

## In-Silico Analysis of Nonsynonymous Single Nucleotide Polymorphism in Human PCSK1 Gene

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### ABSTRACT

The proprotein convertases (PCs) are involved in variety of cellular precursors in the secretory pathway. Polymorphisms in proprotein convertase subtilisin/kexin type 1 (PCSK1) have been associated with adult and childhood obesity. In this work non synonymous SNPs of the PCSK1 gene were retrieved from the dbSNP database. In order to predict the damaging or deleterious nsSNPs, multiple consensus tools were employed by using online tool VEP. Further we also employed SNP-GO tools to predict pathogenic nonsynonymous SNPs. Mutants like D176Y, E345A, G228V, G308E, G310R, G440E, G442R, R110C, S382L, W130S and W404R have shown deleterious and highest pathogenicity. These predicted deleterious and pathogenic nsSNPs are expected to have impending functional influence and may contribute in understanding the functional roles of PCSK1 gene associated with obesity.

**KEY WORDS:** NSSNP, PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 1, NEUROENDOCRINE CONVERTASE 1, IN SILICO ANALYSIS, PCSK1.

### INTRODUCTION

An obesity increasing worldwide and polymorphisms in proprotein convertase subtilisin/kexin type 1 (PCSK1) gene have been associated with adult and childhood obesity. Body mass index variation (risk of common obesity) is associated with more than 60 single-nucleotide polymorphisms (SNPs), identified by genome-wide association studies (Philippe 2015). The proprotein convertases (PCs) are involved in variety of cellular precursors in the secretory pathway and due to homology of their catalytic domains to bacterial subtilisin

and yeast kexin, the genes are known as subtilisin and kexin-like proprotein convertases (PCSKs) (Stijnen, 2016 Loffler, 2016). Human PCSK1 gene consists of 14 exons located on chromosome 5 (Ramos-Molina, 2016), and its promoter contains transcriptional elements CRE-1 and CRE-2 which can be transactivated by CREB-1 and ATF1 transcription factors (Espinosa, 2008; Stijnen, 2016). Analysis of human tissues and cells revealed the presence of a dominant transcript and the major sites of expression being endocrine pancreas, pituitary and brain (Stijnen, 2016). 71% of PCSK1 variant were located in coding region of the catalytic domain and 21% are located on the P domain (Akinci 2019).

Many studies show a strong evidence about rs6232 and rs6235 involving with obesity (Jackson, 2003). association with three variants are found in PCSK1 gene rs6232 encoding by N221D substitution involve in reduce the activity of PC1/3 while rs234 encodes by Q665E compatible with rs6235 that encodes by S690T are essential to form a linkage between PC1/3 and its sorting in secretory granules (Stijnen, 2016; Frank 2013), these

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variants have a significantly role in reducing the level of plasma glucose rapidly and increase serum insulin levels causing a hyperglycemia as type 2 diabetes (T2D) by increasing in glucose production, insulin resistance and a dysfunction in  $\beta$  cell that found in pancreatic cells (Gjesing, 2011), along with the effect of blood pressure and energy ratio, causing a hypertension in the blood vessels which lead to a cardiovascular (Heni, 2010 Pepin et al 2019).

The R405X mutation cause a deletion of P and C-terminal tail domain (Bandsma, 2013). Identified N309K a deleterious in PCSK1 gene which make C-terminal domain incapable of cleave in intermolecular interaction (Wilschanski, 2014). K26E is located before the signal peptide cleavage site, M125I, T175M, N180S, Y181H, G226R and S325N are located in the catalytic domain and the T558A is located in the middle domain. These mutations have an impact on PC1/3 folding and its stability. also, G209R and G593R mutation might affect on the PC1/3 misfolding due to their enzymatic activation (Blanco, 2015). In addition, T175M was defined as induce the inhibition in N-glycosylation site which is responsible for cellular signal and altering the protein maturation (Creemers, 2012 Pepin et al 2019).

