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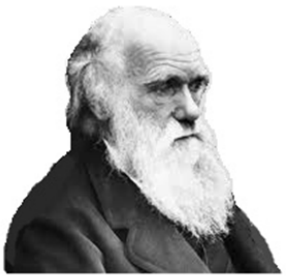

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Computational study of *ACE* and *AGT* gene of RAAS pathway

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ABSTRACT

Renin angiotensin aldosterone system (RAAS) is a hormone regulatory hormone system that regulate blood pressure. The two major genes *ACE* and *AGT* are the players of RAAS pathway. These genes codes for angiotensin convertase enzyme and angiotensinogen protein respectively. The angiotensin convertase enzyme convert inactive angiotensinogen into active angiotensin which further helps in the regulation of blood pressure. Due to imbalance in this pathway may cause hypertension. So in the present study we decided to perform the computational study of *ACE* and *AGT* gene. We evaluated the deleterious/damaging effect of SNPs of *ACE* and *AGT* gene by SIFT and I-Mutant2.0. The total number of SNPs predicted to be deleterious by both tools were 5 (1.83%) and 22 (6.07%) for *AGT* and *ACE* genes respectively. We also studied subcellular location of *ACE* and *AGT* genes and drugs targeting these genes from database GeneCards. Further the result output of both the softwares were also compared.

***Keywords*:** Angiotensin converting enzyme, Hypertension, Renin angiotensin aldosterone system, Angiotensinogen, Single nucleotide polymorphism

1. INTRODUCTION

Hypertension is a condition in which the blood pressure in the arteries is persistently elevated. It is a long term medication condition which is also called as silent killer, if not detected on time can cause severe problems to the major organs of the body such as heart,

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kidneys, eyes and brain. It also leads to cardiovascular diseases which include coronary heart disease, cerebrovascular diseases, rheumatic heart diseases and congenital heart disease (Juan et al., 2013). There are studies which strongly correlates hypertension with adverse outcomes such as stroke, ischemic heart diseases, heart failure and renal diseases (Poulter et al., 2015). According to world health organization, hypertension is one of the most important risk factor for life threatening disorders (Makuc et al., 2017). It is estimated to cause 4.5 % of global disease burden. Worldwide raised blood pressure is estimated to cause 7.5 million deaths, about 13.5% of the total of all deaths (Solomon et al., 2015). In India the prevalence and incidence of hypertension is extensively studied and it was found that the hypertension is 25% prevalent in urban population and 10% prevalent in rural population of India respectively (Anchala et al., 2014; Gupta et al., 2015; Sebastian et al., 2016; Singh et al., 2016; Anuradha et al., 2017). The renin angiotensinogen aldosterone system (RAAS) plays an important role in controlling the blood pressure (Richard et al., 2006). *ACE* and *AGT* are the important genes that major role in RAAS pathway.

The *ACE* (Angiotensin convertase enzyme) converts the inactive angiotensinogen in to active angiotensin (*AGT* II) which further acts as a potent vasoconstrictor. This leads to the contraction of the muscles of the vessels of large arteries and small arterioles thus leading to the high blood pressure. Another gene *AGT* codes for angiotensinogen, the active form of this protein i.e. angiotensin further stimulates the production of aldosterone which triggers the absorption of salts and water by the kidneys. Such increase in the volume of the fluid also increases blood pressure. So the role of these two genes in RAAS pathway revealed the importance of *ACE* and *AGT* in regulating the blood pressure or the condition of hypertension.

Further there are several studies which reports the effect of various SNPs (Single nucleotide polymorphism) of these genes influencing the genetic predisposition towards hypertension (Zhao et al., 2015; Makuc et al., 2017). These shows that the importance of SNPs for their ability to influence drug efficacy, disease risk and susceptibility. Thus there is great need for an effective and efficient method to filter out pathogenic and deleterious from the available pool of variant data and further explore the impact of those selected SNPs at the molecular level. Bioinformatics tools can be used in a cost efficient manner for prioritizing SNPs of likely functional importance, enabling an investigation of the structural basis of disease causing mutations most likely to contribute to an individual's disease susceptibility. So in the present study we decided to perform the computational analysis of non-synonymous SNPs of *ACE* and *AGT*.

