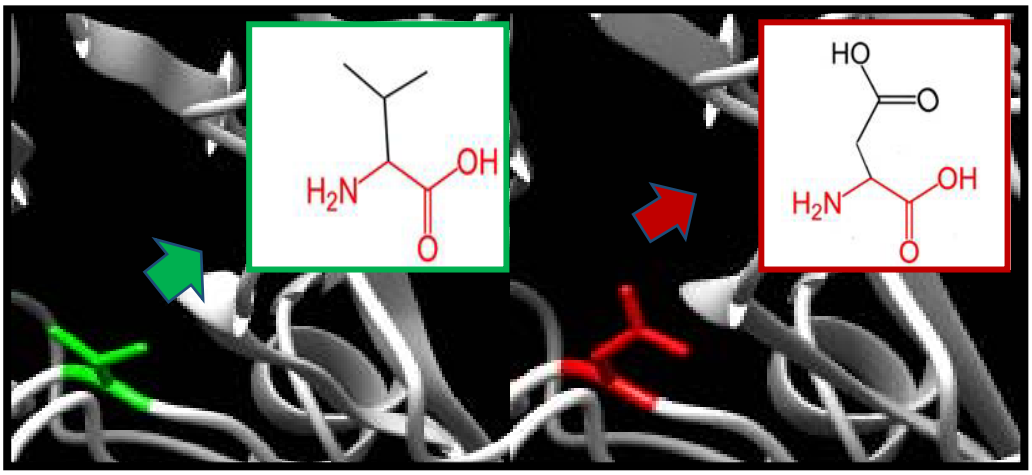

Figure. (6): P197L: amino acid Proline change to Leucine at position 197.

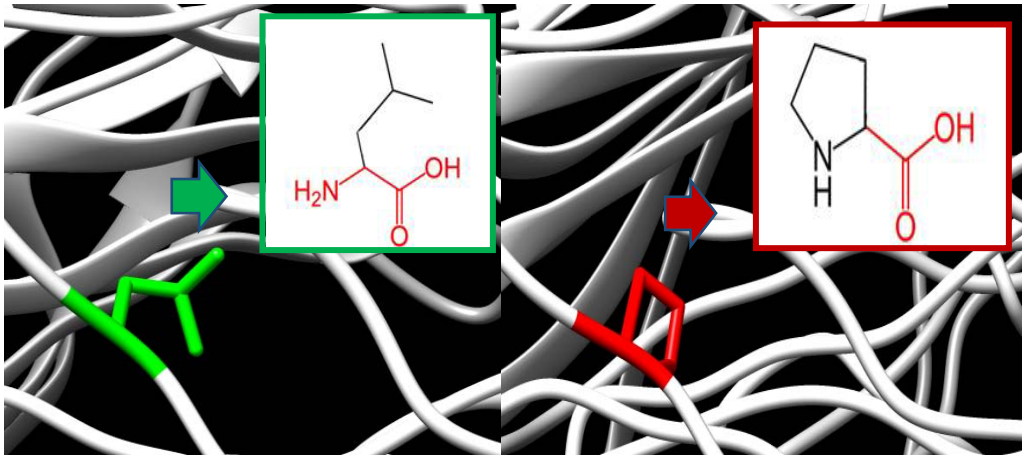

Figure. (7): L204P: aminoacid Leucine change to Proline at position 204.

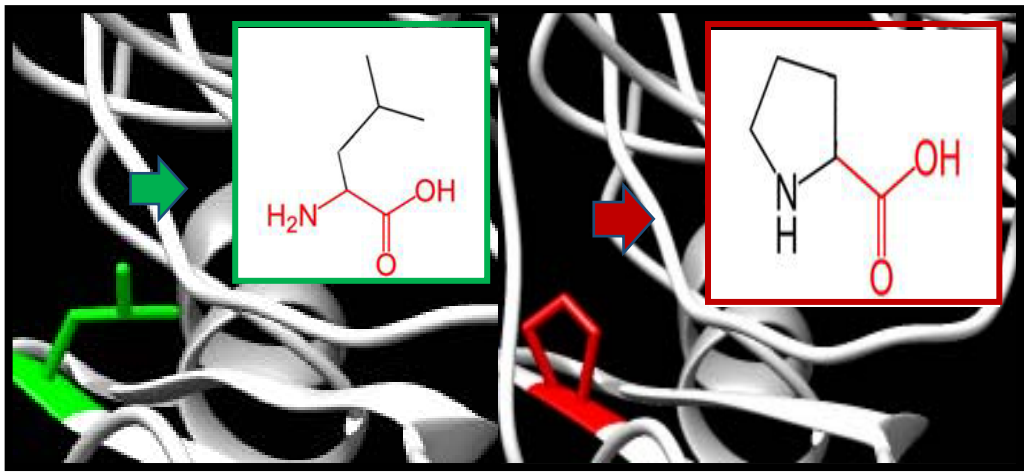

Figure. (8): L224P: amino acid change from Leucine to Proline at position 224.

