Vol. 2 No. 3 (2025)

ONLINE ISSN :3007-309X
PRINT ISSN :3007-3081

Journal of Medical & Health Sciences Review

ASSOCIATION STUDY OF AGT GENE POLYMORPHISM "RS699" WITH HYPERTENSION IN A PAKISTANI POPULATION

Fajar Baig[1], Ayesha Baig[2], Naveed Khan[1]

[1]Department of Biotechnology, Abdul Wali Khan University, Mardan, Pakistan
Email: fajarbaigdec@gmail.com , naveedkhan@awkum.edu.pk

[2]Department of Zoology, Abdul Wali Khan University, Mardan, Pakistan
Email: ayeshabaig7nov@gmail.com

ARTICLE INFO:

Keywords:
Tetra ARMS PCR, AGT, Hypertension, Association study, SNP

Corresponding Author:
Naveed Khan,
Department of Biotechnology, Abdul Wali Khan University, Mardan, Pakistan
Email:

naveedkhan@awkum.edu.pk

Article History:
Published on 11 August 2025

ABSTRACT

Hypertension is a complex genetic disorder with numerous genetic and environmental factors. Essential hypertension is recognized as a global health concern, which remarkably increases the possibility of the development of cardiovascular and kidney diseases. This condition ranks as the 13th leading cause of death in the United States and has a global prevalence rate of 33, with a notably higher rate of 46.2% among the Pakistani population. Among the various biological Pathways, the Renin-Angiotensin-Aldosterone System (RAAS) is one of the most important pathways involved in blood pressure regulation. Being an essential component of RAAS, the Single Nucleotide Polymorphisms (SNPs) in the *AGT* gene are thoroughly studied in connection to hypertension in different populations across the globe. This study aimed to investigate the association between *AGT* polymorphism (rs699) and hypertension using a case-control study in a Pakistani Population from the district Nowshera. Blood samples were collected from 224 study subjects (112 hypertensive patients and 112 controls), and DNA was extracted using a non-enzymatic salting-out method. An Amplification Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR) based genotyping method was developed and utilized to determine the genotypes of the study subjects. The resultant genotypic and allelic frequencies were statistically tested for association with Hypertension through Chi-squared and Fisher's exact tests using Prism-GraphPad software. The study revealed no significant association between the SNP (rs699) and hypertension in our sample set ($P = 0.3802$, $\chi^2 = 1.934$). Including additional SNPs and a large sample size is likely to provide regional insights into the genetic basis of hypertension.

INTRODUCTION

Hypertension, also known as High blood pressure (according to WHO criteria 140/90 mmHg), is a complex disorder that significantly increases the risk of various cardiovascular diseases, dementia, and chronic kidney diseases (Tanira and Al Balushi, 2005, Zhou et al., 2021). Worldwide, Hypertension prevalence has been noticed to be elevated from 32% to 33% in 2019 compared to reports from 1990, accompanied by a two-fold surge in the rate of adult hypertension, i.e. from 650 million to 1.3 billion (Organization, 2023).

Regardless of its etiology, hypertension is divided into two groups: primary or essential hypertension and secondary hypertension. Primary hypertension contributes to almost 90-95% of hypertension cases. It is a term used for elevated blood pressure not secondary to any known disease (monogenic conditions, kidney diseases, neuroendocrine tumors, and Conn's syndrome) (Tanira and Al Balushi, 2005, Carretero and Oparil, 2000). Secondary hypertension refers to high blood pressure due to a known etiology. Accounting for 5-10% of the total hypertension cases, this type of hypertension occurs secondary to renal conditions, disorders of the adrenal glands like Conn's syndrome, hyper- and hypothyroidism, Sleep Apnea, Cardiac and vascular system disorders (Carretero and Oparil, 2000).

