Identification Of Genetic Variants In AGT Gene With Association With Increased Blood Pressure And The Risk Of Hypertension.

In partial fulfillment of the degree, Master of Medical Genetics, Umm Al-Qura University, Department of Medical Genetics, College of Medicine, 2020 – 2021

Faris Khader Hasan Alhassani[1*], Fhaid Atyih Fhaid Mohammad Alghamdi[2], Abdulrahman Raja Awad Alnefaie[3], Wael Salem Zaben Alzyadi[4] ,Mohammed Thakir Salem Alghamdi[5], Marzouq Bin Mohsen Alnefaie [6]

[1*] Taif Health Cluster, Laboratory Specialist, Laboratory Department, Alsahan Bani Saad Hospital
[2]Taif Health Cluster, Laboratory Specialist, Laboratory Department, Alsahan Bani Saad Hospital
[3]Taif health cluster, laboratory technician, laboratory Department, ALSAHAN BANI SAAD HOSPITAL
[4]Taif health cluster, laboratory technician, laboratory Department, ALSAHAN BANI SAAD HOSPITAL
[5]Taif Health Cluster, Laboratory Specialist ,laboratory Department, King Faisal Hospital
[6]NURSE TECHNICIAN, Phlebotomy Department, ALSAHAN BANI SAAD HOSPITAL.

[*]**Corresponding Author:** Faris Khader Hasan Alhassani
[1*] Taif Health Cluster, Laboratory Specialist, Laboratory Department, Alsahan Bani Saad Hospital

Acknowledgment
I would like to thank my supervisor, Dr. Ahmad Mufti, for his guidance and support throughout the course of this research. My appreciation also goes to my friends and colleagues and the department faculty and staff for making my time at Umm Al-Qura University a great experience.
Finally, my heartfelt gratitude is extended to my parents and my family for their encouragement.

Abstract
The present research provides extensive research on the variants of the angiotensinogen (*AGT*) gene, which plays an essential role in the risk factors related to cardiovascular medical conditions and hypertension. The *AGT* gene is used in our body to regulate blood pressure, and it also provides equilibrium to various fluids in the human body. This research aims to identify the common variants that play an essential role in cardiovascular medical conditions. This research also included identifying the impact the variants and their mutation might have on developing essential hypertension. Kurdi, De Mello, and Booz (2005, pp. 1357-1367) stated that the renin-angiotensin-aldosterone system (RAAS) is responsible for the etiology of hypertension. The findings of this research were the focus on the nine SNPs which founded in *AGT* gene tow SNPs present in exon number 2 named rs699 and rs4762 and in the untranslated region found five SNPs named rs5046, rs5049, rs11568020, rs5050, and rs5051. Finally, the intron contains two SNPs named rs2148582 and rs3789679. It determined that *AGT* genes help us better understand the functions and processes of the different variants. Several studies have found a connection between enhanced *AGT* gene variants causing an increase in hypertension and *AGT* plasma. The following research touches on various aspects of angiotensinogen *AGT*, its system, and its impact on various functions in our body.

1. Introduction:

Hypertension is the most common and complex human disease which causes significant morbidity and mortality worldwide because it is considered the risk factor of Cardiovascular disease (CVD) (Zhou *et al.*, 2018). It remains a severe health problem causing a high-cost expenditure because of its fatal complications, such as renal disease, stroke, and heart failure (Fajar *et al.*, 2019).

Hypertension expression is a condition in which the blood vessels have persistently raised pressure and known as high blood pressure according to WHO. The carried blood from the heart to all body parts in the vessels is called the blood cycle. Every moment heartbeats, it pumps blood into the vessels. Blood pressure is formed by blood pushing forcing against the blood arteries walls. When raised blood pressure, the heart pump has become more complex (Oparil et al., 2018).

Hypertension is dividing into two types. The first one is high blood pressure, an unknown cause called essential hypertension. The second type is caused by known or direct causes, which named secondary hypertension. The most hypertension complication is primary risk factor cardiovascular disease, including stroke, heart attack, heart failure, and aneurysm (Oparil *et al.*, 2018). The real and main causes of increased blood pressure are unknown. However, many reasons may play a role, including overweight, lack of physical, smoking, or obesity, activity, too much salt in the diet, and genetics factors.

The mapping studies of various genetic factors, including gene-disease, gene-gene, and gene-environment interaction, are believed to have a reasonable probability of keeping future treatment and preventing the disease. When talking about the genes, In hypertension, studies have focused on the polymorphism of genes in RAAS, the main pathway playing a vital role in developing hypertension.

The blood pressure in the body is primarily regulated by the renin-angiotensin-aldosterone system (RAAS) (Al- Najai et al., 2013). The RAAS contains an Angiotensinogen (*AGT*) element, a protein hormone (Amber and Brown, 1999). This protein hormone gets released in the liver as an angiotensin precursor, which leads to systematic control of blood pressure (Amber and Brown, 1999). The severe complication and high prevalence have made this disease a vital problem in our world (World health organization, 2003; International society of hypertension writing group, 2003). Essential hypertension is a complex disorder because of environmental exposure and genetic history (Willams, 2007). The genetic material is responsible for an estimated 30-50% vulnerability of blood pressure in various populations (Hong et al., 1994). Various studies related to genetic linkage and individual's association with their genes have been carried out, which entails the implication of numerous loci was a link with pathways of blood pressure (Brasier et al., 1986). From these, the system of renin-angiotensin-aldosterone (RAAS) has been readily studied (Bader and Ganten, 2008).

The RAAS is a system that plays a vital role in renal sodium regulation, regulating blood pressure level, absorption of water, and vascular tone as a hormonal and circulating system (Kurdi, De Mello, and Booz, 2005). As the research aims to identify the impact variants of *AGT* had on hypertension, the evidence also stated that the renin- RAAS is responsible for the etiology of hypertension (Kurdi, De Mello and Booz 2005, pp. 1357-1367). It is known that *AGT* participates in the formulation procedure of Angiotensin I (Schaap and Kunst, 2009). Angiotensin I is the major protein of angiotensin II. In the RAAS, angiotensin II persists as a vital energetic element that contacts the vessels that carry blood and increases an individual's blood pressure (Li et al., 2015). The Angiotensinogen gene determines the plasma's concentration level (Li et al., 2015). The mapping of the *AGT* gene has to be done on the 1q42.3 chromosomes, which spread over 13kb and consist of four intros and five exons (Corvol et al., 1999).

One of this paper's objectives is to identify the influence variants of *AGT* had on hypertension. The evidence provided by much research has been doing to assess the link between essential hypertension and polymorphisms in various populations (Li et al., 2015). The first study assessing these links was done on the people of Utah and France. Over a period, the association has studied various populations.

The researchers will mention these populations to clear point the exhaustive search in the world to see all differences that include Europeans (Sarzani et al., 2010; Liao et al., 2014; Bendedetto et al., 2007; Bengra et al., 2002). Research among the population of Saudi Arabia is done by (Al-Najai et al., 2013). Among Indians, research is conducted by (Singh & Karthikeyan, 2014; Padma et al., 2014; Rao et al., 2011; Dhanachandra Singh et al., 2014; Charita et al., 2012; Gopi Chand et al., 2011). In African American population (Fejerman et al., 2006; Kumar & Patil., 2005; Wu et al., 2003; Jain et al., 2005; Markovic et al., 2005; Do An et al., 2014) conducted the research. Among Caucasians (Conen et al., 2008; Mao & Huang, 2014; Markovic et al., 2005; Jain et al., 2010) conducted the research. Similarly, among Chinese (Liu et al., 2004; Wang et al., 2007; Wang et al., 2014; Su Sl et al., 2012; Yu H et al.,2014) conducted the study. Among Turks (Kaplan et al., 2014) assessed the link. In Mongolians (Gui-yan et al., 2006) was first to conduct research related to the association.

Moreover, European Americans (Wang X et al., 2006; Zhu et al., 2003) completed the study. In the Japanese population (Nakamura, 2015; Ono et al., 2011; Takeuchi et al., 2012) conducted research to assess the link. Finally, among Dutch population (Deckers et al., 2015) conducted research. Similarly, among various other ethnic populations, a study assessing the link has been undertaken.

From the research which is done in this field, it was found that a polymorphism mass localized in the *AGT* gene is present. These *AGT* founded were included nine SNPs rs4762 (exon 2), rs699 (exon 2), rs2148582 (intron), rs3789679 (intron), (rs5046 (5'UTR), , rs11568020 (5'UTR), rs5051 (5'UTR), rs5050 (5'UTR), rs5049 (5'UTR). These *AGT* genes variants have a strong link with essential hypertension in several different populations (Purkait *et al.*, 2017).

