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Computational Analysis of Single Nucleotide Polymorphism (SNPs) in Human*SLC5A1* Gene

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Abstract: Glucose galactose malabsorption (GGM) is an autosomal recessive disease manifesting within the first weeks of life. It is characterized by a selective failure to absorb dietary glucose and galactose from the intestine leading to severe life threatening diarrhea and dehydration. Mutations in the Na+/glucose co-transporter gene (*SLC5A1* gene) have been determined to be associated with congenital GGM. In this study different computational tools were used to investigate the nsSNPs (Single nucleotide polymorphisms) in the *SLC5A1* gene and to determine their effects on the protein function and structure. *SLC5A1* gene was investigated in NCBI database and SNPs were analyzed using seven computational software (SIFT, Polyphen-2, PROVEAN, SNPs and GO, PHD-SNPs, I-mutant and MU Pro). The protein structural analysis was done by modeling using Project Hope and Chimera after homology modeling by CPH models 3.2. In addition Gene MANIA software was used to study the association between this gene and related ones. A total of 166 nsSNPs were obtained from the SNPs database in NCBI during 2019. A total of 37 SNP were predicted to be deleterious using SIFT software, while 25 SNPs were predicted to be probably damaging by PolyPhen-2 and 30 SNPs were predicted to be deleterious by PROVEAN. The results of SIFT, PolyPhen-2, PROVEAN, SNPs&GO, PHD-SNP collectively revealed that 16 SNPs were predicted to be highly damaging.

Keywords: Computational Analysis, Glucose–Galactose Malabsorption, *SLC5A1* Gene

1. Introduction

Glucose / galactose malabsorption (GGM) is an autosomal recessive disease manifesting within the first weeks of life andis characterized by a selective failure to absorb dietary glucose and galactose from the intestine [1]. Patients with GGM are presented with the neonatal onset of severe life-threatening watery diarrhea and dehydration [2]. It was first described in 1962 [3]. The diarrhea ceases within one hour after removing oral intake of lactose, glucose, and galactose, but promptly returns with the introduction of one or more of the offending sugars into the diet [4].

Secondary active transport of glucose occurs via symportwith sodium, using SGLT proteins (sodium-glucose transport protein), in the choroid plexus, proximal tubules of kidneys, and the intestine [4]. Mutations in the Na+/glucose co-transporter gene *SLC5A1* (Solute Carrier Family 5 Member 1 (Sodium/Glucose Cotransporter) can cause structural and functional deletion in the SGLT-1 proteins thus glucose and galactose are not absorbed from the intestine leading to clinical manifestations [5]. A total of more than 40 SLC5A1 mutation have been identified in patients with congenital Glucose / galactose malabsorption up to date [6].

The *SLC5A1* gene encoding the SGLT1 membrane protein was cloned and sequenced in 1987 [7]. This gene is located within chromosome 22q13.1 and is composed of 15 exons. Expression of *SLC5A1* gene is mainly in the intestine and kidney. The translated protein is composed of 664 amino acids with a molecular mass of approximately73 kDa, consisting of a core of 13 transmembrane domains [8-9].

For various reasons it might not be feasible to perform

laboratory studies for all SNPs in a specific gene or even the whole genome. Thus computational studies are now becoming indispensable for the identification and prioritization of SNPs with functional importance from an enormous number of non-risk alleles. Computational methods are sufficiently fast and flexible and can provide predictions of functionally significant SNPs with a high accuracy of 80–85% [10] if combined with other techniques such sequencing, structure and phylogenetic relationships

In this study different computational methods were used to identify the SNPs (Single nucleotide polymorphisms)in *SLC5A1* gene and the effects of the predicted mutation on the protein function and structure.

2. Methodology

SLC5A1 gene was investigated in dbSNP/NCBI database using computational analysis. The SNPs and the related ensembles protein (ESNP) were obtained from the SNPs database (dbSNPs) http://www.ncbi.nlm.nih.gov/snp/and Uniprot database during the year 2019. Several software were used for analysis

2.1. GeneMANIA

(http://www.genemania.org). GeneMANIAfinds related genes to the 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. Gene MANIA can be used to find new members of a pathway, additional genes which where missed in screening or find new genes with a specific function [11]. The input was*SLC5A1* gene name and the results are usually shown as a diagram and tables showing the relation between the different genes.

