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RESEARCH ARTICLE

# Mutation in angiotensin II type 1 receptor disrupts its binding to angiotensin II leading to hypotension: An insight into hydrogen bonding patterns

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**Abstract** **To understand the role of angiotensin II type 1 receptor gene (AGTR1) gene products in relation to hypotension we have analyzed the single nucleotide polymorphisms (SNPs) associated with this gene. This can help us to understand the genetic variations that can alter the function of the gene products. In this present study, we report the polymorphic variant associated with AGTR1 and its weak interaction with angiotensin II (AngII) which leads to hypotension. Out of 1318 SNPs, six are found to be non-synonymous, of which rs1064533 shows significant damaging effect. A missense mutation (T1255G), i.e., from thymine to guanine for rs1064533 in AGTR1 gene results in amino acid substitution from cysteine (Cys) to tryptophan (Trp) in the receptor protein. A strong hydrogen bond exists between Cys289 of native AGTR1 protein and glutamine 167 of AngII. Interestingly, it is replaced by a weak hydrogen bond in the mutant protein between Trp289 (mutant residue) and serine 340. Such a substitution from small, hydrophilic to bulky, hydrophobic residue in AGTR1 protein results in reduced binding affinity of the receptor protein with AngII, leading to hypotension. The results presented from this *in silico* study will open up new prospect for genetic analysis of AGTR1 gene and will be beneficial to the researchers for understanding the role played by AGTR1 gene in hypotension disease.**

**Keywords** AGTR1, AngII, hypotension, rs1064533, cysteine, tryptophan

## Introduction

Blood pressure (BP) is highly regulated quantity and is maintained by the integrated physiological systems to secure an adequate blood perfusion in all tissues, despite widely varying metabolic demands (Rosskopf et al., 2007). Humans who have abnormal blood pressures may either have hypertension (high BP) or hypotension (low BP), both of which can be monogenic or polygenic (Sugiyama et al., 2001). Hypotension is defined as a decrease in systolic pressure to less than 100 mmHg (Rout et al., 1993). The genetic basis of hypotension has been studied, providing important insight into the mechanisms of BP regulation. Polymorphisms in the genes underlying this disorder are the potential contributors to BP variation (Tobin et al., 2008). Attention has been focused on non-synonymous single nucleotide polymorphisms (nsSNPs) for an association study of metabolic disorders which can be useful to examine the potential impact an amino acid variant may have on the function of the encoded protein (Johnson et al., 2005). Angiotensin II type 1 (AT1) receptors, member of the G-protein-coupled receptor superfamily (Griendling and Alexander, 1993) mediate most of the physiologic effects of angiotensin II (AngII), an octapeptide that serves as the regulator of BP and fluid homeostasis (Higuchi et al., 2007). The non-synonymous variants of the human angiotensin II type 1 receptor gene (AGTR1) have been analyzed and found to alter the biological properties of the AT1 receptor protein with loss of functional phenotypes (Hansen et al., 2004). Mice with mutant AT1 receptor have been found to develop chronic hypotension due to the functional loss of the receptor (Sugaya et al., 1995). The effect of AngII to drive up BP is achieved through the activation of the AT1 receptor (Berry et al., 2001;

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Leclerc et al., 2006). Bioinformatics tools are used to screen the potentially deleterious SNPs based on the gene of interest (Mah et al., 2011). A computational approach has also been employed to study the protein–protein complexes and their interactions to understand the disease mechanism (Ausiello et al., 1997). Studies on hypotension are necessary for a complete understanding of BP regulation (Xi, 2002). Polymorphism in AGTR1 gene reduces the binding affinity of AT1 receptor to Ang II, disrupting the BP regulation (Hansen et al., 2004; Leclerc et al., 2006). We assume that there are no bioinformatics approaches to document the decreased affinity of mutated AGTR1 gene products to AT1 receptor. This prompted us to carry out the analysis of nsSNPs of AGTR1 gene and the interaction of AT1 receptor protein with Ang II. Our results will provide an insight to the researchers for understanding the regulatory role played by AGTR1 gene and the genetic consequence of hypotension.