Hypertension is a complex disorder, having numerous genetic, environmental, and behavioral risk factors in combination. Additionally, factors like medication use, obesity, and dietary habits contribute to its development (Tanira and Al Balushi, 2005, Rossier *et al*., 2017). Around 30-50% susceptibility to hypertension in various populations is due to genetic mutations (Alhassani *et al*., 2023). Currently, 31 genes having genetic alterations responsible for monogenetic forms of hypertension and more than 1477 single nucleotide polymorphisms have been identified, through GWAS, to be associated with complex multifactorial forms of hypertension (Padmanabhan and Dominiczak, 2021).

To better understand the underlying mechanisms causing hypertension, scientists have thoroughly studied the genetics of the RAAS pathway and the sodium-regulating systems. Several abnormalities of metabolism, such as abnormal Insulin and glucose levels, are also observed to be involved in the pathogenicity of high blood pressure, making hypertension a component of the complex metabolic syndrome. The RAAS pathway is an essential mechanism involved in regulating arterial pressure, systemic vascular resistance, volume of blood, and the balance of electrolytes (Fountain *et al*., 2017). It is the most thoroughly studied pathway responsible for controlling the balance of water and salt in in the body, and today, many available anti-hypertensive drugs are directed against this system (Lupton *et al*., 2011). Some candidate genes involved in the RAAS pathway, such as *AGT*, *ACE*, and *AGTR1*, have formed the basis of various studies aimed at identifying genetic alterations that raise the risk of developing hypertension (Lupton *et al*., 2011).

The association of the *AGT* gene with hypertension was established in 1992, when 15 variants were identified and examined through case-control studies to evaluate their correlation with high blood pressure. It is located on position 1q42.2 of chromosome 1, having four introns and five exons. The *AGT* gene codes for angiotensinogen, a 485-amino-acid-long globular protein that acts as the sole substrate for the production of angiotensin I via the renin enzyme in response to low blood pressure. Missense SNP (rs699) in the *AGT* gene occurs in exon 2, causing an amino acid change from methionine to threonine, likely increasing BP and *AGT* in plasma. Meta-analysis from numerous studies has reported

the notable association between rs699 and hypertension (Chaimati *et al*., 2023). Till now, no study has been reported on the rs699 association with hypertension in the Pakistani population. Therefore, this study aimed to evaluate the resultant allelic and genotypic data for association with hypertension in subjects from District Nowshehra, Khyber Pakhtunkhwa, Pakistan.

MATERIALS AND METHODS

Study Subjects

The study subjects included 224 individuals from district Nowshera, Pakistan. Among these, 112 were hypertension patients sampled from Qazi Hussain Medical Complex, Nowshera, and a private cardiologist clinic in Nowshera, while the other 112 served as gender and age-matched normal controls. Blood samples of 3 ml were collected from both patients and controls in EDTA (ethylenediaminetetraacetic acid) tubes. The blood samples were then stored in a refrigerator at 4℃ until further processing.

Inclusion and exclusion criteria

This study followed WHO criteria for hypertension diagnosis (blood pressure of ≥ 140 mmHg and diastolic pressure of > 90 mmHg, recorded during at least two visits on different days) for selecting hypertensive individuals. Additionally, we primarily included patients who had been on anti-hypertensive drugs for a minimum period of 6 months. In contrast, controls consisted of individuals with no history of high blood pressure or anti-hypertensive drug use. Males and females from various age groups were included in the study to prevent bias in the data. Written consent was obtained from all study subjects.

DNA extraction from blood samples

Using the non-enzymatic salting out method (Bharatha et al., 2014), genomic DNA was isolated from blood samples. The extracted DNA was analysed on 1% agarose gel (Figure 1), followed by checking quality and quantity on NanoDrop.

Tetra- ARMS Primers designing for SNP (rs699)

The *AGT* nucleotide sequence was retrieved from NCBI. The Tetra ARMS-PCR primers were designed and optimized according to the guidelines of Medrano and De Oliveira (2014), and Collins and Ke (2012) (Table 1 shows details of primers used).