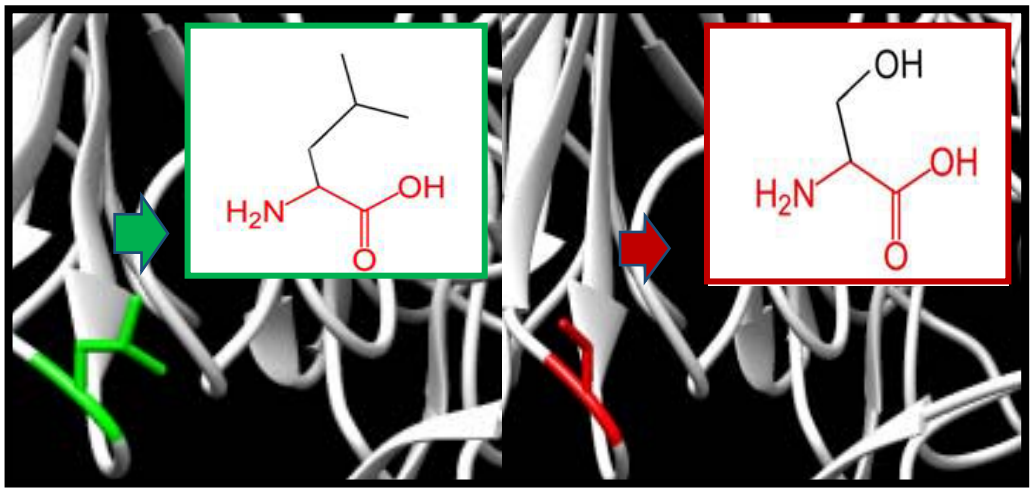

Figure. (9): L433S: amino acid Leucine change to Serine at position 4333.

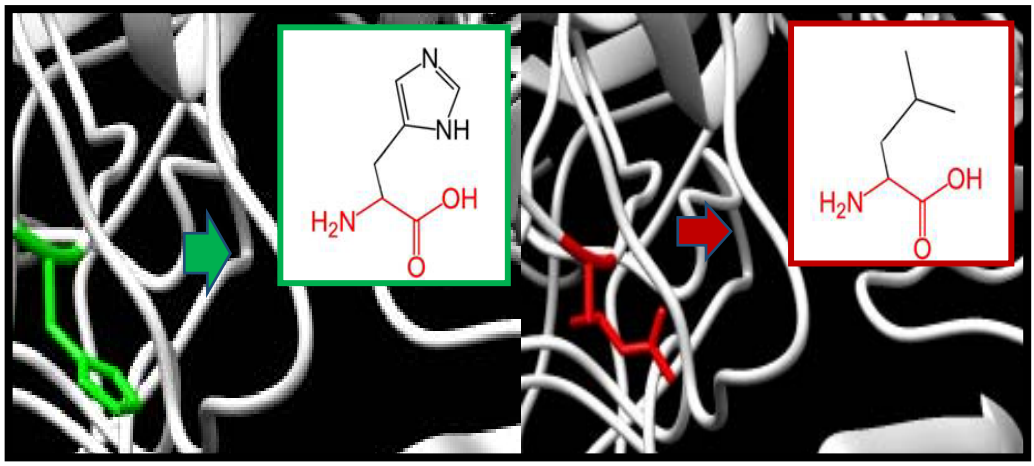

Figure. (10): H478R: amino acid Histidine change to Arginine at position 478.