## Materials and methods

### Data source

The SNPs associated with AGTR1 gene were obtained from the single nucleotide polymorphism database (dbSNP) (Wheeler et al., 2002) and the reference SNP (rs) IDs are listed in the Supplementary table. There were a total of 1318 SNPs associated with AGTR1.

### F-SNP identifies nsSNPs by mining Ensembl database

We used Functional Single Nucleotide Polymorphism (F-SNP) database (Lee and Shatkay, 2009) for selecting nsSNPs of AGTR1 gene. The Ensembl database providing genome annotations with SNP locations and effects related to gene and protein structures (Ramensky et al., 2002; Reumers et al., 2005; Hubbard et al., 2009) was mined by F-SNP to identify nsSNPs.

### Identifying deleterious nsSNPs using F-SNP database

F-SNP database provides the functional information about SNPs with respect to protein coding, splicing regulation, and post-translation effects by mining variety of web services and databases. Several computational algorithms were mined by F-SNP to predict the damaging effect of coding nsSNPs (Lee and Shatkay, 2009) which include Sorting Intolerant from Tolerant (SIFT) (Ng and Henikoff, 2003), Polymorphism Phenotyping (PolyPhen) (Johnson et al., 2005; Zhu et al., 2008), SNPeffect (Reumers et al., 2006), Large scale annotation of coding nsSNPs (LS-SNP) (Karchin et al., 2005; Ryan et al., 2009) and SNPs3D (Yue et al., 2006). Other computational tools e.g., Exonic splicing enhancer (ESEfinder) (Cartegni et al., 2003), RESCUE-ESE (Fairbrother et al., 2004), Exonic splicing regulator (ESRSearch) (Fairbrother et al., 2002) and Putative Exonic splicing enhancer (PESE) (Zhang et al., 2005) were mined to identify SNPs in exonic splice regions. Golden Path was mined to identify SNPs in transcriptional regulatory regions (Kuhn et al., 2007). OGPET (Gerken et al., 2004) was used to examine post-translation modification sites. OGPET predicted the O-glycosylation sites (Lanver et al., 2010). PhastCons program was used to examine the conservation level of genomic sequences (Siepel et al., 2005; Satija et al., 2010).

### Comaprative modeling of wild and mutant AGTR1 proteins

Modeler, comparative modeling software was executed to build protein models from the templates obtained from sequence similarity with the target protein sequence (Fiser and Sali, 2003). The prediction process consisted of target-template alignment, model building, and model evaluation (Eswar et al., 2007).

### Computing disulphide bridges

We employed WHAT IF server (Hekkelman et al., 2010) to locate disulphide bridges in the native protein.

### Computation of cation-π interactions

Cation-π interactions for the mutant protein were computed by the program cation-π trends using realistic electrostatics [CAPTURE] (Gallivan and Dougherty, 1999).

### Interaction of modeled AGTR1protein with AngII

We used Evaluation of Surface Complementarity, Hydrogen bonding, and Electrostatic interaction in molecular Recognition (ESCHER) for docking procedure. ESCHER had three modules: SHAPES, BUMPS and CHARGES (Ausiello et al., 1997). The first module evaluated the geometric complementarity between the protein complexes. The second module, BUMPS identified molecular collisions within them and the CHARGES evaluated their electrostatic complementarity (Ausiello et al., 1997).

## Results

### Identification of nsSNPs and their selection using F-SNP database

The F-SNP database resource (Lee and Shatkay, 2008) lists six nsSNPs out of 1318 SNPs for AGTR1 gene. They are: rs1064533, rs12721226, rs12721225, rs13095608, rs17852013 and rs17852012. The corresponding allele change of these nsSNPs is also obtained from F-SNP. SIFT and PolyPhen tools are employed to obtain the corresponding

changes in the amino acid residues of these nsSNPs. F-SNP calculates a specific functional significance (FS) score for each of these nsSNPs which signifies their damaging effects (Lee and Shatkay, 2009). FS scores computed by F-SNP database are found to be 0.743, 0.374, 0.566, 0.563, 0.479 and 0.400 for the six nsSNPs rs1064533, rs12721226, rs12721225, rs13095608, rs17852013 and rs17852012, respectively, as depicted in Table 1.