2. MATERIALS AND METHODS

2. 1. Prediction for deleterious/ damaging SNPs

For the evaluation of damaging/deleterious status of the SNPs of *ACE* and *AGT* genes, SIFT and I-Mutant 2.0 were used. For this, variants information (SNP IDs) of *ACE* and *AGT* were collected from dbSNP. dbSNP is world's largest database for nucleotide variations, and is part of the National Center for Biotechnology Information (NCBI), an internationally respected resource for molecular biology information.

2. 1. 1. SIFT (Sorting of Intolerance from Tolerance)

It is a sequence homology-based tool that sorts intolerant from tolerant amino acid substitutions and predicts whether an amino acid substitution in a protein will have a phenotypic effect. SIFT is based on the premise that protein evolution is correlated with protein function. Positions important for function should be conserved in an alignment of the protein family, whereas unimportant positions should appear diverse in an alignment.

2. 1. 2. MUTANT 2.0

I-Mutant 2.0 can be used both as a classifier for predicting the sign of the protein stability change upon mutation, and as a regression estimator for predicting the related Delta G (δG) values. Acting as a classifier, I-Mutant 2.0 correctly predicts (with a cross-validation procedure) 80% or 77% of the dataset, depending on the usage of structural or sequence information, respectively.

2. 1. 3. GeneCards

The information regarding subcellular location and drugs targeting *ACE* and *AGT* genes were retrieved from database GeneCards. GeneCards is a searchable, integrative database that provides comprehensive, user-friendly information on all annotated and predicted human genes. It automatically integrates gene-centric data from ~125 web sources, including genomic, transcriptomic, proteomic, genetic, clinical and functional information.

3. RESULTS AND DISCUSSION

3. 1. Distribution of SNPs over *ACE* and *AGT* gene

In the present study the *ACE* and *AGT* genes have following SNPs distribution respectively as shown in figure1. The distribution of SNPs over the gene region also influence its function. The regulatory regions of the gene like 5'UTR, exonic region, intronic region and 3' UTR are hotspot regions where SNPs distribution determines the expressivity of the gene.

5'UTR is a promoter site which determines the binding affinity of polymerases and is very important for the increased/decreases or basal level of the expression of the gene. The mRNA stability is a major prerequisite condition for the optimal gene expression. For this 3' UTR is the region which determine the stability of the mRNA. The SNPs allocation at the intronic region is also very important because it is the major site for splicing and its regulation. Various studies have reported the importance of such SNPs in disease predisposition and its susceptibility (Wolinger et al., 2015). Further the SNPs lying in the exonic region and are directly translating into functional protein are distinctly influencing the expression of the respective gene.

The SNPs in the coding region are of three types i.e. synonymous, non-synonymous and frame-shift (Figure 2). Synonymous SNPs do not affect the protein sequence while non synonymous and frame-shift change the amino acid sequence of the protein.

