ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2023; 28(3): 3990-3997
doi: 10.25083/rbl/28.3/3990.3997

Received for publication, June, 11, 2023
Accepted, December, 30, 2023

Original article

Angiotensinogen: *in-silico* insight to mutations, protein-protein interactions and evolution

MISBAH HUSSAIN[1], SUMAIRA NISHAT[2]

[1] Department of Biotechnology, University of Sargodha, University Road, Sargodha, Pakistan
[2] Department of Computer Sciences, University of Agriculture, Faisalabad, Pakistan.

Abstract

In this era of rapidly evolving genetic and non-genetic diseases, in-silico analysis of disease related proteins has proven beneficial in identification and characterization of potential therapeutic targets. In present study, angiotensinogen (AGT) was studied for its sequence based ortholog and paralogs, structural characterization, mutational analysis, protein interactions and evolutionary changes. Sequence studies identified 194 orthologs and 36 paralogs of AGT. Functional analysis *AGT* polymorphisms revealed the association of M235T with increased AGT stability. Protein-protein interaction analysis exposed the involvement of AGT in metabolic diseases. So, this study provided base for the experimentally proven association of M235T polymorphism with metabolic diseases. Moreover, it presents AGT as strong therapeutic target for the control of these diseases.

Keywords

Angiotensinogen; cardiovascular disease; protein-protein interaction; phylogenetic analysis.

*Corresponding author: Dr.Misbah Hussain, Department of Biotechnology, University of Sargodha, University Road, Sargodha. Pakistan misbahussain@yahoo.com or misbah.hussain@uos.edu.pk

Introduction

Renin angiotensin aldosterone system (RAAS) plays a critical role in controlling blood pressure. Angiotensin II is a main bio-active component of RAAS. Angiotensin II is generated from the angiotensinogen (AGT) by the action of angiotensin converting enzyme with an intermediate step of angiotensin I formation by the cleavage of 10 amino acids from N-terminal of angiotensinogen by renin enzyme [1]. Several hypertension, diabetes and cardiovascular disease associated polymorphisms have been identified in AGT gene. Among these polymorphisms, functionally missense M235T (rs699) and T174M (rs4762) polymorphisms of AGT gene are most widely studied [2].

AGT is a member of non-inhibitory serpin superfamily. NCBI (https://www.ncbi.nlm.nih.gov/gene?Db=gene&Cmd=DetailsSearch&Term=183) states that human AGT gene is a 53 kDa, 485 amino acid long protein, encoded by 2291 bp long transcript of *AGT* gene consisting of 5 exons located on 1q42.2. *AGT* gene is oriented on minus strand with the total size of ~12 kb. In globular form, AGT is inactive [3]. However, its derived peptides are bio-active, control blood pressure. Up-regulation of an octapeptide (Angiotensin II) can lead to atherosclerosis, CAD, diabetes etc [4].

Cleavage of 10 amino acids from N-terminal of AGT by renin is a rate limiting step in synthesis of angiotensin II [3]. N-terminal 10 residues of AGT are highly conserved across all species, cleavage site of renin (Leu10 and Val 11) is buried in 63 amino acid long N-terminal tail of human AGT. Exposure of renin cleavage site and generation of angiotensin I depends on change in folding pattern due to oxidation or reduction of conserved disulfide bridge between cysteine at position 18 and 138 in human. In human, 60% of plasma AGT is oxidized (bridged) which is four folds more efficiently cleaved by receptor bound renin than reduced AGT (unbridged) [5-7]. Renin bound to newly discovered (pro)renin receptor cleaves the angiotensinogen 4 times more efficiently as compared to unbound renin [7]. Other than this, renin acts in specie specific manner, *in vivo* studies have demonstrated the inability of mouse renin to cleave human AGT. The proposed reason behind this renin specie specificity is the presence of human val11-Ile12 vs mouse Leu11-Tyr12 residues adjacent to renin cleavage site [8].

Several studies have delineated the exquisite role of angiotensin II in controlling blood pressure. Pathophysiological role of angiotensin II in progression of atherosclerosis, CAD, diabetes etc. have also been observed [4]. However, association of *AGT* polymorphisms [M235T and T174M] with aforementioned pathologies is heterogeneous [9-11].

Significant role of AGT in hypertension and cardiovascular diseases provides base for this study with an aim to decipher the structural differences and impact of polymorphism on its tertiary structure along with the phylogenetic history of AGT with the help of putative orthologues and paralogues. Moreover, influence of AGT on other metabolic pathways was also studied with the help of protein-protein interaction analysis.