### Functional prediction of nsSNPs using F-SNP database

F-SNP database predicts the deleterious effect of SNPs with respect to protein coding, splicing regulation, transcriptional regulation and post-translation effects (Lee and Shatkay, 2008). To obtain the damaging functional effect of nsSNPs, F-SNP integrates multiple tools that are based on different algorithms and computes the FS score for each of them. The deleterious SNP has a FS score value between 0.5 and 1 (Lee and Shatkay, 2009). Three nsSNPs: rs1064533, rs12721225 and rs13095608 are found to have significant FS scores in the range of 0.5 to 1.

The nsSNP, rs1064533 with FS score of 0.743 is predicted to be deleterious by SIFT, PolyPhen, SNP-effect, LS-SNP and SNPs3D tools which are queried by F-SNP database to predict the functional impact in protein coding region. Splicing regulation system is found to be altered by ESE finder, ESRSearch, and RESCUE-ESE. Golden Path examines a change in transcriptional regulatory region for rs1064533 (Table 2). The nsSNP, rs12721225 with FS score of 0.566 is predicted to be damaging by SIFT, PolyPhen, LS-SNP and SNPs3D tools. A change in splicing regulation region is found by ESRSearch and PESE. Changes in transcriptional regulatory region and in post translational modification site are examined by Golden Path and OGPET, respectively (Table 2). The nsSNP, rs13095608 with FS score of 0.563 is predicted to be damaging by SIFT, PolyPhen, SNPeffect and SNPs3D tools. ESRSearch predicts a change in the splicing regulation system and a change in transcriptional regulatory region is observed by Golden Path. The genomic regions for SNPs rs1064533, rs12721225 and rs13095608 are found to be conserved among all species by PhastCons (Table 2). Among these three nsSNPs, rs1064533 is found to have the highest deleterious FS score of 0.743 which is predicted to be functionally important.

### Wild and mutant protein structures obtained from modeler

The 3-D protein structures of both wild and mutant proteins are obtained by comparative homology modeling (Eswar et al., 2007). The program assigns the target sequence and the database of sequences of known structure from PDB as the input to obtain target-template alignments. A better measure of the significance of the alignment is given by the lower expected value (E-value) of the alignment which helps to choose the suitable template candidates (Eswar et al., 2007). The templates, 3OE0A, 3ODUA and 3OE6A, having lower E-value with better sequence coverage with the query protein are chosen for significant sequence alignments. A dendogram is obtained which calculates a clustering tree from the input matrix of pairwise distances, to understand the differences among the template candidates (Eswar et al., 2007). The most appropriate template, 3OE0A with better sequence identity is selected for target-template alignment and construction of the final model. Here, we have shown the native and mutant protein structures of SNP rs1064533 in Fig. 1, where cysteine (Cys) residue at 289 position in the native protein is substituted by tryptophan (Trp) residue in the mutant protein.

## Disulphide interactions

Cysteine 289 which undergoes mutation is not involved in disulphide interaction in the wild-type protein with any other cysteine residues.

### Cation-π interactions in the mutant protein

The substituted tryptophan at 289 position in the mutant protein is not involved in cation-π interaction with any other π residues.

### Protein–protein docking of wild and mutant AGTR1 protein and AngII

We report the results of docking predictions obtained by

**Table 1** nsSNPs with their corresponding amino acid changes and FS scores

| SNP ID | F-SNP | | | Amino acid change with position | |
|---|---|---|---|---|---|
| | SNP type | Allele change | FS score | SIFT | PolyPhen |
| rs1064533 | Non-synonymous | T/G | 0.743 | Cys(289)Trp | Cys(289)Trp |
| rs12721226 | Non-synonymous | G/A | 0.374 | Ala(163)Thr | Ala(163)Thr |
| rs12721225 | Non-synonymous | G/T | 0.566 | Ala(244)Ser | Ala(244)Ser |
| rs13095608 | Non-synonymous | T/G | 0.563 | Val(41)Gly | Val(41)Gly |
| rs17852013 | Non-synonymous | C/G | 0.479 | Leu(222)Val | Leu(222)Val |
| rs17852012 | Non-synonymous | C/A | 0.400 | Pro(341)His | Pro(341)His |

FS: Functional significance; T: Thymine; G: Guanine; Cys: Cysteine; Trp: Tryptophan; A: Adenine; Ala: Alanine; Thr: Threonine; Ser: Serine; Val: Valine; Gly: Glycine; C: Cytosine; Leu: Leucine; Pro: Proline; His: Histidine.