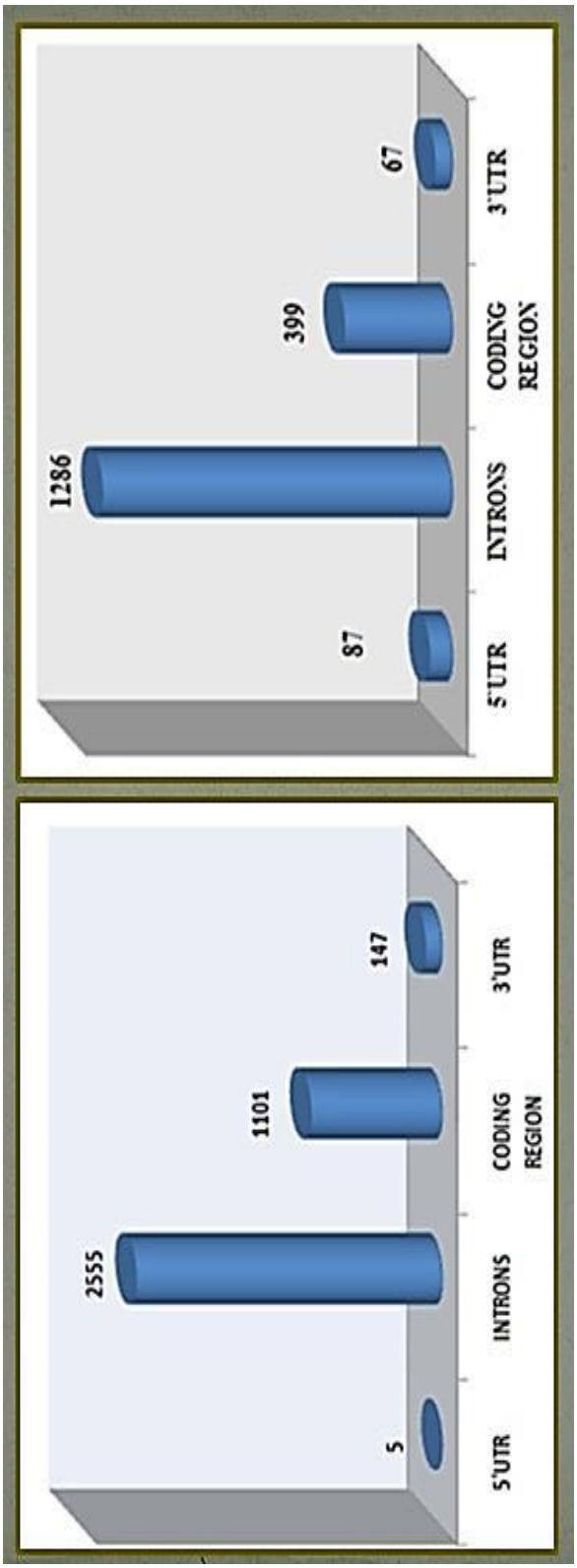

Figure 1. SNPs distribution over *ACE* and *AGT* gene according to dbSNP

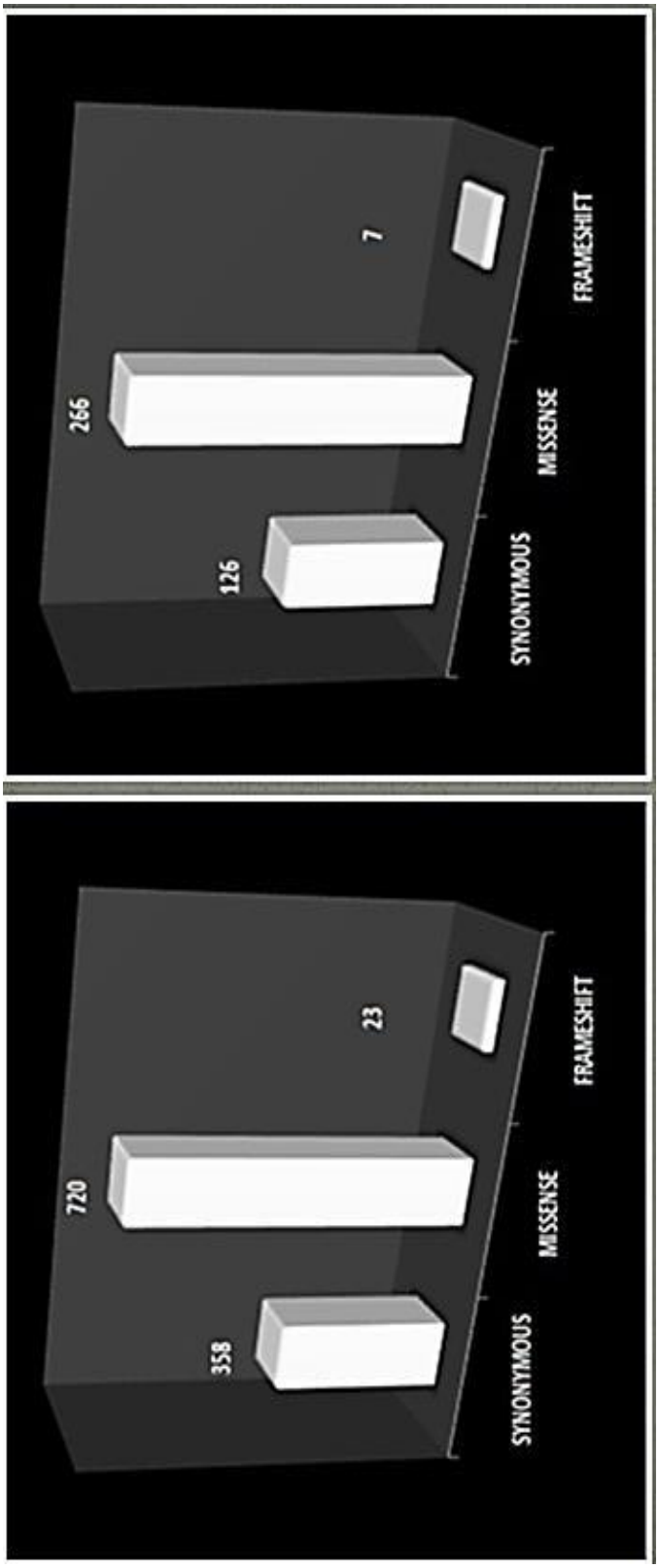

Figure 2. SNPs distribution in the coding region of the *ACE* and *AGT* gene

3. 2. Assessment of deleterious SNPs of *ACE* and *AGT* genes by SIFT and I-Mutant

The non synonymous SNPs of *AGT* and *ACE* genes were evaluated for deleterious status through SIFT and I-Mutant. Out of total (271) nsSNPs of *AGT* gene, 195 (71.95%) and 8 (2.91%) nsSNPs were found to be deleterious by I-Mutant and SIFT respectively. Similarly in case of *ACE* gene, 512(71.11%) and 21 (2.86%) were found to be damaging by I-Mutant and SIFT out of total 743 ns SNPs (Table 1).

Table 1. List of nsSNPs (tolerated/deleterious) of *ACE* and *AGT* genes that were analyzed by computational methods i.e. SIFT and I-Mutant 2.0

