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Computational Analysis of Single Nucleotide Polymorphism (SNPs) in Human GRM4 Gene

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Abstract **Background:** L-glutamate is one of the most common amino acid in nature and it acts as excitatory neurotransmitter in the central nervous system. GRM4 is a large gene located in chromosome 6 and consists of 7217 bp (NCBI) divided into 10 exons. The location of GRM4 (chromosomal segment 6p21.3) is tentative susceptibility loci for Juvenile Myoclonic Epilepsy so many studies investigate the association of GRM4 polymorphism with myoclonic epilepsy juvenile. **Design and methods:** GRM4 gene was investigated in dbSNP/NCBI database NCBI and we used computational analysis approach. Deleterious nsSNPs were predicted by SIFT and Polyphen soft wares then the damaging nsSNPs were submitted to I mutant tool. Protein structural analysis of amino acid variants was performed by Chimera 1.8 and Project Hope. **Results:** We analyze 29854 SNPs from NCBI; 8561 of them found on homosapiens; of which 330 were missense, of which 208 were in the coding region, 334 were non-synonymous SNPs (nsSNPs), 232 were in the 3'un-translated region. These SNPs were analyzed using different soft wares; SIFT, Polyphen-2, Imutant3.0, PhD-SNP, PolymiRTs, Project Hope and GENEMAIA to investigate the effect of SNPs mutations on GRM4 protein structure and function. **Conclusions:** Computational tools were used to analyze deleterious SNPs in GRM4 gene. Out of 8561 SNPs, the SNPs (rs184636998), (rs199744441), (rs199744441), (rs149277708), (rs144660534), (rs139236496) were identified as highly damaging in coding region confirmed by using bioinformatics tools . In 3'un-translated region two SNPs (rs188406833) and (rs192860479) contained (C) allele had 4 miRSite as target binding site can be disrupts a conserved miRNA and one SNPs (rs77415386) contained (D) allele had 6 miRSite as derived allele that disrupts a conserved miRNA site. Further study must be done to detect the effect of these SNPs on the protein structure and function.

Keywords: *glutamate, neurotransmitter, ionotropic, metabotropic, Myoclonic Epilepsy, SNPs*

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1. Introduction

L-glutamate is one of the most common amino acid in nature and it acts as excitatory neurotransmitter in the central nervous system. Glutamatergic neurotransmission is involved in most aspects of normal brain function and can be perturbed in many neuropathologic conditions. The metabotropic glutamate receptors are a family of G protein-coupled receptors that have been divided into 3 groups on the basis of sequence homology, putative signal transduction mechanisms, and pharmacologic properties. Group I includes GRM1 and GRM5 and these receptors have been shown to activate phospholipase C. Group II includes GRM2 and GRM3 while Group III includes GRM4, GRM6, GRM7 and GRM8. [8,9,14]

GRM4 is encoded to Metabotropic glutamate receptor 4 (mGluR4) which is belongs to group III of the metabotropic glutamate receptor family. [9,14] group III receptors are coupled to Gi/o which inhibits adenylyl cyclase, decreasing the formation of Camp and they are involved in spatial learning and memory, and umami taste (mGlu4), stimulation of photoreceptors (mGlu6), long term depression (mGlu7) and presynaptic inhibition in the perforant pathway (mGlu8). [7,8]

GRM4 is a large gene located in chromosome 6 and consists of 7217 bp (NCBI) divided into 10 exons [7,8].

The location of GRM4 (chromosomal segment 6p21.3) is tentative susceptibility loci for Juvenile Myoclonic Epilepsy so many studies investigate the association of GRM4 polymorphism with myoclonic epilepsy juvenile [10,13]. There are also some studies investigate the association of GRM4 polymorphism with schizophrenia, depression, Colon cancer and tumors [7,8].

Single-nucleotide polymorphism (SNPs) most commonly refer to single-base differences in DNA among individuals. SNPs of various types can change the function or the regulation and expression of a protein. There is no studies on GRM4 single nucleotide polymorphism. In this study we will analyze GRM4 SNPs using bioinformatics prediction tools to check the effect of the mutation on the protein structure and function.

2. Material and Methods

GRM4 gene was investigated in dbSNP/NCBI database NCBI (http://www.ncbi.nlm.nih.gov/snp). GRM4 gene contained a total of 29854 SNPs; 8561 of them found on homosapiens; of which 330 were missense, of which 208 were in the coding region, 334 were non-synonymous SNPs (nsSNPs), 232 were in the 3'un-translated region. Predictions of deleterious nsSNPs was performed by SIFT and Polyphen softwares. Damaging nsSNPs by two these servers were submitted to I mutant tool, then the functional impact of the deleterious SNPS was analyzed by project hope. The FASTA format of the protein was obtained from Uniprot at Expassy database. The 3D structure of a 65% identical to protein was retrieved from database by using BAST/NCBI. The protein used as a template is called “Metabotropic glutamate receptor 4 [Homo sapiens]" with ID pdb|3MQ4|. The SNPS at the 3UTR region were analyzed by Polymirt software.

3. Bioinformatics Data Analysis

3.1. Deleterious nsSNP Analyze by the SIFT Program

Sorting Intolerant from Tolerant (SIFT, version 2) [3]. The SIFT prediction, as previously described is based on perform multiple alignments of a number of peptide sequences and it predicts whether substitution with any of the other amino acids is tolerated or deleterious for every position in the submitted sequence. The SIFT prediction was given as a tolerance index (TI) score ranging from 0.0 to 1.0, which was the normalized probability that the amino acid change was tolerated. The SNP with a TI score of <0.05 was considered to be deleterious i.e. an amino acids with probabilities < 0.05 were predicted to be deleterious. (http://blocks.fhcrc.org/sift/SIFT.html).

3.2. PolyPhen -2

Polymorphism Phenotyping v2, [6] this software has position-specific independent count (PSIC) the score of 1.0 is considered to be damaging, and the nsSNP affect protein structure. (PolyPhen-2, v.2.2.2; http://genetics.bwh.harvard.edu/pph).

3.3. Investigation of Mutant Protein Stability by I-Mutant 3.0

I- Mutant 3.0 is a Support Vector Machine-based web server was used to predict protein stability changes upon single-site mutations. The input FASTA sequence of protein along with the residues change was provided for analysis of DDG value (kcal/mol) Also the RI value (reliability index) was computed [4]. (I-Mutant 3.0/I-Mutant3.0.cgi).

3.3. PhD-SNP

An Online support vector machine (SVM) based classifier, which can predict if the new phenotype produced after nsSNP can be related to a genetic disease or as a neutral polymorphism. (http://snps.biofold.org /phd-snp/phd-snp.html [2]

3.4. PolymiRTS

PolymiRTS database [20] (http://compbio.uthsc.edu/miRSNP/) [1] were used to predict the effect of SNPs in the GRm4 mRNA 3′-UTR on the mRNA.

3.5. Project HOPE

Project Hope software [11] revealed many differences between wild and mutant residues as shown in Table 2. Also the project results of the mutation analyses are illustrated with figures of the amino acids. (http://www.cmbi.ru.nl/hope/home).

3.6. Chimera

Chimera [5] is a software for analysis of molecular structures, this software can give the 3D structure of the protein and then changing between native and mutant amino acids with the candidate to display the impact that can be produced. Chimera accepts the input in the form of pdp ID or pdp file. (https://www.cgl.ucsf.edu/chimera/).