2.2. SIFT: "Sorting Intolerant from Tolerant"

(http://siftdna.org/www/SIFT_dbSNP.html) Itis a sequence homology-based tool that presumes important amino acids will be conserved in the protein family. Hence, changes at well-conserved positions tend to be predicted as deleterious or tolerated. A list of nonsynonymous ID (rsID) that were obtained from the dbSNP database were the input for SIFT and then only the deleterious SNPs were chosen for further analysis. The cutoff value in the SIFT program is a tolerance index of ≥0.05. The higher the tolerance index, the less functional impact a particular amino acid substitution is likely to have [12].

2.3. PolyPhen-2(Polymorphism Phenotyping v2)

(http://genetics.bwh.harvard.edu/pph2/). Itis an online bioinformatics program that predicts the possible impact of amino acid substitution on the stability and function of human proteins using structural and comparative evolutionary considerations. This program basically searches for 3D protein structures, multiple alignments of homologous sequences and amino acid contact information in several protein structure databases, then calculates position specific independent count scores (PSIC) for each of the two variants, and then computes the PSIC scores difference between two variants. The higher a PSIC score difference, the higher the functional impact a particular amino acid substitution is likely to have [13]. Prediction outcomes could be classified as benign, possibly damaging or probably damaging. For structural and functional predictions, SNPs that were predicted to be deleterious by SIFT were submitted to PolyPhen-2 as protein sequence in FASTA format (obtained from Expasy), along with the position of the mutation, native and the new substituent amino acids.

2.4. PROVEAN (Protein Variation Effect Analyzer)

(http://provean.jcvi.org/index.php). It is a software tool which predicts the effect of all classes of protein sequence variations such as single amino acid substitutions, insertions, deletions, and multiple substitution on the function of protein. Prediction out comes could be classified as deleterious or neutral [14]. The protein sequence in FASTA was again the input for this software.

2.5. SNPs&GO (Predicting Disease Associated Variations Using GO (Gene Ontology Terms)

SNPs&GO (http://snps.biofold.org /snps-and-go/snps-and-go.html). It is an accurate method that, starting from a protein sequence, can predict whether a mutation is disease related or not by exploiting the protein functional annotation. SNPs&GO collects in unique framework information derived from protein sequence, evolutionary information, and function as encoded in the Gene Ontology terms, and outperforms other available predictive methods [15]. The protein sequence and mutation sites were the input for this software.

2.6. PHD-SNP (Predictor of Human Deleterious Single Nucleotide Polymorphisms)

PHD- SNP is a web-based tool available at (http://snps.biofold.org/phd-snp/phd-snp.html). PhD-SNP is a Support Vector Machines (SVMs) based method that predicts disease associated nsSNPs using sequence information. The protein sequence and mutation positions were the input. For each mutation, PhD-SNP returns an output score (ranging from 0-1) that represents the probability of this nsSNPs being associated with disease. The method considers 0.5 to be the threshold above it the nsSNPs are predicted to be disease-associated [16].

2.7. Protein Stability

In order to predict the effect of single point mutationon the protein stability, two software were used:

2.7.1. I-Mutant Suite

(http://gpcr2.biocomp.unibo.it/cgi/predictors/IMutant3.0.cgi). It is a support vector machine (SVM)-based tool for the automatic prediction of protein stability changes upon single

point mutations. The input was the protein sequence, the position of the SNP in the protein and the new residue. The method allows to predict if a mutation can largely destabilize the protein (Gibbs-free energy change DDG <-0.5 Kcal/mol) or largely stabilize (DDG >0.5 Kcal/mol) or have a weak effect (-0.5≤G≤0.5 Kcal/mol)[17].

2.7.2. MUpro

http://mupro.proteomics.ics.uci.edu/). It is another web server for prediction of protein stability changes upon mutations. It use support vector machines to predict protein stability changes for single-site mutations by using sequence information. The protein sequence and point of mutation was the input and the output is either increased or decreased stability [18].

2.8. Project hope

(http://www.cmbi.ru.nl/hope/). It is a fully automatic program that analyzes the structural and functional effects of point mutations. It builds a report with text, figures, andanimations [19]. The protein sequence in FASTA format, wild and new amino acid and point of substitution were the input for Project hope.

