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Decoding AGT *rs(699)* variant and gene expression in relation to congenital heart defects

Nandini Krishnamurthy[1], Jebaraj Rathinaswamy[2], Ashok Kumar[3], S Shuba[2], Devi Krishna RB[1], Sanjana Murali[1], Revathy Kumar[1], Delphia Arul anto[1], Maria Christina Beuna[1] and Andrea Mary. F[1*]

Abstract

Background Congenital Heart Defects (CHDs) are among the most common birth anomalies worldwide, with genetic and molecular factors contributing significantly to their development. The renin–angiotensin system (RAS) plays a key role in cardiovascular regulation, and polymorphisms in the angiotensinogen (*AGT*) gene, particularly *rs (699)* (M235T), likely influence susceptibility to CHDs. Aim: To investigate the association between the *AGT rs (699)* polymorphism and CHD risk in an Indian pediatric population. Methods: A case-control study was conducted involving 112 children with CHDs and 112 age- and sex-matched healthy controls. Genomic DNA was extracted and genotyped using PCR-RFLP. Genotype and allele frequencies were compared between groups to assess their association with CHD risk. Results: The *TT* genotype was significantly more among CHD cases (44.6%) than controls (10.7%) ($P < 0.0001$), indicating a strong association with disease risk. The *CT* genotype also showed a significant association ($P = 0.0090$; OR = 2.3750, 95% CI: 1.2411–4.5450), while the *CC* genotype was more prevalent in controls. Under the dominant model (*CT* + *TT*), individuals carrying at least one *T* allele had a significantly higher risk of CHD ($P < 0.0001$; OR = 4.2308, 95% CI: 2.3582–7.5903). Allele frequency analysis revealed a higher *T* allele frequency in cases (61.6%) than controls (28.6%) ($P < 0.0001$; OR = 4.0116, 95% CI: 2.7008–5.9587). Additionally, gene expression analysis indicates significant upregulation of *AGT* in CHD cases, with a fold change of 1.773 ($P = 0.032676$), suggesting that the *T* allele may influence both genetic susceptibility and gene activity. Conclusion: The *AGT rs (699) T* allele and *TT* genotype are strongly associated with an increased risk of CHD. These findings suggest that *AGT* gene polymorphisms, potentially along with altered gene expression, may contribute to CHD pathogenesis. Although the difference in expression was minimal, it may indicate a potential role of *AGT* gene activity in CHD pathogenesis. However, due to the limited number of samples, no significant clinical correlation could be established between gene expression levels and specific CHD subtypes. Further studies with larger cohorts are warranted to validate these findings and explore their clinical relevance.

Keywords Congenital heart defects (CHDs), Angiotensinogen *AGT* gene, *Rs (699)* genetic variation, (M235T) variant, Gene expression

*Correspondence:
Andrea Mary. F
andreamary@sriramachandra.edu.in
[1]Sri Ramachandra Institute of Higher Education and Research, Chennai, India
[2]Sri Ramachandra Medical Centre, Chennai, India
[3]Vels Institute of Sciences Technology and Advanced Studies (VISTAS), Chennai, India

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Introduction

Congenital Heart Defects (CHDs) are structural defects in the heart or main blood arteries that occur at birth. These anomalies appear during fetal development and can impair the heart's walls, valves, arteries, or veins. CHDs are among the most common congenital defects, affecting around 1% of all live births. The severity of CHDs varies greatly, from mild flaws that may not require treatment to severe problems that necessitate surgical intervention (https://www.cdc.gov/heart-defects/about/index.html; https://www.verywellhealth.com/heart-disease-4014709). The term "congenital heart defects" (CHDs) refers to a range of structural cardiac issues that exist from birth. The most common kinds are septal defects, which are holes in the heart's septum and comprise atrial septal defect (ASD) and ventricular septal defect (VSD). There are also valve issues, such as atresia (narrowing), regurgitation (leakage), or stenosis (narrowing). Cyanotic abnormalities, like Tetralogy of Fallot, can give the skin a bluish color and cause oxygen-poor blood to circulate throughout the body. Normal blood flow is restricted by obstructive defects, such as coarctation of the aorta, which include blood vessel narrowing (https://www.heart.org/en/health-topics/congenital-heart-defects/about-congenital-heart-defects/common-types-of-heart-defects). The renin-angiotensin system (RAS) contributes to a variety of cardiovascular disorders and is crucial for preserving normal heart function [1]. To date, a number of RAS components have been implicated in the development of cardiovascular diseases, including angiotensin-converting enzyme, angiotensin-converting enzyme 2, angiotensin II (AngII), angiotensin 1–7, angiotensin type I receptor, angiotensin type II receptor, Mas receptor, and so on. These elements may combine to create an intricate regulatory system for heart conditions [2]. As the distinct precursor of RAS, angiotensinogen *AGT* can produce all angiotensin peptide products by successive enzymatic cleavages; as a result, *AGT* can serve as a marker of the functioning of the complete RAS [3]. *AGT*'s role in cardiovascular disorders, however received little attention. *AGT* and its roles in cardiovascular diseases can be better understood with the help of numerous innovative approaches, such as the development of cell-specific genetic mouse models and antisense oligonucleotides or RNA-based therapies that target specific tissues [4, 5]. Since the renin-angiotensin system (RAS) plays a major part in cardiovascular physiology and pathology, we wanted to investigate the genetic variation in the *AGT* gene, specifically the single nucleotide polymorphism (SNP) *rs (699)*, in connection with congenital heart abnormalities (CHDs). The role of *AGT* (angiotensinogen), the primary precursor in the RAS cascade, has not been thoroughly investigated in relation to CHDs, despite the fact that other RAS components have been linked to cardiovascular disorders. The molecular pathways driving congenital heart defects may be better understood by taking into account genetic polymorphisms like *rs (699)*, which may affect *AGT* expression or function.