3.7. Gene MANIA

Gene MANIA an online server gives the function, interaction and the network of the gene based on genomics and proteomics data with a large database and high accuracy [12]. (http://www.genemania.org)

4. Result

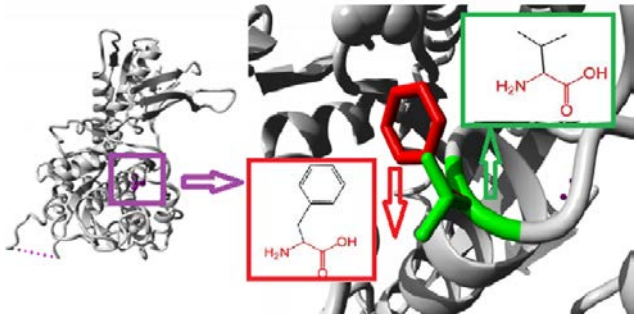

Figure 1. change in the amino acids in positions 144 from valine into phenylalanine

Table 1. shows of nonsynonymous SNPs predicted with SIFT, Polyphen and I mutant programs, chosen SNPs with PSIC SD equal 1 and TOLERANCE INDEX rang (0 – 0.001)

				Sift prediction		POLYPHEN prediction	I mutant				PHD-snp	
SNP	NUCLEOTIDE CHANGE	protein ID	AMINO ACID CHANGE	SIFT RESULT	TOLERANCE INDEX	POLYPHEN prediction	PSIC SD	SVM2	RI	DDG value	Effect	RI
rs184636998	C/T	ENSP00000363292	V737M	DELETERIOUS	0.001	PROBABLY DAMAGING	1	DECREASE	6	-0.76	Disease	4
rs199744441	G/A	ENSP00000440556	P849L	DELETERIOUS	0	PROBABLY DAMAGING	1	DECREASE	5	0.59	Disease	3
rs199744441	G/A	ENSP00000437730	P680L	DELETERIOUS	0	PROBABLY DAMAGING	1	DECREASE	4	-0.55	Disease	3
rs199744441	G/A	ENSP00000363292	P733L	DELETERIOUS	0.001	PROBABLY DAMAGING	1	DECREASE	4	-0.55	Disease	3
rs199744441	G/A	ENSP00000398456	P709L	DELETERIOUS	0.001	PROBABLY DAMAGING	1	DECREASE	4	-0.55	Disease	3
rs149277708	C/T	ENSP00000363292	R507H	DELETERIOUS	0.001	PROBABLY DAMAGING	1	DECREASE	8	-1.18	Disease	7
rs149277708	C/T	ENSP00000440556	R623H	DELETERIOUS	0.001	PROBABLY DAMAGING	1	DECREASE	9	-1.49	Disease	7
rs149277708	C/T	ENSP00000398456	R483H	DELETERIOUS	0.001	PROBABLY DAMAGING	1	DECREASE	8	-1.36	Disease	7
rs149277708	C/T	ENSP00000437925	R490H	DELETERIOUS	0.001	PROBABLY DAMAGING	1	DECREASE	9	-1.49	Disease	7
rs144660534	C/T	ENSP00000437730	R454H	DELETERIOUS	0.001	PROBABLY DAMAGING	1	DECREASE	9	-1.49	Disease	7
rs144660534	G/A	ENSP00000398456	R334C	DELETERIOUS	0.001	PROBABLY DAMAGING	1	Decrease	4	-1	Neutral	4
rs139236496	C/A	ENSP00000363292	V144F	DELETERIOUS	0.001	PROBABLY DAMAGING	1	DECREASE	7	-1.01	Disease	8
rs139236496	C/A	ENSP00000398456	V73F	DELETERIOUS	0.001	PROBABLY DAMAGING	1	Decrease	7	-1.01	Disease	5

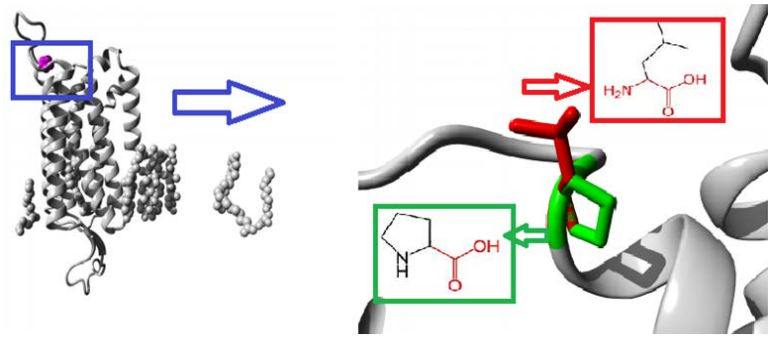

Figure 2. shows change in the amino acids from proline into a leucine in positions (680)

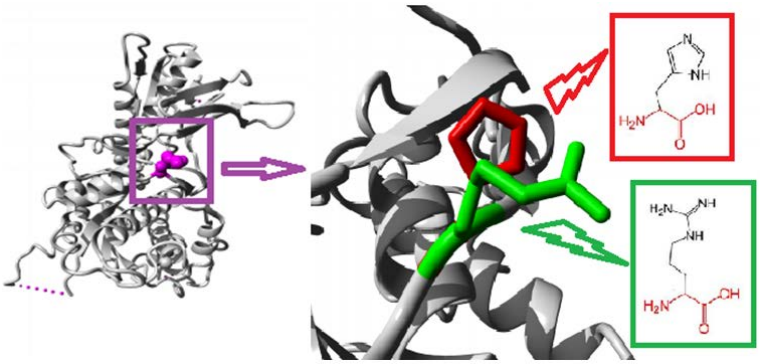

Figure 3. shows change in the amino acid from arginine into histidine in positions (507)

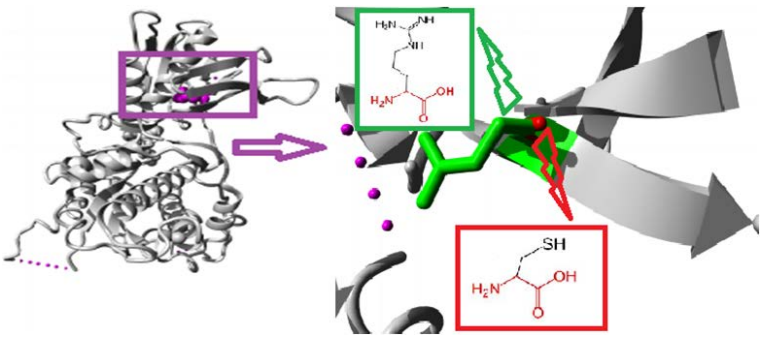

Figure 4. shows change in the amino acid in position 334 from arginine to cysteine

Table 2. shows the SNPs predicted by Polymirt to induce disruption or formation of mirRNA binding site