The variants mentioned not only act as an epidemiological factor for cardiovascular disease (CAD), but it is also responsible for the demonstration in atherosclerosis severity (Lenz et al., 2005). It shows us that the *AGT* gene has severe risk factors concerning these medical conditions (Rodriguez-Perez et al., 2001; Buraczynska et al., 2003; Abboud et al., 2010).

This research paper would aim to review the common variants in cardiovascular patients and identify the possible effect these variants might have on developing hypertension. The RAAS has been widely researched because it correlated with blood pressure control. Its widespread research is because it is said to be the cause of the prognosis of multiple types of hypertension .

The *AGT* gene's role in developing hypertension among humans was founded (Jeunemaitre et al., 1992). Upon research, various kinds of *AGT* genes have been discovered, which play a significant role in developing hypertension (Corvol and Jeunemaitre, 1997). Therefore, the correlation between allying *AGT* gene and essential hypertension prognosis showed.

2. Renin-angiotensin-aldosterone system:

The most important of the endocrine systems consider RAAS, which affects blood pressure control. The place of secreted renin substance from kidney and exactly in juxtaglomerular apparatus in response to glomerular under the minimum salt

intake or perfusion a and released in response to stimulation from the sympathetic nervous system. (Mohammed and Abdelhafiz, 2015).

The angiotensin I is resulting from converting renin substrate (angiotensinogen) by the renin. Angiotensin I is a physiologically inactive substance and rapidly converted to angiotensin II in the lungs by the angiotensin-converting enzyme (ACE). Angiotensin II is an effective role vasoconstrictor and thus causes an increase in blood pressure. Besides, it stimulates aldosterone released from the zona glomerulosa in the adrenal gland resulting in a further rise in blood pressure related to water retention and sodium (Mohammed and Abdelhafiz, 2015) (figure 1).

The circulating RAAS is not responsible for being directly the high blood pressure in essential hypertension. In particular, many patients with hypertensive have low renin levels and angiotensin II (predominantly black and older people), and drugs that block the renin-angiotensin system are not particularly effective (Mohammed and Abdelhafiz, 2015).

There is more proof that there are essential non-circulating "local" renin-angiotensin paracrine or epicene systems, which control blood pressure. It is reported the local renin systems in the kidney, the arterial tree, and the heart. They may have essential roles in regulating regional blood flow (Mohammed and Abdelhafiz, 2015).

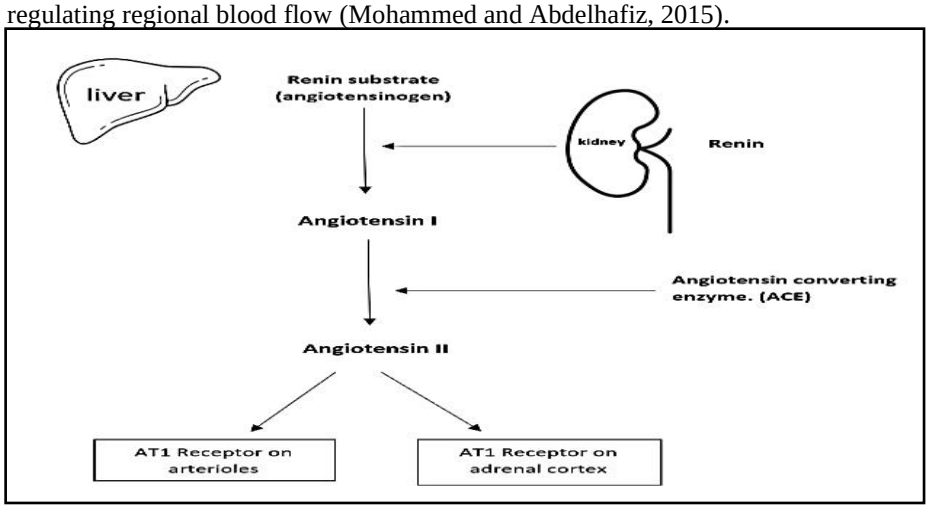


Figure 1: Renin-angiotensin system and effects on blood pressure and aldosterone release.

3. AGT gene:

The *AGT* cDNA is 1,455 nucleotides long in its structure and had a code for 485- amino acid proteins (Kageyama, Ohkubo, and Nakanishi, 1984). As shown in (figure 2), the *AGT* is made of four introns and five exons spread over 13 kb. 37 bp, which is the first exon, is part of the five untranslated regions. The origin of the formation of the peptides of active angiotensin is found on 10 N-terminal amino acids divided by renin, which then provides angiotensin I (Hong et al., 1994). The exons, which are on the second number, entail codes for a sole peptide of wither 24 or 33 residues.

It also entails the residues of a mature protein which are the initial 252 amino acids. The third exon is coded for 90 amino acids, and the fourth exon is coded for 48 residues. The fifth exon consists of 62 amino acids and has a minor sequence of coding. It is followed by a long 3-inch untranslated series that has two signals which are polyadenylation. It accounts for the bearing of dual-species of mRNA, which are differed by their length. The difference in size is by 200 nucleotides (Gaillard, Clasuser, and Corvol, 1989). By using situ hybridization, studies indicate that the human *AGT* gene is located on chromosome 1q42 (Isa et al.1990).

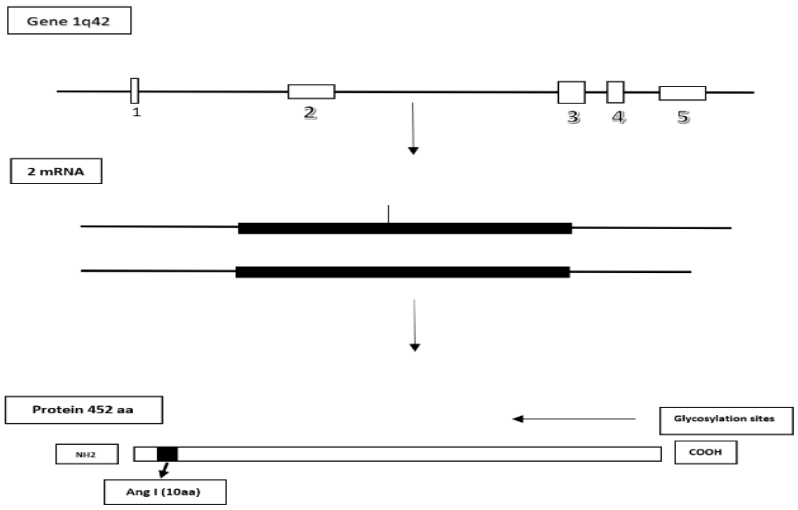


Figure 2: structure of *AGT* Gene, mRNA, and Protein.

3.1. The function of the AGT gene:

The function of *AGT* ensures the regulation of blood pressure. Modification in the gene sequence plays an essential element for the risk factors relating to cardiovascular disease (Kumar, Fejerman, and Bakar, 2008). The pathogenesis of cardiovascular disease and risk factors relating to it includes hypertension and demonstration of coronary artery disease (CAD) (Schluter and Wenzel 2008, pp.311-325). Variants of *AGT* such as rs699 (p.M268T or p.M235T) and rs4762 (p.M207T) are related to play an influence on the hazards factor of the cardiovascular medical condition (Tsai et al., 2003; Ragia et al., 2010; Fang et al., 2010).

3.2. Characteristics of AGT Protein:

The molecular mass of *AGT* between 55 and 65 kDa, and the human *AGT* is a globular glycoprotein depending on its glycosylation state. It contains four putative glycosylation sites linked to which may be the source of glycosylation chains. However, the role of this glycosylation process is not known in humans. An enzymatic assay was usually measured for Ang (I) after the excess renin complete hydrolysis. Direct immunoassays, using against *AGT* the monoclonal and polyclonal antibodies that estimate both its inactive C-terminal part and intact *AGT*, des (Ang I) *AGT*, have also been developed. In the liver, which contains mainly enzymes capable of metabolizing Ang I, it doing this method precisely quantifies *AGT* in tissues making a problematic interpretation of the enzymatic assay (Luft, 1997).