Materials and methods

Study population and compliance with bioethics

A total of 224 participants were enrolled in this case-control study, comprising 112 children diagnosed with congenital heart defects (CHDs) and 112 unrelated healthy controls. All participants were genetically unrelated and belonged to the Indian population. Ethical approval for the study was obtained from the Institutional Ethics Committee of Sri Ramachandra Institute of Higher Education and Research (SRIHER), Chennai, India (IEC Reference Number: IEC-NI/23/AUG/88/50). The study was conducted in accordance with national regulations and international guidelines on biomedical research ethics.

Given the involvement of minors, written informed consent was obtained from the parents or legal guardians, along with assent from the children and completion of patient questionnaires. Blood samples were obtained legally through SRIHER and used solely for research purposes. CHD diagnoses were confirmed using cardiac MRI and echocardiography, and only patients who subsequently underwent surgical intervention were included. A face-to-face interview and structured screening questionnaire were used to collect demographic and clinical information from each participant. A 5 mL venous blood sample was drawn from all subjects for genetic analysis. To reduce potential confounding factors and ensure study validity, individuals with known chromosomal abnormalities or additional congenital disorders were excluded. Control subjects were selected from the same geographic region and matched with cases based on age and sex. Only individuals without congenital anomalies and with no maternal exposure to known teratogenic risks (e.g., pre-existing diabetes, phenylketonuria, or therapeutic drug exposure during pregnancy) were included in the control group.

DNA extraction

Genomic DNA was extracted from peripheral blood samples using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. Briefly, 200 µL of whole blood was mixed with 20 µL of Proteinase K and 200 µL of AL buffer in a sterile microcentrifuge tube. The mixture was incubated at 56 °C for 10 min to ensure complete cell lysis. Subsequently, 200 µL of ethanol was added to the lysate to facilitate DNA binding. The resulting mixture was transferred to the QIAamp spin column, and centrifuged at 8,000 rpm for 1 min. The column was then washed with 500 µL of Wash Buffer 1, followed by centrifugation at 8,000 rpm for 1 min. A

second wash was carried out using 500 µL of Wash Buffer 2, with centrifugation at 8,000 rpm for 3 min to ensure thorough purification. After washing, the spin column was placed in a clean microcentrifuge tube, and 50 µL of Elution Buffer was added directly to the membrane. The DNA was eluted by centrifuging at 10,000 rpm for 1 min and subsequently stored at − 50 °C until further use. The extracted DNA was quantified using a NanoPhotometer® N60/N50 (Implen, Munich, Germany), and samples with an A260/A280 ratio between 1.8 and 2.0 were considered optimal. DNA samples were stored at − 20 °C until use.

Genotyping of the (rs699) AGT gene polymorphism

Genotyping of the AGT gene polymorphism *rs (699)* was performed using polymerase chain reaction (PCR), followed by agarose gel electrophoresis for product visualization. The PCR reaction was set up in a final volume of 30 µL, containing 10X PCR buffer, 2.5 mM of each dNTP, 1.5 mM $MgCl_2$, 10 pmol of each primer, 1 U of Taq DNA polymerase, and 50 ng of genomic DNA. The primers used to amplify the rs699 region were obtained from Thermo Fisher Scientific (Waltham, MA, USA): Forward 5’-TGG ATG CGC ACA AGG TCC TGT-3’ and Reverse 5’-CAG GGT GCT GTC CAC ACT GGC TCG C-3’, producing a 305 bp fragment. PCR amplification was performed under the following thermal cycling conditions: an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 64.7 °C for 1 min, and extension at 72 °C for 1 min. A final extension step was carried out at 72 °C for 5 min, followed by a hold at 4 °C. PCR products were then resolved on a 2% agarose gel stained with ethidium bromide and visualized using a gel documentation system (Bio-Rad, Hercules, CA, USA). A 100 bp DNA ladder (Thermo Fisher Scientific, Waltham, MA, USA) was used as a molecular size marker to confirm the expected 305 bp amplification product.

RFLP for *(rs699)* and Sequencing analysis

Restriction Fragment Length Polymorphism (RFLP) analysis was employed to determine the genotypes of the AGT *rs (699)* polymorphism using a site-specific restriction enzyme. The restriction enzyme Bsh1236I (Thermo Fisher Scientific, Waltham, MA, USA; 5 U) was selected based on in silico analysis using Restriction Mapper 2.0 to ensure precise and efficient digestion of the PCR-amplified 305 bp DNA fragment. The digestion reaction was performed in a total volume of 30 µL containing 10X restriction buffer, 5 U of Bsh1236I, and 10 µL of the purified PCR product. The mixture was incubated at 37 °C for 4 h to allow complete digestion of the DNA fragments. The resulting digested products were separated on a 2% agarose gel stained with ethidium bromide and visualized using a gel documentation system (Bio-Rad, Hercules, CA, USA). A 100 bp DNA ladder (Thermo Fisher Scientific, Waltham, MA, USA) was used as a molecular size marker to determine fragment sizes. Genotypes were determined based on the banding patterns: individuals with the *CC* wild type genotype displayed two fragments of 281 bp and 24 bp, heterozygous *CT* genotype showed three bands at 305 bp, 281 bp, and 24 bp, while homozygous mutant *TT* genotype remained uncut and showed a single 305 bp band. All samples were subsequently sent for sequencing, which confirmed that the frequency of CT alleles was higher in the cases group than in the control group.