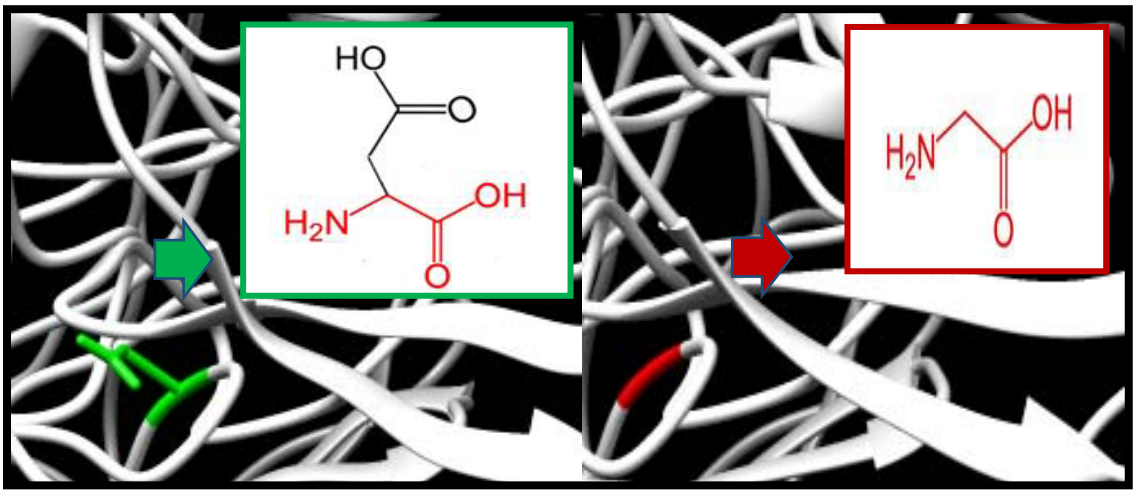

Figure. (11): D483G: amino acid Aspartic acid change to Glycine at position 483.

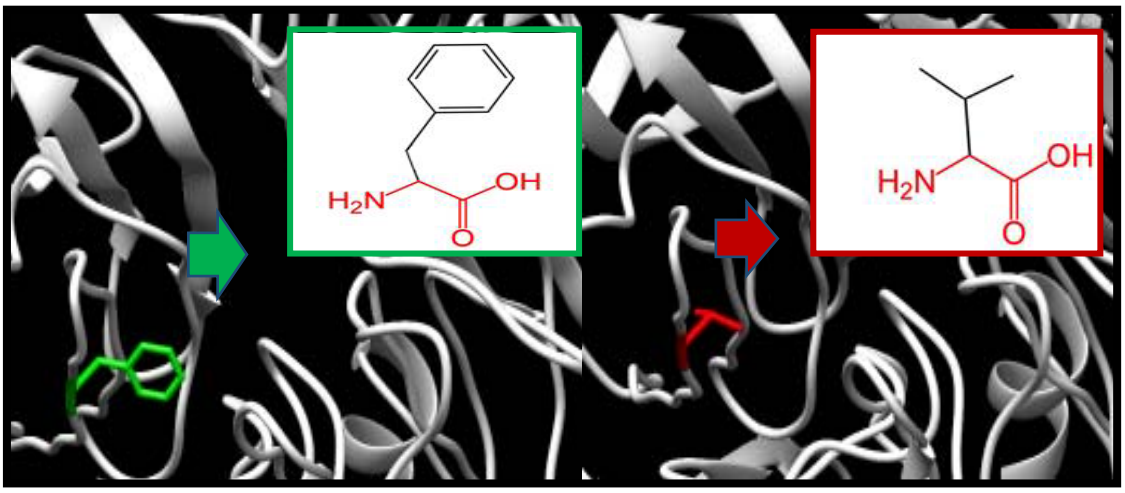

Figure. (12): F498V: amino acid Phenylalanine change to Valine at position 498.

Discussion:

Ten novel mutations have been found that affect the stability, structure and the function of *DCAF17* gene with one associated microRNA in the 3'-UTR region utilizing various *in silico* bioinformatics tools . These methods for prediction used standard tools and multiple disciplines to detect and characterize the missense variation. In order to ensure our results and increase the accuracy; eight web server *in silico* algorithm have been used: SIFT, Proven, Polyphen, SNAP 2, SNP&GO, PHD, Pmut and I mutant.

This gene considered to be with no function but it has relation to the disease Woodhouse-Sakati Syndrome with MIM no 241080 (from NCBI) [13, 45]. Because there is no similar mutation pathogenicity prediction study to this gene; this founding considered to be novel mutation [46] ,[13], [47].

