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INSILICO PREDICTION OF FUNCTIONAL AND STRUCTURAL IMPACT OF NOVEL NONSYNONYMOUS SNPS IN HUMAN ADRENERGIC BETA-RECEPTORS

Praveen P. Balgir*[1]., Rajesh Kumar Goel[2]., Jaspreet Kaur[1].,Maleeka Sharma[1] and Shobha R. Dhiman[3]

[1]Department of Biotechnology, Punjabi University, Patiala, India
[2]Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala, India
[3]Department of Human Genetics, Punjabi University, Patiala, India

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ABSTRACT

G-protein-coupled receptors (GPCRs) are the most common protein target among currently available marketed drugs. As a result, they were among the first group of proteins whose genetic variability was studied extensively. Beta-adrenergic receptors (ADRBs) are most widely studied GPCRs and most commonly used drugs are the β-adrenergic receptor blockers. Defects in phenotypes and functions are due to non-synonymous single nucleotide polymorphisms (nsSNPs), which are crucial to predict the genetic basis of diseases like asthma, hypertension, myocardial infarction and cardiovascular disease. Present study is an attempt, using different in silico tools, to predict all nsSNPs and the plausible effect of all these mutations on the structural and functional conformation of the protein. As per NCBI SNP Database, 39 nsSNPs of adrenoceptor beta 1 (ADRB1) and 43 of adrenoceptor beta 2 (ADRB2) are predicted in coding region of these genes. Out of these 9 nsSNPs of ADRB1 and 5 of ADRB2 lie in the functionally important sites like ligand binding, Phosphorylation, Glycosylation, Disulfide bond formation and Myristolyation. These variations were analyzed using SIFT, PolyPhen 2, MuPro, PANTHER, PROVEAN and MutPred. Thus, we hypothesized that these rare looked upon variations have the potential to result in change at phenotypic level and should be investigated by subsequent imperical approach to predict as no population data is available for many of these SNPs.

INTRODUCTION

Change in expression of Beta-adrenergic receptors (ADRBs) initiates asthma and cardiovascular conditions including hypertension, angina pectoris, arrhythmias, heart failure and enlarged heart muscle (hypertrophic cardiomyopathy. Beta-blockers are first line medication to treat these conditions. Selective β-blockers (atenolol, metoprolol, betaxolol, bisoprolol, and esmolol) act mainly on heart while partial agonists (acebutolol, carteolol, penbutolol, and pindolol) are weak stimulators, but still block the major activity of neurotransmitters like epinephrine and non-epinephrine (Frishman, 2003). Hence in the cardiovascular system, genes are utmost decisive factor for pharmacogenetics.

ADRBs are G-protein-coupled receptors which comprise the biggest superfamily of signaling molecules. They consists of an extracellular amino terminus, seven transmembrane-spanning α helical regions, three extracellular loops, three intracellular loops, and a carboxy-terminal intracellular tail. They carry out signaling via coupling to guanine nucleotide binding proteins (G-proteins). Every cell type or organ expresses one or more of the nine ADRBs subtypes. ADRBs are critical for the maintenance of body homeostasis at resting state, or during stress such as exercise, exhaustion and fatigue (Mason *et al,* 1999). Stimulation of ADRBs in the heart leads to increased ionotropy, lusitropy and chronotropy via the Gs pathway, resulting in raised cAMP levels. Protein Kinase A (PKA) is activated by cAMP. PKA phosphorylates proteins important for cardiomyocyte function. ADRBs have also been shown to activate PKA by pathways independent of GPCRs (Zhu *et al,* 2003; Sucharov *et al,* 2006). They bind to epinephrine and norepinephrine as well as exogenously administered drugs. In heart failure arising from almost any cause, catecholamines (particularly norepinephrine) are elevated, representing the systems attempt to increase cardiac output via cardiac β1-ARs, for which norepinephrine is the ligand (http://rgd.mcw.edu/).