Table 1. Primers used in the study

SNP	SEQUENCE	Primer	bp	GC (%)
rs699	5` GACTGGCTGATCTCAGCTACACATT 3`	Forward Outer	25	48
	5` GCCTCTCTCTATCTGGGAGCCTT 3`	Reverse outer	23	56.5
	5`GCTGTCCACACTGGCTCACA 3´	Forward Inner	20	60
	5`GGAAGACTGGCTGCTCCCTTAC 3`	Reverse Inner	22	59.1

SNP Genotyping

Tetra-ARMS PCR-based genotyping was performed for the AGT A>G rs699 polymorphism. For this purpose, four primers were used: two outer primers (forward and reverse) and two inner primers (forward and reverse). In PCR tubes, a reaction mixture of 10µl was prepared. This 10µl mixture consisted of 1µl (70-130 ng/µl) DNA sample, 5 µl master mix (Fine Biotech life sciences, UK), 0.2 µl (0.2µM final concentration) forward outer primer, 0.2 µl (0.2µM final concentration) reverse outer primer, 0.6 µl (0.6µM final concentration) forward inner primer, 0.6 µl (0.6µM final concentration) reverse inner primer, and 2.4 µl PCR-grade water. Amplification was conducted in a 96-well thermal cycler using the following thermal cycling profile: an initial denaturation step at 95°C for 3 minutes, followed by denaturation at 95°C for 30 seconds in the subsequent cycles, annealing at 61°C for 40 seconds, and extension at 72°C for 1 minute, repeated for 35 cycles. A final extension was performed at 72°C for 5 minutes. All study

subjects, including cases and controls, were genotyped under these conditions. The specificity of the PCR was validated through Sanger sequencing.

Statistical Analysis

After complete genotyping of all cases and controls, the results were statistically analyzed using GraphPad Prism software. Statistical tests, including Fisher's exact test and the Chi-square test, were applied to assess the distribution of alleles and genotypes between hypertensive patients and controls and to evaluate whether rs699 is significantly associated with the hypertension phenotype. Numerous genetic models- comprising co-dominant, homozygous recessive, homozygous dominant, heterozygous dominant, and additive models- were used to assess the allelic association, with a P-value <0.05 considered statistically significant.

RESULTS

The SNP (rs699) was genotyped using Tetra ARMS PCR in 112 hypertensive patients and 112 age- and gender-matched normal controls, and the resulting genotypic and allelic frequencies were statistically evaluated for association with hypertension (Table 2 and Figure 2). The genotype frequencies in the cases were reported as follows: homozygous AA 24%, homozygous GG 35%, and heterozygous AG 41%, while in the controls, the frequencies were reported as: homozygous AA 19.6%, homozygous GG 43.7%, and heterozygous AG 36.6% (Figure.4). The study results showed no significant difference (P=0.3802, $\chi 2$ = 1.934, 2) between genotype distributions among cases and controls (Table 3). Moreover, the frequencies of the A and G alleles were also determined; the A allele had a frequency of 44.64% in cases and 37.95% in controls, while the G allele was present in 55.36% of cases and 62.05% of controls (Figure.5). The allelic frequencies revealed no significant difference between cases and controls with a P value of 0.1791 and OR= 1.319 (0.9122-1.915). The rs699 SNP showed no correlation with the development of hypertension in any of the statistical models tested, with p values of 0.2181, 0.5182, 0.5836, and 0.1791 observed in the homozygous dominant, homozygous recessive, heterozygous dominant, and additive models, respectively (Table.3). The results indicated no association between the *AGT* gene variant rs699 and hypertension development in the study population.