Materials and Methods

Data collection

Sequence data of human AGT protein was collected from Universal protein resource knowledgebase (UniProtKB) with accession number P01019. Significant orthologous and paralogue of *AGT* gene were determined from the National Center for Biotechnology Information (NCBI) and ENSEMBL Genome Browser. These genome databases identified 194 orthologues, 36 paralogues (SERPIN1-12), SERPIN B1-B13, SERPINC1, SERPIND1, SERPIN E1-E33, SERPIN F1,F2, SERPIN G1, SERPINH1).

Mutational analysis

For mutational analysis of AGT, crystal structure of human AGT (5M3Y.pdb) was retrieved from RCSB Protein Data Bank (PDB). PDB-sum was used to obtain graphical view of secondary structure. SwissPDB viewer was used to insert misssense mutations M235T and T174M in human AGT (5M3Y). Moreover, Chimera was used to visualize and superimpose the normal and mutated human AGT. SNAP2 (https://rostlab.org/services/snap2web/), I-Mutant (http://folding.biofold.org/i-mutant/i-mutant2.0.html) and PhD-SNP (http://snps.biofold.org/phd-snp/phd-snp.html) were used to check the functional effect of mutations on AGT protein. ProtParam (http://web.expasy.org/protparam/) was used to check the effect of aforementioned missense mutations on physical and chemical properties of AGT.

Protein-protein interaction analysis

The protein-protein interaction (PPI) analysis of AGT was carried out using sequence search module of STRING database (https://string-db.org/), which envisage all known and predicted physical and functional protein-protein associations[12].

Phylogenetic analysis

The phylogenetic tree of AGT was reconstructed by using Molecular Evolution Genetics Analysis (MEGA) version 7, through the neighbor-joining (NJ) method.

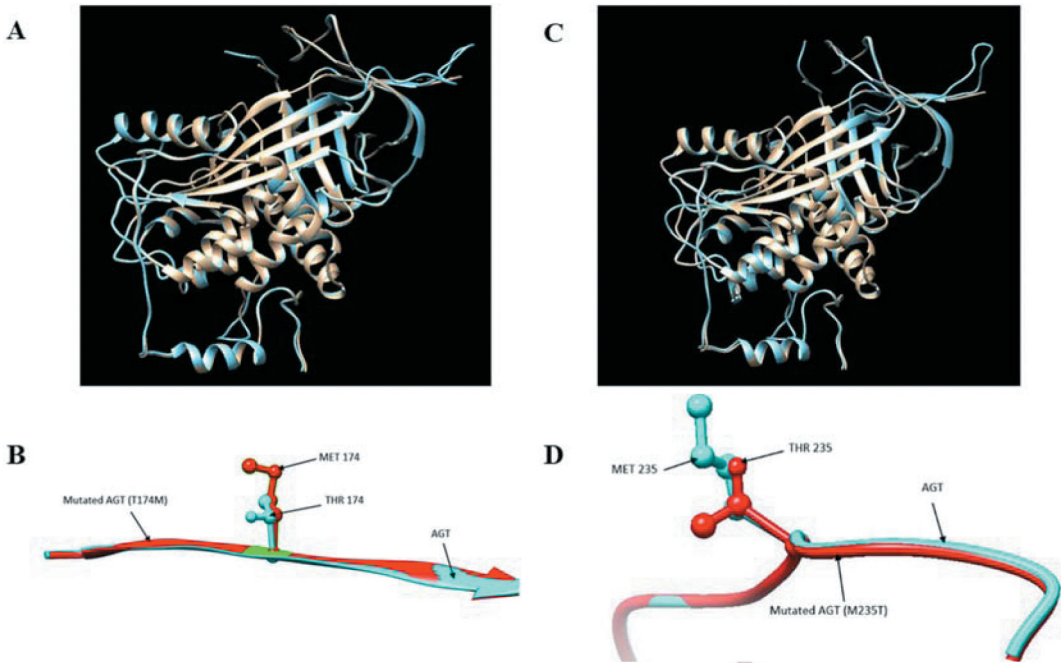

Fig. 1. [A and B] Superimposition of 174T (blue) and 174M (brown and red), [C and D] Superimposition of 235M (blue) and 235T (brown and red)