2.9. CPHmodels3.2

(http://www.cbs.dtu.dk/services/CPHmodels/) is a web server predicting protein 3D structure by using asingle template homology modeling. The template recognition is based on profile-profile alignment guided by secondary structure and exposure predictions [20].

2.10. Chimera

(http://www.cgl.ucsf.edu/chimera). It is used to generate the mutated 3D model models of each protein [21].

3. Results and Discussion

The goal of this study was to analyze the nsSNPs in *SLC5A1* gene and the effect of predicted mutations at the proteomic level. *SLC5A1* gene plays a vital role in human body and it was found to be co-expressed and shared domains with 11 genes as predicted by GeneMANIA (Figure 1 and Table 1).

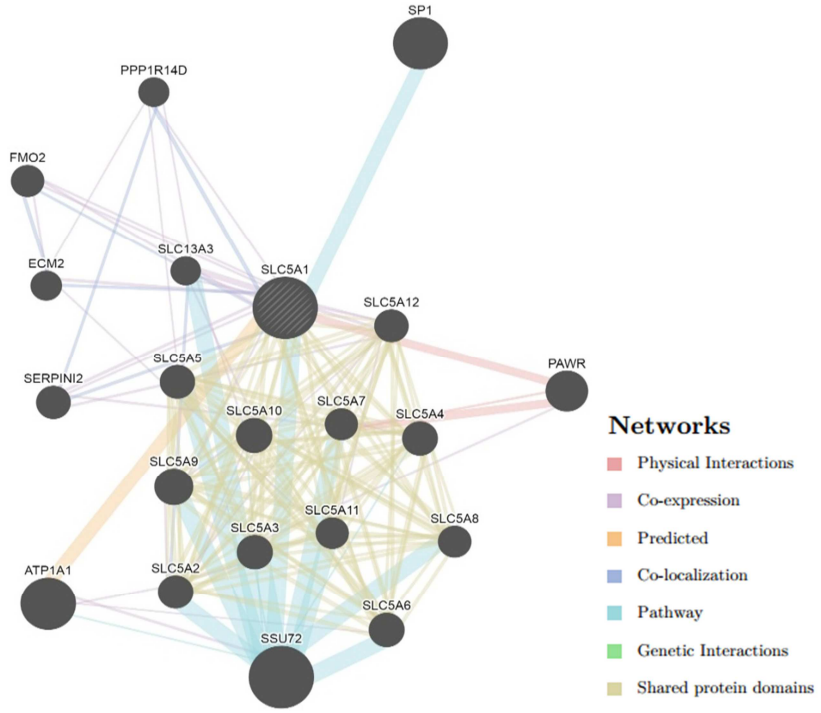


Figure 1. *Genetic interactions, pathways, co-expression, co-localization and protein domain similarity of SLC5A1 gene.*

Table 1. *SLC5A1 functions and its appearance in network and genome.*

Function	FDR	Genes in network	Genes in genome
Solute:Sodium Symporter Activity	9.42E-06	4	12
Sodium Ion Transmembrane Transporter Activity	6.59E-05	5	65
Symporter Activity	0.00027572	4	36
Solute:Cation Symporter Activity	0.00027572	4	35
Active Transmembrane Transporter Activity	0.00039644	5	111
Secondary Active Transmembrane Transporter Activity	0.00194446	4	64
Monovalent Inorganic Cation Transmembrane Transporter Activity	0.0031379	5	180
Metal Ion Transmembrane Transporter Activity	0.01081159	5	238
Inorganic Cation Transmembrane Transporter Activity	0.01651465	5	266
Alcohol Transmembrane Transporter Activity	0.12788567	2	12

A total of 166 ns SNPs were obtained from the SNPs database (dbSNPs) in NCBI. Following analysis using SIFT software, 37 SNPs were predicted to be deleterious. A total of 25 SNPs were predicted to be probably damaging by PolyPhen-2 and 30 SNPs were predicted to be deleterious by PROVEAN as shown in (Tables 2, Appendix A1).

Table 2. *The results SIFT, Polyphen-2, and PROVEAN.*

SOFT WARE	RESULTS
SIFT	Total:166 ns SNPs. 37 deleterious.
PolyPhen-2	i 25 probably damaging. ii 9 possibly damaging. iii 3 Benign.
PROVEAN	i 30 Deleterious. ii 7 Neutral

Analysis with SNPs &GO and PHD-SNP showed different results, 23 SNPs were predicted to be disease related with SNPs &GO compared to 32 with PHD-SNP (Figure 2, Appendix A2).