3.3. Tissue and Cellular Distribution of AGT gene:

Like other components of the renin-angiotensin system that are present in tissues involved in cardiovascular functions, AGT is synthesized in many tissues other than the liver (Ferrario et al., 2014). Even though these tissues produce less than in the liver, AGT appears to be an important component of the extravascular local renin-angiotensin system. Since the Km of renin for AGT is relatively high, the rate of Ang I production by these systems is probably controlled by the local AGT concentration rather than by the extravascular renin concentration (Nehme et al., 2019). It has been suggested that the AGT synthesized by glial cells is secreted and then taken up by neurons, thus forming a paracrine renin-angiotensin system (Corvol and Jeunemaitre, 1992).

The principal AGT mRNA in the rat aorta seems to be in the brown adipose tissue. Most of the AGT mRNA in the heart is in the atria, and it is less abundant in the ventricles. The presence of mRNA for the various components of the renin-angiotensin system, including Ang II receptors, in the heart, is good evidence that the renin-angiotensin system has a physiological role in cardiac function. Cardiac AGT mRNA is also increased in animals placed on a low-salt diet and decreased in animals treated with ACE inhibitors (Ribeiro-Oliverira et al., 2008).

AGT mRNA has been detected in the kidney, mostly in proximal tubule cells. The amounts of AGT protein and mRNA in retroperitoneal depots decline with age, suggesting that AGT is essential for the growth of adipose tissue (Dzau, 1993). Finally, AGT mRNA has been detected in several other human tissues, such as the adrenal gland, where it is present in both the cortex and medulla. It could contribute, together with locally produced renin, to the paracrine generation of Ang II and the regulation of aldosterone secretion (Bunnemann, Fuxe, and Ganten, 1993).

4. AGT variants with hypertension:

Various studies have reported links between different types of phenotypes and blood pressure. The differing Variety of phenotypes includes plasma parameters. However, this connection is only assumed to some extent, either secondary or primary, with increased blood pressure. The link and relationship among them can only be clarified with the help of genetic studies.

4.1. Association studies of AGT polymorphism and high blood pressure:

Hypertension is a medical issue that can severely decrease the mortality rate and its link with various other diseases like cardiovascular (Lawes, Vander and Rodgers 2001). It was found that it is a complex medical condition based on multiple factors in the environment (Whelton et al., 2002). Many studies also found that interaction among genes and numerous elements in the domain results in hypertension (Williams et al., 2007). It has been seen that the system of the RAS takes part in a vital role in blood pressure regulation and the cause of hypertension. Therefore, *AGT*, angiotensinogen-modifying enzyme (ACE), and the angiotensinogen II form-one receptor (AGTR1) have been studied to a great extent to find their association with hypertension (Zhu et al., 2003).

Various studies found the RAAS gene polymorphism like the single nucleotide polymorphism (SNP) (M235T), the modification in the ACE by polymorphism (either removal or insertion), and the A1166c single nucleotide polymorphism (SNP) in AGTR1. All have some form of risk associated with hypertension of diseases related to hypertension (Kim et al., 2015). An old study has found that a specific type of genotype in *AGT*, TT genotype, is linked with increased risk of lacunar infarction (24) and hypertension history (Ishikawa et al., 2001). (He et al., 2011) Found that A1166C plays a vital role in AGTR1 in knowing hypertension one has, and (Jin et al., 2012) found that the enhancement in the left ventricular concentration index is correlated to a genotype in the AGTR1 called CC genotype.

4.2. AGT variants with coronary heart disease:

The variants of the RAAS have a relative risk of cardiovascular diseases conferred according to several studies. The evaluated studies are many which appear the relative risk of cardiovascular diseases conferred by the renin-angiotensin system and its gene variants (Cambien et al., 1992). One reported in the "Etude Cas-Te'moin de l'Infarctus du Myocarde" (ECTIM) mention the link myocardial infarction in Caucasian patients and variants of the ACE gene, which considered at little risk of cardiovascular diseases according to their body mass index and lipid profile (Luft, 1997).

Were used the same control populations and case (630 patients who lived a myocardial infarction and 741 controls) to discover the role of the T174M and M235T *AGT* gene variants in coronary disease pathogenesis. At the end of the result is no difference in the *AGT* genotype distributions in the controls and cases. Whereas they found the M174 allele in carriers individuals were more often receiving antihypertensive treatment, a more significant proportion of them required several drugs. Three studies have shown a link of the M235T *AGT* allele with coronary heart disease (Luft, 1997).

There will be mention three studies that have shown an association between the M235T *AGT* allele and coronary heart disease. The first one, using a limited case-control study (82 cases and 160 controls) which found a limited extent higher frequency in Japanese patients to detect the 235T allele with coronary atherosclerosis (at least one coronary artery with more than 25% luminal diameter reduction) (Ishigami T et al., 1995). The second one reported that a group from New Zealand, which contains 422 patients, was at greater risk of coronary heart disease than 406 matched controls. The result of this report was that the 235T allele increased the risk of CHD 2- to 6-fold and the risk of myocardial infarction 3- to 4-fold, after adjustment for several other risk factors (Katsuya T et al., 1995). Finally, Japanese patients found the 235TT genotype was less frequent with a control group than in myocardial infarction. The odds ratio in the ACE DD genotype was more increased in patients carrying this genotype. (Kamitani A et al., 1995).

A large study in Caucasians was planned to detect an association between the renin-angiotensin system genes variants. The coronary artery stenosis degree shows no correlation with the ACE I/D polymorphism M235T variants (Jeunemaitre X et al., 1992).

4.3. AGT variants and complication of diabetes:

The more complications of insulin-dependent diabetes mellitus (IDDM) are Degenerative retinopathy and nephropathy. These complications influenced by several factors such as blood glucose control, the duration time of IDDM, and genetic factors (Luft, 1997). In diabetic nephropathy, the genes of the renin-angiotensin system, in particular ACE, may be implicated because high glomerular concentrations of angiotensin II can support glomerular hypertension and contribute to renal deterioration (Marre M, 1996).

The evaluation of the link between diabetic nephropathy and *AGT* variants in patients with IDDM has been done. They found that the patients with nephropathy who were homozygous for the 235T allele had a higher level of systolic blood pressure than those carrying the T235 $_{M}$ or the M235 $_{M}$ genotype. For one study on 423 Caucasian patients with type 1 diabetes and 663 with type 2 diabetes was no relationship between them. The lack of connections between diabetic nephropathy and the M235T variant does not finally keep out a role for the *AGT* gene (Luft, 1997).

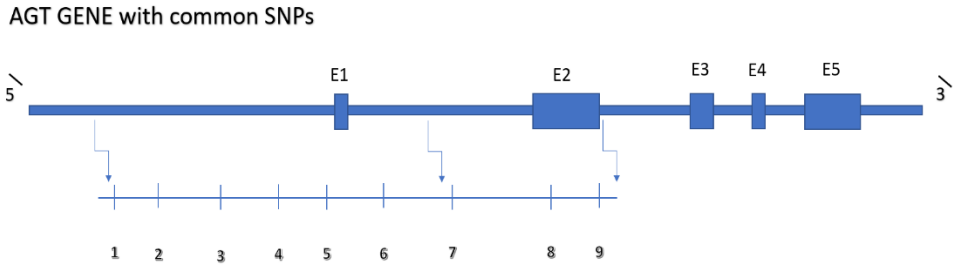
4.4. AGT, obesity, and blood pressure:

AGT is local synthesis and present in adipose tissue, and the *AGT* maybe is a portion of a paracrine system that affected the release of Ang II in the near of the vascular smooth muscle cells. The nutritional status in people also influences the blood supply to adipose tissue and affects vascular resistance and blood pressure in obese individuals. The concentration of plasma *AGT* strongly correlated with blood pressure during weight loss (Luft, 1997).

In obese Japanese women, were study the 67 women state showed that hypertensive has a higher intra-abdominal/subcutaneous fat index than the normotensive. (Luft, 1997). The question here is the association between intraabdominal fat accumulation and hypertension is involved in the pathogenesis of hypertension in obese individuals. One study found that the M174T *AGT* variant was associated with hypertension only in thin individuals (Luft, 1997)

5. Statistical analysis:

For this research paper, we will discuss one study that focuses on the *AGT* gene, especially of our interest region, the 5' UTR, exon, and intron region of the *AGT* gene. So, the study searched through the Ensemble genome browser, NCBI SNPs database, which included Nine SNPs rs4762 (exon 2), rs699 (exon 2), rs2148582 (intron), rs3789679 (intron), rs5046 (5'UTR), rs5049 (5'UTR), rs11568020 (5'UTR), rs5051 (5'UTR), rs5050 (5'UTR) by Statistical analysis and reviews all results which appears the relationship between *AGT* variants and hypertension (Figure. 3) (Purkait *et al.*, 2017).