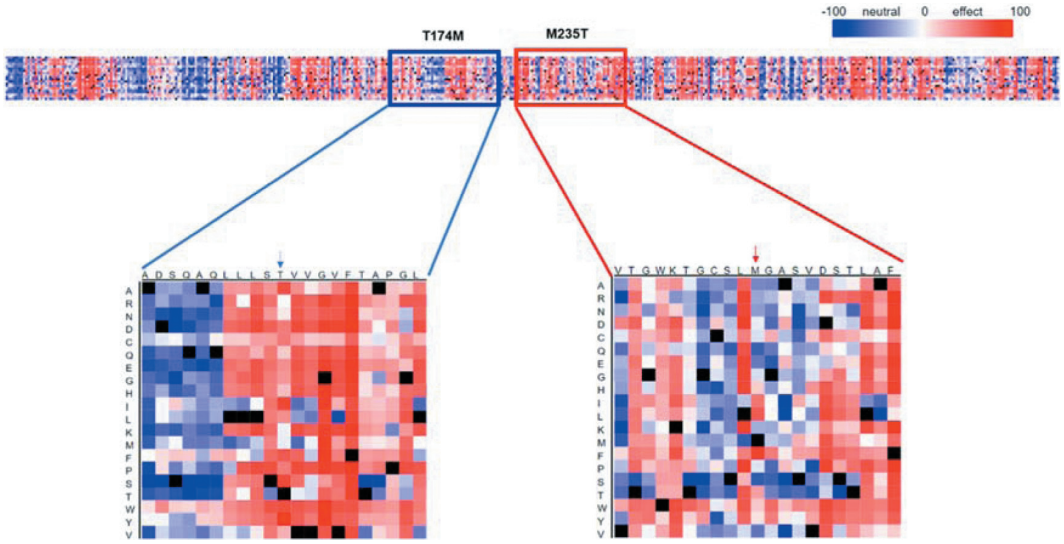

Fig. 2. SNAP2 prediction score for T174 M and M235T mutations in human angiotensinogen protein shown as heatmap. Arrow indicates the location of mutation. Functional effect score is shown for wild type amino acids (x-axis) to any other amino acid (y-axis), it is replaced with.

Results

Mutational analysis

For mutational analysis of AGT, crystal structure of human AGT (5M3Y.pdb) was retrieved from RCSB Protein Data Bank (PDB) (Figure 2A). The mutational analysis of AGT was carried out on the basis of two known missense mutations, T174M and M235T. SwissPDB viewer was used to insert reported mutations in human AGT as Swiss-Pdb-Viewer allows to mutate a residue by searching its rotamer library. Best rotamer model having fewer steric clashes was selected for further analysis while Chimera was used to view these mutations structurally (Figure 1).

Genetic polymorphisms, M235T and T174M, are located in exon 2 which is a coding region and contribute in protein structure. M235 and T174 plays an important role in maintaining structure of angiotensinogen protein. Methionine is sulfur containing hydrophobic amino acid which plays an important role in maintaining protein structure. The unbranched side chain of methionine give it extra flexibility, which is unique to only methionine among other hydrophobic residues. While, Threonine is a polar amino acid. Thus,

the substitution of Methionine with Threonine at 235 amino acid and replacement of Threonine with Methionine at residue 174 can affect the protein structure owing to their different biochemical properties.

Neural network based SNAP2 predicts the functional effects of mutations and score their effect from -100 (strong neutral) to +100 (strong effect). SNAP2 indicated that functional effect of methionine substitution at residue 174 is slightly neutral (score -5), while the replacement of methionine with threonine at amino acid 235 effect the function of AGT (score 37) (Figure 2).

The functional effect analysis of AGT by I-Mutant and PhD-SNP slightly contradict the findings of SNAP2. I-Mutant described that both mutations decrease the protein stability while PhD-SNP revealed no effect of described mutations on protein function (Table 1). For I-Mutant analysis binary classification was used (ΔΔG <0: decreased stability, ΔΔG >0 = increased stability), but when analyzed under ternary classification (ΔΔG <-0.5: large decrease of stability, ΔΔG >0.5: large increase of stability, -0.5<= ΔΔG <=0.5: neutral stability) the predicted effect of T174M shifted from decrease in stability to neutral.

To resolve this contradiction, ProtParam was used to check the impact of T174 M and M235T on physio-chemical properties of AGT. Results of ProtParam were in parallel with SNAP2 and showed that Thr>Met substitution at residue 174 does not affect the protein stability, while Met>Thr substitution at residue 235 draw a significant impact on protein stability. However, instead of decreasing the AGT stability, M235T slightly increase the protein stability (Table 2). One more factor, affecting the AGT stability could be the decrease in hydrophobicity (Table 2) due to the substitution of methionine (hydrophobic) with threonine (polar), which can make it more soluble and stable.