## MATERIAL AND METHODS

**Datasets:** The SNPs of the PCSK1 gene were retrieved from the dbSNP database (Sherry, 2001). Keyword “Human PCSK1” used as our search term. Furthermore, it is filtered by selecting variation class as SNV, function class as missense. The protein sequences (P29120) were retrieved from the UniProt (<https://www.uniprot.org>).

**Predicting deleterious and damaging nsSNPs:** In order to predict the damaging or deleterious nsSNPs, multiple consensus tools were employed by using online tool VEP (<http://www.ensembl.org/Tools/VEP>). VEP advantages include: it uses latest human genome assembly GRCh38.p10, and can predict thousands of SNPs from multiple tools including SIFT, PROVEAN, Condel, and PolyPhen-2, at a time. nsSNP rs-ids were uploaded to VEP tool to get the prediction results

**SIFT:** The algorithm predicted that the tolerant and intolerant coding base substitution based upon properties of amino acids and homology of sequence (Choi Y, 2015). The tool considered that vital positions in the protein sequence have been conserved throughout evolution and therefore substitutions at conserved alignment position is expected to be less tolerated and affect protein function than those at diverse positions., SIFT predicted substituted amino acid as damaging at default threshold score  $<0.05$ , while score  $0.05$  is predicted as tolerated.

**PolyPhen-2:** This tool is predicting the structural and functional consequences of a particular amino acid substitution in human protein (Adzhubei, 2010). Prediction of PolyPhen-2 is based on a number of features including information of structural and sequence

comparison. The PolyPhen-2 score varies between 0.0 (benign) to 10.0 (damaging). The PolyPhen-2 prediction output categorizes the SNPs into three basic categories, benign (score  $<0.2$ ), possibly damaging, (score between 0.2 and 0.96), or probably damaging (score  $>0.96$ ).

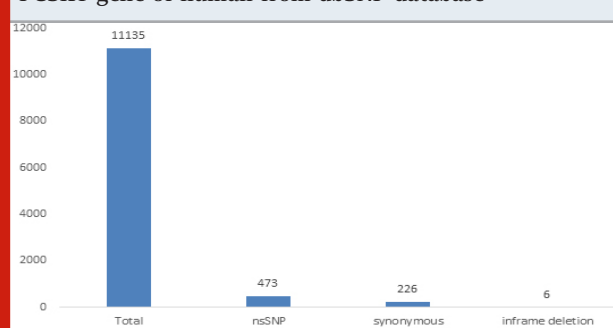
**PROVEAN:** This tool (<http://provean.jcvi.org/>) uses an alignment-based scoring method for predicting the functional consequences of single and multiple amino acid substitutions, and in-frame deletions and insertions (Choi, 2015). The tool has a default threshold score, i.e.  $-2.5$ , below which a protein variant is predicted as deleterious, and above that threshold, a protein variant is neutral.

**CONDEL (CONsensus DEleteriousness):** This tool evaluates the probability of missense single nucleotide variants (SNVs) deleterious. it computes a weighted average of the scores of SIFT, PolyPhen2, MutationAssessor and FatHMM (Hecht, 2015).

### Predicting disease associated nsSNPs

**SNPs&GO:** A web server predicting whether an amino acid substitution is associated to a disease or not (<http://snps.biofold.org/snps-and-go> ). It is a SVM (Support Vector Machine) based tool which takes features of protein sequence, evolutionary information, and functional annotation according to Gene Ontology terms. We input isoform 1 of Swiss-Prot Code of LSP1 (P33241) and provided the list of amino acid mutations. The results predicted the probability for the polymorphisms of helicase whether being disease- associated or not by three methods: (a) SNPs&GO, (b) PhD-SNP, and (c) PANTHER. Probability score  $>0.5$  is predicted as disease associated variation (Calabrese, 2015).

Figure 1: Number of SNPs in different function class of PCSK1 gene of human from dbSNP database



## RESULTS AND DISCUSSION

473 nsSNP ids of human PCSK1 gene was downloaded from dbSNP database of NCBI (Supplementary Table 1), after filtering variation class SNV, function class missense, there were 473 SNP mapped to missense, 226 SNPs mapped to synonymous and 6 SNPs mapped to inframe deletion, while 11135 mapped to total SNPs of different variation class (Figure 1).