Location	dbSNP ID	Variant type	Wobble base pair	Ancestral Allele	Allele	miR ID	Conservation	miRSite	Function Class	Exp Support	context+ score change
33989624	rs149624269	SNP	Y	G	A	hsa-miR-6741-5p	19	GCACCCAg	C	N	-0.33
						hsa-miR-6776-5p	19	gCACCCAG	C	N	-0.233
33989848	rs113261322	SNP	Y	G	G	hsa-miR-4767	10	cctgtgGCCCGCA	D	N	-0.228
					A	hsa-miR-3621	10	cctgtGACCCGCA	C	N	-0.503
						hsa-miR-3656	10	cctgtgACCCGCA	C	N	-0.19
						hsa-miR-513a-5p	7	CCTGTGAcccgca	C	N	-0.026
33989853	rs34678276	INDEL	N	-	-	hsa-miR-211-3p	6	gtGTCCCTGtggc	O	N	-0.137
						hsa-miR-7845-5p	6	gTGTCCCTgtggc	O	N	-0.083
					C						
33990114	rs182834289	SNP	N	C	C	hsa-miR-1255a	3	tCTCATCCtcttt	D	N	-0.145
						hsa-miR-1255b-5p	3	tCTCATCCtcttt	D	N	-0.145
						hsa-miR-3605-5p	2	tctCATCCTCttt	D	N	-0.147
						hsa-miR-583	2	tctcatCCTCTTT	D	N	-0.032
					T	hsa-miR-3123	2	tctcATTCTCTtt	C	N	0.032
						hsa-miR-4311	2	tctcatTCTCTTT	C	N	-0.031
33990136	rs78795224	SNP	N	T	T	hsa-miR-31-5p	4	ggcTCTTGCCtcc	D	N	-0.047
						hsa-miR-335-5p	4	gGCTCTTGcctcc	D	N	-0.059
33990137	rs76783395	SNP	N	T	T	hsa-miR-31-5p	4	tggcTCTTGCCtc	D	N	-0.047
						hsa-miR-335-5p	4	tgGCTCTTGcctc	D	N	-0.059
					A	hsa-miR-6807-5p	3	TGGCTCAtgcctc	C	N	-0.109
33990180	rs188406833	SNP	Y	G	A	hsa-miR-4274	2	tccccGACTGCTt	C	N	-0.102
						hsa-miR-542-5p	2	TCCCCGActgctt	C	N	-0.195
						hsa-miR-6777-5p	2	TCCCCGActgctt	C	N	-0.175
						hsa-miR-6889-5p	2	TCCCCGActgctt	C	N	-0.172
33990234	rs192860479	SNP	Y	G	G	hsa-miR-4524b-3p	8	agcCCTGTCTttc	D	N	-0.113
					A	hsa-miR-3158-3p	2	AGCCCTAtctttc	C	N	-0.154
						hsa-miR-4446-3p	2	AGCCCTAtctttc	C	N	-0.184
						hsa-miR-4662a-3p	10	agccCTATCTTtc	C	N	-0.04
						hsa-miR-5088-5p	2	AGCCCTAtctttc	C	N	-0.154
33990290	rs77415386	SNP	N	C	C	hsa-miR-149-3p	2	atctctCCCTCCC	D	N	-0.19
						hsa-miR-4728-5p	2	atctctCCCTCCC	D	N	-0.209
						hsa-miR-6785-5p	2	atctctCCCTCCC	D	N	-0.218
						hsa-miR-6797-5p	2	atctcTCCCTCCc	D	N	-0.141
						hsa-miR-6883-5p	2	atctctCCCTCCC	D	N	-0.199
					T	hsa-miR-7106-5p	2	atctctTCCTCCC	C	N	-0.223
						hsa-miR-766-5p	2	atctcTTCCTCCc	C	N	-0.095
33990393	rs185477653	SNP	Y	G	G	hsa-miR-4707-5p	4	ggGCCGGGGcggt	D	N	-0.213
					A	hsa-miR-6075	4	GGGCCGAggcggt	C	N	-0.223
33990402	rs75362579	SNP	Y	G	G	hsa-miR-4655-3p	5	gaggACGAGGGgc	D	N	-0.164
						hsa-miR-4749-3p	6	gaggacGAGGGGC	D	N	-0.141
						hsa-miR-6795-3p	5	gaggaCGAGGGGc	D	N	-0.172
					A	hsa-miR-2355-3p	2	gAGGACAAggggc	C	N	-0.05
						hsa-miR-3909	2	GAGGACAaggggc	C	N	-0.114
						hsa-miR-676-3p	2	gAGGACAAggggc	C	N	-0.078
						hsa-miR-6780b-3p	5	gaggACAAGGGgc	C	N	-0.068
						hsa-miR-6852-3p	2	GAGGACAaggggc	C	N	-0.128
33990447	rs2229901	SNP	Y	G	A	hsa-miR-3652	8	CCAGCCAtcactg	C	N	-0.123
						hsa-miR-4430	8	CCAGCCAtcactg	C	N	-0.123
						hsa-miR-4505	8	CCAGCCAtcactg	C	N	-0.204
						hsa-miR-5787	8	CCAGCCAtcactg	C	N	-0.204
						hsa-miR-6842-3p	8	CCAGCCAtcactg	C	N	-0.191
						hsa-miR-889-5p	8	cCAGCCATcactg	C	N	-0.073
					G	hsa-miR-5190	8	ccagccGTCACTG	D	N	-0.094
33990499	rs190359317	SNP	N	C	C	hsa-miR-6721-5p	9	CCTGCCCgtgggc	D	N	-0.145
						hsa-miR-7112-5p	10	cCTGCCCGtgggc	D	N	-0.203
					T	hsa-miR-4514	10	cCTGCCTGtgggc	C	N	-0.081
						hsa-miR-4692	10	cCTGCCTGtgggc	C	N	-0.081
						hsa-miR-6808-5p	9	CCTGCCTgtgggc	C	N	-0.132
						hsa-miR-6893-5p	9	CCTGCCTgtgggc	C	N	-0.122
						hsa-miR-940	9	CCTGCCTgtgggc	C	N	-0.132
33990557	rs139823405	SNP	N	C	C	hsa-miR-1178-5p	2	cgTGACCCTgtgg	D	N	-0.111

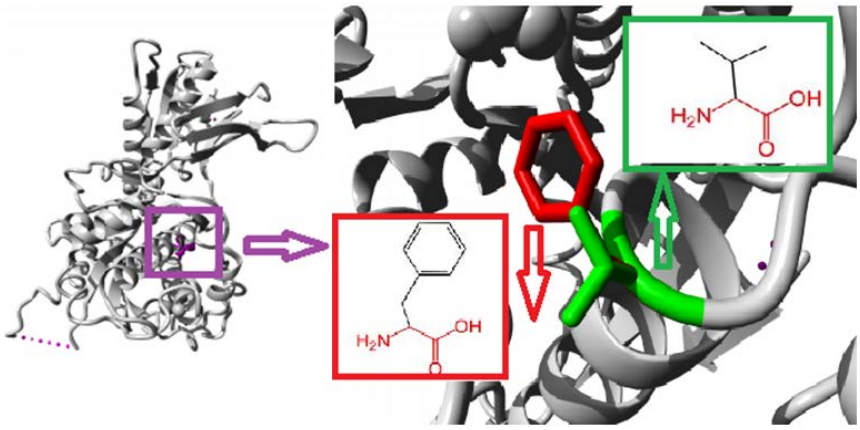

Figure 5. shows change in the amino acids in positions 73 7 from valine into a methionine