The three beta-adrenergic receptor subtypes

There are three subtypes of ADRBs (ADRB1, ADRB2 and ADRB3) encoded by three different genes. ADRB1 and

*Corresponding author: **Praveen P. Balgir***
Department of Biotechnology, Punjabi University, Patiala, India

ADRB2 have been well studied as they have important effects on cardiac physiology. ADRB1 is predominantly expressed subtype in the heart, which mediates increase in dromotropy (Fustrer *et al,* 2004). ADRB2 is prominently present in bronchial smooth muscle (Gauthier *et al*, 2000). ADRB1 and ADRB2 encode 477 and 413 amino-acid proteins, respectively and are intronless. To date ADRB3 has been least studied and its role on cardiovascular disease is not well known (Taylor and Bristow, 2004).

ADRB1 polymorphisms

As per NCBI SNP Database, 234 polymorphisms of ADRB1 have been predicted and 39 of these predict amino-acid changes in the ADRB1 protein. The Arg389Gly mutation was the first most studied polymorphism of ADRB1 present in the intracellular cytosplasmic tail (Mason *et al,* 1999). The Ser49Gly mutation affects ligand binding and is located in the extracellular amino terminus (Johnson and Terra 2002). Several other rare polymorphisms of ADRB1 have not been widely studied. Variants lying in intracellular carboxy terminus and involved in disulfide bonding, phosphorylation, myristoylation could modulate the function of protein which needs more exploration.

ADRB2 polymorphisms

As per NCBI SNP Database, there are 209 polymorphisms reported for the ADRB2 and out of it 43 are present in coding region. Gly16Arg, Gln27Glu and Thr164Ile: these missense polymorphisms have been mostly studied. They are located in the amino-terminal region of the receptor and consist of arginine (Arg) to glycine (Gly) substitution at position 16 (Arg16Gly) and glutamine (Gln) to glutamic acid (Glu) substitution at position 27 (Gln27Glu). In vitro studies have shown that these two polymorphisms may affect ADRB2 susceptibility to agonist-induced desensitization. The Gly16 variant has been associated with enhanced agonist-induced downregulation (Green *et al,* 1994). In contrast; the Glu27 allele has been associated to resistance to down regulation, as compared to the wild-type Gln27 allele (Bruck *et al,* 2003).

Much remains to be learned about more nsSNPs, which may alter the protein structure and function that ultimately affect the pharmacogenetics interaction among the drug and receptor. So other polymorphisms except widely studied should also be validated, which may ultimately be more fruitful in the field of precision medicine.

The road next remains challenging for rendering these sorts of data into clinical practice. Thus, attention has been focused on identifying non-synonymous single nucleotide polymorphisms (nsSNPs) with potential impact on structure and function of the encoded protein (Johnson *et al,* 2005). Present study was designed to screen and predict potentially deleterious mutations present in the protein using multiple bio-informatics tools available freely on the internet.

MATERIALS AND METHODS

Data for identification of nsSNPs

Human ADRB1 and ADRB2 gene data were obtained from OMIM (+109630,+109690 - http://www.ncbi.nlm.nih.gov/omim) and Entrez on the National Center for Biotechnology Information (NCBI) website. The Uniprot accession number (P08588, P07550) was obtained in the Swissprot database (http://expasy.org), fasta format of proteins were downloaded from Uniprot (http://www.uniprot.org/).

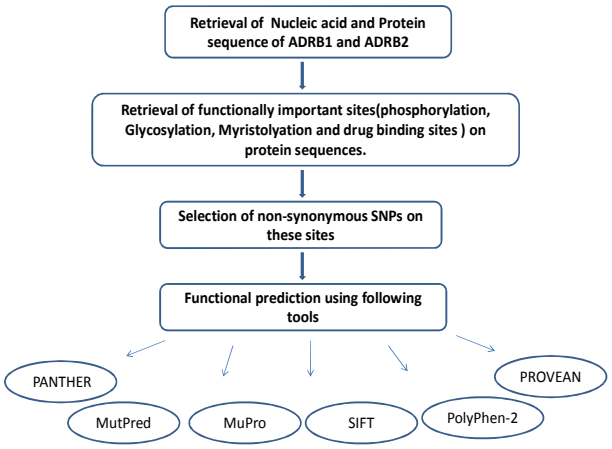

Figure 1 A stepwise computational approach employed to study the amino acid sites that are potent drug targets and involved in protein stability.