Figure 1: Frequency of aggressive behavior of patients/attendants faced by study subjects.

SNP-ids	AA-change	SIFT (score)	PolyPhen (score)	Condel (score)	PROVEAN (score)	PANTHER Prediction	RI
rs759379849	D193G	*(0)	#(0.999)	*(0.935)	*(0.92173)	Disease	8
rs1561374455	D195G	*(0)	#(0.96)	*(0.848)	*(0.91956)	Disease	8
rs752416942	D272G	*(0)	#(1)	*(0.945)	*(0.93175)	Disease	8
rs749888385	T353I	*(0.02)	#(0.967)	*(0.792)	*(0.78636)	Disease	8
rs762403860	A213V	*(0)	#(0.998)	*(0.919)	*(0.71639)	Disease	9
rs1475050973	C212R	*(0)	#(1)	*(0.945)	*(0.99425)	Disease	9
rs552958813	D176N	*(0)	#(1)	*(0.945)	*(0.80172)	Disease	9
rs752416942	D272V	*(0)	#(1)	*(0.945)	*(0.9783)	Disease	9
rs1363728113	G155D	*(0)	#(0.98)	*(0.869)	*(0.90023)	Disease	9
rs1382566997	G155S	*(0)	#(0.986)	*(0.879)	*(0.83899)	Disease	9
rs1490377137	G158A	*(0)	#(0.999)	*(0.935)	*(0.873)	Disease	9
rs768031892	G209R	*(0)	#(1)	*(0.945)	*(0.95336)	Disease	9
rs142673134	G279A	*(0.04)	#(0.959)	*(0.752)	*(0.86296)	Disease	9
rs1312543959	G298A	*(0)	#(0.999)	*(0.935)	*(0.87223)	Disease	9
rs778681269	G311R	*(0)	#(1)	*(0.945)	*(0.95276)	Disease	9
rs567641208	G390S	*(0)	#(0.999)	*(0.935)	*(0.88839)	Disease	9
rs1389330621	N180K	*(0)	#(0.999)	*(0.935)	*(0.85994)	Disease	9
rs1269967613	N429K	*(0)	#(0.994)	*(0.897)	*(0.87835)	Disease	9
rs1246203022	P280S	*(0)	#(1)	*(0.945)	*(0.96058)	Disease	9
rs775618000	P341L	*(0)	#(1)	*(0.945)	*(0.98437)	Disease	9
rs775136858	P386L	*(0)	#(0.998)	*(0.919)	*(0.98692)	Disease	9
rs1332430207	Q408R	*(0)	#(1)	*(0.945)	*(0.73267)	Disease	9
rs748072514	R110H	*(0)	#(0.999)	*(0.935)	*(0.76822)	Disease	9
rs768934109	R296I	*(0)	#(1)	*(0.945)	*(0.95246)	Disease	9
rs1421014042	S186N	*(0)	#(0.996)	*(0.906)	*(0.59873)	Disease	9
rs137852824	S307L	*(0)	#(0.999)	*(0.935)	*(0.86222)	Disease	9
rs1166018774	T210S	*(0)	#(0.999)	*(0.935)	*(0.71762)	Disease	9
rs1303515025	T276I	*(0)	#(0.996)	*(0.906)	*(0.84742)	Disease	9
rs766414747	T375K	*(0)	#(0.998)	*(0.919)	*(0.88839)	Disease	9
rs766414747	T375M	*(0)	#(0.993)	*(0.895)	*(0.88839)	Disease	9
rs1346360455	T381I	*(0)	#(1)	*(0.945)	*(0.88839)	Disease	9
rs1434467255	W130L	*(0)	#(1)	*(0.945)	*(0.99433)	Disease	9
rs868424536	W152L	*(0)	#(0.985)	*(0.877)	*(0.99023)	Disease	9
rs1245583638	W342G	*(0)	#(0.998)	*(0.919)	*(0.99704)	Disease	9
rs1246742230	W98R	*(0.02)	#(0.994)	*(0.835)	*(0.99587)	Disease	9
rs552958813	D176Y	*(0)	#(1)	*(0.945)	*(0.97364)	Disease	10
rs864309557	E345A	*(0)	#(1)	*(0.945)	*(0.873)	Disease	10
rs747169606	G228V	*(0)	#(1)	*(0.945)	*(0.97617)	Disease	10
rs990328651	G308E	*(0)	#(1)	*(0.945)	*(0.95246)	Disease	10
rs748808191	G310R	*(0)	#(1)	*(0.945)	*(0.95276)	Disease	10
rs865777271	G440E	*(0)	#(1)	*(0.945)	*(0.95665)	Disease	10
rs761336991	G442R	*(0)	#(0.999)	*(0.935)	*(0.95665)	Disease	10
rs774036542	R110C	*(0)	#(1)	*(0.945)	*(0.93582)	Disease	10
rs1561368007	S382L	*(0)	#(0.998)	*(0.919)	*(0.88839)	Disease	10
rs1434467255	W130S	*(0)	#(1)	*(0.945)	*(0.99699)	Disease	10
rs1180593976	W404R	*(0)	#(0.998)	*(0.919)	*(0.99969)	Disease	10