S.No	rs number	Base change	Type of mutation	Amino acid change	I- Mutant DDG (Kcal/mol)	SIFT Damaging SNPs
			ACE(Angiotensin convertase enzyme)			
1.	rs75214560	T>C	Fwd	S>P	-0.86	Damaging
2.	rs4314	C>T	Fwd	R>W	-0.48	Damaging
3.	rs12720746	T>C	Fwd	V>A	-2.57	Damaging
4.	rs28730839	C>G	Fwd	P>H	-1.68	Damaging
5.	rs2229839	C>T	Fwd	P>L	-0.59	Damaging
6.	rs13306087	G>A	Fwd	A>T	-0.70	Damaging
7.	rs35141294	C>T	Fwd	R>W	-3.08	Damaging
8.	rs4303	G>A	Fwd	A>T	-1.49	Damaging
9.	rs3730025	G>A	Fwd	Y>C	-1.46	Damaging
10.	rs77294580	G>T	Fwd	D>N	-1.34	Damaging
11.	rs117647476	A>G	Fwd	I>V	-0.35	Damaging
12.	rs4976	T>C	Fwd	I>T	-0.88	Damaging
13.	rs4977	T>G	Fwd	F>V	-2.54	Damaging
14.	rs4364	C>A	Fwd	R>S	-1.04	Damaging
15.	rs4981	A>C	Fwd	Q>P	-2.11	Damaging
16.	rs200649158	C>T	Fwd	R>W	-0.89	Damaging
17.	rs111998398	T>C	Fwd	W>R	-1.44	Damaging
18.	rs3730043	C>T	Fwd	T>M	-0.67	Damaging