In order to identify the ligand binding sites in the downloaded sequence, literature for ADRB1 and ADRB2 respectively was searched (Kleinau *et al,* 2011; Warne *et al,* 2012). The complete PDB structure of adrenoceptors is not available. Buthuman protein sequence of ADRB1 and ADRB2 has good similarity with turkey. So, the equivalent residues of both adrenoceptors in human that interacts with different ligands are shown in Table no.1

Table 1 Interaction of amino acid residues with different ligands.

Turkey β1 amino acid residue	2° structure	Ligands that interact with amino acid	Equivalent residues in human β receptors	
			β1	β2
Gly 98	H2	Dob	Gly 115	Gly 90
Leu 101	H2	Dob	Ile 118	His 93
Val 102	H2	Dob	Val 119	Ile 94
Trp 117	H3	Cyp, Iso, Sal, Dob, Car	Trp 134	Trp 109
Thr 118	H3	Cyp, Sal, Dob	Thr 135	Thr 110
*Asp 121	H3	Cyp, Iso, Sal, Dob, Car	Asp 138	Asp 113
Val 122	H3	Cyp, Iso, Sal, Dob, Car	Val 139	Val 114
Val 125	H3	Cyp, Iso, Sal, Dob, Car	Val 142	Val 117
Cys 199	EL2	Car	Cys 216	Cys 191
Asp 200	EL2	Car	Asp 217	Asp 192
Phe 201	EL2	Cyp, Sal, Dob, Car	Phe 218	Phe 193
Thr 203	EL2	Cyp, Car(Car via water)	Thr 220	Thr 195
Ala 208	H5	Cyp, Car	Ala 225	Ala 200
*Ser 211	H5	Cyp, Iso, Sal, Dob, Car	Ser 228	Ser 203
*Ser 215	H5	Cyp, Iso, Sal, Dob, Car	Ser 232	Ser 207
Trp 303	H6	Cyp	Trp 337	Trp 286
Phe 306	H6	Cyp, Iso, Sal, Dob, Car	Phe 340	Phe 289
Phe 307	H6	Cyp, Iso, Sal, Dob, Car	Phe 341	Phe 290
*Asn 310	H6	Cyp, Iso, Sal, Dob, Car	Asn 344	Asn 293
*Asn 329	H7	Cyp, Iso, Sal, Dob, Car	Asn 363	Asn 312
*Trp 330	H7	Dob	Trp 364	Trp 313
Tyr 333	H7	Cyp, Iso, Sal, Dob, Car	Tyr 367	Tyr 316
Phe 325	H7	Car	Phe 359	Tyr 308
Val 326	H7	Car, Dob	Tyr 360	Ile 309

Equivalent residues in the human β1, β2 receptors are shown along with their secondary structure position. Residues with * interact via polar contacts, whilst all other contacts are

predicted to be via van der Waals interactions. Ligands are abbreviated as follows: cyanopindolol, Cyp; isoprenaline, Iso; salbutamol, Sal; dobutamine, Dob; Carmoterol, Car.

For Other sites like phosphorylation, Glycosylation, Myristolyation, Uniprot was searched. The information on 39 nsSNPs of ADRB1 and 43 of ADRB2 lying in coding region of these genes was collected from dbSNP (http://www.ncbi.nlm.nih.gov/snp) including SNP ID, amino acid change and functional consequences, when available.

Functional analysis prediction

To predict the effect nsSNPs present on functionally important sites, several computational algorithms like Sorting Intolerant from Tolerant (SIFT) (Ng and Henikoff, 2003) and Polymorphism; Phenotyping (PolyPhen-2) were used. Various other bioninformatics tools like PANTHER, MuPro, PROVEAN and MutPred were used.