**Table 2** Functional prediction of nsSNPs by F-SNP database

| Functional category | Prediction tools | Prediction results of SNPs | | |
|---|---|---|---|---|
| | | rs1064533<br>FS score: 0.566 | rs12721226<br>FS score: 0.374 | rs12721225<br>FS score: 0.566 |
| Protein- coding | PolyPhen | Damaging | Benign | Damaging |
| | SIFT | Damaging | Tolerated | Damaging |
| | SNP-effect | Deleterious | Deleterious | Benign |
| | LS-SNP | Deleterious | Deleterious | Deleterious |
| | SNPs3D | Deleterious | Benign | Deleterious |
| | Ensembl | Non-synonymous | Non-synonymous | Non- synonymous |
| Splicing regulation | ESEfinder | Changed | Changed | Not changed |
| | ESRSearch | Changed | Not changed | Changed |
| | PESE | Not changed | Not changed | Changed |
| | RESCUE- ESE | Changed | Changed | Not changed |
| Transcriptional regulation | Golden Path | Exist | Exist | Exist |
| Post-translation | OGPET | Not exist | Exist | Exist |
| Conserved | PhastCons | Conserved | Conserved | Conserved |
| Functional category | Prediction tools | Prediction results of SNPs | | |
| | | rs13095608<br>FS score: 0.56 | rs17852013<br>FS score: 0.479 | rs17852012<br>FS score: 0.400 |
| Protein- coding | PolyPhen | Damaging | Damaging | Damaging |
| | SIFT | Damaging | Not found | Not found |
| | SNP-effect | Deleterious | No entry | No entry |
| | LS-SNP | Benign | Benign | Benign |
| | SNPs3D | Deleterious | Deleterious | Benign |
| | Ensembl | Non-synonymous | Non-synonymous | Non-synonymous |
| Splicing regulation | ESEfinder | Not changed | Changed | Changed |
| | ESRSearch | Changed | Changed | Not changed |
| | PESE | Not changed | Changed | Changed |
| | RESCUE-ESE | Not changed | Not changed | Not changed |
| Transcriptional regulation | Golden Path | Exist | Exist | Exist |
| Post-translation | OGPET | Not exist | Not exist | Not exist |
| Conserved | PhastCons | Conserved | Conserved | Conserved |

FS: Functional significance.