GeneMANIA did not indicated specific function for *DCAF17* gene , but there is interaction with another genes [47], [15], [48], [14]. The function of the gene is not clear until now [13].

Project HOPE server was used to predict the effect of the amino acid substitutions on the molecular level of the protein. The ten SNPs were submitted to hope server individually and the obtained result indicated a change in the size, hydrophobicity and charge of the final protein, all the SNPs were found in Ddb1- And Cul4-Associated Factor 17 IPR031620 domain region in the gene, which further confirm its pathogenicity.

All the mutant amino acids except three (R72W , V186D, P197L) were found to be smaller in size in comparison to the wild type which may lead to loss of interactions with other molecules or residues. The hydrophobicity of the wild-type and mutant residue differs , there were 6 SNPs mentioned in project hope report (D69V, R72W, V186D, L433P, H478L, D483G) that has mutant amino acid with more hydrophobicity that could lead to loss of hydrogen bonds and/or disturb correct folding. There is a difference in charge between the wild-type and mutant amino acid , with three SNPS(D69V, R72W, D483G) mentioned in the report to have different nerutral charge and one SNPs (V186D) with negative charge in the mutant amino acids which could cause a loss of interactions with other molecules or residues.

Different biological processes are controlled by transcriptional regulation done by non-coding RNA molecules (miRNA) consisting of 18–24 nucleotides in length which could activate and/or suppress protein translation inside the cells at post-transcriptional level.[49-52] it was found in gene card site (https://www.genecards.org/) that *DCAF17* gene may be dysregulated by miRNA, so we analyzed the microRNA in the 3'-UTR region of *DCAF17* gene using multiple softwares (ensemble, regRNA2, miRmap and mirbase). We found one relevant miRNA; (hsa-miR-4436b-3p) with PhyloP conservation score of 0.71 and after analyzing its annotation through miRmap using sequence from 5 species human,mouse,worm,fly and Arabidopsis it appeared to have a role in inhibiting adipose triglyceride lipase, carcinogenesis and other possible roles .

This study was limited as it depended in computerized software and it need further wet lab studies for confirmation.

We now appreciate the regulatory role of the 3'-UTR in the function of DCAF17 protein and with this 10 novel mutation we could develop a precise biomarker for Woodhouse-Sakati Syndrome in the future .

Conclusion:

Ten novel SNPs were found to be deleterious in this paper with possible role as future biomarker for Woodhouse-Sakati syndrome, and one microRNA was found in the 3'-UTR to regulate the function of the protein.

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Conflict of Interest:

No conflict of interest to declare.

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References:

1. Rachmiel M, Bistritzer T, Hershkoviz E, Khahil A, Epstein O, Parvari R. Woodhouse-Sakati syndrome in an Israeli-Arab family presenting with youth-onset diabetes mellitus and delayed puberty. Horm Res Paediatr. 2011;75(5):362-6.
2. Hdiji O, Turki E, Bouzidi N, Bouchhima I, Damak M, Bohlega S, et al. Woodhouse-Sakati Syndrome: Report of the First Tunisian Family with the C2orf37 Gene Mutation. J Mov Disord. 2016;9(2):120-3.
3. Abdulla MC, Alazami AM, Alungal J, Koya JM, Musambil M. Novel compound heterozygous frameshift mutations of C2orf37 in a familial Indian case of Woodhouse-Sakati syndrome. J Genet. 2015;94(3):489-92.
4. Agopiantz M, Corbonnois P, Sorlin A, Bonnet C, Klein M, Hubert N, et al. Endocrine disorders in Woodhouse-Sakati syndrome: a systematic review of the literature. J Endocrinol Invest. 2014;37(1):1-7.
5. Abusrair AH, Bohlega S, Al-Semari A, Al-Ajlan FS, Al-Ahmadi K, Mohamed B, et al. Brain MR Imaging Findings in Woodhouse-Sakati Syndrome. AJNR Am J Neuroradiol. 2018;39(12):2256-62.
6. Al-Swailem SA, Al-Assiri AA, Al-Torbak AA. Woodhouse Sakati syndrome associated with bilateral keratoconus. Br J Ophthalmol. 2006;90(1):116-7.
7. Ben-Omran T, Ali R, Almureikhi M, Alameer S, Al-Saffar M, Walsh CA, et al. Phenotypic heterogeneity in Woodhouse-Sakati syndrome: two new families with a mutation in the C2orf37 gene. Am J Med Genet A. 2011;155A(11):2647-53.