	1	2	3	4	5	6	7	8	9
rs. No	rs5046	rs5049	rs11568020	rs5050	rs5051	rs2148582	rs3789679	rs4762	rs699
SNP LOCATION	-532	-217	-152	-20	-6	68	172	174;3389	235;4072
ALLELE	G>A	C>T	C>A C>T	T>C T>G	C>A C>G C>T	A>C A>G A>T	G>A G>T	G>A	A>G
Position	5'UTR	5'UTR	5'UTR	5'UTR	5'UTR	Intron	Intron	Exon 2	Exon 2

Figure 3: AGT with common SNPs

5.1. The Study patients:

In West Bengal in Eastern India, the study chooses the ethnic Bengali speaking population of Kolkata city and surrounding area to do a cross-sectional case-control study consisted of 256 hypertensive patients and 158 controls (Purkait *et al.*, 2017).

5.2. Method uses:

In reviewing this study, we see the researcher was done a genotype analysis of the *AGT* gene about hypertension to ensure the linkage in an Indian population. To achieve this was done the specific SNPs analysis and through *AGT* and gene region promoter in 414 subjects (158 controls against 256 Hypertensive cases) (Purkait *et al.*, 2017).

5.3. Subject's characteristics:

The descriptive statistics of metric variables were done in this study as per the disease. Comparatively, the researcher found many factors such as blood pressure, the mean age, low-density lipoproteins, Uric acid, Urea, Blood urea nitrogen, chloride, glucose, and cholesterol in the normotensive group were significantly less than the hypertensive group, as shown in Table 1 (Purkait *et al.*, 2017).

Table 1: The study group's clinical characteristics (Purkait et al., 2017).

Variables	Hypertensive ($n = 256$)		Normotensive ($n = 158$)		*t*-test (*p*-value)
	Mean	SE	Mean	SE	
Glucose(mg/dl)	132.50	3.70	120.77	3.71	**0.035**
Age (YEAR)	56.45	0.51	53.03	0.41	**0.000**
SBP (mm of mercury)	161.38	1.08	105.37	0.61	**0.000**
DBP (mm of mercury)	91.98	0.74	75.51	0.67	**0.000**
Body Mass Index (BMI) (Kg/m^2)	24.22	0.28	23.45	0.31	**0.075**
Triglycerides (mg/dl)	162.83	5.08	153.38	5.81	0.233
Cholesterol (mg/dl)	177.82	2.70	167.19	2.75	**0.009**
LDL (mg/dl)	97.66	2.03	90.79	1.94	**0.022**
HDL (mg/dl)	47.71	1.07	45.73	1.40	0.259
Urea(mg/dl)	46.44	2.28	22.40	1.25	**0.000**
BUN (mg/dl)	21.69	1.06	10.46	0.58	**0.000**
Chloride (mmol/L)	110.10	0.84	103.92	0.76	**0.000**
Uric Acid (mg/dl)	6.10	0.10	5.48	0.11	**0.000**

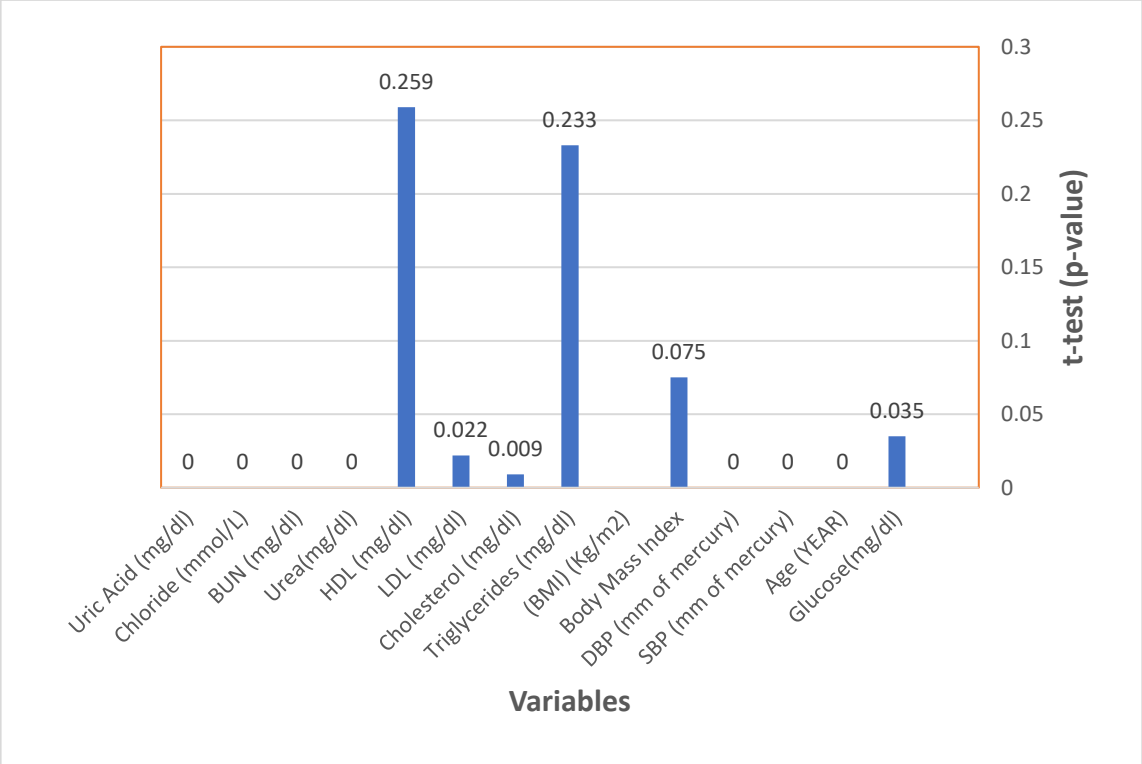


Figure 4: chart which appears the p. value of variables.

5.4. analyses of AGT polymorphism and hypertension:

In Hardy-Weinberg equilibrium among the Normotensive control group, all the SNPs were polymorphic with minor allele frequencies > 5%. Genotype distributions in agreement shown in (Table 2). The non-synonymous mutations show in two types of SNPs, rs4762 and rs699. While remaining SNPs were in an intron or the untranslated regions of a gene. The association analysis of hypertension (158 controls versus 256 cases) shows variants with the disease in the causative associations of the rs4762 T [p-value= 0.034 and Odds ratio =1.57], the rs5050 C [p-value= 0.000 and Odds ratio= 2.808] and the rs11568020 A [p-value= 0.003 and Odds ratio= 6.382] as in (Table 3) (Purkait *et al.*, 2017).

Table 2: All nine diallelic polymorphisms in the *AGT* Gene with genotype distribution and HWE tests (Purkait *et al.*, 2017).

dbSNP ID	Minor Allele (A1)	A2	Group	Genotype Distribution		HWE		
				GENO	**p-value**	O(HET)	E(HET)	**p-value**
rs5046	T	C	Hypertensive	14/90/152	0.655	0.3516	0.3547	0.861
			Normotensive	12/52/94		0.3291	0.3653	0.198
rs5049	A	G	Hypertensive	14/94/148	0.684	0.3672	0.363	1
			Normotensive	12/56/90		0.3544	0.3781	0.406
rs11568020	A	G	Hypertensive	0/20/236	***0.004***	0.07812	0.07507	1
			Normotensive	0/2/156		0.01266	0.01258	1
rs5050	C	A	Hypertensive	34/74/148	***0.000***	0.2891	0.4008	***0.000***
			Normotensive	4/30/124		0.1899	0.2116	0.244
rs5051	G	A	Hypertensive	28/90/138	0.292	0.3516	0.4077	***0.031***
			Normotensive	12/66/80		0.4177	0.4074	0.846
rs2148582	T	C	Hypertensive	26/90/140	0.170	0.3516	0.4008	0.060
			Normotensive	10/68/80		0.4304	0.4019	0.433
rs3789679	T	C	Hypertensive	0/50/206	*	0.1953	0.1762	0.145
			Normotensive	2/24/132		0.1519	0.1615	0.342
rs4762	T	C	Hypertensive	8/70/178	***0.000***	0.2734	0.2795	0.659
			Normotensive	0/36/122		0.2278	0.2019	0.224
rs699	T	C	Hypertensive	28/88/140	***0.023***	0.3438	0.4043	**0.019**
			Normotensive	8/72/78		0.4557	0.4019	0.114

Significance values are written by italicized and bold, Chi-Sq = 4.384, Level of significance <0.05; Degrees of freedom (DF) = 2; *Chi-Square approximation probably invalid; 2 cells with expected counts less than 5.0; 1 cell with expected counts less than 1.0

Table 3: Fisher exact test for the study group Normotensive and Hypertensive (Purkait et al., 2017).