19.	rs12720742	C>G	Fwd	T>R	-0.92	Damaging
20.	rs56038824	C>G	Fwd	V>E	-1.25	Damaging
			AGT(Angiotensinogen)			
S. No.	rs number	Base pair change	Type of mutation	Amino acid change	I-Mutant score DDG (Kcal mol^{-1})	SIFT
1.	rs61751076	C>T	Fwd.	A>V	0.37*	Damaging
2.	rs61751077	C>T	Rev.	S>F	-0.37*	Damaging
3.	rs617562527	C>G	Rev.	P>A	-2.29*	Damaging
4.	rs17856352	C>T	Rev.	P>S	-2.77*	Damaging
5.	rs61731497	T>C	Rev.	C>R	-0.35*	Damaging

There is a distinctive difference between the output of I- Mutant and SIFT. The standard deviation alongwith standard error (SD±SE) have been calculated for the output data have shown in Figure 3.

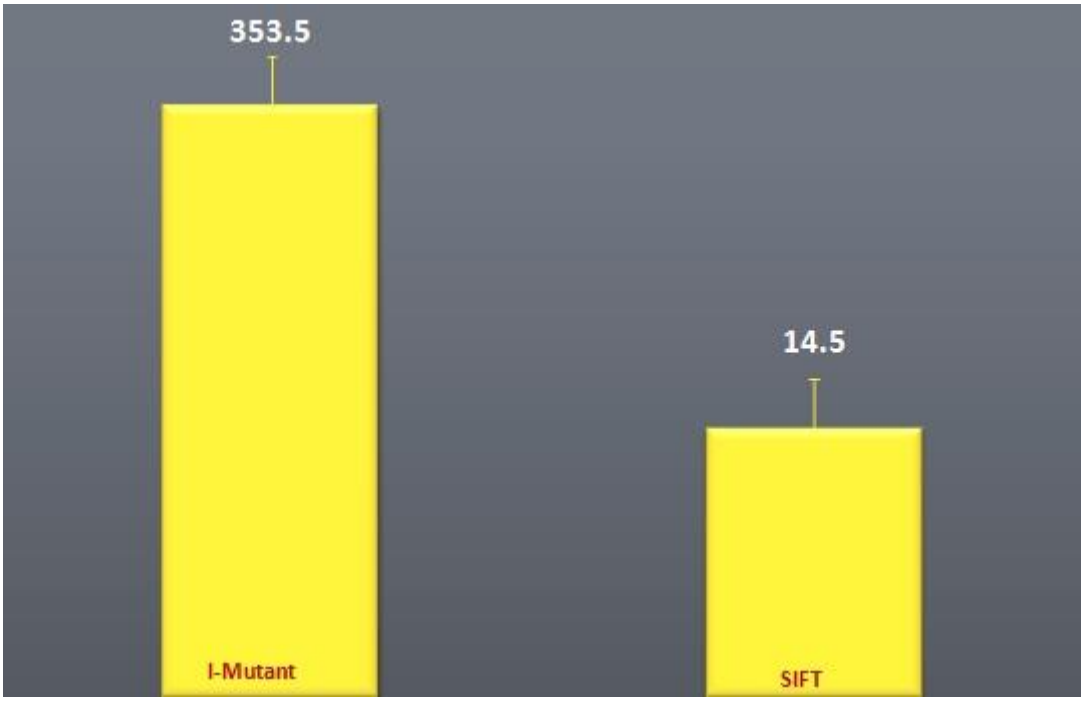

Figure 3. Comparison of output of I-Mutant and SIFT in terms of SD±SE

Chi square testing (P>0.05, 95% CI) have also shown that the method of assessment of damaging status of SNPs is independent for both tools. SIFT use sequence homology and predicts scores on the bases of position scoring, While the I-Mutant calculates the Gibb's free energy for amino acid change at particular position of the protein sequence.