Gene expression using RT PCR

Total RNA was isolated from peripheral blood samples using the TRIzol™ Reagent (Takara Bio, Kusatsu, Japan; RNAiso Plus) according to the manufacturer’s instructions. The isolated RNA was reverse-transcribed into complementary DNA (cDNA) using the PrimeScript™ 1st Strand cDNA Synthesis Kit (Takara Bio, Kusatsu, Japan; High-Capacity cDNA Conversion Kit). Quantitative real-time PCR (qPCR) was performed using SYBR Green chemistry (Applied Biosystems, Foster City, CA, USA) with the following gene-specific primers obtained from Thermo Fisher Scientific (Waltham, MA, USA): Target gene: Forward 5′-GGAAGATGAAGGGCTTCT CC-3′ and Reverse 5′-CGGGGAGATAGTTTCTTCA T-3′. β-actin (reference gene): Forward 5′-CACCATTG GCAATGAGCGGTTC-3′ and Reverse 5′-AGGTCTTT GCGGATGTCCACGT-3′. The qPCR amplification was carried out under standard thermal cycling conditions: initial denaturation at 94 °C for 10 min, followed by 40 cycles of 94 °C for 20 s and 60 °C for 20 s. Data acquisition was performed using Rotor-Gene 2.1.0.9 software (Qiagen, Hilden, Germany). Relative gene expression levels were calculated using the ΔCt method, with β-actin serving as an endogenous control to normalize target gene expression.

Statistical analysis

Demographic characteristics were compared between cases and controls using a two-sided Student’s t-test for continuous variables such as age, weight, height, and maternal age, and a Chi-square (χ^2) test for categorical variables such as gender, consanguinity, and family history of congenital heart disease (CHD). All statistical analyses were performed using IBM SPSS Statistics software (version 26.0; IBM Corp., Armonk, NY, USA). The genotype distribution in the control group followed the Hardy–Weinberg equilibrium ($p = 0.19$).

Allele and genotype frequencies, as well as relative gene expression data from qPCR, were analyzed using the same software. Relative expression levels were calculated using the ΔCt method, and differences between groups

were assessed using the ΔΔCt approach, with β-actin as the endogenous control. A p-value < 0.05 was considered statistically significant.

Sample size calculation

The sample size was calculated using the Two-Proportion comparison method in IBM SPSS Statistics software (version 26.0; IBM Corp., Armonk, NY, USA). Population 1 represented patients with the genetic variant, and Population 2 represented controls without the variant. Based on previous literature and pilot observations, the expected proportions were set as $P_1 = 0.86$ and $P_2 = 0.61$, with an estimated risk difference of 0.25. Considering a 5% alpha error (two-sided) and 99% power, the required sample size was computed as 109.48 per group, which was rounded up to 112 per arm to account for potential attrition, yielding a total of 224 samples for the study.

Two Proportion	
Proportion in group I	0.86
Proportion in group II	0.61
Estimated risk difference	0.25
Power (1- beta) %	99
Alpha error (%)	5
1 or 2 sided	2
Required sample size for each arm	112

For cases (*n* = 112)

Inclusion criteria

Children aged between 0 and 18 years of both genders, diagnosed with septal defects and attending the Department of Pediatric Cardiology, Sri Ramachandra Medical College (SRMC), Porur, Chennai, were included in the case group.

Table 1 Demographic and clinical characteristics of cases and controls

Variables	Cases (*n* = 112)	Controls (*n* = 112)	*P* value
Age (years, mean ± SD)	7.0 ± 4.20	6.4 ± 3.9	0.2324
Gender			
Male	67 (59.82%)	64 (57.14%)	0.8034
Female	45 (40.18%)	48 (42.86%)	
Weight (Kg, mean ± SD)	30.8 ± 11.27	31.3 ± 11.06	0.3985
Height (cm, mean ± SD)	155.6 ± 37.0	151.2 ± 43.5	0.6342
Family history of CHD	11 (9.82%)	0 (0%)	0.001
Maternal age at delivery (mean ± SD)	27.8 ± 4.5	26.9 ± 3.9	0.278
Parental consanguinity			
Present	38 (33.9%)	14 (12.5%)	0.002
Types of CHD			
Ventricular Septal Defect (VSD)	56 (50.00%)	–	–
Atrial Septal Defect (ASD)	34 (30.36%)	–	–
Tetralogy of Fallot (TOF)	22 (19.64%)	–	–

Exclusion criteria

Children with associated chromosomal abnormalities, metabolic disorders, or other affected with genetic syndromes were excluded from the study.

For controls (*n* = 112)

Inclusion criteria

Age- and sex-matched healthy volunteers aged between 0 and 18 years with no history or clinical evidence of cardiac disease were included as controls. The study population selection was independent of socio-economic status.

Exclusion criteria

Children with a history of preterm birth, chronic illness, congenital anomalies (non-cardiac), or recent hospitalization for severe infection were excluded from the control group.

Results

Distribution of variables

The distribution of demographic characteristics between cases and controls was analyzed using a two-sided Student's t-test for continuous variables such as age, weight, height, and maternal age, and a Chi-square (χ^2) test for categorical variables such as gender, consanguinity, and family history of congenital heart disease (CHD). The distribution of CHD subtypes among the cases is also presented. The summarized findings are shown in Table 1.