SNPs	Minor allele	Frequency		Odds Ratio (95% CI)	*p*-value
		Hypertensive	Normotensive		
rs5049	A	0.2383	0.2532	0.9228 (0.6668-1.277)	0.677
rs11568020	A	0.03906	0.006329	6.382 (1.482-27.49)	***0.003***
rs5046	T	0.2305	0.2405	0.9458 (0.68 - 1.315)	0.736
rs3789679	T	0.09766	0.08861	1.113 (0.6851-1.809)	0.714
rs5051	G	0.2852	0.2848	1.002 (0.7342-1.367)	1
rs2148582	T	0.2773	0.2785	0.9943 (0.7272-1.36)	1
rs4762	T	0.168	0.1139	1.57 (1.034-2.383)	***0.034***
rs699	T	0.2812	0.2785	1.014 (0.7418-1.386)	1
rs5050	C	0.2773	0.1203	2.808 (1.9-4.148)	***0.000***

Level of significance < 0.05. Significance values are italicized and bold.

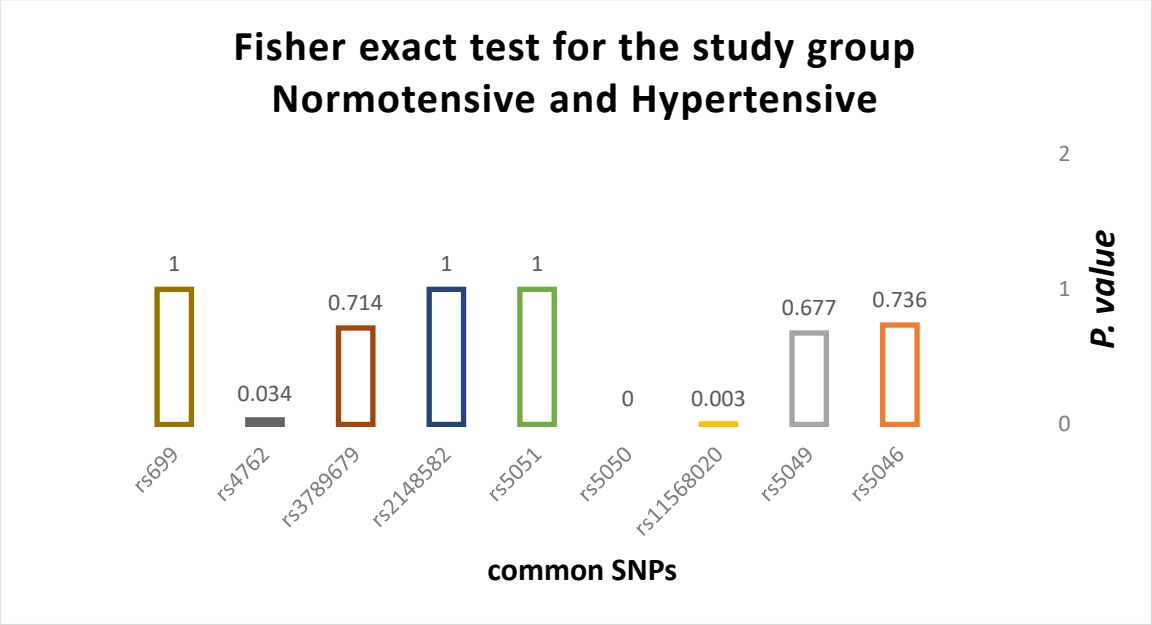


Figure 5: Chart shows the P. value to all nine SNPs.

The linkage was confirmed with regression analysis through three genotypic model tests. The first one is named recessive model (REC), the second one dominant model (DOM), and the third one additive model (ADD), which all uses to confirm the predictive association between both study groups. In DOM and ADD models showed significant association with hypertension for the SNP rs11568020 (ADD: OR = 6.61, p = 0.012; DOM: OR = 6.61, P =0.012) and rs4762 (ADD: OR = 1.587, p = 0.032), while the SNP rs699 (OR = 2.303; p = 0.04417) shows significant association and risk with the hypertension in recessive model. The SNP rs5050 is showing association with hypertension in all three genotypic models (REC: OR = 5.896; p = 0.001, ADD: OR = 2.339, p = 0.000 and DOM: OR = 2.661, p = 0.000) see (Table 4).

Table 4: Logistic regression analysis between Normotensive and Hypertensive group (Purkait *et al.*, 2017).

Test	SNP	A1	OR	P
Dominant model	rs5046	T	1.005	0.981
	rs5049	A	0.9658	0.865
	rs11568020	A	*6.61*	*0.011*
	rs5050	C	*2.661*	*0.000*
	rs5051	G	0.877	0.517
	rs2148582	T	0.8498	0.422
	rs3789679	T	1.232	0.432
	rs4762	T	*1.485*	*0.090*
	rs699	T	0.8079	0.292
Recessive model	rs5046	T	0.7039	0.388
	rs5049	A	0.7039	0.388

	rs11568020	A	NA	NA
	rs5050	C	*5.896*	*0.001*
	rs5051	G	1.494	0.265
	rs2148582	T	1.673	0.183
	rs3789679	T	0.000	0.999
	rs4762	T	0.000	0.998
	rs699	T	*2.303*	*0.044*
Additive model	rs5046	T	0.948	0.745
	rs5049	A	0.9241	0.631
	rs11568020	A	*6.61*	*0.011*
	rs5050	C	*2.339*	*0.000*
	rs5051	G	1.002	0.991
	rs2148582	T	0.9946	0.972
	rs3789679	T	1.119	0.657
	rs4762	T	*1.587*	*0.032*
	rs699	T	1.013	0.932

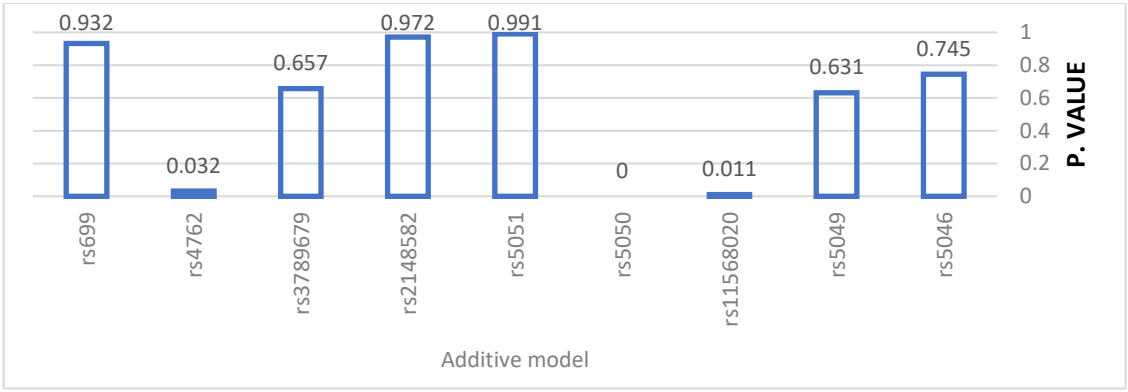


Figure 6: Chart shows the P. value in the additive model in nine SNPs**.**

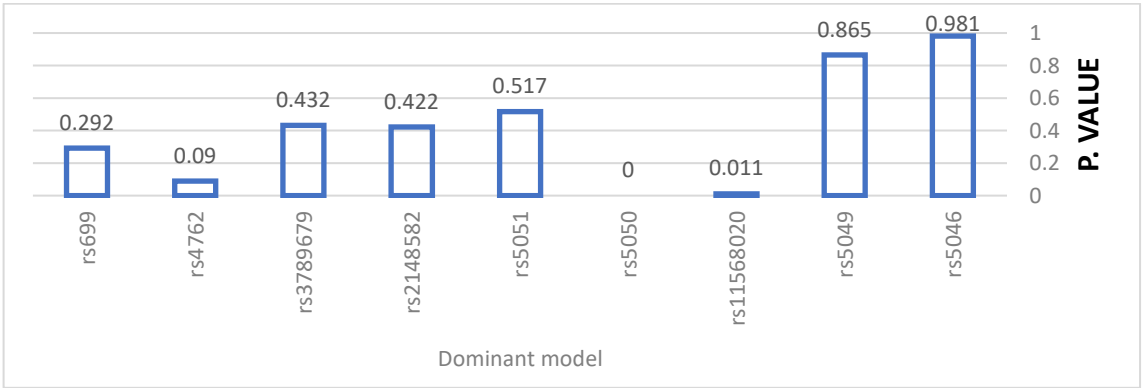


Figure 7: Chart shows the P. value in the dominant model in nine SNPs.