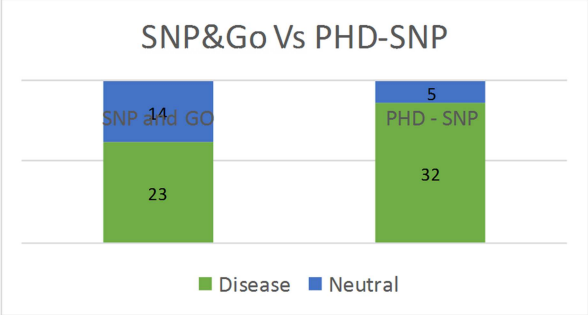


Figure 2. *The result of SNPs & GO compared to PHD-SNP*

From the results of all five software (SIFT, PolyPhen-2, PROVEAN, SNPs&GO and PHD-SNP) 16 SNPs were predicted to be highly damaging (Table 3).

Table 3. *Prediction results of nsSNPs using five different softwares.*

No	SNP ID	Amino acid change	SIFT prediction	Polyphen-2Predication	Provean Predication	SNP and GO	PHD SNP
1	rs33939896	T76M	deleterious	probably damaging	Deleterious	Disease	Disease
2	rs121912669	D28G	deleterious	probably damaging	Deleterious	Disease	Disease
3	rs199573966	R287H	deleterious	probably damaging	Deleterious	Disease	Disease
4	rs199872285	T81M	deleterious	probably damaging	Deleterious	Disease	Disease
5	rs200004849	V298A	deleterious	probably damaging	Deleterious	Disease	Disease
6	rs200304934	S393F	deleterious	probably damaging	Deleterious	Disease	Disease
7	rs200406921	A341E	deleterious	probably damaging	Deleterious	Disease	Disease
8	rs201079555	R173C	deleterious	probably damaging	Deleterious	Disease	Disease
9	rs201216997	L211V	deleterious	probably damaging	Deleterious	Disease	Disease
10	rs201271081	R443C	deleterious	probably damaging	Deleterious	Disease	Disease
11	rs201598524	R287C	deleterious	probably damaging	Deleterious	Disease	Disease
12	rs202070786	K321N	deleterious	probably damaging	Deleterious	Disease	Disease
13	rs370932142	T144M	deleterious	probably damaging	Deleterious	Disease	Disease
14	rs371505974	G191R	deleterious	probably damaging	Deleterious	Disease	Disease
15	rs372081140	T421I	deleterious	probably damaging	Deleterious	Disease	Disease
16	rs373203939	R8Q	deleterious	probably damaging	Deleterious	Disease	Disease

Regarding protein stability, the stability was found to be decreased in all SNPs except in two SNPs: rs200304934 and rs202070786which showed increased stability when I-mutant software has been used, and only one mutation:rs199872285 showed increase protein stability. The prediction accuracy based on sequence information alone is close to the accuracy of methods that depend on tertiary structure information. MUpro software overcomes one important shortcoming of approaches that require tertiary structures to make accurate predictions. Thus, this method can be used on a genomic scale to predict the stability changes for large numbers of proteins with unknown tertiary structure [18].

The SNPs were further submitted to the Project Hope software to see the effect of amino acid substitution on protein structure. Each amino acid has its own specific size, charge, and hydrophobicity value and the wild type residue and newly introduced mutant residue often differ in these properties. Differences in size in all predicted SNPs can affect the contact with the lipid-membrane. In addition, differences in hydrophobicity can affect the hydrophobic interactions with the membrane lipids and can result in loss of hydrogen bonds and/or disturb correct folding. This was predicted for SNPs: rs121912669, rs33939896, rs199872285, rs200304934, rs201079555, rs201598524, rs371505974, rs201271081, rs370932142) (Table 4).

Difference in charge between wild-type and mutant residue can also affect protein function can cause loss of interactions with other molecules or residues. This was predicted for SNPs: (rs121912669, rs201079555 rs199573966, rs201598524, rs371505974, rs373203939, rs202070786, rs201271081) (Table 4).