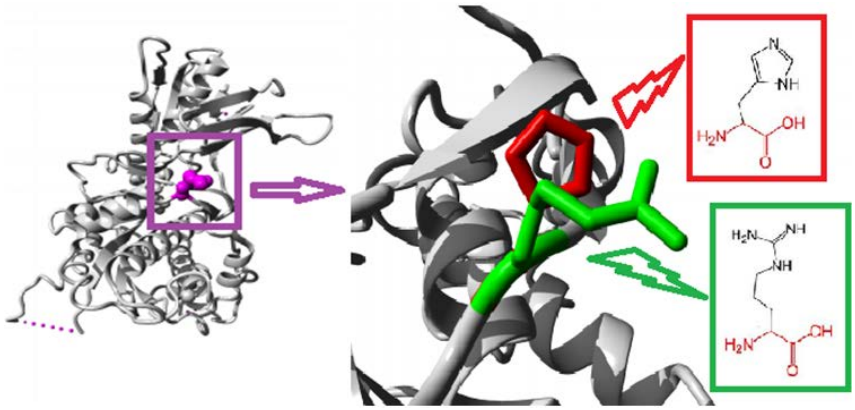

Figure 6. change in the amino acid in position 454 from arginine into histidine

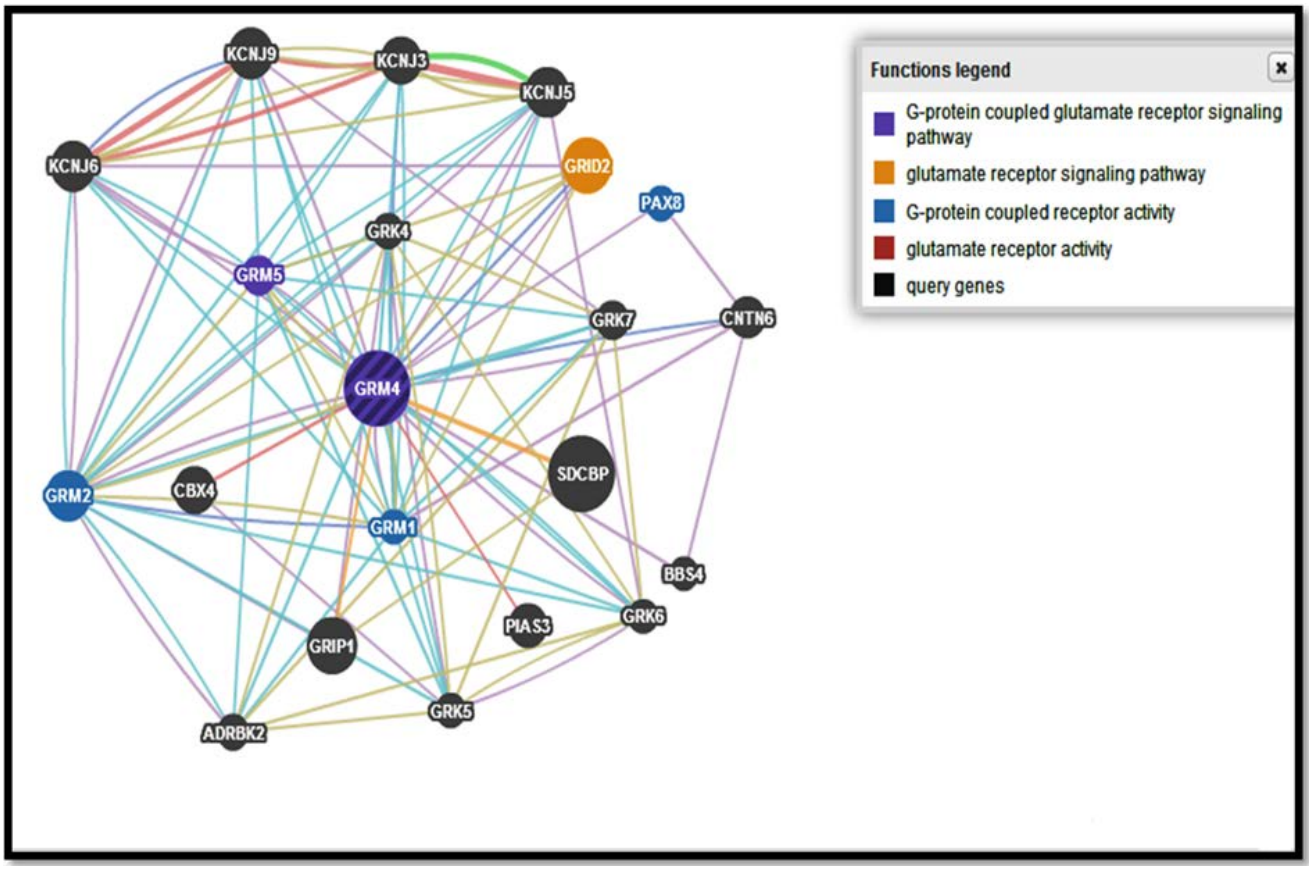

Figure 7. shows functional interaction between GRM4 and its related genes

Table 3. shows the genes co-expressed and share a domain with GRM4

Gene symbol	Description	Co-expression	shared domain
GRID2	glutamate receptor, ionotropic, delta 2	yes	yes
BBS4	Bardet-Biedl syndrome 4	yes	no
PAX8	paired box 8	yes	no
CNTN6	contactin 6	yes	no
GRM2	glutamate receptor, metabotropic 2	yes	yes
GRM5	glutamate receptor, metabotropic 5	yes	yes
GRM1	glutamate receptor, metabotropic 1	yes	yes
GRK6	G protein-coupled receptor kinase 6	yes	yes
KCNJ6	potassium inwardly-rectifying channel, subfamily J, member 6	yes	no
KCNJ9	potassium inwardly-rectifying channel, subfamily J, member 9	yes	no
KCNJ5	potassium inwardly-rectifying channel, subfamily J, member 5	yes	no
CNTN6	potassium inwardly-rectifying channel, subfamily J, member 6	no	no
GRID2	glutamate receptor, ionotropic, delta 2	no	no
KCNJ9	potassium inwardly-rectifying channel, subfamily J, member 9	no	no
KCNJ3	potassium inwardly-rectifying channel, subfamily J, member 3	no	no
GRK7	G protein-coupled receptor kinase 7	no	yes
ADRBK2	adrenergic, beta, receptor kinase 2	no	yes
GRK5	G protein-coupled receptor kinase 5	no	yes
GRK4	G protein-coupled receptor kinase 4	no	yes
CBX4	chromobox homolog 4	no	no
PIAS3	protein inhibitor of activated STAT, 3	no	no
SDCBP	syndecan binding protein (syntenin)	no	yes
GRIP1	glutamate receptor interacting protein 1	no	yes

Table 4. shows the GRM4 functions and its appearance in network and genome

Feature	FDR	Genes in network	Genes in genome
glutamate receptor activity	2.69E-07	5	20
inward rectifier potassium channel activity	4.82E-03	3	15
G-protein coupled receptor activity	2.16E-02	5	222
voltage-gated potassium channel complex	3.18E-02	3	39
potassium channel complex	3.18E-02	3	39
ion channel complex	3.18E-02	4	121
transmembrane transporter complex	3.33E-02	4	134
glutamate receptor signaling pathway	5.71E-02	3	52
G-protein coupled glutamate receptor signaling pathway	9.31E-02	2	10
potassium ion transmembrane transport	9.31E-02	3	68
cellular potassium ion transport	9.31E-02	3	68

FDR: False discovery rate is greater than or equal to the probability that this is a false positive.

5. Discussion

5.1. Investigating the Desired Gene Using dbSNP/NCBI:

GRM4 gene was investigated in dbSNP/NCBI (http://www.ncbi.nlm.nih.gov/snp). This gene containing a total of 29854 SNPs, 8561 SNPs of them found on Homo sapiens; of which 313 were missense, 192 synonymous, 3 nonsense, 7 frame shift and 289 were in the non-coding region (3'un-translated region) and the rest of the SNPs lies at 5'UTR and in the introns.

5.2. Predicting Damaging Amino Acid Substitutions Using SIFT (v5.1) and Prediction of Functional Modification Using Polyphen-2 (Polymorphism Phenotyping v2)

Only SNPs that are found on the coding region and 3'UTR SNPs were selected for computational analysis. SNPs lies on coding region are Predicted by SIFT and Polyphen: Predictions of deleterious nsSNPs was performed by SIFT and Polyphen software; only 225 were predicted to be deleterious by sift. While 168 were predicted to be damaging by both servers. First, we submitted batch nsSNPs (rs SNPs) to SIFT server; then the resultant damaging nsSNPs were submitted to Polyphen as query sequences in FASTA Format, it traced 142 probably damaging nsSNPs, the other 25 nsSNPs were scored as possibly damaging are reported in Table 1.