SIFT (Sorting Intolerant from Tolerant) is a program for predicting a SNPs effect on protein structure. SIFT assumes that sequences of proteins have been conserved throughout evolution and therefore any ns-SNP at these sites may potentially affect the protein function. Thus SIFT uses sequence homology to predict effects of substitutions at each position of the protein. SIFT is available as an online tool (http://sift.jcvi.org) ((Ng and Henikoff, 2003).

PolyPhen2 (Polymorphism Phenotyping2) is the most commonly used algorithm which predicts all possible effects of an amino acid substitution on the stability and function of human proteins using physical, structural and comparative evolutionary (genetics.bwh.harvard.edu/pph2/) ((Johnson *et al,* 2005; Zhu *et al,* 2008).

PANTHER: The deleterious effects are predicted based on scores given by these tools. In case of Panther, the probability that a given variant will cause a deleterious effect on protein function is estimated by $P_{deleterious,}$ such that asubPSEC score of -3 corresponds to a $P_{deleterious}$of 0.5 It calculates the subPSEC (substitution position-specific evolutionary conservation) scores that are continuous values from 0 (neutral) to about 10 (most likely to be deleterious) based on the alignment of evolutionarily related proteins.. $P_{deleterious}$calculates the probability of a mutation being deleterious, where score 1 is deleterious and 0 indicates non deleterious (http://pantherdb.org/tools/csnpScoreForm.jsp) (Thomas *et al*, 2003).

MuPro: comprises of a set of machine learning programs which predict how a single-site amino acid mutation affects protein stability. The advantage of this method is that to predict protein stability changes no tertiary structure of the protein is required as the prediction accuracy using sequence formation is comparable to that of using tertiary structures. Hence the server's prediction is rather accurate (http://www.ics.uci.edu/~baldig/mutation.html) (Cheng *et al,* 2006).

PROVEAN is a sequence based predictor using a clustering method where top 30 clusters are averaged to generate a final PROVEAN score that estimates the effect of amino acid variation on protein function A variant is hypothesized to be "deleterious" if the final score is below a certain threshold value (default -2.5) and is hypothesized to be "neutral" if the score is above the threshold (http://provean.jcvi.org/index.php) (Choi *et al,* 2012).

Table 2 Prediction scores from SIFT, PolyPhen2, PANTHER and MuPro tools of the nsSNPs selected for ADRB1 gene.

Ligand Binding Sites (218-367)	SNP ID	Amino Acid Change	SIFT	PolyPhen 2	PANTHER		MuPro		
					SubPSEC	$P_{deleterious}$	Confidence Score (Neural Network)	Confidence Score (SVM)	Effect on stability of protein
Topological domain (Extracellular)	rs373885952	V219I	0.42 (tolerated)	0.007 (benign)	-1.00523	0.11975	-0.800806003119735	-0.53570604	Decrease
Topological domain (Extracellular)	rs200385012	R222P	0.24 (tolerated)	0.862 (Possibly Damaging)	-3.16897	0.54214	0.636636206752852	0.37698163	Increase
Transmembrane (helical)	rs180897	A343T	0.05 (Deleterious)	0.763 (Possibly Damaging)	-2.60864	0.40339	-0.804319530912265	-1	Decrease
Transmembrane (helical)	rs138212934	W364C	0 (Deleterious)	0.999 (Probably Damaging)	-10.30463	0.99933	-0.914447019984403	-1	Decrease
Disulfide Bond (131-215)									
Transmembrane (helical)	rs373548972	T135S	0.01 (Deleterious)	0.945 (Probably Damaging)	-3.36327	0.58983	-0.630933675201507	-0.48138877	Decrease
Transmembrane (helical)	rs145117867	S145I	0 (Deleterious)	1 (Probably Damaging)	-7.4751	0.98874	0.7452566098874149	0.95269365	Increase
Topological domain (Cytoplasmic)	rs370777515	I160N	0 (Deleterious)	0.99 (Probably Damaging)	-3.95711	0.72254	-0.999873714145792	-1	Decrease
Transmembrane (helical)	rs138952486	A175G	0 (Deleterious)	0.998 (Probably Damaging)	-4.15679	0.76075	-0.902479640153902	-0.89327247	Decrease
Transmembrane (helical)	rs527899303	R176Q	0 (Deleterious)	**0.994** (Probably Damaging)	-2.04318	0.27752	-0.75306271185866	-0.15575163	Decrease