Association of (rs699) variant among cases and controls

Our study analyzed the genotype and allele frequencies of the *(rs699)* polymorphism in both cases and controls to investigate its potential association with susceptibility to congenital heart diseases (CHDs).

In this present study comparing 112 cases and 112 controls, the distribution of genotypes for a specific polymorphism was analyzed. The PCR amplification yielded a distinct band of 305 bp corresponding to the expected product size. Subsequent RFLP digestion using the *Bsh1236I* enzyme revealed three distinct genotypic patterns homozygous wild-type, heterozygous, and homozygous mutant as illustrated in Fig. 1A and B. The heterozygous genotype exhibited both 305 bp, 281 bp, 24 bp fragments, confirming enzyme digestion, while the homozygous mutant (*T* allele) displayed a single 281 bp band (Fig. 2). To ensure accuracy, representative samples from each genotype were validated through Sanger sequencing, which confirmed the presence of the expected nucleotide substitutions (Fig. 3). Among the cases, the *CC* genotype was observed in 21% (24 individuals), *CT* in 34% (38 individuals), and *TT* in 45% (50 individuals). In contrast, the control group showed a higher frequency of the *CC* genotype at 53% (60 individuals),

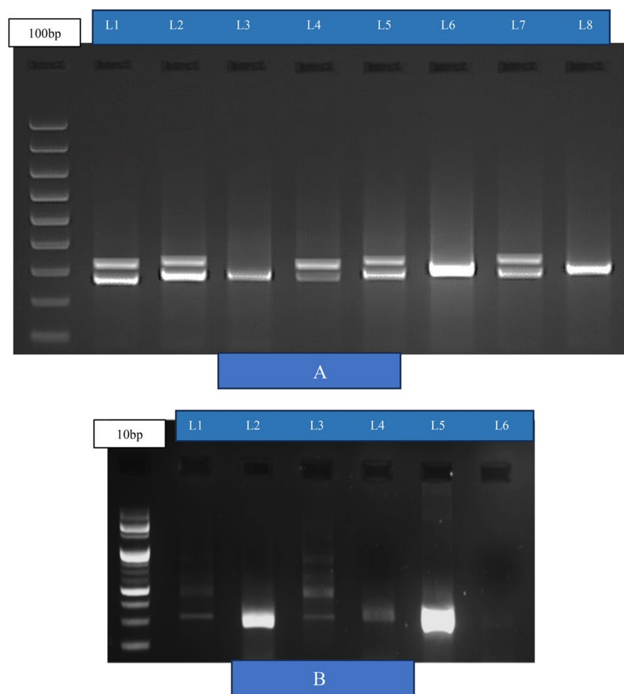

Fig. 1 For RFLP analysis, the PCR products were digested using Bsh1236I enzyme (5 Units, Thermo Scientific). The lanes L1, L2, L4, L5, and L7 displayed fragments of 305 bp and 281 bp, indicating the presence of heterozygous alleles, while the lanes L3, L6, and L8 showed a single homozygous mutant (T allele) band pattern, as represented in Fig. 1 **A**. The heterozygous samples were further electrophoresed using a 10 bp DNA ladder to visualize the presence of the 24 bp fragment, as depicted in Fig. 1 **B**. All RFLP products were visualized on a 3% agarose gel

while *CT* and *TT* genotypes were present in 36% (40 individuals) and 11% (12 individuals), respectively. Using the *CC* genotype as the reference, the *CT* genotype was associated with a significantly increased risk, with an odds ratio (OR) of 2.3750 (95% CI: 1.2411–4.5450; $p = 0.0090$). The *TT* genotype showed an even stronger association with disease risk, with an OR of 10.4167 (95% CI: 4.7367–22.9079; $p < 0.0001$). When the *CT* and *TT* genotypes were combined, the association remained highly significant, with an OR of 4.2308 (95% CI: 2.3582–7.5903; $p < 0.0001$). Allele frequency analysis revealed that the *C* allele was more frequent among controls (71%) compared to cases (38%), while the *T* allele was more common in cases (62%) than in controls (29%). The presence of the *T* allele was significantly associated with an increased risk of disease (OR: 4.0116; 95% CI: 2.7008–5.9587; $p < 0.0001$) (Table 2).

Gene expression analysis

RNA was extracted from the blood samples of CHD patients and controls, converted into complementary DNA (cDNA), and subjected to reverse transcriptase polymerase chain reaction (RT-PCR) for gene expression analysis. β-actin was used as the internal reference gene for normalization. The comparative double delta Ct (ΔΔCt) value between cases and controls was −0.827, corresponding to a 1.773-fold increase in gene expression in the patient samples (Fig. 3). The significance of this difference ($p = 0.032676$) was confirmed using the Student's *t*-test. However, a significant clinical correlation between gene expression levels and specific subgroups of congenital heart defects could not be established due to the limited sample size.

Hap map analysis of allele frequency in various population

The allele frequency data from the HapMap database provides a comprehensive overview of the global distribution of the *T* (alternative) and *C* (reference) alleles of the *AGT* ((M235T)) polymorphism across various populations. Globally, the *T* allele was more prevalent (54.5%) compared to the *C* allele (45.5%), indicating a slight dominance of the *T* allele in the general population. This trend was also observed in the European population, where the *T* allele had a higher frequency (57.9%) than the *C* allele (42.1%). In contrast, a strikingly different pattern emerged in African and Asian populations. Among Africans, the *C* allele was predominant, occurring in 81.9% of individuals compared to only 18.1% for the *T* allele. A similar distribution was observed in the Asian population, where the *T* allele was present in just 17.5% of individuals, while the *C* allele accounted for 82.5%. These results suggest a strong population specific distribution of this polymorphism, which could reflect evolutionary, environmental, or genetic drift factors affecting allele frequencies in different ethnic groups. Interestingly, in our patient sample (PS), the *T* allele was found at a notably higher frequency (71%) compared to the *C* allele (28%), which aligns more closely with the global and European trends but diverges sharply from the African and Asian patterns. This observation may indicate a potential genetic predisposition linked to the *T* allele in the context of our study population. The elevated frequency of the *T* allele in the PS group could suggest its involvement in disease susceptibility, warranting further investigation into its functional implications in the pathophysiology of congenital heart defects or related conditions (Fig. 4).