8. Gurbuz F, Desai S, Diao F, Turkkahraman D, Wranitz F, Wood-Trageser M, et al. Novel inactivating mutations of the DCAF17 gene in American and Turkish families cause male infertility and female subfertility in the mouse model. Clin Genet. 2018;93(4):853-9.
9. Koshy G, Danda S, Thomas N, Mathews V, Viswanathan V. Three siblings with Woodhouse-Sakati syndrome in an Indian family. Clin Dysmorphol. 2008;17(1):57-60.
10. Medica I, Sepcic J, Peterlin B. Woodhouse-Sakati syndrome: case report and symptoms review. Genet Couns. 2007;18(2):227-31.
11. Alazami AM, Al-Saif A, Al-Semari A, Bohlega S, Zlitni S, Alzahrani F, et al. Mutations in C2orf37, encoding a nucleolar protein, cause hypogonadism, alopecia, diabetes mellitus, mental retardation, and extrapyramidal syndrome. Am J Hum Genet. 2008;83(6):684-91.
12. Schneider SA, Bhatia KP. Dystonia in the Woodhouse Sakati syndrome: A new family and literature review. Mov Disord. 2008;23(4):592-6.
13. Almeqdadi M, Kemppainen JL, Pichurin PN, Gavrilova RH. Phenotypic Variability of c.436delC DCAF17 Gene Mutation in Woodhouse-Sakati Syndrome. The American journal of case reports. 2018;19:347-53.
14. Sheridan MB, Wohler E, Batista DA, Applegate C, Hoover-Fong J. The Use of High-Density SNP Array to Map Homozygosity in Consanguineous Families to Efficiently Identify Candidate Genes: Application to Woodhouse-Sakati Syndrome. Case Rep Genet. 2015;2015:169482.
15. Dastsooz H, Nemati H, Fard MAF, Fardaei M, Faghihi MA. Novel mutations in PANK2 and PLA2G6 genes in patients with neurodegenerative disorders: two case reports. BMC Med Genet. 2017;18(1):87.
16. Nishizaki SS, Boyle AP. Mining the Unknown: Assigning Function to Noncoding Single Nucleotide Polymorphisms. Trends in genetics : TIG. 2017;33(1):34-45.
17. Li L, Chen Q, Wei DQ. Prediction and functional analysis of single nucleotide polymorphisms. Current drug metabolism. 2012;13(7):1012-23.
18. Murray D, Doran P, MacMathuna P, Moss AC.
19. Habib R, Basit S, Khan S, Khan MN, Ahmad W. A novel splice site mutation in gene C2orf37 underlying Woodhouse-Sakati syndrome (WSS) in a consanguineous family of Pakistani origin. Gene. 2011;490(1-2):26-31.
20. Nanda A, Pasternack SM, Mahmoudi H, Ishorst N, Grimalt R, Betz RC. Alopecia and hypotrichosis as characteristic findings in Woodhouse-Sakati syndrome: report of a family with mutation in the C2orf37 gene. Pediatr Dermatol. 2014;31(1):83-7.
21. Wang T, Mao J, Liu MJ, Choy KW, Li HB, Cram DS, et al. A patient with five chromosomal rearrangements and a 2q31.1 microdeletion. Clin Chim Acta. 2014;430:129-33.
22. Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations using PolyPhen-2. Curr Protoc Hum Genet. 2013;Chapter 7:Unit7 20.
23. Ng PC, Henikoff SJGr. Accounting for human polymorphisms predicted to affect protein function. 2002;12(3):436-46.
24. Manickam M, Ravanan P, Singh P, Talwar P. In silico identification of genetic variants in glucocerebrosidase (GBA) gene involved in Gaucher's disease using multiple software tools. Front Genet. 2014;5:148.
25. Ng PC, Henikoff SJARGHG. Predicting the effects of amino acid substitutions on protein function. 2006;7:61-80.
26. Schneider G, Hu J, Sim N-L, Kumar P, Henikoff S, Ng PC.