MutPred works by a random forest algorithm based on many features of protein structure and function like the probabilities of gain or loss of properties including loss of solvent accessibility, loss of catalytic residue, loss of stability, and gain of methylation site. The MutPred score is the prediction of the probability that a single amino acid change is deleterious/disease-associated. A missense mutation with a MutPred score >0.5 is considered as "harmful", while a Mutpred score >0.75 is considered as a high confidence "harmful" prediction (http://mutpred.mutdb.org/) (Li *et al*, 2009).

RESULTS

ADRBs being GPCRs acts by activating intracellular G proteins upon binding with catecholamine agonist ligands such as adrenaline and noradrenaline (Rosenbaum *et al*, 2009; Evans *et al,* 2010). Also these receptors are the targets for many drugs that act either by activating or inhibiting βARs for the treatment of hypertension, asthma or cardiac dysfunction (Warne *et al,* 2011) Inherited disease susceptibility and genetic variation in coding and noncoding regions in humans is mostly associated with SNPs whose variability leads in differences in drug toxicity and efficacy (Johnson and Ligget, 2011). In the present study we have analyzed the structural and functional effect of missense mutations arising from SNPs. Different bioinformatics tools were used to identify the deleterious mutations. NCBI database records 39 nsSNPs of ADRB1 and 43 of ADRB2 present in coding region of the gene out of which 9 nsSNPs of ADRB1 and 5 of ADRB2 are present on the ligand binding and disulfide bonds formation sites and hence these SNPs are susceptable to cause a direct affect on protein structure. However none of the nsSNPs were present on phosphorylation, Glycosylation and Myristolyation sites.

In SIFT 7 nsSNPs of ADRB1 and 2 of ADRB2 were identified to be deleterious with a tolerance index score ≤0.05.In PolyPhen 2, a total of 8 nsSNPs of ADRB1 and 1 nsSNP of ADRB2 were predicted as damaging (PSIC > 0.5); 6 of these nsSNPs of ADRB1 and 1 of ADRB2 were predicted to be probably damaging, with a PSIC score of > 0.9.The PANTHER software estimates the likelihood that the nsSNPs will affect the function of the protein. The calculated subPSECs were equal to or lower than-3, resulting in a probability of deleterious effect higher than 0.5 for 6 nsSNPs. In MuPro score less than 0 means the mutation decreases the protein stability. The smaller the score, the more confident the prediction is. 7 of nsSNPs of ADRB1 and 4 of ADRB2 have been predicted to decrease the stability of protein by Mupro.

The PROVEAN score was lower than -2.5 for 7 nsSNPs in ADRB1 and 2 in ADRB2, indicating that these variants do affect the protein function and are likely to be deleterious. In the MutPred analysis, 8 nsSNPs of ADRB1 and 3 of ADRB2 showed a probability of being a deleterious mutation, with g scores higher than 0.5. For 2 of these nsSNPs of ADRB1 and ADRB2 the program indicated an actionable or confident hypothesis (p score < 0.05) that the molecular mechanism would be disrupted.

Table 3 Prediction of changes in structural and functional properties of ADRB1 gene by PROVEAN and MutPred