Protein-protein interaction analysis

Protein-protein interaction (PPI) analysis were carried out to understand the involvement of AGT in other pathophysiological pathways. PPI was studied by using a biological database STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) which is linked to several sources like GO, KEGG, BioGRID, NCI-Nature pathway interaction database etc. As angiotensinogen is a main component of RAAS pathway, STRING database showed that it mainly interacts with Renin (gene: REN), angiotensin converting enzyme (gene: ACE), angiotensin converting enzyme 2 (gene: ACE2), angiotensin II type 1 receptor (gene: AGTR1) and angiotensin II type 2 receptor (gene: AGTR2). Further broadening the PPI analysis revealed that AGT also interacts with renin/prorenin receptor (a.k.a. ATPase, H+ transporting, lysosomal accessory protein 2. Gene: ATP6AP2), endothelin 1 (gene: EDN1), chymase (gene: CMA1), thrombin (a.k.a. Coagulation factor II. Gene: F2) and kininogen (gene: KNG1) (Figure 3). Biological functions of these interacting proteins, their respective pathways and associated diseases are described in table 3.

Evolutionary analysis

To study the evolutionary relationship of AGT and its orthologues, initially, alignment of AGT protein and its orthologues was carried out by using ClustalX, then the phylogenetic tree was constructed by MEGA 7 software using distance matrix method (Neighbor joining method) (Figure 4). Phylogenetic analysis classified its orthologues into primates and glires. High sequence similarity and bootstrap value at multiple nodes showed that in each cluster the orthologues are closely related to AGT, which is an indicator of common ancestors before divergence into different

Table 1. SNAP2, I-Mutant and PhD-SNP predicted functional effects of T174M and M235T on angiotensinogen

SNAP2			
Protein phenotype	**Predicted effect**	**Score**	**Expected accuracy (%)**
T174M	Neutral	-5	53
M235T	effect	37	66
I-Mutant			
Protein phenotype	**Predicted effect**	**ΔΔ*G* value prediction**	**Reliability index**
T174M	Decrease stability	-0.18 Kcal/mol	0
M235T	Decrease stability	-0.83 Kcal/mol	6
PhD-SNP			
Protein phenotype	**Effect**	**Reliability index**	
T174M	Neutral	6	
M235T	Neutral	6	

Table 2. Physical and chemical properties of AGT with T174M and M235T polymorphisms

Protein phenotype	**Molecular weight**	**pI**	**Estimated half-life**	**Instability index**	**Aliphatic index**	**GRAVY**
174T+235M	53154	5.87	30 hours	41.16	99.77	0.065
174M	53184	5.87	30 hours	41.16	99.77	0.070
235T	53124	5.87	30 hours	40.99	99.77	0.059
174M+235T	53154	5.87	30 hours	40.99	99.77	0.065

pI, Isoelectric point; GRAVY, grand average of hydropathicity.