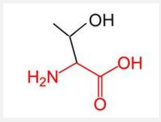
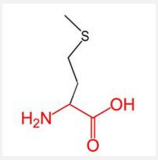
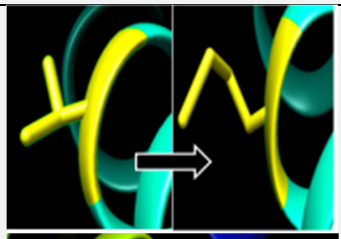
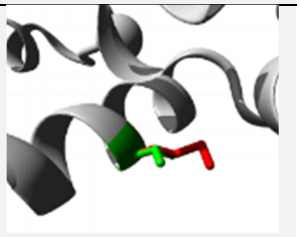
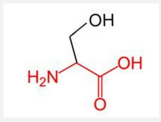
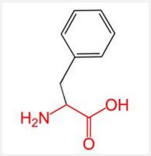
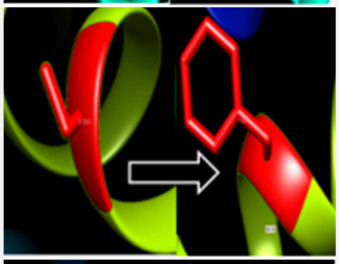
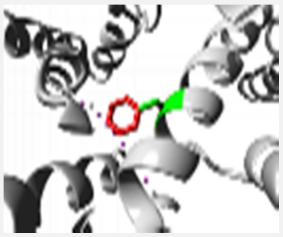
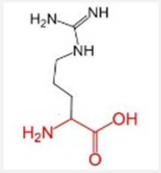
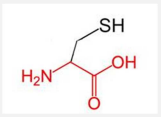
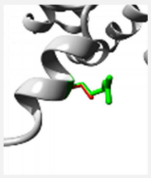
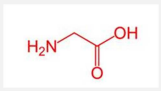
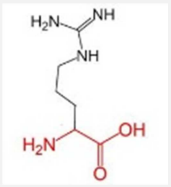
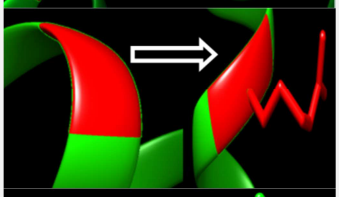
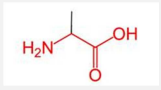
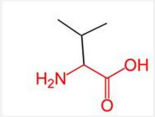
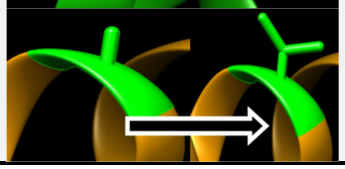
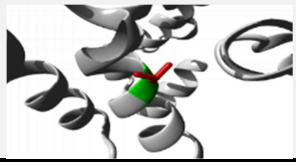
Three SNPs namely (rs121912669), (rs371505974) and (rs200406921) have been reported in previous studies [8, 22] to be associated with SGLT and in this study they were predicted to be highly damaging by all software. Also, a recent study revealed two novel SNPs among Saudi population suffering from congenital Glucose galactose malabsorption(G89R and G435D) [8].

Another SNP (rs121912668)has also been reported to be disease related in a previous study [1] while in the current

study it was predicted to be highly damaging by all software programs except in PolyPhen-2 it predicted to be possibly damaging with high score 0.936.

It is thus important todifferentiate between disease associated and neutral SNPs since this will help in understanding the relationship between the genotype and phenotype and provide a better diagnosis strategies.

Table 4. *3D model by Chimera and project hope for SGLT1 protein.*

SNP ID	Amino acid change	Wild type	mutant type	3D structure Wild type ⟶ mutant type	Project hope Wild type (green) mutant type (red)
rs33939896	T76M				
rs200304934	S393F				
rs201079555	R173C				
rs371505974	G191R				No result
rs200406921	A341V				

4. Conclusions

In this study found 16 ns SNPs were identified inmutationsSLC5A1 gene. Three of the predicted SNPs were also reported in clinical trials, while the others need further confirmative studies. Predicting the phenotypic effect of nsSNPs using computational algorithms will also help in better understanding of the genetic variations in response to diseases, albeit that computation prediction need further conformation using clinical studies.