S.No.	SNP ID	Amino-acid Change	PROVEAN		MutPred		Molecular Mechanism Disrupted(P)
			SCORE	PREDICTION	SCORE	PREDICTION	
1.	rs373885952	V219I	-0.340	Neutral	0.403	Neutral	
2.	rs200385012	R222P	-2.900	Deleterious	0.502	Harmful mutation	
3.	rs180897	A343T	-1.780	Neutral	0.574	Harmful mutation	
4.	rs138212934	W364C	-11.925	Deleterious	0.891	High Confidence	Gain of catalytic residue at L365 (P = 0.0457)
5.	rs73548972	T135S	-3.718	Deleterious	0.623	Harmful mutation	
6.	rs145117867	S145I	-5.762	Deleterious	0.842	High Confidence	
7.	rs370777515	I160N	-6.663	Deleterious	0.854	High Confidence	
8.	rs138952486	A175G	-3.886	Deleterious	0.835	High Confidence	
9.	rs527899303	R176Q	-2.629	Deleterious	0.672	Harmful mutation	Loss of methylation at R176 (P = 0.002) Loss of MoRF binding (P = 0.0159)

Table 4 Prediction scores from SIFT, PolyPhen2, PANTHER and MuPro tools of the nsSNPs selected for ADRB2 gene.

Ligand Binding Sites (193-316)	SNP ID	Amino Acid Change	SIFT	PolyPhen2	PANTHER		MuPro		Effect on stability of protein
					SubPSEC	$P_{deleterious}$	Confidence Score (Neural Network)	Confidence Score (SVM)	
Transmembrane (helical)	rs201318801	F290S	0 (Deleterious)	1 (Probably Damaging)	0.94577	0.00206	-0.971762991241848	-1	Decrease
Transmembrane (helical)	rs375254430	V292I	0.25 (tolerated)	0.264 (benign)	0.45946	0.1423	-0.774375054036382	-0.4322405	Decrease
Disulfide Bond (106-190)									
Transmembrane (helical)	rs149199162	A128S	0.03 (Deleterious)	0.871 (Possibly Damaging)	0.62449	0.05567	-0.987763617458521	-1	Decrease
Topological domain (Extracellular)	rs148196791	Q179E	1 (tolerated)	0 (benign)	0.14915	0.12747	0.6846455260431004	0.88195115	Increase
Topological domain (Extracellular)	rs200042760	N187S	0.08 (tolerated)	0.007 (benign)	0.10456	0.05866	-0.68801061896647	-0.67245644	Decrease

Table 5 Prediction of change in structural and functional properties of ADRB2 gene by PROVEAN and MUTPRED

S.No.	SNP ID	Amino-acid Change	PROVEAN		MutPred		
			SCORE	PREDICTION	Score	Prediction	Molecular Mechanism Disrupted(P)
1.	rs201318801	F290S	-7.680	Deleterious	0.808	High Confidence	loss of stability (P=0.0086) gain of glycosylation (P=0.0247)
2.	rs375254430	V292I	-0.507	Neutral	0.631	Harmful mutation	Loss of catalytic residue at V292 (P = 0.02)
3.	rs149199162	A128S	-2.677	Deleterious	0.606	Harmful mutation	
4.	rs148196791	Q179E	0.728	Neutral	0.348	Neutral	
5.	rs200042760	N187S	-1.936	Neutral	0.489	Neutral	

Table 6 Population data of SNPs of ADRB1 (www.ncbi.nlm.nih.gov/snp/)

rs ID	Amino-acid Change	Population group	Genotype detail	
rs180897	A343T	European	C	T
		Asian	0.973	0.027
		Sub Saharan	0.982	0.018
		African	1.000	0.000
rs145117867	S145I	ESP Cohort	G	T
			1.000	0.000
rs138952486	A175G	ESP Cohort	C	G
			1.000	0.000

The deleterious scores from SIFT, PolyPhen2, PROVEAN, MutPred and PANTHER provide a numerical value associated with the prediction. In Polyphen2 and MutPred higher scores indicate damaging mutations, while in SIFT, PROVEAN, PANTHER lower or negative scores correspond to damaging SNPs.