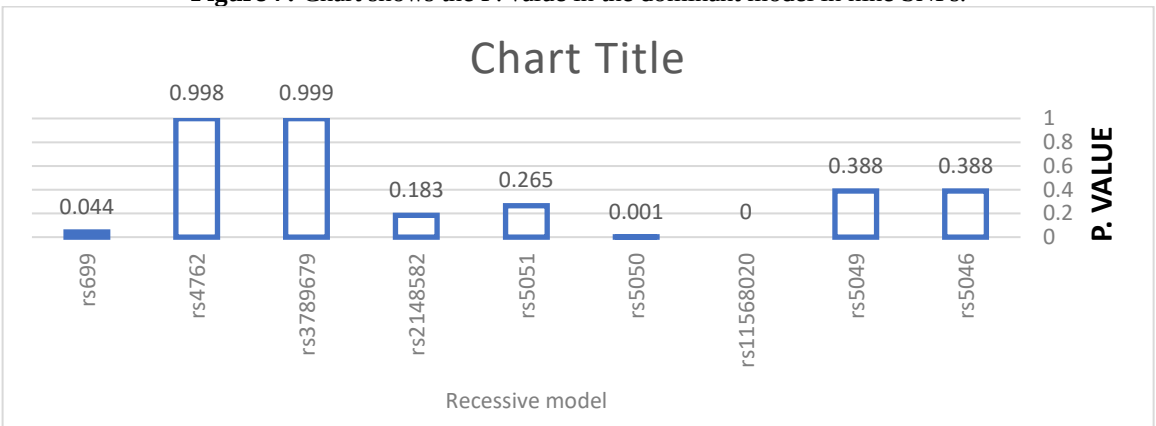


Figure 8: Chart shows the P. value in the recessive model in nine SNPs.

6. Discussion:

Genetic research is present to detect genes responsible for the specific disease that is the main objective. These gene results should concentrate on the illness pathway to treating and preventing them in the best possible way.

There are two types of diseases according to the caused. The first one, the conditions with a comparatively straightforward genetic basis, i.e., the single-gene disorders, the gene detection methods, is adequate to find the genes involved; however, the issue formed with the multi-genetic disorders' conditions detection. The most common illnesses such as stroke, heart disease, cancers, diabetes, or psychiatric disorders have complexity in their genetic control. That means we need more investigation to study all variations caused by these diseases.

7. Conclusion:

According to all information, data, Statistical analysis, and the results obtained from previous research, there is a relationship between some mutations in the *AGT* gene and hypertension.

Identifying genes linked to complex disorders is a more complicated process and very difficult to be sure it. The *AGT* gene needs more investigation and searches to know more details on the vast population and large-sized samples. We also need to use modern technologies such as next-generation sequences (NGS) or whole-exome sequencing (WES).

From this study, it has been found and assessed that the angiotensinogen (*AGT*) gene plays a vital function in the epidemiology of essential hypertension and blood pressure increase. Variants of the *AGT* gene, especially 235T, are extensively studied, and it has been found that it is linked with a higher rate of *AGT* gene correspondence. This Correspondence then increases the concentration of plasma and tissue present in the *AGT* gene. The risk connected to a cardiovascular medical condition is between 20%-30% due to the *AGT* gene. Moreover, it was also found that in ANG II generation, the increase of *AGT* in one's tissue led to regulation of one's blood pressure. *AGT* variants are usually impacted due to various environmental factors and the interaction among genes such as the ACE gene. However, extensive research is required in this domain to obtain a better understanding.

8. References

1. Abboud, N., Ghazouani, L., Kaabi, B., Ben-Hadj-Khalifa, S., Addad, F., Marwen, M., Almawi, W.Y. and Mahjoub, T., 2010. Evaluation of the contribution of renin-angiotensin system polymorphisms to the risk of coronary artery disease among Tunisians. *Genetic testing and molecular biomarkers*, *14*(5), pp.661-666.
2. Al-Najai, M., Muiya, P., Tahir, A.I., Elhawari, S., Gueco, D., Andres, E., Mazhar, N., Altassan, N., Alshahid, M. and Dzimiri, N., 2013. Association of the angiotensinogen gene polymorphism with atherosclerosis and its risk traits in the Saudi population. *BMC cardiovascular disorders*, *13*(1), pp.1-12.
3. Bader, M. and Ganten, D., 2008a. Update on tissue renin-angiotensin systems. *Journal of Molecular Medicine*, *86*(6), p.615.
4. Benedetto, C., Marozio, L., Ciccone, G., Chieppa, G., Quaglia, M., Matullo, G., Bertola, L., Guarrera, S., Carturan, S. and Stratta, P., 2007. Synergistic effect of the renin-angiotensin system and nitric oxide synthase genes polymorphisms in pre-eclampsia. *Acta obstetricia et Gynecologica Scandinavica*, *86*(6), pp.678-682.
5. Bengra, C., Mifflin, T.E., Khripin, Y., Manunta, P., Williams, S.M., Jose, P.A. and Felder, R.A., 2002. Genotyping of essential hypertension single-nucleotide polymorphisms by a homogeneous PCR method with universal energy transfer primers. *Clinical Chemistry*, *48*(12), pp.2131-2140.
6. Bouhnik, J., Clauser, E., Gardes, J., Corvol, P. and Menard, J., 1982. Direct radioimmunoassay of rat angiotensinogen and its application to rats in various endocrine states. *Clinical Science*, *62*(4), pp.355-360.
7. Brasier, A.R., Philippe, J., Campbell, D.J. and Habener, J.F., 1986. Novel expression of the angiotensinogen gene in a rat pancreatic islet cell line. Transcriptional regulation by glucocorticoids. *Journal of Biological Chemistry*, *261*(34), pp.16148-16154.
8. Bunnemann, B., Fuxe, K. and Ganten, D., 1993. The renin-angiotensin system in the brain: an update 1993. *Regulatory peptides*, *46*(3), pp.487-509.
9. Buraczyńska M, Pijanowski Z, Spasiewicz D, Nowicka T, Sodolski T, Widomska - Czekajska T, Ksiazek A, 2003. Renin-angiotensin system gene polymorphisms: assessment of the risk of coronary heart disease. Kardiol Pol. Jan;58(1):1-9
10. Conen, D., Glynn, R.J., Buring, J.E., Ridker, P.M. and Zee, R.Y., 2008. Association of renin-angiotensin and endothelial nitric oxide synthase gene polymorphisms with blood pressure progression and incident hypertension: prospective cohort study. *Journal of hypertension*, *26*(9), p.1780.
11. Corvol P, Persu A, Gimenez-Roqueplo AP, et al. Seven lessons from two candidate genes in human essential hypertension: angiotensinogen and epithelial sodium channel. Hypertension 1999; 33: 1324–1331.
12. Chand, M.G., Srinath, J., Rao, R.S., Lakkakula, B.V., Kumar, S. and Rao, V.R., 2011. Association between the M268T polymorphism in the angiotensinogen gene and essential hypertension in a South Indian population. *Biochemical genetics*, *49*(7-8), pp.474-482.
13. Charita, B., Padma, G., Sushma, P., Deepak, P., and Padma, T., 2012. Estimation of risk and interaction of single nucleotide polymorphisms at angiotensinogen locus causing susceptibility to essential hypertension: a case-control study. *Journal of the Renin-Angiotensin-Aldosterone System*, *13*(4), pp.461-471.