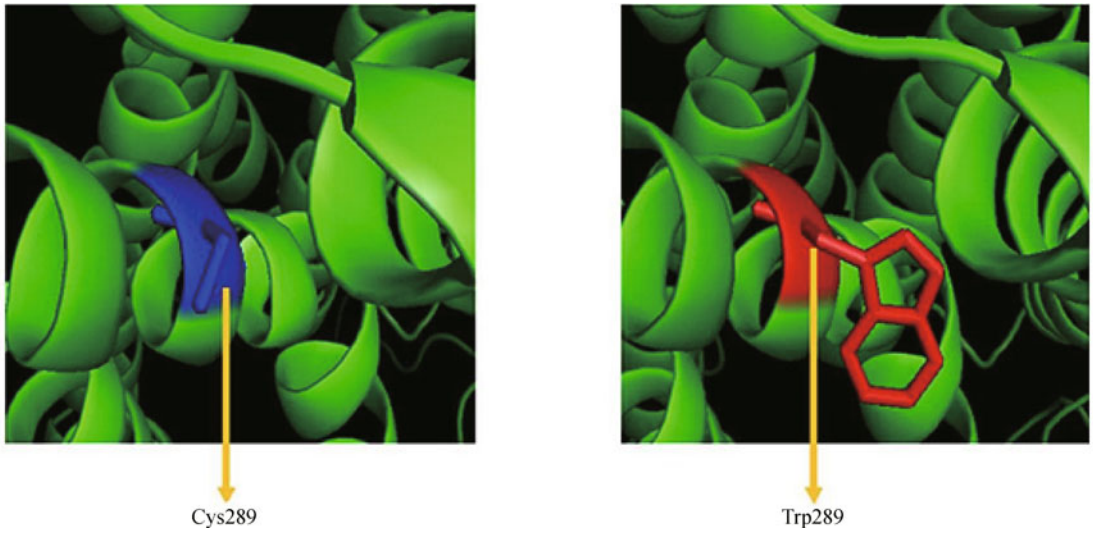


**Figure 1** The native protein structure of AGTR1with Cys (289) and mutant protein structure of AGTR1 with Trp (289) for SNP rs1064533.

ESCHER program (Ausiello et al., 1997). To rank the predictions ESCHER uses a combination of three modules that work in series: SHAPES, BUMPS and CHARGES. It obtains a list of solutions, each identified by the translations and rotations to be applied to the probe (AngII) with respect to the target (AGTR1). ESCHER procedure is applied to construct the protein–protein complex of native AGTR1 and AngII (2WXW). Protein–protein complex of native AGTR1 and AngII (2WXW) shows the interaction between Cys289 of AGTR1 and Gln167 of AngII as depicted in Fig. 2. Protein–protein complex of mutant AGTR1 (rs1064533) and AngII (2WXW) shows the interaction between Trp289 of AGTR1 and Ser340 of AngII as depicted in Fig. 2.

## Discussion

We retrieved a total of 1318 SNPs associated with AGTR1 gene and identified six SNPs to be non-synonymous (rs1064533, rs12721226, rs12721225, rs13095608, rs17852013 and rs17852012). Among these, three nsSNPs (rs1064533, rs12721225 and rs13095608) are found to have significant FS scores of 0.743, 0.566 and 0.563, respectively. The SNP, rs1064533 has the highest deleterious FS score of 0.743 and hence, it is predicted to be functionally important. The other three nsSNPs (rs12721226, rs17852013 and rs17852012) having low FS scores are not considered for our study.

The significant change in protein coding region is found for rs1064533 which replaces cysteine289 in AGTR1 protein with tryptophan due to the nucleotide change at 1255th position where TG**T** is replaced with TG**G**, i.e., **T** (thymine) to **G** (guanine). This indicates a change from a smaller hydrophilic amino acid to a bulkier hydrophobic amino acid. (The base represented in bold caption is the nsSNP.) Putative ESEs are also predicted for rs1064533 by the change in splicing regulation region. ESEs are short oligonucleotide sequences other than splice sites that enhance splicing from an exonic location. ESEs are recognized by proteins of the SR (serine-arginine) family, which recruit components of the core splicing machinery to nearby splice sites (Fairbrother et al., 2004). Numerous disease-associated polymorphisms exert their effects by disrupting the activity of ESEs (Smith et al., 2006). Conservation in the genome region is functionally important (Satija et al., 2010). Any change in this region might affect the protein function. The nsSNP, rs1064533 is found to be conserved among all species. The functional effects of nsSNPs have also been reported in other genetic disorder like dementia (Reynolds et al., 2009) in which bioinformatics tools are used for analysis.

Structural information is needed to fully understand the effects and consequences of mutations (Khan and Vihinen, 2007). The 3-D protein structures of both wild and mutants of AGTR1 are obtained by executing comparative modeling (Fiser and Sali, 2003; Eswar et al., 2007). The C289W substitution for SNP, rs1064533 is localized in the helix region of the modeled protein as shown in Fig. 1. α-helix, a secondary structure element, constitutes one of the major macromolecular building blocks of all "well-ordered" proteins (Benner et al., 1997). It forms one of the basic structural components of protein scaffolds (Khan and Vihinen, 2007). Disulfide bonds are covalent links between pairs of cysteine residues which stabilize the native conformation of a protein and maintain the protein integrity (Wetzel, 1987; Hogg, 2003). Hence we thought it would be useful to compute the role of cysteine 289 residue in disulphide interaction in the wild-type protein. But our result doesn't show any disulphide interaction for this residue.