Figure 1. Gel picture of Genomic DNA

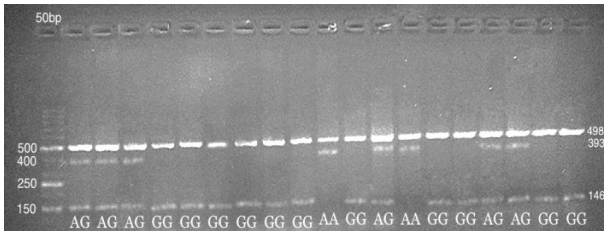

Figure 2. Gel picture of PCR results of *AGT* variant rs699. The first well contains a 50bp DNA ladder. The size of the PCR amplicons is shown on the right side. The genotype of each sample is mentioned at the bottom. The sample (in the 6th well from the left) with the GG genotype was Sanger sequenced (see figure 3)

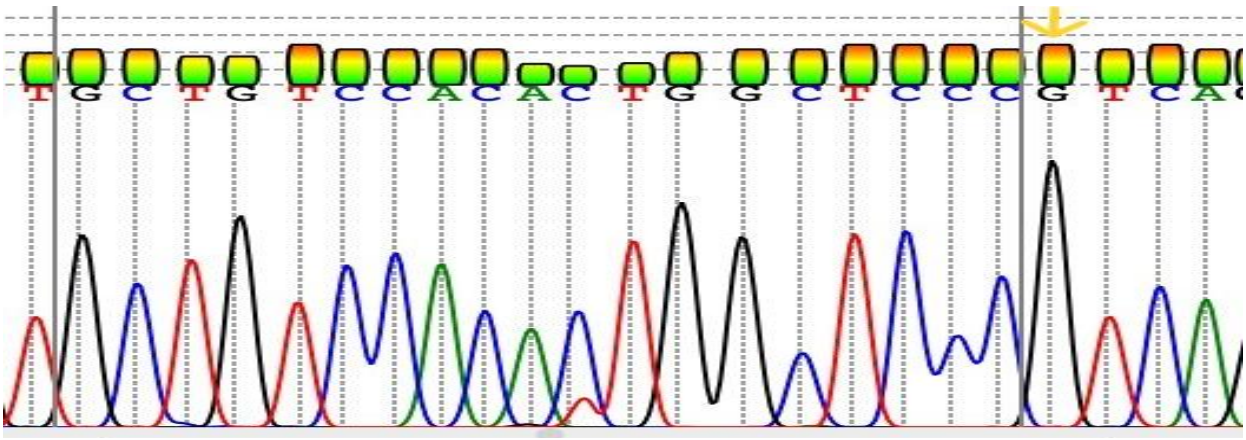

Figure 3. Sanger Sequencing results. The yellow arrow indicates the sample is homozygous for the G allele at the rs699 locus, consistent with the PCR results.

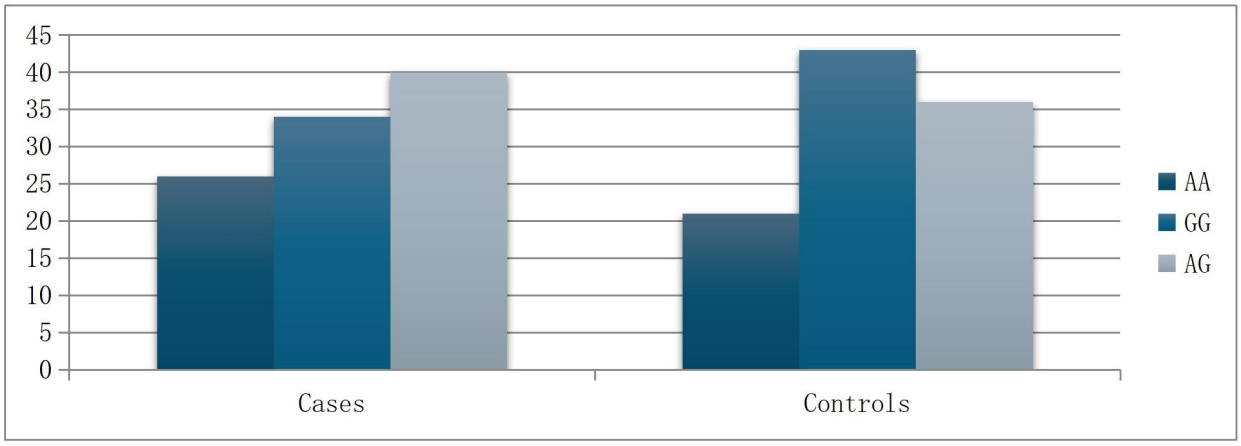

Figure 4. *AGT* gene variant (rs699) genotypic distribution in hypertension cases and controls