5.3. Prediction of Change in Stability due to Mutation Used I-Mutant 2.0 Server

I mutant 2.0 (http://folding.biofold.org/i-mutant/i-mutant2.0.html) results demonstrated that protein stability with related free energy had changed due to mutation. The nine mutations (P→L, R→C, R→H, R→S, A→P, A→T, V→M, D→H, R→C) in GRM4 gene decrease effective stability of the protein, five of them with PSIC SD equal 1 and TOLERANCE INDEX rang (0 – 0.001) listed in Table 1, but the mutation (S→L,) increases effect stability. (Table 6).

For modeling by project hope we choose SNPs with **PSIC SD** equal 1 and **TOLERANCE INDEX** rang (0 – 0.001) chosen SNPs are listed in the Table 1.

5.4. Association of nsSNPs to Disease

PHD-snp software was used to predict if the SNP is disease- related (disease) or neutral polymorphism. We found that only SNP in position 334 R → C was predicted to be neutral polymorphism. All other SNP V737M, P680L, P849L, P733L, P709L, R507H, R623H, R483H, R490H, R454H, V144F, V73F were predicted to be disease related. (Table 1)

5.5. Prediction of SNPS at the 3UTR Region

58 functional SNPs was predicted, among the 58 SNPs, 23 allele disrupted a conserved miRNA site and 35 derived allele created a new site of miRNA. RS188406833 and RS192860479 SNPs contained (C) allele had 4 miRSite as target binding site can be disrupts a conserved miRNA. RS77415386 SNP contained (D) allele had 6 miRSite as derived allele that disrupts a conserved miRNA site.

The table below demonstrates the SNPs predicted by Polymirt to induce disruption or formation of mirRNA binding site. (Table 2)

5.6. Protein Modeling using Project Hope:

Project Hope (http://www.cmbi.ru.nl/hope/input) revealed the 3D structure for the truncated proteins with its new candidates; in addition, it described the reaction and physiochemical properties of these candidates. Here we present the results upon each candidate and discus the conformational variations and interactions with the neighboring amino acids:

C/A mutation (rs139236496) caused mutation of a valine into a phenylalanine at position 144. The wild-type and mutant amino acids differ in size. The mutant residue is bigger, this might lead to bumps. (Figure 1)

G/A mutation (rs199744441) caused mutation of a proline into a leucine at position 680, 733, 709 and 849. The wild-type and mutant amino acids differ in size. The mutant residue is bigger, this might lead to bumps. Prolines are known to have a very rigid structure, sometimes forcing the backbone in a specific conformation. Possibly, the mutation changes a proline with such a function into another residue, thereby disturbing the local structure. (Figure 2)

C/T mutation (rs149277708) caused mutation of arginine into a histidine at position 507, 623, 438, 490 and 454. There is a difference in charge between the wild-type and mutant amino acid. The charge of the wild-type residue is lost by this mutation. This can cause loss of interactions with other molecules. The wild-type and mutant amino acids differ in size. The mutant residue is smaller than the wild-type residue. This will cause a possible loss of external interactions. (Figure 3)

G/A mutation (rs139236496) caused mutation of arginine into a cysteine at position 334. There is a difference in charge between the wild-type and mutant amino acid. The charge of the wild-type residue is lost by this mutation. This can cause loss of interactions with other molecules. The wild-type and mutant amino acids differ in size. The mutant residue is smaller than the wild-type residue. This will cause a possible loss of external interactions. The hydrophobicity of the wild-type and mutant residue differs. (Figure 4)

C/T mutation (rs184636998) caused mutation of a valine into a methionine at position 737. The wild-type and mutant amino acids differ in size. 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. (Figure 5)

C/A mutation (rs144660534) cased mutation of arginine into a histidine at position 454. There is a difference in charge between the wild-type and mutant amino acid. The charge of the wild-type residue is lost by this mutation. This can cause loss of interactions with other molecules. The wild-type and mutant amino acids differ in size. The mutant residue is smaller than the wild-type residue. This will cause a possible loss of external interactions. (Figure 6)

5.7. Functions and Interaction of GRM4 with Functional Similar Gene

It was predicted that GRM4 gene shares the same protein domain with 11 genes (SDCBP, GRIP1, GRK7, ADRBK2, GRK5, GRK4, GRM2, GRM5, GRM1, GRK6 and GRID2). GRM4 gene is similar in its expression level with many genes most of them are glutamate receptors. (Table 3)

6. Conclusions

GRM4 SNPs were analyzed using bioinformatics prediction tools to check the effect of the mutation on the protein structure and function. Five SNPs were detected to be highly damaging in coding region while three SNPs were detected to be deleterious in 3'un-translated region.

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Appendix

Table 5. list of nonsynonymous SNPs with SIFT and POLYPHEN results

GENE NAME	SNP	CHROMOSOME LOCATION/ COORDENATE	NUCLEOTIDE CHANGE	AMINO ACID CHANGE	POLYPHEN-2 RESULT	PSIC SD	SIFT RESULT	TOLERANCE INDEX
GRM4	rs146041340	6/33995965	G/A	T734M	PROBABLY DAMAGING	0.957	DELETERIOUS	0.017
	rs146041340	6/33995965	G/A	T705M	POSSIBLY DAMAGING	0.598	DELETERIOUS	0.019
	rs146041340	6/33995965	G/A	T741M	POSSIBLY DAMAGING	0.791	DELETERIOUS	0.02
	rs146041340	6/33995965	G/A	T874M	POSSIBLY DAMAGING	0.874	DELETERIOUS	0.021
	rs376715421	6/3399602	G/A	R740C	PROBABLY DAMAGING	0.997	DELETERIOUS	0.002
	rs376715421	6/3399602	G/A	R716C	PROBABLY DAMAGING	0.999	DELETERIOUS	0.002
	rs376715421	6/3399602	G/A	R856C	PROBABLY DAMAGING	0.997	DELETERIOUS	0.003
	rs376715421	6/3399602	G/A	R723C	PROBABLY DAMAGING	0.989	DELETERIOUS	0.003
	rs376715421	6/3399602	G/A	R687C	PROBABLY DAMAGING	0.972	DELETERIOUS	0.003
	rs184636998	6/33996029	C/A	V737L	PROBABLY DAMAGING	0.993	DELETERIOUS	0.004
	rs184636998	6/33996029	C/T	V737M	PROBABLY DAMAGING	1	DELETERIOUS	0.001
	rs184636998	6/33996029	C/T	V853M	PROBABLY DAMAGING	0.645	DELETERIOUS	0.001
	rs184636998	6/33996029	C/T	V713M	PROBABLY DAMAGING	0.966	DELETERIOUS	0.001
	rs184636998	6/33996029	C/T	V720M	PROBABLY DAMAGING	0.992	DELETERIOUS	0.001
	rs184636998	6/33996029	C/T	V684M	POSSIBLY DAMAGING	0.863	DELETERIOUS	0.001
	rs199744441	6/3399604	G/A	P849L	PROBABLY DAMAGING	1	DELETERIOUS	0
	rs199744441	6/3399604	G/A	P680L	PROBABLY DAMAGING	1	DELETERIOUS	0
	rs199744441	6/3399604	G/A	P733L	PROBABLY DAMAGING	1	DELETERIOUS	0.001
	rs199744441	6/3399604	G/A	P709L	PROBABLY DAMAGING	1	DELETERIOUS	0.001
	rs199744441	6/3399604	G/A	P716L	PROBABLY DAMAGING	1	DELETERIOUS	0.002
	rs373592485	6/33996094	G/A	S691L	PROBABLY DAMAGING	0.998	DELETERIOUS	0.004
	rs373592485	6/33996094	G/A	S831L	PROBABLY DAMAGING	0.998	DELETERIOUS	0.007
	rs370375147	6/34003455	G/A	S671L	PROBABLY DAMAGING	0.999	DELETERIOUS	0.005
	rs370375147	6/34003455	G/A	S811L	PROBABLY DAMAGING	0.992	DELETERIOUS	0.006
	rs370375147	6/34003455	G/A	S678L	POSSIBLY DAMAGING	0.880	DELETERIOUS	0.007
	rs370375147	6/34003455	G/A	S642L	POSSIBLY DAMAGING	0.766	DELETERIOUS	0.007
	rs375956059	6/34003836	C/T	G568D	PROBABLY	1	DELETERIOUS	0.019