Appendix

Table A1. *Analysis of SLC5A1 nsSNPs predicted with SIFT, P-2olyphen, and PROVEAN programs.*

SNP ID	amino acid change	SIFT score	PROTEIN ID	SIFT prediction	Polyphen -2 prediction	Polyphen score	PROVEAN prediction	Score
rs33915717	R15W	0.03	ENSP00000266088	deleterious	probably damaging	0.958	neutral	-1.21
rs33939337	L527F	0.022	ENSP00000266088	deleterious	probably damaging	0.998	deleterious	-3.56
rs33939896	T76M	0.025	ENSP00000444898	deleterious	probably damaging	1	deleterious	-5.88
rs33978633	N354S	0.037	ENSP00000444898	deleterious	Benign	0.06	deleterious	-3.69
rs111735032	M201V	0.044	ENSP00000444898	deleterious	Benign	0.058	neutral	-1.65
rs121912668	D28N	0	ENSP00000266088	deleterious	possibly damaging	0.936	deleterious	-4.38

SNP ID	amino acid change	SIFT score	PROTEIN ID	SIFT prediction	Polyphen -2 prediction	Polyphen score	PROVEAN prediction	Score
rs121912669	D28G	0	ENSP00000266088	deleterious	probably damaging	0.996	deleterious	-6.04
rs142230209	G185C	0.003	ENSP00000444898	deleterious	possibly damaging	0.731	deleterious	-3.08
rs143443198	R267Q	0.021	ENSP00000266088	deleterious	possibly damaging	0.811	deleterious	-3.52
rs144006333	V655M	0.005	ENSP00000266088	deleterious	probably damaging	0.997	neutral	-1.92
rs199573966	R287H	0.001	ENSP00000444898	deleterious	probably damaging	1	deleterious	-4.78
rs199872285	T81M	0.001	ENSP00000444898	deleterious	probably damaging	1	deleterious	-5.33
rs199936890	G382R	0.014	ENSP00000444898	deleterious	possibly damaging	0.747	deleterious	-4.23
rs199996478	V517M	0.047	ENSP00000444898	deleterious	possibly damaging	0.923	neutral	-2.17
rs200004849	V298A	0.011	ENSP00000266088	deleterious	probably damaging	1	deleterious	-3.75
rs200118751	I79T	0.002	ENSP00000266088	deleterious	probably damaging	0.996	deleterious	-3.78
rs200304934	S393F	0.001	ENSP00000266088	deleterious	probably damaging	1	deleterious	-5.7
rs200352654	R63Q	0.011	ENSP00000266088	deleterious	probably damaging	0.999	deleterious	-3.21
rs200401846	Q330R	0.004	ENSP00000444898	deleterious	probably damaging	0.958	deleterious	-3.21
rs200406921	A341E	0.017	ENSP00000444898	deleterious	probably damaging	0.969	deleterious	-3.18
rs200562349	A312T	0.033	ENSP00000444898	deleterious	possibly damaging	0.84	neutral	-1.84
rs200684333	P512A	0.022	ENSP00000444898	deleterious	possibly damaging	0.943	deleterious	-6.02
rs200727862	V183M	0.047	ENSP00000444898	deleterious	probably damaging	0.989	neutral	-2.14
rs201079555	R173C	0	ENSP00000444898	deleterious	probably damaging	1	deleterious	-7.76
rs201216997	L211V	0.04	ENSP00000444898	deleterious	probably damaging	1	deleterious	-2.71
rs201271081	R443C	0.017	ENSP00000444898	deleterious	probably damaging	0.999	deleterious	-6.2
rs201383366	M65T	0	ENSP00000266088	deleterious	possibly damaging	0.866	deleterious	-4.39
rs201507039	C355S	0.003	ENSP00000266088	deleterious	Benign	0.036	deleterious	-9.3
rs201598524	R287C	0	ENSP00000444898	deleterious	probably damaging	1	deleterious	-7.65
rs201799893	R558H	0.043	ENSP00000266088	deleterious	probably damaging	0.983	deleterious	-4.43
rs202033427	K122N	0.019	ENSP00000266088	deleterious	probably damaging	0.997	neutral	-1.97
rs202070786	K321N	0.001	ENSP00000266088	deleterious	probably damaging	1	deleterious	-4.85
rs367741549	M198I	0.043	ENSP00000444898	deleterious	possibly damaging	0.455	deleterious	-3.14
rs370932142	T144M	0.024	ENSP00000444898	deleterious	probably damaging	0.97	deleterious	-4.66
rs371505974	G191R	0.001	ENSP00000444898	deleterious	probably damaging	1	deleterious	-7.68
rs372081140	T421I	0	ENSP00000444898	deleterious	probably damaging	0.999	deleterious	-5.7
rs373203939	R8Q	0	ENSP00000444898	deleterious	probably damaging	1	deleterious	-3.89