14. Deckers, I.A., van den Brandt, P.A., van Engeland, M., van Schooten, F.J., Godschalk, R.W., Keszei, A.P. and Schouten, L.J., 2015. Polymorphisms in genes of the renin-angiotensin-aldosterone system and renal cell cancer risk: Interplay with hypertension and intakes of sodium, potassium, and fluid. *International journal of cancer*, *136*(5), pp.1104-1116.
15. Dhanachandra Singh, K., Jajodia, A., Kaur, H., Kukreti, R. and Karthikeyan, M., 2014. Gender-specific association of RAS gene polymorphism with essential hypertension: a case-control study. *BioMed research international*, *2014*.
16. Dzau, V.J., 1993. Tissue renin-angiotensin system in myocardial hypertrophy and failure. *Archives of internal medicine*, *153*(8), pp.937-942.
17. Fajar, J. K. *et al.* (2019) 'The association between angiotensin II type 1 receptor A1166C gene polymorphism and the risk of essential hypertension: a meta-analysis', *Egyptian Journal of Medical Human Genetics*, 20(1), pp. 1–13. doi: 10.1186/s43042-019-0016-3.
18. Fang, Y.J., Deng, H.B., Thomas, G.N., Tzang, C.H., Li, C.X., Xu, Z.L., Yang, M. and Tomlinson, B., 2010. Linkage of angiotensinogen gene polymorphisms with hypertension in a sibling study of Hong Kong Chinese. *Journal of hypertension*, *28*(6), p.1203.
19. Fejerman, L., Wu, X., Adeyemo, A., Luke, A., Zhu, X., Hicks, C. and Cooper, R.S., 2006. The effect of genetic variation in angiotensinogen on serum levels and blood pressure: a comparison of Nigerians and US blacks. *Journal of human hypertension*, *20*(11), pp.882-887.
20. Ferrario, C.M., Ahmad, S., Nagata, S., Simington, S.W., Varagic, J., Kon, N. and Dell'Italia, L.J., 2014. An evolving story of angiotensin-II-forming pathways in rodents and humans. *Clinical Science*, *126*(7), pp.461-469.
21. Gaillard, I., CLAUSER, E. and CORVOL, P., 1989. Structure of human angiotensinogen gene. *Dna*, *8*(2), pp.87-99.
22. Gui-Yan, W., Yan-hua, W., Qun, X., Wei-Jun, T., Ming-Ling, G., Jian, W., Ming-Wu, F. and Yong-hong, Z., 2006. Associations between RAS gene polymorphisms, environmental factors, and hypertension in Mongolian people. *European journal of epidemiology*, *21*(4), pp.287-292.
23. He, J., Gu, D., Kelly, T.N., Hixson, J.E., Rao, D.C., Jaquish, C.E., Chen, J., Zhao, Q., Gu, C., Huang, J. and Shimmin, L.C., 2011. Genetic variants in the renin-angiotensin-aldosterone system and blood pressure responses to potassium intake. *Journal of hypertension*, *29*(9), pp.1719-1730.
24. Hong, Y., De Faire, U., Heller, D.A., McClearn, G.E. and Pedersen, N., 1994. Genetic and environmental influences on blood pressure in elderly twins. *Hypertension*, *24*(6), pp.663-670.
25. Isa, M.N., Boyd, E., Morrison, N., Harrap, S., Clauser, E. and Connor, J.M., 1990. Assignment of the human angiotensin gene to chromosome 1q42–q43 by nonisotopic in situ hybridization. *Genomics*, *8*(3), pp.598-600.
26. Ishigami, T., Umemura, S., Iwamoto, T., Tamura, K., Hibi, K., Yamaguchi, S., Nyuui, N., Kimura, K., Miyazaki, N. and Ishii, M., 1995. A molecular variant of the angiotensinogen gene is associated with coronary atherosclerosis. *Circulation*, *91*(4), pp.951-954.
27. Ishikawa, K., Baba, S., Katsuya, T., Iwai, N., Asai, T., Fukuda, M., Takiuchi, S., Fu, Y., Mannami, T., Ogata, J. and Higaki, J., 2001. T+ 31C polymorphism of angiotensinogen gene and essential hypertension. *Hypertension*, *37*(2), pp.281-285.
28. Jain S, Tillinger A, Mopidevi B, et al., 2010. Transgenic mice with -6A haplotype of the human angiotensinogen gene have increased blood pressure compared with the -6G haplotype. J Biol Chem; 285: 41172–41186.
29. Jain, S., Li, Y., Patil, S. and Kumar, A., 2005. A single-nucleotide polymorphism in the human angiotensinogen gene is associated with essential hypertension and affects glucocorticoid-induced promoter activity. *Journal of molecular medicine*, *83*(2), pp.121-131.
30. Jeunemaitre, X., Soubrier, F., Kotelevtsev, Y.V., Lifton, R.P., Williams, C.S., Charru, A., Hunt, S.C., Hopkins, P.N., Williams, R.R., Lalouel, J.M. and Corvol, P., 1992. Molecular basis of human hypertension: role of angiotensinogen. *Cell*, *71*(1), pp.169-180.
31. Jin, Y., Kuznetsova, T., Thijs, L., Schmitz, B., Liu, Y., Asayama, K., Brand, S.M., Heymans, S., Brand, E., Fagard, R. and Staessen, J.A., 2012. Association of left ventricular mass with the AGTR1 A1166C polymorphism. *American journal of hypertension*, *25*(4), pp.472-478.
32. Kageyama, R., Ohkubo, H. and Nakanishi, S., 1984. The primary structure of human preangiotensinogen is deduced from the cloned cDNA sequence. *Biochemistry*, *23*(16), pp.3603-3609.
33. Kaplan, İ., Sancaktar, E., Ece, A., Şen, V., Tekkeşin, N., Basarali, M.K., Kelekci, S. and Evliyaoglu, O., 2014. Gene polymorphisms of adducin GLY460TRP, ACE I/D, AND AGT M235T in pediatric hypertension patients. *Medical science monitor: international medical journal of experimental and clinical research*, *20*, p.1745.
34. Kim, H.K., Lee, H., Kwon, J.T. and Kim, H.J., 2015. A polymorphism in AGT and AGTR1 gene is associated with lead-related high blood pressure. *Journal of the Renin-Angiotensin-Aldosterone System*, *16*(4), pp.712-719.
35. Kløverpris, S., Skov, L.L., Glerup, S., Pihl, K., Christiansen, M. and Oxvig, C., 2013. The formation of high-molecular-weight angiotensinogen during pregnancy is a result of competing for redox reactions with the proform of eosinophil major basic protein. *Biochemical Journal*, *449*(1), pp.209-217.
36. Kumar, A., Li, Y., Patil, S. and Jain, S., 2005. A haplotype of the angiotensinogen gene is associated with hypertension in African Americans. *Clinical and experimental pharmacology and physiology*, *32*(5-6), pp.495-502.
37. Kumar, R., Singh, V.P. and Baker, K.M., 2008. The intracellular renin-angiotensin system: implications in cardiovascular remodeling. *Current opinion in nephrology and hypertension*, *17*(2), pp.168-173.