rs375956059	6/34003836	C/T	G544D	DAMAGING PROBABLY DAMAGING	1	DELETERIOUS	0.023
rs375956059	6/34003836	C/T	G551D	PROBABLY DAMAGING	0.978	DELETERIOUS	0.023
rs375956059	6/34003836	C/T	G515D	PROBABLY DAMAGING	0.851	DELETERIOUS	0.029
rs375956059	6/34003836	C/T	G684D	PROBABLY DAMAGING	1	DELETERIOUS	0.03
rs369988009	6/34003861	G/A	R676C	PROBABLY DAMAGING	1	DELETERIOUS	0.028
rs369988009	6/34003861	G/A	R560C	PROBABLY DAMAGING	1	DELETERIOUS	0.035
rs369988009	6/34003861	G/A	R543C	PROBABLY DAMAGING	1	DELETERIOUS	0.037
rs369988009	6/34003861	G/A	R536C	PROBABLY DAMAGING	1	DELETERIOUS	0.038
rs369988009	6/34003861	G/A	R507C	PROBABLY DAMAGING	1	DELETERIOUS	0.04
rs368285953	6/34003896	C/T	S548N	PROBABLY DAMAGING	0.993	DELETERIOUS	0.041
rs368285953	6/34003896	C/T	S524N	PROBABLY DAMAGING	0.996	DELETERIOUS	0.049
rs149277708	6/34004019	C/T	R507H	PROBABLY DAMAGING	1	DELETERIOUS	0.001
rs149277708	6/34004019	C/T	R623H	PROBABLY DAMAGING	1	DELETERIOUS	0.001
rs149277708	6/34004019	C/T	R483H	PROBABLY DAMAGING	1	DELETERIOUS	0.001
rs149277708	634004019	C/T	R490H	PROBABLY DAMAGING	1	DELETERIOUS	0.001
rs149277708	6/34004019	C/T	R454H	PROBABLY DAMAGING	1	DELETERIOUS	0.001
rs375967226	6/34004055	C/T	R495H	PROBABLY DAMAGING	1	DELETERIOUS	0.03
rs375967226	6/34004055	C/T	R471H	PROBABLY DAMAGING	1	DELETERIOUS	0.047
rs370298804	6/34004056	G/T	R495S	PROBABLY DAMAGING	0.999	DELETERIOUS	0.013
rs370298804	6/34004056	G/T	R471S	PROBABLY DAMAGING	0.998	DELETERIOUS	0.019
rs370298804	6/34004056	G/T	R478S	PROBABLY DAMAGING	0.994	DELETERIOUS	0.024
rs370298804	6/34004056	G/T	R611S	PROBABLY DAMAGING	0.999	DELETERIOUS	0.041
rs377004554	6/34004122	C/T	V449M	PROBABLY DAMAGING	1	DELETERIOUS	0.003
rs377004554	6/34004122	C/T	V589M	PROBABLY DAMAGING	1	DELETERIOUS	0.004
rs377004554	6/34004122	C/T	V456M	POSSIBLY DAMAGING	0.892	DELETERIOUS	0.004
rs377004554	6/34004122	C/T	V420M	POSSIBLY DAMAGING	0.892	DELETERIOUS	0.005
rs377004554	6/34004122	C/T	V473M	PROBABLY DAMAGING	0.999	DELETERIOUS	0.007
rs201804932	6/34004325	C/T	S405N	PROBABLY DAMAGING	0.997	DELETERIOUS	0.001
rs201804932	634004325	C/T	S521N	PROBABLY DAMAGING	0.999	DELETERIOUS	0.001
rs201804932	6/34004325	C/T	S381N	PROBABLY DAMAGING	0.996	DELETERIOUS	0.001
rs201804932	6/34004325	C/T	S388N	POSSIBLY DAMAGING	0.832	DELETERIOUS	0.001
rs201804932	6/34004325	C/T	S352N	POSSIBLY DAMAGING	0.932	DELETERIOUS	0.001
rs369112240	6/34004374	G/A	R365W	PROBABLY DAMAGING	0.991	DELETERIOUS	0.015
rs369112240	6/34004374	G/A	R389W	PROBABLY DAMAGING	0.996	DELETERIOUS	0.016
rs369112240	6/34004374	G/A	R372W	PROBABLY DAMAGING	0.98	DELETERIOUS	0.016
rs369112240	6/34004374	G/A	R505W	PROBABLY DAMAGING	0.964	DELETERIOUS	0.017
rs369112240	6/34004374	G/A	R336W	POSSIBLY DAMAGING	0.951	DELETERIOUS	0.017
rs188910868	6/34008014	G/A	R367C	PROBABLY DAMAGING	0.983	DELETERIOUS	0.049
rs144660534	6/34008041	G/A	R474C	PROBABLY DAMAGING	0.999	DELETERIOUS	0
rs144660534	6/34008041	G/A	R358C	PROBABLY	0.999	DELETERIOUS	0.001

rs144660534	6/34008041	G/A	R334C	DAMAGING PROBABLY DAMAGING	1	DELETERIOUS	0.001
rs144660534	6/34008041	G/A	R341C	POSSIBLY DAMAGING	0.92	DELETERIOUS	0.001
rs144660534	6/34008041	G/A	R305C	POSSIBLY DAMAGING	0.866	DELETERIOUS	0.001
rs376872551	6/34008439	C/G	A279P	PROBABLY DAMAGING	1	DELETERIOUS	0.002
rs376872551	6/34008439	C/G	A303P	PROBABLY DAMAGING	1	DELETERIOUS	0.003
rs376872551	6/34008439	C/G	A419P	PROBABLY DAMAGING	1	DELETERIOUS	0.003
rs376872551	6/34008439	C/G	A286P	PROBABLY DAMAGING	1	DELETERIOUS	0.003
rs376872551	6/34008439	C/G	A250P	PROBABLY DAMAGING	1	DELETERIOUS	0.003
rs145098725	6/34008451	C/T	A299T	PROBABLY DAMAGING	0.992	DELETERIOUS	0.026
rs145098725	6/34008451	C/T	A275T	PROBABLY DAMAGING	0.997	DELETERIOUS	0.029
rs145098725	6/34008451	C/T	A282T	PROBABLY DAMAGING	0.999	DELETERIOUS	0.033
rs145098725	6/34008451	C/T	A415T	PROBABLY DAMAGING	0.992	DELETERIOUS	0.034
rs145098725	6/34008451	C/T	A246T	PROBABLY DAMAGING	0.999	DELETERIOUS	0.039
rs137969888	6/34008457	C/T	V297M	PROBABLY DAMAGING	1	DELETERIOUS	0.017
rs137969888	6/34008457	C/T	V280M	PROBABLY DAMAGING	1	DELETERIOUS	0.021
rs137969888	6/34008457	C/T	V413M	PROBABLY DAMAGING	1	DELETERIOUS	0.022
rs137969888	6/34008457	C/T	V273M	PROBABLY DAMAGING	1	DELETERIOUS	0.022
rs137969888	6/34008457	C/T	V244M	PROBABLY DAMAGING	1	DELETERIOUS	0.023
rs199952154	6/34008472	A/G	F268L	PROBABLY DAMAGING	0.997	DELETERIOUS	0.002
rs199952154	6/34008472	A/G	F292L	PROBABLY DAMAGING	0.992	DELETERIOUS	0.003
rs199952154	6/34008472	A/G	F275L	POSSIBLY DAMAGING	0.921	DELETERIOUS	0.003
rs199952154	6/34008472	A/G	F408L	PROBABLY DAMAGING	0.999	DELETERIOUS	0.004
rs199952154	6/34008472	A/G	F239L	POSSIBLY DAMAGING	0.692	DELETERIOUS	0.004
rs200372182	634024456	C/G	D345H	PROBABLY DAMAGING	0.988	DELETERIOUS	0.03
rs200372182	6/34024456	C/G	D205H	PROBABLY DAMAGING	1	DELETERIOUS	0.034
rs199793377	6/34029697	A/G	I213T	PROBABLY DAMAGING	0.975	DELETERIOUS	0.008
rs199793377	6/34029697	A/G	I142T	PROBABLY DAMAGING	0.989	DELETERIOUS	0.008
rs199793377	6/34029697	A/G	I149T	PROBABLY DAMAGING	0.976	DELETERIOUS	0.01
rs199793377	6/34029697	A/G	I282T	PROBABLY DAMAGING	0.98	DELETERIOUS	0.011
rs199793377	6/34029697	A/G	I113T	PROBABLY DAMAGING	0.988	DELETERIOUS	0.017
rs200915496	6/34029715	G/A	S207L	POSSIBLY DAMAGING	0.947	DELETERIOUS	0.007
rs200915496	6/34029715	G/A	S136L	POSSIBLY DAMAGING	0.948	DELETERIOUS	0.009
rs200915496	6/34029715	G/A	S143L	POSSIBLY DAMAGING	0.885	DELETERIOUS	0.011
rs200915496	6/34029715	G/A	S276L	PROBABLY DAMAGING	0.958	DELETERIOUS	0.013
rs200915496	6/34029715	G/A	S107L	PROBABLY DAMAGING	0.985	DELETERIOUS	0.014
rs376441438	6/34029731	G/A	R202C	PROBABLY DAMAGING	1	DELETERIOUS	0.006
rs376441438	6/34029731	G/A	R131C	PROBABLY DAMAGING	1	DELETERIOUS	0.007
rs376441438	6/34029731	G/A	R271C	PROBABLY DAMAGING	1	DELETERIOUS	0.01
rs148055958	6/34029733	C/T	R201H	PROBABLY DAMAGING	0.995	DELETERIOUS	0.041
rs148055958	6/34029733	C/T	R130H	PROBABLY	0.997	DELETERIOUS	0.048