MolProbity analysis using Swiss-model

To better understand how this genetic variation affects protein structure and function, computational models like SWISS-MODEL were used. SWISS-MODEL provides a reliable homology-based modeling approach to predict the 3D structure of the *AGT* protein, allowing for the analysis of potential structural alterations caused by the SNP.

MolProbity result provides a detailed analysis of the protein structure and can be crucial for understanding the impact of the *(rs699) AGT* SNP (methionine-to-threonine

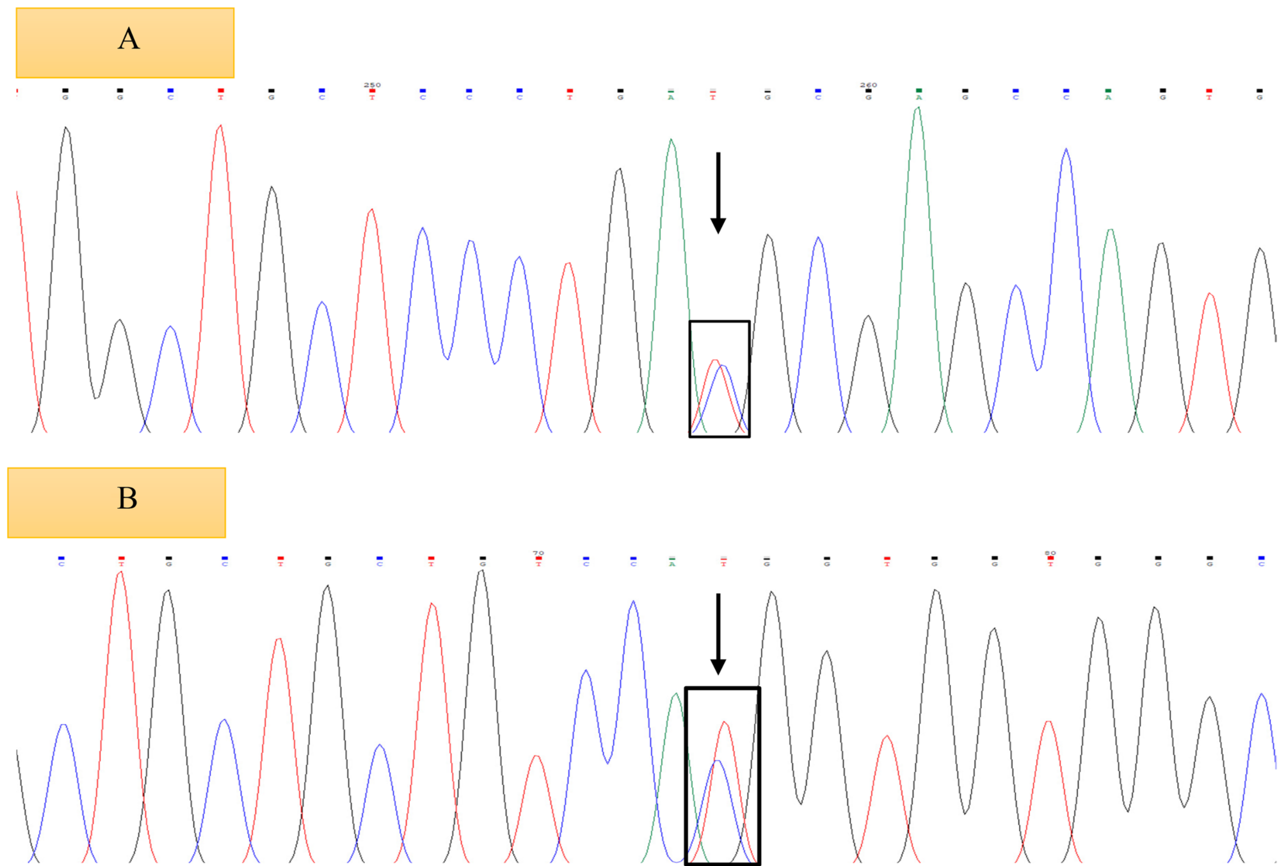

Fig. 2 The amplified products were subsequently validated by Sanger sequencing to confirm the accuracy of the genotyping results. Representative samples from each observed genotype pattern were selected for sequencing, and the obtained chromatograms were analyzed to verify the presence of the expected nucleotide substitutions corresponding to the identified alleles (Fig. 2 **A** and **B**). The sequencing results were consistent with the RFLP findings, thereby confirming the reliability and specificity of the amplification and restriction digestion process, as illustrated in Fig. 2