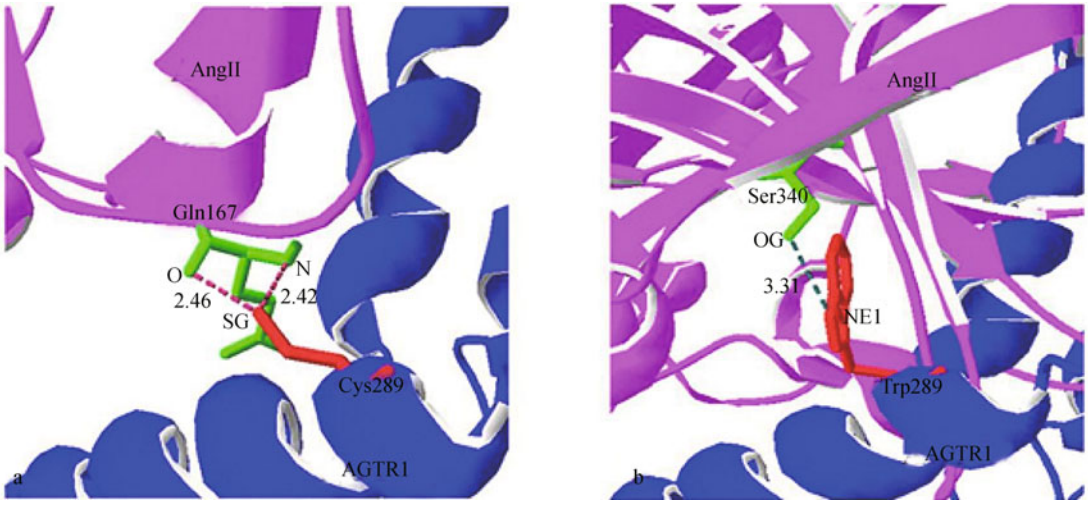


**Figure 2** Protein–protein interactions of AGTR1 and AngII. (A) Hydrogen bond with distance of 2.46 Å between sulfhydryl group at gamma position (SG) of Cys289 in native AGTR1 and oxygen (O) of Gln167 in AngII. Another hydrogen bond with distance of 2.42 Å between SG of Cys289 in native AGTR1 and nitrogen (N) of Gln167 in AngII. (B) Hydrogen bond with distance of 3.31Å between nitrogen at epsilon1 position (NE1) of Trp289 in mutant AGTR1 and hydroxyl group at gamma position (OG) of Ser340 in AngII.

Though cysteine 289 is not involved in disulphide interaction, the evidence for the importance of this residue has been provided in a recent study that analyzed the SNPs in the human AGTR1 gene (Leclerc et al., 2006). It has been proposed that cysteine 289 lies in a region of transmembrane seven (TM7) domain of AGTR1 protein that mediates binding of the AngII polypeptide (Perodin et al., 2002). α-helical TM proteins contain several functionally and structurally important types of substructures. They play vital role in endocytosis and exocytosis, intracellular transport, cell-cell interaction mechanisms and receptor-mediated signaling (Viklund et al., 2006). The C289W variant in the receptor protein displays reduced surface expression and decreases in binding affinities to threefold. Therefore, it has been reported that the presence of a bulky tryptophan residue may alter receptor confirmation sufficiently enough to disturb the interaction with AngII polypeptide (Hansen et al., 2004).