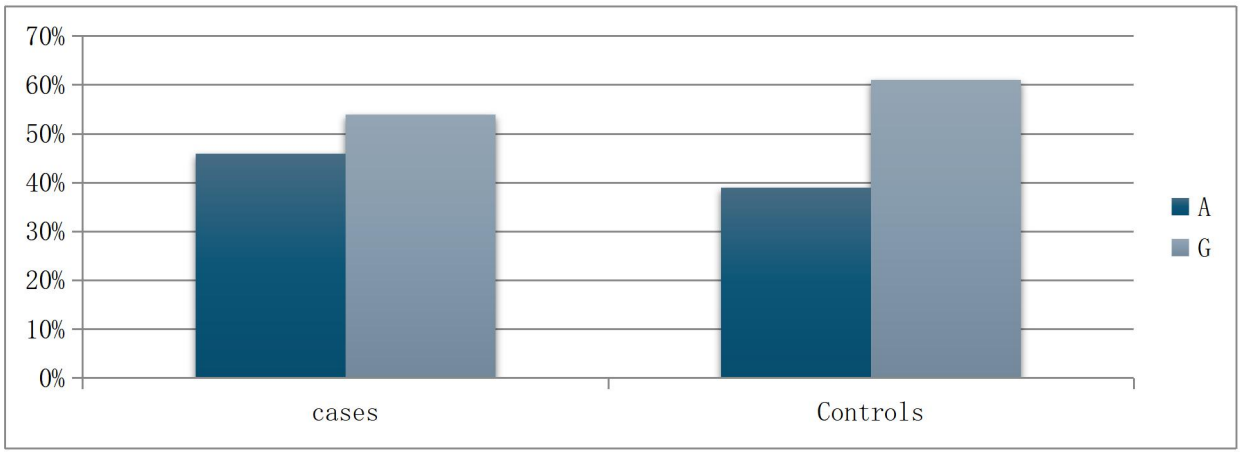

Figure 5. *AGT* gene variant (rs699) Allelic frequencies distribution across cases and controls

Table 2. Demographic characteristics of hypertension cases and controls

Parameters		Cases (Hypertension) 112		Controls (Non-hypertension) 112	
		N	%	N	%
Gender	Female	62	55.36%	62	55.36%
	Male	50	44.64%	50	44.64%
Age	25-30	1	0.89%	1	0.89%
	31-35	3	2.67%	3	2.67%
	36-40	17	15.17%	17	15.17%
	41-45	14	12.55%	14	12.55%
	46-50	18	16.07%	18	16.07%
	51-55	19	16.96%	19	16.96%
	56-60	20	17.85%	20	17.85%
	61-65	12	10.71%	12	10.71%
	66-70	6	5.35%	6	5.35%
	71+	2	1.78%	2	1.78%

Table 3. *AGT* gene variant (rs699) genotype and allele distribution across cases and controls