3. 3. Subcellular localization of *ACE* and *AGT* gene

The cells of eukaryotic organisms are elaborately subdivided into functionally distinct membrane-bound compartments which are known as subcellular organelles within the cell. The below diagrams shows the subcellular location of *ACE* and *AGT* genes and the confidence number given to each organelle is according to the differential amount of gene expression present in them. The highest number depicts the largest amount of *ACE* and *AGT* gene expression and the lowest confidence depicts the least amount of gene expression in the organelle (Figure 4).

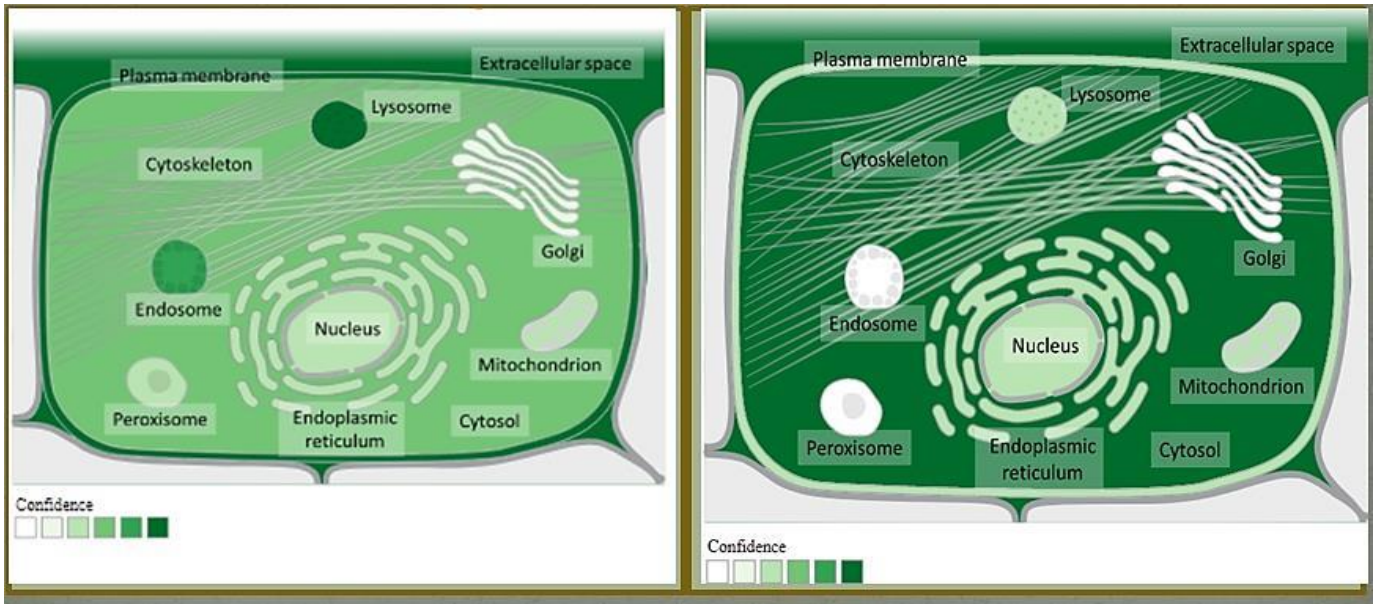

Figure 4. Differential abundance of *ACE* and *AGT* expression in various organelles and the intensity of color depicts the relative abundance of *ACE* and *AGT* in different organelles

The cells of eukaryotic organisms are elaborately subdivided into functionally distinct membrane-bound compartments which are known as subcellular organelles within the cell. The below diagrams shows the subcellular location of *ACE* and *AGT* genes and the confidence number given to each organelle is according to the differential amount of gene expression present in them. The highest number depicts the largest amount of *ACE* and *AGT* gene expression and the lowest confidence depicts the least amount of gene expression in the organelle (Figure 4).