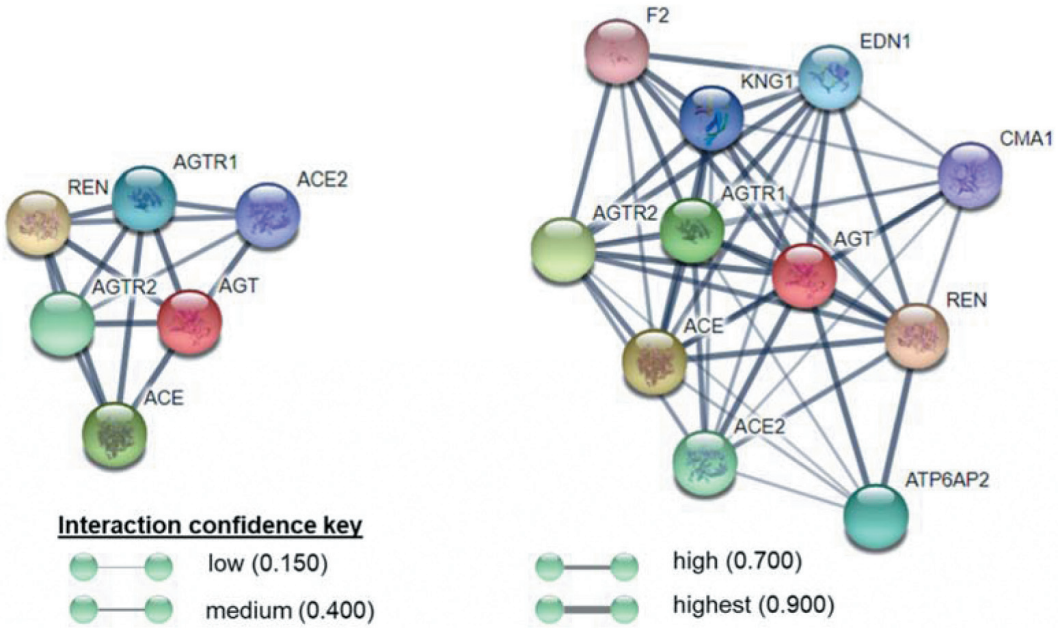

Fig. 3. Angiotensinogen (AGT) protein-protein interaction map using STRING database. *Each node is representing a gene of angiotensinogen (AGT) interacting protein.*

Table 3. Angiotensinogen (AGT) interacting proteins, their biological functions, pathways and associated diseases

Sr. No	Protein	Function	**Pathway**	**Disease**
1.	Renin	Converts angiotensinogen to angiotensin I	RAAS	Hypertension, renal tubular dysgenesis, cardiovascular diseases
2.	Angiotensin converting enzyme	Converts angiotensin I to angiotensin II (peptidase activity), degrade bradykinin (kininase activity), GPIase activity	RAAS	Hypertension, renal tubular dysgenesis, heart disease, microvascular complications of diabetes
3.	Angiotensin converting enzyme 2	Converts angiotensin I to angiotensin (1-9), converts angiotensin II to angiotensin (1-7), Inactivate pyr-apelin 13 and apelin 17	RAAS	Severe acute respiratory syndrome, neurogenic hypertension
4.	Angiotensin II type 1 receptor	Binds to angiotensin II and transactivate growth pathways	RAAS	Cardiovascular diseases, hypertension, renal tubular dysgenesis
5.	Angiotensin II type 2 receptor	Binds to angiotensin II, activates cardioprotective cell signals	RAAS, Adrenergic signaling in cardiomyocytes	Vesicoureteral reflux
6.	Renin/prorenin receptor	Act as renin and prorenin receptor, mediate renin-dependent cellular responses by activating ERK1/2	RAAS, Innate immune system	Mental retardation, epilepsy
7.	Chymase	regulation of submucosal gland secretion, extracellular matrix degradation, in heart and vasculature, converts angiotensin I to angiotensin II	Extracellular matrix degradation pathway, RAAS	Urticaria pigmentosa. Urticaria. Hypertension, Atherosclerosis
8.	Endothelin-1	Potent vasoconstrictor	Endothelin-1/EDNRA signaling pathway, Peptide ligand-binding receptor	Cardiac and vascular diseases, Renal diseases, Pulmonary arterial and renal hypertension
9.	Thrombin	Converts fibrinogen to fibrin, help in wound healing, function in inflammation and blood homeostasis, maintains vascular integrity, activates factor V, VII, VIII, XIII	Peptide ligand-binding receptor, Gamma carboxylation, hypusine formation and arylsulfatase activation pathway	Thromboembolism, Hypoprothrombinemia, recurrent pregnancy loss
10.	Kininogen	High molecular weight kininogen (HMWK) help in blood coagulation, inhibits thrombin and plasmin induced thrombocyte aggregation, bradykinin (released from HMWK) induce natriuresis, diuresis, vasodilation, hypoglycemia and effect smooth muscle cell contraction	kinin–kallikrein system, RET (rearrangement during transfection) signaling	High molecular weight kininogen deficiency, diffuse scleroderma, Bradykinin mediated angioedema

GPIase activity: glycosylphosphatidylinositol (GPI)-anchored protein releasing activity.