Statistical models	Genotypes	Cases (Hypertension)	Controls (non-hypertensive)	OR 95% CI	$\chi 2$ value	P value
Co-dominant	AA	27 (24%)	22 (19.6%)	-	1.934,2	0.3802
	GG	39 (35%)	49 (43.7%)			
	AG	46 (41%)	41 (36.6%)			
Homozygous dominant	GG	39 (34.82%)	49 (43.75%)	0.6869 (0.4011 to 1.161)	-	0.2181
	AA+AG	73 (65.18%)	63 (56.25%)			
Homozygous recessive	AA	27 (24.1%)	22 (19.64%)	1.299 (0.6926 to 2.509)	-	0.5182
	GG+AG	85 (75.89%)	90 (80.35%)			
Heterozygous dominant	AG	46 (41%)	41 (36.6%)	1.207 (0.7131 to 2.056)		0.5836
	AA+GG	66 (59%)	71 (63.39%)			
Additive	A	100 (44.64%)	85 (37.95%)	1.319 (0.9122 to 1.915)		0.1791
	G	124 (55.36%)	139 (62.05%)			

DISCUSSION

Hypertension is a complex genetic disorder resulting from the interplay of numerous determinants, including gender, age, genetic, vascular, and environmental factors (Kuneš and Zicha, 2009, Singh *et al*., 2016). Genes leading to hypertension might contain allelic variants resulting in high blood pressure (Weder, 2007). SNPs are the incredibly common form of genetic variations that exist in populations, occurring in both protein-coding and non-coding regions. SNPs located in coding regions result in amino acid changes that could lead to alterations in protein function, while SNPs situated in non-coding regions near coding sequences might exert their effect by disrupting the expression patterns of genes (Smithies *et al*., 2000).

RAAS pathway genes (*AGT, ACE, and AGTR1*) have been reported in numerous studies to be involved in regulating blood pressure and the development of hypertension in various populations. One of the SNPs found in the *AGT* gene results in an amino acid change from methionine to threonine, thereby increasing angiotensinogen levels in plasma, which leads to hypertension (Ruppert and Maisch, 2003, Lupton *et al.*, 2011). This SNP has been reported in several populations as being associated with numerous complex diseases, including visceral obesity, cardiovascular diseases, and hypertension. However, no studies have been reported on the Pakistani population to identify its association with hypertension. Therefore, our study aimed to investigate the association of the *AGT* gene variant rs699 with hypertension through a case-control study in Nowshera, Pakistan.

This study involved 112 cases of hypertension, comprising 62 females and 50 males, alongside 112 normal controls to investigate the association between rs699 and hypertension. Care was taken to minimize biases and errors in the data by maintaining a similar age and gender ratio among the subjects (Table 1). After genotyping and statistical analysis, we found no significant differences in the distribution of genotypic and allelic frequencies among cases and controls, and hence no significant association between rs699 and hypertension in the study subjects. However, in contrast to our finding, studies from different regions, including the Jammu Kashmir population, South Africa, Turkey, Tunisia, Japan, and Egypt, reported that the rs699 variant increases the risk of hypertension development (Sharma et al., 2024, Kalideen et al., 2024, Becer and Özkan, 2022, Mehri et al., 2012, Nakamura et al., 2007, El-Garawani et al., 2021).

Similar to our findings, studies conducted in Azerbaijan, among the Jordanian population, in West Africa, and Southern Nigeria reported no correlation between the *AGT* variant rs699 and the risk of hypertension (Abaszade *et al*., Alhawari *et al*., 2024, Tchelougou *et al*., 2015, Kooffreh *et al*., 2013). A Possible explanation for this could be a small sample size, population-specific genetic differences, as well as changes in environmental and lifestyle factors.

CONCLUSION

This is the first study conducted in the Pakistani population to check for the association between the risk of hypertension and AGT variant rs699. No significant association was found between the rs699 SNP and hypertension in the study population. Further studies with larger sample sizes and additional SNPs are recommended to get a regional insight to the underlying genetic basis of hypertension.