rs147191760	6/34029784	G/A	S184L	DAMAGING PROBABLY DAMAGING	0.991	DELETERIOUS	0.003
rs147191760	6/34029784	G/A	S253L	PROBABLY DAMAGING	0.996	DELETERIOUS	0.003
rs147191760	6/34029784	G/A	S113L	PROBABLY DAMAGING	0.993	DELETERIOUS	0.003
rs147191760	6/34029784	G/A	S120L	POSSIBLY DAMAGING	0.931	DELETERIOUS	0.003
rs147191760	6/34029784	G/A	S84L	POSSIBLY DAMAGING	0.812	DELETERIOUS	0.004
rs144674222	6/340298	C/T	V108M	PROBABLY DAMAGING	0.985	DELETERIOUS	0.004
rs144674222	6/340298	C/T	V248M	PROBABLY DAMAGING	0.988	DELETERIOUS	0.004
rs144674222	6/340298	C/T	V79M	PROBABLY DAMAGING	0.985	DELETERIOUS	0.005
rs144674222	6/340298	C/G	V179M	PROBABLY DAMAGING	0.969	DELETERIOUS	0.006
rs375096027	6/340597	C/G	E163D	PROBABLY DAMAGING	0.992	DELETERIOUS	0.005
rs375096027	6/340597	C/G	E99D	POSSIBLY DAMAGING	0.788	DELETERIOUS	0.006
rs375096027	6/340597	C/G	E63D	POSSIBLY DAMAGING	0.53	DELETERIOUS	0.007
rs375096027	6/340597	C/G	E232D	POSSIBLY DAMAGING	0.863	DELETERIOUS	0.008
rs139236496	6/34059759	C/A	V144F	PROBABLY DAMAGING	1	DELETERIOUS	0.001
rs139236496	6/34059759	C/A	V73F	PROBABLY DAMAGING	1	DELETERIOUS	0.001
rs139236496	6/34059759	C/A	V213F	PROBABLY DAMAGING	1	DELETERIOUS	0.002
rs139236496	6/34059759	C/A	V80F	PROBABLY DAMAGING	0.998	DELETERIOUS	0.002
rs139236496	6/34059759	C/A	V44F	PROBABLY DAMAGING	0.999	DELETERIOUS	0.002
rs368047897	6/34059819	C/T	D124N	PROBABLY DAMAGING	0.999	DELETERIOUS	0.023
rs368047897	6/34059819	C/T	D53N	PROBABLY DAMAGING	0.999	DELETERIOUS	0.026
rs368047897	6/34059819	C/T	D60N	PROBABLY DAMAGING	0.755	DELETERIOUS	0.026
rs368047897	6/34059819	C/T	D24N	PROBABLY DAMAGING	0.865	DELETERIOUS	0.029
rs368047897	6/34059819	C/T	D193N	PROBABLY DAMAGING	0.999	DELETERIOUS	0.034
rs375789000	6/34100765	C/T	R170H	PROBABLY DAMAGING	0.999	DELETERIOUS	0.003
rs452752	6/34100769	G/A	L169F	PROBABLY DAMAGING	0.999	DELETERIOUS	0.002
rs367998394	6/34100964	G/A	R104C	PROBABLY DAMAGING	1	DELETERIOUS	0.006
rs371898377	6/34100967	C/T	A103T	PROBABLY DAMAGING	0.971	DELETERIOUS	0.027
rs146834171	6/34101101	T/C	H58R	PROBABLY DAMAGING	0.981	DELETERIOUS	0.002
rs146041340	6.3399597	G/A	T758M	POSIBBLY DAMAGING	0.874	DELETERIOUS	0.014
rs146041340	6.3399597	G/A	T741M	POSIBBLY DAMAGING	0.791	DELETERIOUS	0.02
rs184636998	6.3399603	C/A	V720L	BENIGN	0.029	DELETERIOUS	0.005
rs184636998	6.3399603	C/T	V720M	PROBABLY DAMAGING	0.992	DELETERIOUS	0.001
rs139612028	6.3399608	T/C	M703V	BENIGN	0.036	DELETERIOUS	0.046
rs373592485	6.3399609	G/A	S715L	POSIBBLY DAMAGING	0.95	DELETERIOUS	0.004
rs373592485	6.3399609	G/A	S662L	BENIGN	0.381	DELETERIOUS	0.008
rs370375147	6.3400346	G/A	S695L	PROBABLY DAMAGING	0.996	DELETERIOUS	0.004
rs370375147	6.3400346	G/A	S678L	POSIBBLY DAMAGING	0.880	DELETERIOUS	0.007
rs369988009	6.3400386	G/A	R543C	PROBABLY DAMAGING	1	DELETERIOUS	0.037
rs149277708	6.3400402	C/T	R490H	PROBABLY DAMAGING	1	DELETERIOUS	0.001
rs370298804	6.3400406	G/T	R442S	PROBABLY DAMAGING	0.983	DELETERIOUS	0.034
rs377004554	6.3400412	C/T	V456M	POSIBBLY DAMAGING	0.892	DELETERIOUS	0.004