27. 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.
28. Kumar P, Mahalingam K. In silico approach to identify non-synonymous SNPs with highest predicted deleterious effect on protein function in human obesity related gene, neuronal growth regulator 1 (NEGR1). 3 Biotech. 2018;8(11):466.
29. Ramensky V, Bork P, Sunyaev S. Human non-synonymous SNPs: server and survey. Nucleic acids research. 2002 Sep 1;30(17):3894-900.
30. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. Nature methods. 2010;7(4):248-9.
31. Calabrese R, Capriotti E, Fariselli P, Martelli PL, Casadio RJHm. Functional annotations improve the predictive score of human disease-related mutations in proteins. 2009;30(8):1237-44.
32. López-Ferrando V, Gazzo A, de la Cruz X, Orozco M, Gelpí JLJNar. PMut: a web-based tool for the annotation of pathological variants on proteins, 2017 update. 2017;45(W1):W222-W8.
33. Capriotti E, Fariselli P, Casadio RJNar. I-Mutant2. 0: predicting stability changes upon mutation from the protein sequence or structure. 2005;33(suppl_2):W306-W10.
34. Venselaar H, te Beek TA, Kuipers RK, Hekkelman ML, Vriend GJBb. Protein structure analysis of mutations causing inheritable diseases. An e-Science approach with life scientist friendly interfaces. 2010;11(1):548.
35. Warde-Farley D, Donaldson SL, Comes O, Zuberi K, Badrawi R, Chao P, et al. The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. 2010;38(suppl_2):W214-W20.
36. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, et al. UCSF Chimera—a visualization system for exploratory research and analysis. 2004;25(13):1605-12.
37. Cunningham F, Achuthan P, Akanni W, Allen J, Amode MR, Armean IM, et al. Ensembl 2019. Nucleic Acids Res. 2019;47(D1):D745-D51.
38. Frankish A, Vullo A, Zadissa A, Yates A, Thormann A, Parker A, et al.
39. Huang HY, Chien CH, Jen KH, Huang HD. RegRNA: an integrated web server for identifying regulatory RNA motifs and elements. Nucleic Acids Res. 2006;34(Web Server issue):W429-34.
40. Chang TH, Huang HY, Hsu JB, Weng SL, Horng JT, Huang HD. An enhanced computational platform for investigating the roles of regulatory RNA and for identifying functional RNA motifs. BMC Bioinformatics. 2013;14 Suppl 2:S4.
41. Vejnar CE, Zdobnov EM.
42. Kozomara A, Birgaoanu M, Griffiths-Jones S. miRBase: from microRNA sequences to function. Nucleic Acids Res. 2019;47(D1):D155-D62.
43. Kozomara A, Griffiths-Jones S. miRBase: annotating high confidence microRNAs using deep sequencing data. Nucleic Acids Res. 2014;42(Database issue):D68-73.
44. Kozomara A, Birgaoanu M, Griffiths-Jones S.
45. Ali A, Mistry BV, Ahmed HA, Abdulla R, Amer HA, Prince A, et al. Publisher Correction: Deletion of DDB1- and CUL4- associated factor-17 (Dcaf17) gene causes spermatogenesis defects and male infertility in mice. Scientific reports. 2018;8(1):11779.

46. Ali A, Mistry BV, Ahmed HA, Abdulla R, Amer HA, Prince A, et al. Deletion of DDB1- and CUL4- associated factor-17 (Dcaf17) gene causes spermatogenesis defects and male infertility in mice. Scientific reports. 2018;8(1):9202.
47. Arber CE, Li A, Houlden H, Wray S. Review: Insights into molecular mechanisms of disease in neurodegeneration with brain iron accumulation: unifying theories. Neuropathology and applied neurobiology. 2016;42(3):220-41.
48. Gispert S, Brehm N, Weil J, Seidel K, Rub U, Kern B, et al. Potentiation of neurotoxicity in double-mutant mice with Pink1 ablation and A53T-SNCA overexpression. Human molecular genetics. 2015;24(4):1061-76.
49. Kamaraj B, Gopalakrishnan C, Purohit R. In silico analysis of miRNA-mediated gene regulation in OCA and OA genes. Cell Biochem Biophys. 2014;70(3):1923-32.
50. Kristjansdottir K, Fogarty EA, Grimson A. Systematic analysis of the Hmga2 3' UTR identifies many independent regulatory sequences and a novel interaction between distal sites. RNA. 2015;21(7):1346-60.
51. Liang B, Zhao J, Wang X. A three-microRNA signature as a diagnostic and prognostic marker in clear cell renal cancer: An In Silico analysis. PLoS One. 2017;12(6):e0180660.
52. Yu L, Xiang L, Feng J, Li B, Zhou Z, Li J, et al.