Conflict of Interest: The Authors declared no conflict of interest

Ethical Approval: Approved by the Ethical Review Committee, Department of Biotechnology, Abdul Wali Khan University, Mardan and informed consent was obtained from all participants

Funding: RIF of Abdul Wali Khan University, Mardan

AUTHORS' CONTRIBUTIONS:

Concept and design: Naveed Khan; **Samples collection**: Fajar Baig, Ayesha Baig; **Lab work and Analysis**: Fajar Baig with inputs from Naveed Khan; **Manuscript drafting:** Fajar Baig, Ayesha Baig, with inputs from Naveed Khan**; Editing and Revision**: Naveed Khan

REFRENCES

ABASZADE, Z., GASıMOV, K. & SHAHMURADOV, I. Study of single nucleotide polymorphism in the encoding exons of the AGT gene of an arterial hypertension patient from Azerbaijan. *AND BIOMEDICINE***,** 11.

ALHASSANI, F. K. H., ALGHAMDI, F. A. F. M., ALNEFAIE, A. R. A., ALZYADI, W. S. Z., ALGHAMDI, M. T. S. & ALNEFAIE, M. B. M. 2023. Identification Of Genetic Variants In AGT Gene With Association With Increased Blood Pressure And The Risk Of Hypertension. *Journal of Survey in Fisheries Sciences,* 10**,** 184-196.

ALHAWARI, H., JARRAR, Y., ZIHLIF, M., WAHBEH, A., ALSHELLEH, S., ALHABAHBEH, R. & ABDELRAZAQ, D. 2024. The Association of M235T Genetic Polymorphism in Angiotensinogen Gene and Other Non-Genetic Factors with Essential Hypertension among Jordanian Patients. *Journal of Personalized Medicine,* 14**,** 273.

BECER, S. & ÖZKAN, D. 2022. Angiotensinogen (AGT) gene screening and nutrigenetic approaches in patients diagnosed with hypertension. *Turkish Journal of Clinics and Laboratory,* 13**,** 316-321.

BHARATHA, A., SUGUNA, S. & KUNKULOL, N. D. S. K. R. 2014. Genomic DNA isolation from human whole blood samples by non enzymatic salting out method.

CARRETERO, O. A. & OPARIL, S. 2000. Essential hypertension: part I: definition and etiology. *Circulation,* 101**,** 329-335.

CHAIMATI, S., SHANTAVASINKUL, P. C., SRITARA, P. & SIRIVARASAI, J. 2023. Effects of AGT and AGTR1 Genetic Polymorphisms and Changes in Blood Pressure Over a Five-Year Follow-Up. *Risk Management and Healthcare Policy***,** 2931-2942.

COLLINS, A. & KE, X. 2012. Primer1: primer design web service for tetra-primer ARMS-PCR.

EL-GARAWANI, I. M., SHAHEEN, E. M., EL-SEEDI, H. R., KHALIFA, S. A., MERSAL, G. A., EMARA, M. M. & KASEMY, Z. A. 2021. Angiotensinogen gene missense polymorphisms (rs699 and rs4762): the association of end-stage renal failure risk with type 2 diabetes and hypertension in Egyptians. *Genes,* 12**,** 339.

FOUNTAIN, J. H., KAUR, J. & LAPPIN, S. L. 2017. Physiology, renin angiotensin system.

JEUNEMAITRE, X., INOUE, I., WILLIAMS, C., CHARRU, A., TICHET, J., POWERS, M., SHARMA, A. M., GIMENEZ-ROQUEPLO, A.-P., HATA, A. & CORVOL, P. 1997. Haplotypes of angiotensinogen in essential hypertension. *The American Journal of Human Genetics,* 60**,** 1448-1460.

KALIDEEN, K., RAYNER, B. & RAMESAR, R. 2024. Genetic Factors Contributing to the Pathogenesis of Essential Hypertension in Two African Populations. *Journal of Personalized Medicine,* 14**,** 323.

KOOFFREH, M. E., ANUMUDU, C. I., AKPAN, E. E., IKPEME, E. V. & KUMAR, P. L. 2013. A study of the M235T variant of the angiotensinogen gene and hypertension in a sample population of Calabar and Uyo, Nigeria. *Egyptian Journal of Medical Human Genetics,* 14**,** 13-19.