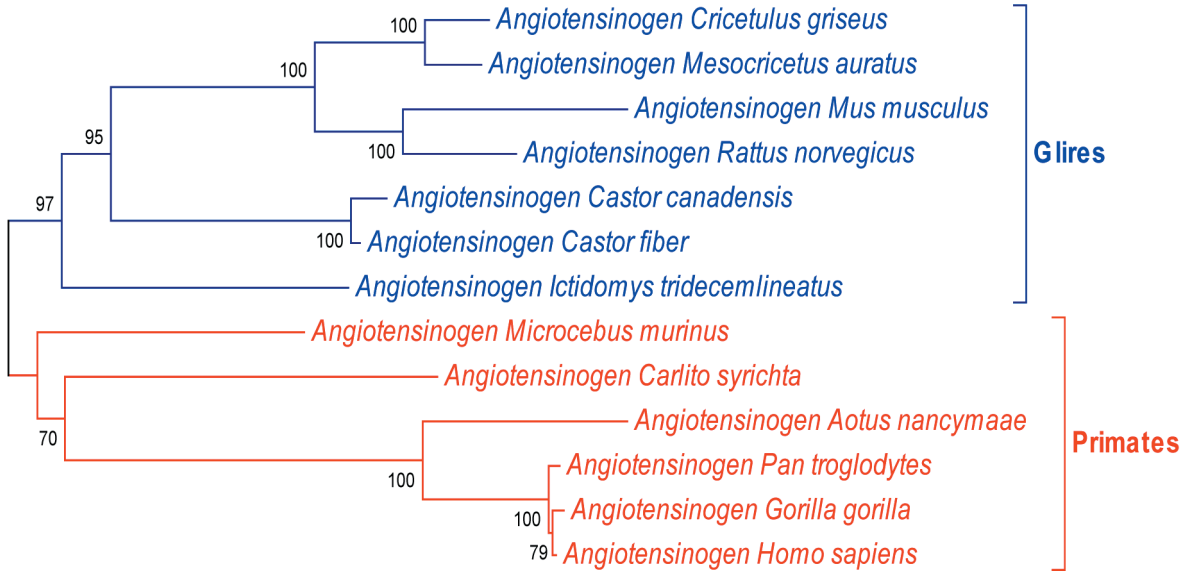

Fig. 4. Phylogenetic tree of Angiotensinogen and its orthologues. Relationship derived through Neighbor-Joining method using MEGA7. Bootstrap values are shown next to branches.

species. This phylogram indicates primates are evolving at slower rate than glires.

Discussion

Continuously increasing knowledge about gene annotation and development of high throughput techniques for sequencing has enforced the bioinformaticians to develop tools for processing this large amount of data. Bioinformatic tools has enabled the scientist to understand function of important genes, their evolution and involvement in human diseases. Angiotensinogen (*AGT*)gene is also one such gene which plays a central role in hypertension, cardiovascular and renal diseases [13, 14].

Mutations in *AGT* gene significantly influence the pathological activation of physiological pathways which critically control the body functions. This increased stability could be a cause of increased plasma AGT levels, as experimentally proven by Kooffreh *et al.*[15]. The pathological studies of M235T polymorphism have described its strong association with essential hypertension [16, 17]. This association is also evident from the direct involvement of renin angiotensin aldosterone system in blood pressure regulation [18]. Further broadening the hypertension associated disease panel indicated the association of M235T with increased risk of arterial fibrillation [19], myocardial infarction [20], coronary artery disease [21-23] and its severity [24]. Yang *et al.* have also demonstrated the weak association of M235T with type 2 diabetes [25]. *In-silico* studies presented in this study also support the findings of above mentioned experimental studies by showing that change of methionine with threonine decrease the protein stability. Aledo JC reviewed the role of methionine in proteins and stated that methionine is very important proteionogenic amino acid. Its unbranched side chain and sulfur molecule give flexibility to proteins and help them maintaining their structure [26].

Protein-protein analysis by STRING database showed that AGT interacts with 10 proteins including renin, angiotensin AGT, renin, ACE, ACE2, AGT1R and AGT2R are all main components of RAAS pathway [1]. All these components directly or indirectly influence the effect of other components. They also work in feedback mechanism and maintains blood pressure homeostasis.

Other than above-mentioned proteins, renin/prorenin receptor, chymase, endothelin-1, kininogen and thrombin also indirectly influence the blood pressure homeostasis by interacting with RAAS components. Renin/prorenin receptor increases the catalytic activity of renin enzyme and indirectly affect the angiotensinogen hydrolysis [27]. Chymase shares its biological function with ACE and ACE2 . It plays role in converting the AGT derived angiotensin (1-12) to angiotensin II [Reviewed in 28], which acts as vasoconstrictor peptide like endothelin-1 (encoded by EDN1 gene). Endothelin-1 is a member of endothelin-1/EDNRA signaling pathway, which shares mechanistic and functional similarities with RAAS. Kininogen is a group of high or low molecular weight proteins, including bradykinin and kallidin respectively. These are main components of kinin-kallikrein pathway and act as vasodilator peptides. ACE directly influence this pathway by degrading bradykinin which will eventually cause vasoconstriction. Kininogens also inhibit the thrombin induced platelet aggregation by inactivating the ca^{+} dependent calpain protein [29]. The functional association between AGT and thrombin could be understood by studying the calpain and its Ca^{2+} dependency. AGT increases

the intracellular ca^+ concentration by increasing ca^+ influx via ca^+ channels and stimulating its release by sarcoplasmic reticulum [30]. These elevated ca^+ levels can activate the calpain protein leading to increased thrombin-induced platelet aggregation.