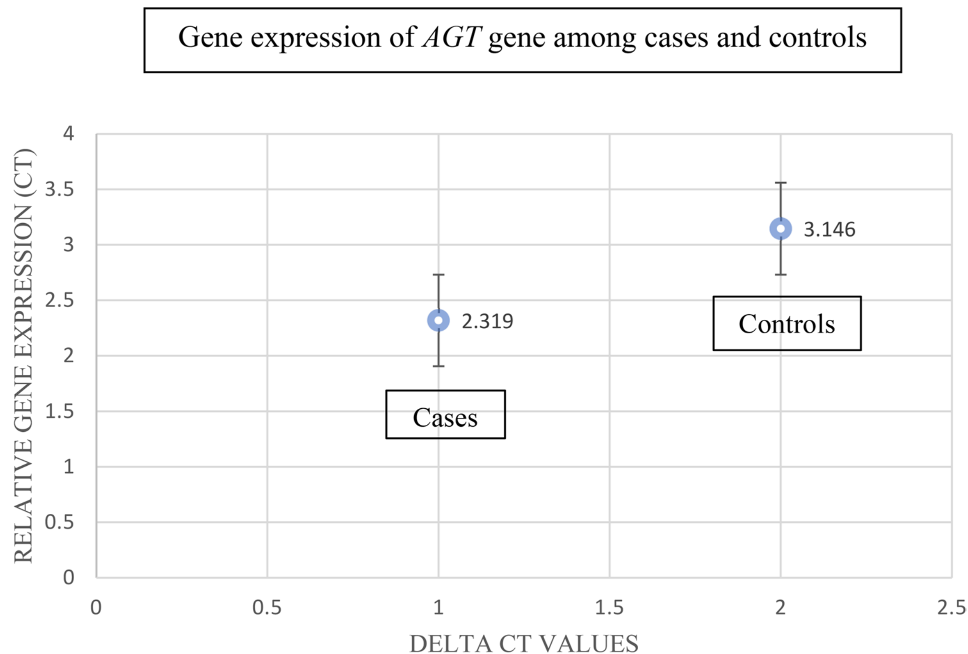

Fig. 3 Scatter plot depicting the Delta Ct values of Cases and Controls against relative gene expression (ct)

Table 2 Allelic frequency between cases and control group

Genotype/Allele	Cases ($n=112$)	Controls ($n=112$)	*P*-value	OR (95% CI)
CC	24 (21%)	60 (53%)	Ref	–
CT	38 (34%)	40 (36%)	0.0090	2.3750 (1.2411 to 4.5450)
TT	50 (45%)	12 (11%)	$P<0.0001$	10.4167 (4.7367 to 22.9079)
CT+TT	88 (79%)	52 (46%)	$P<0.0001$	4.2308 (2.3582 to 7.5903)
C allele frequency	86 (38%)	160 (71%)	Ref	
T allele frequency	138 (62%)	64 (29%)	$P<0.0001$	4.0116 (2.7008 to 5.9587)

shift) on the angiotensinogen *AGT* protein. The MolProbity score of 2.46 suggests that the overall protein model is of good quality, but there is room for further refinement. The Clash Score of 11.62 indicates that there are steric clashes between certain residues (e.g., A416 SER-A419 VAL, A99 VAL-A353 ILE), which may be caused by the structural changes induced by the SNP. The Ramachandran plot results are favorable, with 94.66% of the residues in the favored regions, indicating that the backbone conformation is highly stable and unlikely to be significantly disrupted by the SNP (Fig. 4). There are no Ramachandran outliers (0.00%), which suggests that the protein's 3D fold is unlikely to be adversely affected by the methionine-to-threonine substitution at position 235. However, rotamer outliers (4.68%), such as A228 TRP, A392 LEU, and A137 CYS, indicate that some side chains deviate from their ideal conformations, which could be relevant for the protein's interactions or stability, especially in the context of altered binding affinity due to the SNP. The absence of bad bonds (0/3330) and bad angles (0/4533) further confirms the structural integrity of the protein (Fig. 5). Overall, these results suggest that while the methionine-to-threonine shift may lead to some minor steric clashes and side-chain deviations, the protein's overall fold remains stable, with no significant backbone issues. This structural stability could be important for understanding the potential functional effects of this SNP on angiotensinogen's role in the renin-angiotensin system (RAS).

Discussion

The chromosomes 1q42–43 contain the angiotensinogen *AGT* gene. There are many variations in this gene. The non-synonymous (M235T) variant is one of the main functional *AGT* variants. At nucleotide 704, thymine is changed to cytosine, resulting in an amino acid substitution at position 235 from methionine (M) to threonine (T) [6]. It has a broad range of cell specificity and is present in many tissues, such as the liver, adipose tissue, heart, vascular wall, brain, and kidney [7]. There are 33 amino acid signal peptides among the 485 amino acids in human *AGT*. Renin produces angiotensin I (Ang I), the precursor of several active angiotensin peptides, by cleaving the ten amino acids from the N-terminal. Renin, a part of the Renin-Angiotensin-Aldosterone System (RAAS), cleaves the N-terminal amino acids of mature *AGT* produced by