DISCUSSION

In ADRB1 4 Out of 9nsSNPs compiled in table 1, are predicted to be deleterious by all the above mentioned tools. Both V219I and R222PnsSNPs lies in the extracellular topological domain of the protein and are also involved in ligand binding. At V219I, all 4 tools except SIFT and MuPro gives neutral results. As Valine is an aliphatic, hydrophobic, amino acid, it prefers substitution with other amino acids of the same type like Isoleucine, and Threonine so this change may not significantly affect the protein structure. In case of R222P, SIFT categorized the mutation as tolerable, whereas rest of the tools categorized it as deleterious. A343T and W364C lie in transmembrane helix and involved in ligand binding. At A343T all tools except PROVEAN and PANTHER predicts the substitution as deleterious.W364C is a change from Tryptophan, which is hydrophobic amino acid containing nitrogen in the aromatic ring system, being substituted by cysteine a neutral, small and polar amino acid. (http://www.russelllab.org/aas/Trp.html).Crystallographic studies show that Tryptophan forms hydrogen bond with ligand Dobutamine (Warne *et al,* 2012). A change from tryptophan to cysteine at this position might alter drug binding affinity and could impact the structure with decreased /increased stability and functional implications. All tools predicts this change as deleterious and MutPred referred it as confident hypotheses with molecular mechanisms disrupted (g score >0.5 and p score <0.05) i.e. gain of catalytic residue at L365.

T135S, S145I, A175G and R176Q lies in transmembrane helix and I160N lies in the cytoplasmic domain and are involved in disulfide bond formation.T135 site is involved in binding with Cyanopindolol, Carmoterol, Isoprenaline, Dobutamine and Salbutamol via Vander-wall interaction (Warne *et al,* 2012).

These nsSNPs are predicted to be deleterious by all the tools. So these changes alter the structural and functional properties of the protein. At 176 position MutPred predicts a loss of methylation at R176 (P = 0.002) and loss of MoRF binding (P = 0.0159) impacting protein stability.

Moreover for only three SNPs population of ADRB1 gene study has been carried out and data is tabulated below amongst the discussed SNPs.

In ADRB2 two nsSNPs lying in transmembrane helix and are engaged in ligand binding. F290S was predicted to be deleterious by all the tools except PANTHER. Mutpred predicts that the change at F290 results in loss of stability (P=0.0086) and gain of glycosylation (P=0.0247). In V292I Mutpred and MuPro predictions indicate this variant as deleterious whereas other tools indicate this as a benign amino acid exchange. MutPred predicts a high probability for this variant to be deleterious as the change at V292 results in loss of catalytic residue (P = 0.02).

A128S, Q179E and N187S are involved in disulfide bond formation. A128S lying in transmembrane helix is predicted to be deleterious by all tools except panther. Population data for only two SNPs A128S and Q179E is available in ESP cohort showing them to be monomorphic (www.ncbi.nlm.nih.gov/snp/). As MutPred does not predicts any loss or gain in structural and functional properties but may alter the protein stability. Q179E and N187S present in extracellular domain are hypothesized as neutral and tolerated.

CONCLUSION

The analysis of the nsSNPs involved in the determination of variation in genotypes is a challenge and requires different approaches as they can alter the individual's drug response. Such amino acid variants are important indicators of potential therapeutic approaches and effective action sites. Present study used different tools to predict the most damaging mutations in the ADRB1 and ADRB2 genes, the key protein in control of hypertension and drug targets in humans. Although some of the polymorphisms found in these two Beta-Receptors have been studied in the laboratory, many others have no population data available with respect to their possible damaging effects caused by the mutations on protein structure and function. The tools and softwares used here are based on evolutionary, physicochemical properties and computational methods. Computational prediction tools need to be trained as they are generally based on machine learning algorithms. Thus for an efficient and accurate prediction of functional nsSNPs and their linkage to the disease knowledge of protein structure is crucial. In this study the selected most-probably damaging nsSNPs could be prioritized in further studies of the functional

properties of the mutated receptor. In particular, the W364C, R176Q of ADRB1 and F290S, V292I of ADRB2 SNPs, as indicated to be the most deleterious by different tools.

Finally, these results may contribute to the understanding the causes and pharmacogenetics of complex diseases such as hypertension, diabetes and cardiovascular disease. This will help in an appropriate interpretation of pharmacogenetic implication of antihypertensive drugs specially beta-blockers at population level, with relevant information for individual patients and drug developers.

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