Current study was mainly focusing critical central molecule of RAAS pathway; AGT is thought to first emerged in cartilage fish and made its way to other descending species [31]. AGT has emerged as non-inhibitory serpin protein which has serpin domain to help its binding to proteases like renin for its cleavage to angiotensin peptide.

In present study, almost 194 orthologues of *AGT* gene were identified in various species of fish, primates, glires etc. Evidences from evolutionary analysis concluded that rate of *AGT* evolution is slower in primates as compared to glires which further have heterogenous rates of evolution [32]. Evolutionary rate of *AGT* in different species is quite high however the sequence of functional domain is conserved [33].

Conclusion

In summary, *AGT* gene has been studied for its structural characterization, mutational analysis, protein-protein interactions, and phylogenetic analysis. Phylogenetic analysis revealed that structure of *AGT* gene is highly conserved among various species. Furthermore, the protein-protein interaction analysis supported the role of *AGT* gene in hypertension and cardiovascular disease promoting pathways, this study supports the *AGT* gene as a strong therapeutic target for the control of multiple metabolic diseases.

Conflict of Interest

All authors declare that there is no conflict of interest.

References

1. Hussain M, Awan FR. Hypertension regulating angiotensin peptides in the pathobiology of cardiovascular disease. Clinical and Experimental Hypertension. 2017:1-9.
2. ud Din UA, Khan WA, Shahzad K, Ali B, Hussain M, Awan FR. Genetic Polymorphism of ACE (I/D) is Associated with Diabetic Nephropathy in Pakistani Subjects. Pakistan Journal of Zoology. 2023;55(4):1537.
3. Lu H, Cassis LA, Vander Kooi CW, Daugherty A. Structure and functions of angiotensinogen. Hypertension Research. 2016;39(7):492-500.
4. Shahid M, Rehman K, Akash MSH, Suhail S, Kamal S, Imran M, et al. Genetic polymorphism in angiotensinogen and its association with cardiometabolic diseases. Metabolites. 2022;12(12):1291.
5. Lu H, Cassis LA, Vander Kooi CW, Daugherty A. Structure and functions of angiotensinogen. Hypertension Research. 2016.
6. Zhou A, Carrell RW, Murphy MP, Wei Z, Yan Y, Stanley PL, et al. A redox switch in angiotensinogen modulates angiotensin release. Nature. 2010;468(7320):108-11.
7. Ramkumar N, Stuart D, Peterson CS, Hu C, Wheatley W, Min Cho J, et al. Loss of soluble (pro) renin receptor attenuates angiotensin-II induced hypertension and renal injury. Circulation Research. 2021;129(1):50-62.
8. Burton J, Quinn T. The amino-acid residues on the C-terminal side of the cleavage site of angiotensinogen influence the species specificity of reaction with renin. Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology. 1988;952:8-12.
9. Isordia-Salas I, Santiago-Germán D, Cerda-Mancillas MC, Hernández-Juárez J, Bernabe-García M, Leaños-Miranda A, et al. Gene polymorphisms of angiotensin-converting enzyme and angiotensinogen and risk of idiopathic ischemic stroke. Gene. 2019;688:163-70.
10. 10.Riad M, Adhikari P, Bhattarai S, Gupta A, Ali E, Ali M, et al. Risk assessment using the association between renin-angiotensin genes polymorphisms and coronary artery disease. Cureus. 2021;13(3).
11. Trainor PJ, Brambatti M, Carlisle SM, Mullick AE, Shah SJ, Kahlon T, et al. Blood levels of angiotensinogen and hypertension in the Multi-Ethnic Study of Atherosclerosis (MESA). Journal of the American College of Cardiology. 2023;81(13):1248-59.
12. Szklarczyk D, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, et al. The STRING database in 2021: customizable protein–protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic acids research. 2021;49(D1):D605-D12.
13. Cruz-López EO, Ye D, Wu C, Lu HS, Uijl E, Mirabito Colafella KM, et al. Angiotensinogen Suppression: A New Tool to Treat Cardiovascular and Renal Disease. Hypertension. 2022:10.1161/HYPERTENSIONAHA.122.18731.
14. Hussain M, Awan FR. Hypertension regulating angiotensin peptides in the pathobiology of cardiovascular disease. Clinical and experimental hypertension. 2018;40(4):344-52.
15. Kooffreh ME, Anumudu CI. Increased plasma Angiotensinogen levels and its association with the M235T gene polymorphism and hypertension in Calabar and Uyo cities, Nigeria. Asian Journal of Biomedical and Pharmaceutical Sciences. 2013;3(27):15.
16. Shamaa MM, Fouad H, Haroun M, Hassanein M, Hay MAA. Association between the Angiotensinogen (AGT)