The level of expression of *ACE* gene was found out to be maximum in plasma membrane, extracellular matrix and lysosomes as the *ACE* protein is an integral type of membranal protein. It catalyzes the angiotensin I into angiotensin II in the blood, hence the

ACE occurrence is also reported abundantly in extracellular matrix. The *ACE* enzyme acts as a protease that is why it is abundant in lysosome also. Besides this, the *ACE* is also found to be expressed in nucleus, endoplasmic reticulum and cytosol as given in Table 2.

Table 2. The confidence of the presence of the *ACE* and *AGT* genes in the subcellular compartments.

***ACE* (Angiotensin convertase enzyme)**		
S. No.	**COMPARTMENT**	**CONFIDENCE**
1	Plasma membrane	5
2	Extracellular region	5
3	Lysosome	5
4	Endosome	4
5	Cytosol	3
6	Cytoskeleton	2
7	Mitochondrion	2
8	Peroxisome	2
9	Nucleus	2
10	Endoplasmic reticulum	2
11	Golgi apparatus	1
AGT(Angiotensinogen)		
S. No.	**COMPARTMENT**	**CONFIDENCE**
1.	Extracellular	5
2.	Cytosol	5
3.	Plasma membrane	2
4.	Cytoskelton	2
5.	Mitochondria	2
6.	Nucleus	2
7.	Endoplasmic reticulum	2

Nucleus is the site where *ACE* gets transcribed and translation takes place in the cytosol. The *ACE* expression is also present in endoplasmic reticulum but in lesser amount because it is the minor site of post translational modification of *ACE* protein. In case of *AGT* gene, the extracellular space and cytosol have abundant presence because the major role of *AGT* is to facilitate the reabsorption of Na^+ and Cl^- in the blood stream and the active form of *AGT* is available in the extracellular space. Cytosol is the site where translation of *AGT* gene takes place that is why, *AGT* expression is found to be maximum in extracellular matrix and in cytosol. Besides this, the *AGT* is also found to be expressed in nucleus and endoplasmic reticulum in lesser amount as given in Table 2. Nucleus is the site where *AGT* gets transcribed. The *AGT* expression is also present in endoplasmic reticulum but in lesser amount because it is the site of post translational modification of *AGT* protein.

3. 4. Drugs targeting *ACE* and *AGT* gene

There are various drugs which are used to target the *AGT* gene out of which, 10 drugs are approved, 2 are under investigation and 9 drugs are under clinical trials. Some drugs target the genes involved in the *AGT* pathway so indirectly increase the expression of *AGT*. Drugs along with their target, mechanism of action and their pharmacological action are given below Table 3.

Table 3. Different drugs targeting *ACE* gene

S. No	Drug Name	Target	Mechanism of action	Role
1.	Ramipril	Angiotensin converting enzyme, B1 bradykinin receptor	Competitive inhibitor of both C and N domains of ACE gene	Inhibition of *ACE* gene
2.	Perindopril	Angiotensin converting enzyme	Competitive inhibitor of both C and N domains of ACE gene	Inhibition of *ACE* gene
3.	Captopril	Angiotensin converting enzyme, B1 bradykinin receptor	Competitive inhibitor of both C and N domains of ACE gene	Inhibition of *ACE* gene
4.	Lisinopril	Angiotensin converting enzyme, Angiotensin converting enzyme 2	Competitive inhibitor of both C and N domains of ACE gene	Inhibition of *ACE* gene
5.	Benazepril	Angiotensin converting enzyme	Competitive inhibitor of both C and N domains of ACE gene and degrade bradykinin(vasodilator)	Inhibition of *ACE* gene