38. Kurdi, M., De Mello, W.C. and Booz, G.W., 2005a. Working outside the system: an update on the unconventional behavior of the renin-angiotensin system components. *The international journal of biochemistry & cell biology*, *37*(7), pp.1357-1367.
39. Lenz, J.R., Pereira, A.C., Lemos, P.A., Martinez, E. and Krieger, J.E., 2005. Angiotensinogen M235T polymorphism is associated with coronary artery disease severity. *Clinica chimica acta*, *362*(1-2), pp.176-181.
40. Lawes CM, Vander Hoorn S and Rodgers A. , 2001 Global burden of blood-pressure-related disease. Lancet 2008; 371: 1513–1518
41. Liao, X., Yang, Z., Peng, D., Dai, H., Lei, Y., Zhao, Q., Han, Y. and Wang, W., 2014. Association of T174M polymorphism of angiotensinogen gene with essential hypertension: A meta-analysis. *Genetics and molecular biology*, *37*(3), pp.473-479.
42. Liu, Y., Jin, W., Jiang, Z.W., Zhang, K.X., Sheng, H.H., Jin, L., Sheng, Y.Y., Huang, W. and Yu, J.D., 2004. Relationship between six single nucleotide polymorphisms of the angiotensinogen gene and essential hypertension. *Chinese journal of medical genetics*, *21*(2), pp.116-119.
43. Luft, F. C. (1997) 'Molecular genetics of human hypertension', *Internist*, 62(3), pp. 223–235. doi: 10.1007/s00108-021-00979-1.
44. Mao, S. and Huang, S., 2014. Association of angiotensinogen gene M235T polymorphism with the risk of IgA nephropathy: a meta-analysis. *Renal failure*, *36*(3), pp.466-472.
45. Markovic, D., Tang, X., Guruju, M., Levenstien, M.A., Hoh, J., Kumar, A. and Ott, J., 2005. Association of angiotensinogen gene polymorphisms with essential hypertension in African-Americans and Caucasians. *Human Heredity*, *60*(2), pp.89-96.
46. Mehri, S., Koubaa, N., Hammami, S., Mahjoub, S., Chaaba, R., Nakbi, A., Zouari, B., Abid, M., Arab, S.B., Baudin, B. and Hammami, M., 2010. Genotypic interactions of renin-angiotensin system genes with diabetes type 2 in a Tunisian population. *Life sciences*, *87*(1-2), pp.49-54.
47. Mohammed, M. and Abdelhafiz, K. (2015) 'Autoantibodies in the sera of breast cancer patients: Antinuclear and anti-double stranded DNA antibodies as example', *Journal of Cancer Research and Therapeutics*, 11(2), pp. 341–344. doi: 10.4103/0973-1482.157314.
48. Nakamura A., 2015. Genotypes of the renin-angiotensin system and glucocorticoid complications. Pediatr Int.;57(1):72-8.
49. Nehme, A., Zouein, F.A., Deris Zayeri, Z. and Zibara, K., 2019. An update on the tissue renin-angiotensin system and its role in physiology and pathology. *Journal of cardiovascular development and disease*, *6*(2), p.14.
50. Nguyen, G., Delarue, F., Burcklé, C., Bouzhir, L., Giller, T. and Sraer, J.D., 2002. The pivotal role of the renin/prorenin receptor in angiotensin II production and cellular responses to renin. *The Journal of clinical investigation*, *109*(11), pp.1417-1427.
51. Oparil, S. *et al.* (2018) 'Hypertension', *Nature Reviews Disease Primers*, 4. doi: 10.1038/nrdp.2018.14.
52. Padma, G., Swapna, N., Mamata, M., Charita, B. and Padma, T., 2014. The risk conferred by tagged SNPs of AGT gene in causing susceptibility to essential hypertension. *Clinical and Experimental Hypertension*, *36*(8), pp.579-585.
53. Paul, M., Poyan Mehr, A. and Kreutz, R., 2006. Physiology of local renin-angiotensin systems. *Physiological Reviews*, *86*(3), pp.747-803.
54. Poirier, P., Giles, T.D., Bray, G.A., Hong, Y., Stern, J.S., Pi-Sunyer, F.X. and Eckel, R.H., 2006. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. *Circulation*, *113*(6), pp.898-918.
55. Purkait, P., Halder, K., Thakur, S., Roy, A.G., Raychaudhuri, P., Bhattacharya, S., Sarkar, B.N. and Naidu, J.M., 2017. Association of angiotensinogen gene SNPs and haplotypes with risk of hypertension in eastern Indian population. *Clinical hypertension*, *23*(1), pp.1-10.
56. Purkait, P. *et al.* (2017)' Association of angiotensinogen gene SNPs and haplotypes with risk of hypertension in eastern Indian population', *Clinical Hypertension*, 23(1), pp. 1–10. doi: 10.1186/s40885-017-0069-x.
57. Ragia, G., Nikolaidis, E., Tavridou, A., Arvanitidis, K.I., Kanoni, S., Dedoussis, G.V., Bougioukas, G. and Manolopoulos, V.G., 2010. Renin-angiotensin-aldosterone system gene polymorphisms in coronary artery bypass graft surgery patients. *Journal of the Renin-Angiotensin-Aldosterone System*, *11*(2), pp.136-145.
58. Sarzani, R., Bordicchia, M., Marcucci, P., Minardi, D., Muzzonigro, G., Dessì-Fulgheri, P. and Rappelli, A., 2010. Angiotensinogen promoter variants influence gene expression in human kidney and visceral adipose tissue. *Journal of human hypertension*, *24*(3), pp.213-219.
59. Schaap, M.M. and Kunst, A.E., 2009. Monitoring of socio-economic inequalities in smoking: learning from the experiences of recent scientific studies. *Public health*, *123*(2), pp.103-109.
60. Schlüter, K.D. and Wenzel, S., 2008. Angiotensin II: a hormone involved in and contributing to pro-hypertrophic cardiac networks and target of anti-hypertrophic cross-talks. *Pharmacology & therapeutics*, *119*(3), pp.311-325.
61. Singh, K.D. and Karthikeyan, M., 2014. Combined sequence and sequence-structure-based methods for analyzing RAAS gene SNPs: a computational approach. *Journal of Receptors and Signal Transduction*, *34*(6), pp.513-526.

62. Su, S.L., Lu, K.C., Lin, Y.F., Hsu, Y.J., Lee, P.Y., Yang, H.Y. and Kao, S.Y., 2012. Gene polymorphisms of angiotensin-converting enzyme and angiotensin II type 1 receptor among chronic kidney disease patients in a Chinese population. *Journal of the Renin-Angiotensin-Aldosterone System*, *13*(1), pp.148-154.
63. Takeuchi, F., Yamamoto, K., Katsuya, T., Sugiyama, T., Nabika, T., Ohnaka, K., Yamaguchi, S., Takayanagi, R., Ogihara, T. and Kato, N., 2012. Reevaluation of the association of seven candidate genes with blood pressure and hypertension: a replication study and meta-analysis with a larger sample size. *Hypertension Research*, *35*(8), pp.825-831.
64. Tsai, C.T., Fallin, D., Chiang, F.T., Hwang, J.J., Lai, L.P., Hsu, K.L., Tseng, C.D., Liau, C.S. and Tseng, Y.Z., 2003. Angiotensinogen gene haplotype and hypertension: interaction with ACE gene I allele. *Hypertension*, *41*(1), pp.9-15.
65. Wang, L., Zhang, B., Li, M., Li, C., Liu, J., Liu, Y., Wang, Z., Zhou, J. and Wen, S., 2014. Association between single-nucleotide polymorphisms in six hypertensive candidate genes and hypertension among northern Han Chinese individuals. *Hypertension Research*, *37*(12), pp.1068-1074.
66. Wang, T., Chen, Z.B., Jin, S.J. and Su, Q.J., 2007. Correlation between angiotensinogen gene and primary hypertension with cerebral infarction in the Li nationality of China. *Neuroscience bulletin*, *23*(5), pp.287-292.
67. Wang, X., Zhu, H., Dong, Y., Treiber, F. A., & Snieder, H. (2006). Effects of angiotensinogen and angiotensin II type I receptor genes on blood pressure and left ventricular mass trajectories in multiethnic youth. *Twin research and human genetics : the official journal of the International Society for Twin Studies*, *9*(3), 393–402.
68. Whelton, P.K., He, J., Appel, L.J., Cutler, J.A., Havas, S., Kotchen, T.A., Roccella, E.J., Stout, R., Vallbona, C., Winston, M.C. and Karimbakas, J., 2002. Primary prevention of hypertension: clinical and public health advisory from The National High Blood Pressure Education Program. *Jama*, *288*(15), pp.1882-1888.
69. Williams, S.S., 2007. Advances in genetic hypertension. *Current opinion in pediatrics*, *19*(2), pp.192-198.
70. World Health Organization and International Society of Hypertension Writing Group, 2003. 2003 World Health Organization (WHO)/International Society of Hypertension (ISH) statement on management of hypertension. *Journal of hypertension*, *21*(11), pp.1983-1992.
71. Wu, C., Lu, H., Cassis, L.A. and Daugherty, A., 2011. Molecular and pathophysiological features of angiotensinogen: a mini review. *North American journal of medicine & science*, *4*(4), p.183.
72. Yu, H., Lin, S., Zhong, J., He, M., Jin, L., Zhang, Y. and Liu, G., 2014. A core promoter variant of angiotensinogen gene and interindividual variation in response to angiotensin-converting enzyme inhibitors. *Journal of the Renin-Angiotensin-Aldosterone System*, *15*(4), pp.540-546.
73. Zhou, B., Wen, M., Mi, L., Hu, C.J., Zhang, Y., Wang, J.T. and Tang, L., 2018. Associations between angiotensinogen M235T polymorphisms and the risk of diabetic nephropathy: A meta-analysis. *Diabetes research and clinical practice*, *142*, pp.26-36.
74. Zhu, X., Chang, Y. P., Yan, D., Weder, A., Cooper, R., Luke, A., Kan, D., & Chakravarti, A. 2003. Associations between hypertension and genes in the renin-angiotensin system. *Hypertension (Dallas, Tex. : 1979)*, *41*(5), 1027–1034.
75. Zhou, D. *et al.* (2018) 'Uncontrolled hypertension increases risk of all-cause and cardiovascular disease mortality in US adults: The NHANES III Linked Mortality Study', *Scientific Reports*, 8(1), pp. 1–7. doi: 10.1038/s41598-018-27377-2.