gene (M235T) polymorphism and Essential Hypertension in Egyptian patients. The Egyptian Heart Journal. 2015;67(1):1-5.
17. Say Y-H, Ling K-H, Duraisamy G, Isaac S, Rosli R. Angiotensinogen M235T gene variants and its association with essential hypertension and plasma renin activity in Malaysian subjects: a case control study. BMC cardiovascular disorders. 2005;5(1):7.
18. Hussain M, Awan FR, Gujjar A, Hafeez S, Islam M. A case control association study of ACE gene polymorphism (I/D) with hypertension in Punjabi population from Faisalabad, Pakistan. Clinical and Experimental Hypertension. 2017:1-6.
19. Wang Q-s, Li Y-g, Chen X-d, Yu J-f, Wang J, Sun J, et al. Angiotensinogen polymorphisms and acquired atrial fibrillation in Chinese. Journal of electrocardiology. 2010;43(4):373-7.
20. Raygan F, Karimian M, Rezaeian A, Bahmani B, Behjati M. Angiotensinogen-M235T as a risk factor for myocardial infarction in Asian populations: a genetic association study and a bioinformatics approach. Croatian medical journal. 2016;57(4):351-62.
21. Niemiec P, Zak I, Wita K. The M235T polymorphism of the AGT gene modifies the risk of coronary artery disease associated with the presence of hypercholesterolemia. European journal of epidemiology. 2008;23(5):349-54.
22. Wang Y-J, Pan Y. Angiotensinogen gene M235T polymorphism and risk of coronary artery disease: a meta-analysis. Molecular medicine reports. 2012;6(4):884-8.
23. Borai IH, Hassan NS, Shaker OG, Ashour E, Badrawy ME, Fawzi OM, et al. Synergistic effect of ACE and AGT genes in coronary artery disease. Beni-Suef University Journal of Basic and Applied Sciences. 2017.
24. Lanz JR, Pereira AC, Lemos PA, Martinez E, Krieger JE. Angiotensinogen M235T polymorphism is associated with coronary artery disease severity. Clinica chimica acta. 2005;362(1):176-81.
25. Yang J-K, Zhou J-B, Xin Z, Zhao L, Yu M, Feng J-P, et al. Interactions among related genes of renin-angiotensin system associated with type 2 diabetes. Diabetes Care. 2010;33(10):2271-3.
26. Aledo JC. Methionine in proteins: The Cinderella of the proteinogenic amino acids. Protein Science. 2019;28(10):1785-96.
27. Ichihara A, Yatabe MS. The (pro) renin receptor in health and disease. Nature Reviews Nephrology. 2019;15(11):693-712.
28. Ferrario CM, Groban L, Wang H, Sun X, VonCannon JL, Wright KN, et al. The renin–angiotensin system biomolecular cascade: a 2022 update of newer insights and concepts. Kidney International Supplements. 2022;12(1):36-47.
29. Crawford C. Protein and peptide inhibitors of calpains. Intracellular calcium-dependent proteolysis: CRC Press; 2019. p. 75-89.
30. Touyz RM. Reactive oxygen species as mediators of calcium signaling by angiotensin II: implications in vascular physiology and pathophysiology. Antioxidants & redox signaling. 2005;7(9-10):1302-14.
31. Fournier D, Luft FC, Bader M, Ganten D, Andrade-Navarro MA. Emergence and evolution of the renin–angiotensin–aldosterone system. Journal of molecular medicine. 2012;90(5):495-508.
32. Beklemisheva VR, Romanenko SA, Biltueva LS, Trifonov VA, Vorobieva NV, Serdukova NA, et al. Reconstruction of karyotype evolution in core Glires. I. The genome homology revealed by comparative chromosome painting. Chromosome Research. 2011;19(4):549-65.
33. Wong M. Chapter 29-renin-angiotensin system. Handbook of Hormones, Y Takei, H Ando, and K Tsutsui, Eds. 2016:253-4.