(\*Deleterious, #Probably Damaging)

Predicting deleterious and damaging pathogenic nsSNPs: In order to predict the damaging or deleterious nsSNPs multiple consensus tools were employed. Initially, online tool VEP was used (McLaren, 2016). VEP advantages include: it uses latest human genome assembly GRCh38.p10, and can predict thousands of SNPs from multiple tools including SIFT, Condel, and PolyPhen-2, at a time. 473 nsSNP rsids were uploaded to VEP tool and the prediction results were taken on default scores of consensus tools based on sequence and structure homology methods: (a) SIFT (score <-0.5) (b) Polyphen (score >0.96) (c) PROVEAN (score < 2.5) and Condel (score >0.522).

In order to get a very high confident nsSNPs impacting structure and function of PCSK1 gene, 46 nsSNPs out of 473 nsSNP (Table 1) were found to be deleterious by all four tools and predicted disease by panther tools, and these eleven nsSNPs rs552958813 of mutation D176Y, rs864309557, of mutation E345A, rs747169606 of mutation G228V, rs990328651 of mutation G308E, rs748808191 of mutation G310R, rs865777271 of mutation G440E, rs761336991 of mutation G442R, rs774036542 of mutation R110C, rs1561368007 of mutation S382L, rs1434467255 of mutation W130S and rs1180593976 of mutation W404R were predicted highly pathogenic with more than 9 RI score (Table-1).

Studies show a strong evidence about variants are found in PCSK1 gene involving with obesity, association with variants N221D, S690T and Q665E substitutions found in PCSK1 gene involve in reduce the activity of PC1/3, linkage between PC1/3 and its sorting in secretory granules (Jackson 2003, Stijnen 2016; Frank 2013), Identified N309K a deleterious in PCSK1 gene which make C-terminal domain incapable of cleave in intermolecular interaction (Wilschanski, 2014). K26E is located before the signal peptide cleavage site, M125I, T175M, N180S, Y181H, G226R and S325N are located in the catalytic domain and the T558A is located in the middle domain.

These mutations have an impact on PC1/3 folding and its stability. also, G209R and G593R mutation might affect on the PC1/3 misfolding due to their enzymatic activation (Blanco EH, 2015). In addition, T175M was defined as induce the inhibition in N-glycosylation site which is responsible for cellular signal and altering the protein maturation (Creemers, 2012). Pickett had proposed that R80Q have the most influence part in PC1/3 maturation and its activity (Pickett, 2013). In another report, the S357G mutant that low the calcium dependence and highly resistance the peptide inhibitors (Blanco, 2015).

## CONCLUSION

Our investigation shows mutants D176Y, E345A, G228V, G308E, G310R, G440E, G442R, R110C, S382L, W130S and W404R with deleterious and highest pathogenicity, and may offer valuable information in selecting SNPs that are expected to have impending functional influence

and pathogenicity also eventually may contribute in understanding the functional roles of PCSK1 gene associated with obesity.

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