All the above mentioned drugs play a vital role either on the action of *AGT* gene or pathways of *AGT* gene. Lisinopril acts as an *ACE* inhibitor. Amlodipine reduces the *AGT* expression in kidney through inhibition of vicious cycle. Atenolol decreases the blood pressure by suppressing the *AGT* expression. Irbesartan act as ARB which reduces the blood

pressure and urinary level of *AGT* gene. Simvastatin inhibits the expression of *AGT* gene via JAK-STAT pathway. Various drugs are also reported which are used to target the *ACE* gene. Some drugs target the genes involved in the *ACE* pathway so indirectly increase the expression of *ACE*. Some drugs are given below along with their target, mechanism of action and their pharmacological action Table 4.

Table 4. Drugs targeting *AGT* gene

S. No.	Drug name	Target	Mechanism of action	Pharmacological action
1.	Lisinopril	Angiotensin-converting enzyme	Decreasing Ang II levels in the body decreases blood pressure by inhibiting the effects of AngT II	Inhibitor
2.	Amlodipine	Voltage-dependent L-type calcium channel.	Amlodipine decreases arterial smooth muscle contractility and subsequent vasoconstriction.	Inhibitor
3.	Atenolol	Beta-1 adrenergic receptor	Atenolol competes with sympathomimetic and neurotransmitters such as catecholamines for binding at beta(1)-adrenergic receptors .	Antagonist
4.	Benazepril	Angiotensin-converting enzyme	Benazeprilat, competes with angiotensin I for binding at the angiotensin-converting enzyme and block the conversion of angiotensin I to angiotensin II.	Inhibitor
5.	Irbesartan	Type-1 angiotensin II receptor	an angiotensin II antagonist that selectively blocks the binding of angiotensin II to the AT1 receptor.	Antagonist
6.	Simvastatin	3-hydroxy-3-methylglutaryl-coenzyme A reductase	It competes with HMG-CoA for its reductase which interfere with the activity of its enzyme reduces the precursor of cholesterol.	Inhibitor

8.	Quinapril	Angiotensin-converting enzyme	It is the principle active metabolite of quinapril, competes with ATI for binding to *ACE*	Inhibitor
9.	Atorvastatin	3-hydroxy-3-CH3glutaryl-coenzyme A reductase	Selectively and competitively inhibits the hepatic enzyme HMG-CoA reductase.	Inhibitor
10.	CYT006-AngQb	Type-1 angiotensin II receptor	Induce angiotensin II specific antibodies that should inhibit binding of angiotensin II to its receptors and thus reduce the narrowing of blood vessels.	Unknown

Above described drugs are competitive inhibitors of *ACE* gene and play a major role in lowering down the blood pressure by inhibiting the function of *ACE* which leads to the formation of potent vasoconstrictor and breakdown of vasodilators (Bradykinin).

4. CONCLUSIONS

We targeted the nsSNPs of *ACE* and *AGT* gene for the evaluation of their deleterious/damaging status by two tools i.e. SIFT and I-Mutant. In the present study, we found the distinct differences between the evaluation efficiency of the both tools. The differences between the tools was further supported by chi square test and SD ± SE values. The SIFT work based on sequence homology with protein alignment and identify the functional mutation based on evolutionary fitness while I-Mutant gives its prediction on the basis of Gibb's free energy change during amino acid substitution. nsSNPs of *ACE* and *AGT* genes that were predicted to be deleterious by these two tools are 20 (2.69%) and 5 (1.83%). Further the structural and functional impact of these SNPs should also be studied at protein level. We have also reported the information about drugs targeting *ACE* and *AGT* gene expression and subcellular location of their gene expression alongwith confidence number. These information were retrieved from GeneCards databases. This databases contains the genomics and proteomics information, which are helpful in knowing the multi-dimensional aspect of the gene.

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