Table A2. *ns SNPs in SLC5A1 gene predicted with SNPs and GO and PHD SNPs programs.*

SNP ID	Amino Acid Change	Protein ID	SNP and GO	RI	Prop-ability	PHD SNP	RI	Prop-ability
rs33915717	R15W	ENSP00000266088	Neutral	7	0.145	Neutral	1	0.43
rs33939337	L527F	ENSP00000266088	Neutral	1	0.431	Disease	3	0.651
rs33939896	T76M	ENSP00000444898	Disease	6	0.817	Disease	8	0.902
rs33978633	N354S	ENSP00000444898	Neutral	5	0.251	Disease	3	0.66
rs111735032	M201V	ENSP00000444898	Disease	0	0.501	Disease	2	0.599
rs121912668	D28N	ENSP00000266088	Disease	5	0.759	Disease	6	0.82
rs121912669	D28G	ENSP00000266088	Disease	7	0.844	Disease	8	0.909
rs142230209	G185C	ENSP00000444898	Disease	2	0.59	Disease	5	0.774
rs143443198	R267Q	ENSP00000266088	Disease	6	0.792	Neutral	3	0.328
rs144006333	V655M	ENSP00000266088	Neutral	3	0.334	Disease	4	0.697
rs199573966	R287H	ENSP00000444898	Disease	3	0.656	Disease	5	0.734
rs199872285	T81M	ENSP00000444898	Disease	5	0.747	Disease	1	0.549
rs199936890	G382R	ENSP00000444898	Disease	0	0.506	Disease	6	0.79
rs199996478	V517M	ENSP00000444898	Neutral	6	0.191	Disease	4	0.706
rs200004849	V298A	ENSP00000266088	Disease	3	0.634	Disease	2	0.61
rs200118751	I79T	ENSP00000266088	Neutral	7	0.161	Neutral	1	0.463
rs200304934	S393F	ENSP00000266088	Disease	5	0.77	Disease	6	0.799
rs200352654	R63Q	ENSP00000266088	Neutral	2	0.377	Disease	5	0.727
rs200401846	Q330R	ENSP00000444898	Neutral	3	0.348	Disease	6	0.806
rs200406921	A341E	ENSP00000444898	Disease	1	0.551	Disease	7	0.847
rs200562349	A312T	ENSP00000444898	Neutral	3	0.325	Neutral	0	0.491
rs200684333	P512A	ENSP00000444898	Neutral	6	0.213	Disease	4	0.689
rs200727862	V183M	ENSP00000444898	Neutral	5	0.268	Disease	0	0.51
rs201079555	R173C	ENSP00000444898	Disease	8	0.876	Disease	9	0.953
rs201216997	L211V	ENSP00000444898	Disease	3	0.658	Disease	5	0.763
rs201271081	R443C	ENSP00000444898	Disease	1	0.569	Disease	5	0.735
rs201383366	M65T	ENSP00000266088	Neutral	2	0.378	Disease	5	0.773
rs201507039	C355S	ENSP00000266088	Disease	2	0.621	Neutral	2	0.406
rs201598524	R287C	ENSP00000444898	Disease	6	0.781	Disease	7	0.87
rs201799893	R558H	ENSP00000266088	Neutral	2	0.385	Disease	1	0.531

SNP ID	Amino Acid Change	Protein ID	SNP and GO	RI	Prop-ability	PHD SNP	RI	Prop-ability
rs202033427	K122N	ENSP00000266088	Neutral	5	0.266	Disease	3	0.65
rs202070786	K321N	ENSP00000266088	Disease	1	0.547	Disease	1	0.528
rs367741549	M198I	ENSP00000444898	Disease	0	0.514	Disease	4	0.68
rs370932142	T144M	ENSP00000444898	Disease	2	0.584	Disease	0	0.5
rs371505974	G191R	ENSP00000444898	Disease	7	0.847	Disease	8	0.925
rs372081140	T421I	ENSP00000444898	Disease	5	0.762	Disease	8	0.889
rs373203939	R8Q	ENSP00000444898	Disease	6	0.799	Disease	8	0.897

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