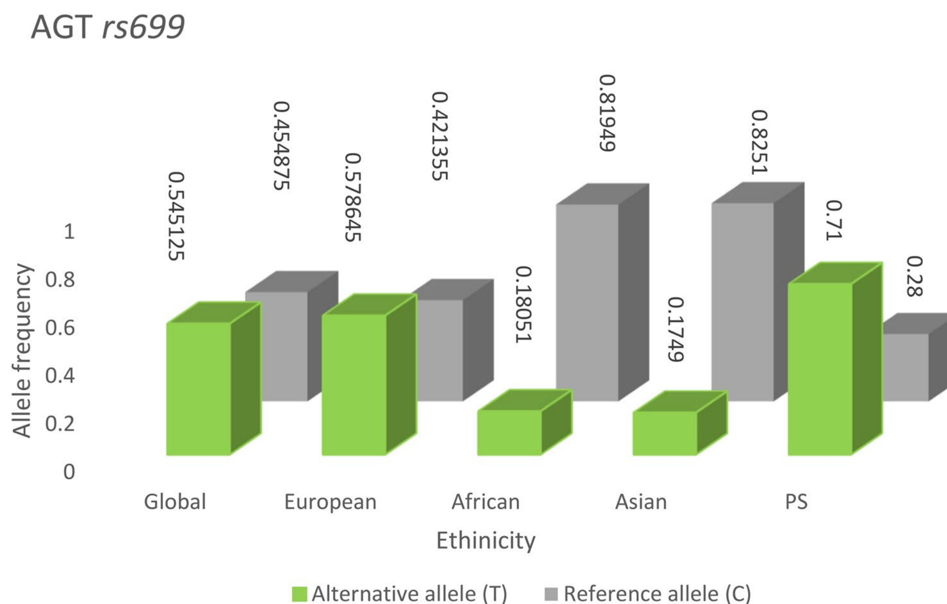

Fig. 4 Allele frequency distribution of the *AGT* (M235T) polymorphism (T and C alleles) across global populations based on HapMap data. The T allele was more frequent globally and among Europeans, while the C allele predominated in African and Asian populations. In the patient sample (PS), the T allele was notably more common (71%), aligning with global trends and suggesting possible disease association

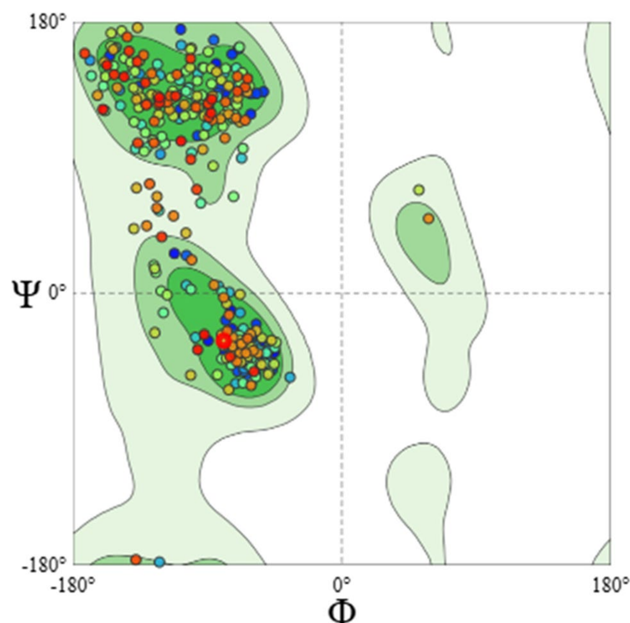

Fig. 5 Structural validation of the modeled *AGT* protein using MolProbity and Ramachandran plot analysis. The modeled structure of the *AGT* protein, generated via SWISS-MODEL to study the methionine-to-threonine substitution, was evaluated for stereochemical quality. The MolProbity score was 2.46, with a clash score of 11.62 indicating moderate steric clashes between select residue pairs. Ramachandran plot analysis showed 94.66% of residues in favored regions and 0.00% outliers, suggesting a stable backbone conformation. Rotamer outliers (4.68%) were observed in a few side chains, while no bad bonds, angles, or C-beta deviations were reported, confirming overall structural integrity

hepatocytes intravenously. Juxtaglomerular cells secrete renin, which first cleaves to produce angiotensin-I decapeptide. Then, angiotensin-converting enzyme (ACE) is responsible for producing angiotensin II octapeptide. The renin-*AGT* enzymatic reaction is the rate-limiting step of the RAAS cascade, which controls plasma *AGT* levels and is necessary for blood pressure maintenance [8, 9]. Angiotensin II uses G-protein-coupled receptors on cell membranes to carry out its actions. 30% of the amino acid sequences of the two distinct angiotensin-II receptor types that are expressed by the kidneys are similar, with 359 and 363 amino acids, respectively. These receptors, called AT1R and AT2R, are located in the kidneys, heart, brain, adipose tissue endothelium, adrenal glands, and vascular smooth muscle [10]. An enhancer was discovered in the 3′ surrounding regions immediately following the second polyadenylation site, and the promoter and neighboring elements are the regions of *AGT* that have been identified as important regulators of *AGT* expression. These regions are located approximately 1.2 kb upstream of exon 1 [11]. The two most prevalent and important *AGT* polymorphisms are *AGT* (M235T) *(rs699)* and T174M (rs4762). The RAAS enzyme has been implicated in a number of cardiovascular diseases. The pathophysiology of numerous cardiovascular diseases, such as myocardial infarction, ischemic heart disease, and coronary artery disease, is significantly influenced by the *AGT* (M235T) polymorphism. A study suggested that an *AGT* polymorphism may cause vasoconstriction, which accelerates the development of atherosclerosis [12]. Renin-angiotensin-aldosterone system (RAAS) abnormalities can cause vascular diseases to begin and worsen. Angiotensin I, the precursor hormone of angiotensin II, is produced through the interaction of renin and angiotensinogen *AGT*, one of the system's first constituents. Genetic variations in the *AGT* gene change the plasma concentration of *AGT*, which may contribute to the development of myocardial infarction, coronary heart disease, and hypertension [13].