	rs137969888	6.3400846	C/T	V280M	PROBABLY DAMAGING	1	DELETERIOUS	0.021
	rs200372182	6.3402446	C/G	D37H	ERROR	ERROR	DELETERIOUS	0.002
	rs200372182	6.3402446	C/G	D212H	BENIGN	0.187	DELETERIOUS	0.033
	rs200915496	6.3402972	G/A	S143L	POSIBBLY DAMAGING	0.885	DELETERIOUS	0.011
	rs376441438	6.3402973	G/A	R138C	BENIGN	0.411	DELETERIOUS	0.008
	rs147191760	6.3402978	G/A	S120L	POSIBBLY DAMAGING	0.931	DELETERIOUS	0.003
	rs140826793	6.3402979	C/A	A118S	BENIGN	0.12	DELETERIOUS	0.029
	rs144674222	6.340298	C/T	V115M	PROBABLY DAMAGING	0.984	DELETERIOUS	0.005
	rs139236496	6.3405976	C/T	V80F	PROBABLY DAMAGING	0.996	DELETERIOUS	0.002
GENE NAME	SNP	CHROMOSOME LOCATION/ COORDENATE	NUCLEOTIDE CHANGE	AMINO ACID CHANGE	POLYPHEN-2 RESULT	PSIC SD	SIFT RESULT	TOLERANCE INDEX
GRM4	rs146041340	6/33995965	G/A	T734M	PROBABLY DAMAGING	0.957	DELETERIOUS	0.017
	rs146041340	6/33995965	G/A	T705M	POSSIBLY DAMAGING	0.598	DELETERIOUS	0.019
	rs146041340	6/33995965	G/A	T741M	POSSIBLY DAMAGING	0.791	DELETERIOUS	0.02
	rs146041340	6/33995965	G/A	T874M	POSSIBLY DAMAGING	0.874	DELETERIOUS	0.021
	rs376715421	6/3399602	G/A	R740C	PROBABLY DAMAGING	0.997	DELETERIOUS	0.002
	rs376715421	6/3399602	G/A	R716C	PROBABLY DAMAGING	0.999	DELETERIOUS	0.002
	rs376715421	6/3399602	G/A	R856C	PROBABLY DAMAGING	0.997	DELETERIOUS	0.003
	rs376715421	6/3399602	G/A	R723C	PROBABLY DAMAGING	0.989	DELETERIOUS	0.003
	rs376715421	6/3399602	G/A	R687C	PROBABLY DAMAGING	0.972	DELETERIOUS	0.003
	rs184636998	6/33996029	C/A	V737L	PROBABLY DAMAGING	0.993	DELETERIOUS	0.004
	rs184636998	6/33996029	C/T	V737M	PROBABLY DAMAGING	1	DELETERIOUS	0.001
	rs184636998	6/33996029	C/T	V853M	PROBABLY DAMAGING	0.645	DELETERIOUS	0.001
	rs184636998	6/33996029	C/T	V713M	PROBABLY DAMAGING	0.966	DELETERIOUS	0.001
	rs184636998	6/33996029	C/T	V720M	PROBABLY DAMAGING	0.992	DELETERIOUS	0.001
	rs184636998	6/33996029	C/T	V684M	POSSIBLY DAMAGING	0.863	DELETERIOUS	0.001
	rs199744441	6/3399604	G/A	P849L	PROBABLY DAMAGING	1	DELETERIOUS	0
	rs199744441	6/3399604	G/A	P680L	PROBABLY DAMAGING	1	DELETERIOUS	0
	rs199744441	6/3399604	G/A	P733L	PROBABLY DAMAGING	1	DELETERIOUS	0.001
	rs199744441	6/3399604	G/A	P709L	PROBABLY DAMAGING	1	DELETERIOUS	0.001
	rs199744441	6/3399604	G/A	P716L	PROBABLY DAMAGING	1	DELETERIOUS	0.002
	rs373592485	6/33996094	G/A	S691L	PROBABLY DAMAGING	0.998	DELETERIOUS	0.004
	rs373592485	6/33996094	G/A	S831L	PROBABLY DAMAGING	0.998	DELETERIOUS	0.007
	rs370375147	6/34003455	G/A	S671L	PROBABLY DAMAGING	0.999	DELETERIOUS	0.005
	rs370375147	6/34003455	G/A	S811L	PROBABLY DAMAGING	0.992	DELETERIOUS	0.006
	rs370375147	6/34003455	G/A	S678L	POSSIBLY DAMAGING	0.880	DELETERIOUS	0.007
	rs370375147	6/34003455	G/A	S642L	POSSIBLY DAMAGING	0.766	DELETERIOUS	0.007
	rs375956059	6/34003836	C/T	G568D	PROBABLY DAMAGING	1	DELETERIOUS	0.019
	rs375956059	6/34003836	C/T	G544D	PROBABLY DAMAGING	1	DELETERIOUS	0.023
	rs375956059	6/34003836	C/T	G551D	PROBABLY DAMAGING	0.978	DELETERIOUS	0.023
	rs375956059	6/34003836	C/T	G515D	PROBABLY DAMAGING	0.851	DELETERIOUS	0.029
	rs375956059	6/34003836	C/T	G684D	PROBABLY DAMAGING	1	DELETERIOUS	0.03
	rs369988009	6/34003861	G/A	R676C	PROBABLY	1	DELETERIOUS	0.028

rs369988009	6/34003861	G/A	R560C	DAMAGING PROBABLY DAMAGING	1	DELETERIOUS	0.035
rs369988009	6/34003861	G/A	R543C	PROBABLY DAMAGING	1	DELETERIOUS	0.037
rs369988009	6/34003861	G/A	R536C	PROBABLY DAMAGING	1	DELETERIOUS	0.038
rs369988009	6/34003861	G/A	R507C	PROBABLY DAMAGING	1	DELETERIOUS	0.04
rs368285953	6/34003896	C/T	S548N	PROBABLY DAMAGING	0.993	DELETERIOUS	0.041
rs368285953	6/34003896	C/T	S524N	PROBABLY DAMAGING	0.996	DELETERIOUS	0.049
rs149277708	6/34004019	C/T	R507H	PROBABLY DAMAGING	1	DELETERIOUS	0.001
rs149277708	6/34004019	C/T	R623H	PROBABLY DAMAGING	1	DELETERIOUS	0.001
rs149277708	6/34004019	C/T	R483H	PROBABLY DAMAGING	1	DELETERIOUS	0.001
rs149277708	634004019	C/T	R490H	PROBABLY DAMAGING	1	DELETERIOUS	0.001
rs149277708	6/34004019	C/T	R454H	PROBABLY DAMAGING	1	DELETERIOUS	0.001
rs375967226	6/34004055	C/T	R495H	PROBABLY DAMAGING	1	DELETERIOUS	0.03
rs375967226	6/34004055	C/T	R471H	PROBABLY DAMAGING	1	DELETERIOUS	0.047

Table 6. Prediction result of I-Mutant software

SNP ID	Temp	PH	WT	MT	Amino acid position	SVM2 prediction effect	DDG value prediction Kcal/mol	RI
rs199744441	25	7	P	L	716	DECREASE	-0.58	2
rs373592485	25	7	S	L	831	INCREASE	0.38	3
rs369988009	25	7	R	C	676	DECREASE	-1.01	4
rs375967226	25	7	R	H	471	DECREASE	-1.42	8
rs370298804	25	7	R	S	471	DECREASE	-1.43	8
rs377004554	25	7	V	M	473	DECREASE	-1.17	7
rs377004554	25	7	R	C	358	DECREASE	-1.31	6
rs376872551	25	7	A	P	419	DECREASE	-0.39	3
rs145098725	25	7	A	T	282	DECREASE	-0.63	3
rs137969888	25	7	V	M	244	DECREASE	-1.11	7
rs200372182	25	7	D	H	205	DECREASE	-0.75	6
rs200915496	25	7	S	L	207	INCREASE	-0.31	1
rs376441438	25	7	R	C	131	DECREASE	-0.73	2