KUNEŠ, J. & ZICHA, J. 2009. The interaction of genetic and environmental factors in the etiology of hypertension. *Physiological research,* 58**,** 33-42.

LUPTON, S. J., CHIU, C. L. & LIND, J. M. 2011. A hypertension gene: are we there yet? *Twin Research and Human Genetics,* 14**,** 295-304.
MARTEAU, J.-B., ZAIOU, M., SIEST, G. & VISVIKIS-SIEST, S. 2005. Genetic determinants of blood pressure regulation. *Journal of hypertension,* 23**,** 2127-2143.
MEDRANO, R. F. V. & DE OLIVEIRA, C. A. 2014. Guidelines for the tetra-primer ARMS–PCR technique development. *Molecular biotechnology,* 56**,** 599-608.
MEHRI, S., MAHJOUB, S., HAMMAMI, S., ZAROUI, A., FRIH, A., BETBOUT, F., MECHMECHE, R. & HAMMAMI, M. 2012. Renin-angiotensin system polymorphisms in relation to hypertension status and obesity in a Tunisian population. *Molecular biology reports,* 39**,** 4059-4065.
NAKAMURA, Y., TABARA, Y., MIKI, T., TAMAKI, S., KITA, Y., OKAMURA, T. & UESHIMA, H. 2007. Both angiotensinogen M235T and α-adducin G460W polymorphisms are associated with hypertension in the Japanese population. *Journal of human hypertension,* 21**,** 253-255.
ORGANIZATION, W. H. 2023. *Global report on hypertension: the race against a silent killer*, World Health Organization.
PADMANABHAN, S. & DOMINICZAK, A. F. 2021. Genomics of hypertension: the road to precision medicine. *Nature Reviews Cardiology,* 18**,** 235-250.
ROSSIER, B. C., BOCHUD, M. & DEVUYST, O. 2017. The hypertension pandemic: an evolutionary perspective. *Physiology,* 32**,** 112-125.
RUPPERT, V. & MAISCH, B. 2003. Genetics of human hypertension. *Herz,* 28**,** 655.
SHARMA, M., RAINA, J. K., BHAGAT, M., SUDERSHAN, A., PANJALIYA, R. K., KOTWAL, S. & KUMAR, P. 2024. Enlighten the association of Angiotensinogen gene (AGT) polymorphisms and hypertension in Jammu region of north Indian population: A case-control study. *Human Gene,* 39**,** 201242.
SINGH, M., SINGH, A., PANDEY, P., CHANDRA, S., SINGH, K. & GAMBHIR, I. 2016. Molecular genetics of essential hypertension. *Clinical and experimental hypertension,* 38**,** 268-277.
SMITHIES, O., KIM, H.-S., TAKAHASHI, N. & EDGELL, M. H. 2000. Importance of quantitative genetic variations in the etiology of hypertension. *Kidney international,* 58**,** 2265-2280.
TANIRA, M. & AL BALUSHI, K. 2005. Genetic variations related to hypertension: a review. *Journal of human hypertension,* 19**,** 7-19.
TCHELOUGOU, D., KOLOGO, J. K., KAROU, S. D., YAMÉOGO, V. N., BISSEYE, C., DJIGMA, F. W., OUERMI, D., COMPAORE, T. R., ASSIH, M. & PIETRA, V. 2015. Renin-angiotensin system genes polymorphisms and essential hypertension in Burkina Faso, West Africa. *International journal of hypertension,* 2015**,** 979631.
WEDER, A. B. 2007. Genetics and hypertension. *The Journal of Clinical Hypertension,* 9**,** 217-223.
ZHOU, B., PEREL, P., MENSAH, G. A. & EZZATI, M. 2021. Global epidemiology, health burden and effective interventions for elevated blood pressure and hypertension. *Nature Reviews Cardiology,* 18**,** 785-802.