Cysteine 289 is also found to be highly conserved residue among all the different animal species in which the AT1 receptor was analyzed (Leclerc et al., 2006). So, a mutation occurring in the conserved residue of TM helix region of AGTR1 protein can lead to functional alteration in the receptor phenotype. A missense mutation in a TM alpha-helix of ATP binding cassette transporter A1 (ABCA1) gene causing Tangier disease has been reported by Maekawa et al., (2009). It has been found that cation-π interactions play stability roles in various protein molecules (Gallivan and Dougherty, 1999; Anbarasu et al., 2006; Anbarasu et al., 2007; Anbarasu and Sethumadhavan, 2007). Since the amino acid change is from cysteine to tryptophan, we thought it would be useful to compute the role of tryptophan residue in cation-π interaction. But the mutant residue is not involved in cation-π interaction, so it may not provide any stability to the protein molecule.

Structural knowledge of interactions between biomolecules is fundamental to understand the complex regulatory and metabolic pathways that occur in living organisms (Fernandez-Recio et al., 2002). Protein–protein interaction is the key process by which most of the proteins fulfill their function (Gavin and Superti-Furga, 2003). A protein–protein docking procedure is subjected to evaluate the interaction between the modeled AGTR1 protein and AngII (2WXW). Cys289 of native AGTR1 protein interacts with Gln167 of AngII. A missense mutation (T1255G) in AGTR1 gene leads to amino acid substitution from Cys289 to Trp289 in AGTR1 protein which shows interaction with Ser340 of AngII (Fig. 2). Hydrogen bonds are major contributor to strong physical interactions and play a central role in protein binding. The binding interfaces of protein are generally more hydrophilic and tend to form more hydrogen bonds than protein interiors (Meyer et al., 1996). To understand the interactions, we have carried out an analysis of hydrogen bonds between the protein–protein complexes. The hydrogen-acceptor distance is one of the measurements of hydrogen bond strength. The shorter the distance, the stronger is the hydrogen bond (Xu et al., 1997). We have found strong hydrogen bonding in the protein–protein complex of native AGTR1 and AngII with a distance of 2.46Å between sulfhydryl group at gamma position (SG) of Cys289 in AGTR1 and oxygen (O) of Gln167 in AngII. Another strong hydrogen bond is found in this complex with a distance of 2.42Å between SG of Cys289 in AGTR1 and nitrogen (N) of Gln167 in AngII. On the other hand, a weak hydrogen bond is found in the protein–protein complex of mutant AGTR1 and AngII with a distance of 3.31Å between nitrogen at epsilon1 position (NE1) of Trp289 in AGTR1 and hydroxyl group at gamma position (OG) of Ser340 in AngII (Fig. 2). Hydrogen-acceptor distance in the range of 2.2–2.5 is considered as strong hydrogen bonds and hydrogen-acceptor distance in the range of 3.2–4.0 Å as weaker ones (Jeffrey and Saenger, 1991). Thus a substitution from hydrophilic to hydrophobic residue with a weak hydrogen bond can disrupt the binding affinity of AGTR1 protein with AngII and fail to regulate the blood pressure, leading to hypotension (Hansen et al., 2004; Leclerc et al., 2006). Most genetic disorders are linked to missense mutations as even minor changes in the size or properties of an amino acid can alter or prevent the function of the protein (Khan and Vihinen, 2007). Our result also supports the evidence provided by Hansen et al. (2004) which describes that a substitution of bulky tryptophan residue may alter confirmation of AGTR1, disturbing the interaction with AngII polypeptide.

Based on the overall results from this study, we can ascertain that the mutation from cysteine to tryptophan at the residue position 289 in the AGTR1 native protein is a potential candidate for the cause of hypotension by AGTR1 gene. Hence, our study demonstrates that nsSNPs resulting in gene alterations can have a functional effect on protein phenotype, leading to alteration in protein–protein interactions. It has also been reported that SNPs in postmeiotic segregation increased 2 (PMS2) gene cause defective protein–protein interactions with MLH1, leading to hereditary nonpolyposis colorectal cancer (HNPCC) (Yuan et al., 2002). The result reported from our study with AGTR1 gene associated with hypotension is well supported by the evidence provided by Hansen et al. (2004). Thus the predicted results of the nsSNP of AGTR1 gene obtained from our study might be considered to be significant, deregulating the interaction of the receptor protein with AngII and leading to the development of hypotension. Our results obtained from these in-silico studies, might provide an insight into the genetic mechanism of the disease.

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