A study found no significant differences between groups in terms of *AGT* (M235T) genotype and allele frequencies. As a result, there was no significant correlation between the *AGT* T allele and PAH. The study also examined the association between *AGT* (M235T) polymorphism and CHD. No significant differences in genotype or allele frequencies were observed when comparing the PAH group to the CHD group, or between the PAH group and healthy controls [14].

Only limited data are available on the relationship between *AGT ((rs699))* and congenital heart defects. A study reported that the *AGT* (TT) genotype was significantly more prevalent in cases of persistent pulmonary hypertension associated with congenital diaphragmatic hernia in newborns, compared to controls [15]. Another study. demonstrated that interactions between *AGT* gene polymorphisms and conventional risk factors may influence the development of hypertension [16]. Conversely, a study found no association between *AGT* (M235T) polymorphisms and blood pressure [17]. To our knowledge, this is the first study to evaluate the correlation between rs (699) polymorphism in congenital heart defects. We decided to look for *(rs699)* genetic variant as children with CHD, especially those with heart failure or specific shunts, may exhibit elevated plasma renin activity (PRA) [18, 19]. As part of the Renin-Angiotensin-Aldosterone System (RAAS), renin cleaves the angiotensinogen *AGT* gene. The kidneys release an enzyme called renin, which selectively targets the N-terminus of *AGT* to produce angiotensin I (AngI). Following this, the Angiotensin-Converting Enzyme (ACE) further transforms this AngI into Angiotensin II (AngII) [20, 21]. Therefore, shift in amino acid sequence may result in uncleaving site of *AGT* gene which may further serve as an risk factor to CHDs. MolProbity results also suggest that the *AGT* protein model maintains good structural integrity, with minimal steric clashes and no backbone or bond geometry violations.

The present study highlights a potential association between the AGT gene (rs699, C > T) polymorphism

and increased susceptibility to congenital heart disease (CHD). The observed genotypic and allelic distributions suggest that the *T* allele may act as a genetic risk factor, whereas the *C* allele appears to confer a protective effect. The AGT gene encodes angiotensinogen, a key precursor in the renin–angiotensin system that regulates vascular tone, blood pressure, and fluid balance. The rs699 polymorphism (Met235Thr substitution) has been reported to alter plasma renin and angiotensinogen levels, potentially contributing to endothelial dysfunction and abnormal cardiac morphogenesis. Similar associations have been described in previous studies investigating cardiovascular disorders, hypertension, and left ventricular hypertrophy, suggesting that the functional consequences of this variant extend beyond acquired heart disease to congenital structural defects as well. In the current study, expression profiling of the AGT gene revealed a modest but statistically significant increase in mRNA levels among CHD cases compared to controls. This upregulation, though limited in magnitude, may reflect the downstream transcriptional effects of the rs (699) variant. The elevated AGT expression could enhance local angiotensin II production, thereby influencing cardiac tissue remodeling and oxygen delivery during development. However, the absence of a significant correlation between gene expression levels and specific CHD subtypes may be attributed to the limited sample size or subtype heterogeneity.

Conclusion

Our study highlights a significant association between the *AGT* (M235T) polymorphism and the disease, with the T allele and TT genotype more commonly observed in affected individuals. This suggests a possible genetic contribution to disease risk. The combined presence of CT and TT genotypes further strengthened this association, indicating that individuals carrying the T allele may have increased susceptibility. The genotype distribution in the control group was found to be in Hardy–Weinberg equilibrium (χ^2 = 1.71, $p = 0.19$), indicating no significant deviation between the observed and expected genotype frequencies. To support these findings, we also examined *AGT* gene expression levels in blood samples. The results showed a modest but statistically significant increase in expression among cases compared to controls, suggesting that the polymorphism likely influence not only genetic risk but also gene activity. However, due to the limited sample size, especially within subgroups, we could not establish a clear relationship between expression levels and specific clinical forms of the disease. Together, the genotype and expression data support an association between AGT genetic variation, altered gene expression, and disease development. Further studies involving larger populations are needed to confirm these findings and to explore the biological pathways involved.

Abbreviations

AGT	Angiotensinogen
CHD	Congenital Heart Defect
DNA	Deoxyribonucleic Acid
RFLP	Restriction Fragment Length Polymorphism
SNP	Single Nucleotide Polymorphism
RAAS	Renin–Angiotensin–Aldosterone System
ACE	Angiotensin–Converting Enzyme
Ang I	Angiotensin I
Ang II	Angiotensin II
PRA	Plasma Renin Activity
SRIHER	Sri Ramachandra Institute of Higher Education and Research
IEC	Institutional Ethics Committee
SD	Standard Deviation
OR	Odds Ratio
CI	Confidence Interval
SWISS	MODEL–Automated protein structure homology–modeling server
MolProbity	All–atom structure validation tool
kb	Kilobase
bp	Base Pair

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Authors' contributions

NK designed the study and analyzed data. JR, AK, SS and AMF supervised the research and statistical analysis. DK, SM, AAD helped in review. RK and MCB helped in data collection. All authors contributed to the article.

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Data availability

SNP and gene expression data findings support that the study findings contributes to the pathogenesis of CHDs.

Declarations

Ethics approval and consent to participation

The present study was approved by the Institutional Ethics Committee of Sri Ramachandra Institute of Higher Education and Research (SRIHER), Porur, Chennai, Tamil Nadu, India (Ref No: IEC-NI/23/AUG/88/50). Written informed consent was obtained from the parents or legal guardians of all participating children prior to sample collection and inclusion in the study.

Consent for publication

Not applicable.

Conflict of interest

Authors does not have any conflict of interest.

Competing interests

The authors declare no competing interests.

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