Available online on www.ijpqa.com
International Journal of Pharmaceutical Quality Assurance 2018; 9(1); 6-10
doi: 10.25258/ijpqa.v9i01.11352
ISSN 0975 9506

Research Article

The Higher Frequency of G Allele Found in the -152 Site of *Agt* Gene But was Not Aligned with the Angiotensinogen Levels

Susanto A H[1*], Rohman M S[2]

[1]*Basic Nursing Department, School of Nursing, Faculty of Medicine, Universitas Brawijaya, Malang 65145, Indonesia.*
[2]*Department of Cardiology and Vascular Medicine, Faculty of Medicine, Universitas Brawijaya- Saiful Anwar General Hospital 65145, Malang, Indonesia.*

Received: 4th Oct, 17; Revised: 5th Jan, 18; Accepted:10th Jan, 18; Available Online:25th March, 2018

ABSTRACT

Pathogenesis of essential hypertension involves an interaction between genetic and environmental factors. Genetic variant G-152A of angiotensinogen (*AGT*) promoter is thought to affect *AGT* gene transcription and angiotensinogen levels. Angiotensinogen is an important substrate for renin in the RAS that is finally converted into angiotensin II that plays a key role in the control of blood pressure. However, the studies that discuss the single nucleotide polymorphism (SNP) G-152A of *AGT* gene are still limited, especially in Indonesian hypertensive patients. Therefore, this study was designed to detect the SNP G-152A of *AGT* and reported its serum angiotensinogen levels. The variants were identified in 62 patients in Malang, Indonesia with *essential hypertension* by PCR and further identified by automated sequencing. Serum samples were collected to analyze the angiotensinogen levels by a sandwich ELISA. The data showed that the *AGT* promoter in our *patients* had genetic variants -152G (G allele/GG genotype) and -152A (A allele/AG genotype) with a frequency of 0.92 : 0.08, respectively. There were no patients with AA genotype. In the analysis of serum angiotensinogen levels (mean ± SD), it was found that A allele had an angiotensinogen level of 371.30 ± 69.92 (ng/mL) and G allele was 343.53 ± 74.95 (ng/mL). The A allele had slightly higher angiotensinogen level than G allele with no significant difference (P=0.437). Thus, in our study is found a genetic variant G-152A of *AGT* gene represented the GG, AG genotypes in the (-152) site and it has higher frequency of G allele. Further research is still needed to determine that G/A allele directly affects on the occurrence of essential hypertension.

Keywords: *Genetic Variant, The SNP G-152A, AGT Gene, Serum Angiotensinogen, Essential Hypertension.*

INTRODUCTION

Essential hypertension is the most common type of hypertension (more than 95%) and becomes a serious health problem worldwide[1]. Essential hypertension affects more than 20% of the adult population and has a vague etiology. The complex pathogenesis of essential hypertension involves the interaction between genetic and environmental factors[2]. The Renin-Angiotensin (RAS) system plays an important role in the maintenance of blood pressure[3]. The molecular variants of the angiotensinogen gene (*AGT*), the important component of the renin-angiotensin system, are considered as a genetic risk factor for the occurrence of essential hypertension[4]. Human *AGT* gene is located on chromosome 1 (1q42.2), containing 5 exons and produces protein angiotensinogen. Angiotensinogen is the *prohormone* of *angiotensin II*, secreted from the liver that interacts with renin to produce angiotensin I which is a major effector molecule of RAS[4,5]. Several variations especially in the promoter area of *AGT* gene are known to affect transcriptional activity of the gene in the production of its protein. The single nucleotide polymorphisms (SNPs) at position -152 in the *AGT* promoter is thought to be a functional site in affecting basal transcriptional activity directly related to increased angiotensinogen levels[6,7]. In general, the studies have discussed the SNP -152 of *AGT* and the serum angiotensinogen levels are still limited. Thus, the aims of the study were to describe the presence and the frequency of genotypes in the -152 site and to analyze the differences of serum angiotensinogen levels between each allele.

MATERIALS AND METHODS

Subjects

Sixty two outpatients with essential hypertension in Saiful Anwar General Hospital Malang, Indonesia were enrolled for this study. Essential hypertension was defined as systolic blood pressure of ≥140mmHg, diastolic blood pressure of ≥90mmHg, or the use of at least one class of antihypertensive agent. Patients who had (1) secondary hypertension (2) massive bleeding, liver problems and kidney failure (3) pregnancy (4) estrogen or corticosteroid therapy were excluded. The age, gender, weight, height (was used to calculate Body mass index-BMI), smoker, ureum, creatinin, blood glucose, cholesterol were also recorded.

Single nucleotide polymorphism (*SNP) Detection*

Blood samples from patients were prepared and genomic DNA was isolated using a DNA extraction kit

**Author for Correspondence: susanto.akhiyan@ub.ac.id*

Table 1: Baseline characteristics.

Variable	SNP of -152 *AGT*		*P**
	G allele (n=57)	A allele (n=5)	
Age (year)	59.12±8.15	55.40±4.1	0.122
Sex (M/F)	28/29	4/1	0.178**
Weight (kg)	66.66±10.99	73.4±10.36	0.226
Height (cm)	159.16±7.76	161.4±2.31	0.424
BMI (m^2)	26.47±3.9	28.18±3.8	0.384
Creatinin (mg/dl)	2.08±7.81	1.16±0.304	0.379
Ureum (mg/dl)	29.66±13.11	32.04±11.51	0.681
Blood Glucose (mg/dl)	98.81±19.01	97.2±11.05	0.781
Smoking (%)	1(1.75%)	1(20%)	0.127**
Colesterol (mg/dl)	185.46±41.85	184.2±36.21	0.944

BMI: body mass index; $*p$ value ≤ 0.05: significantly different between groups; ***Chi-Square Test*

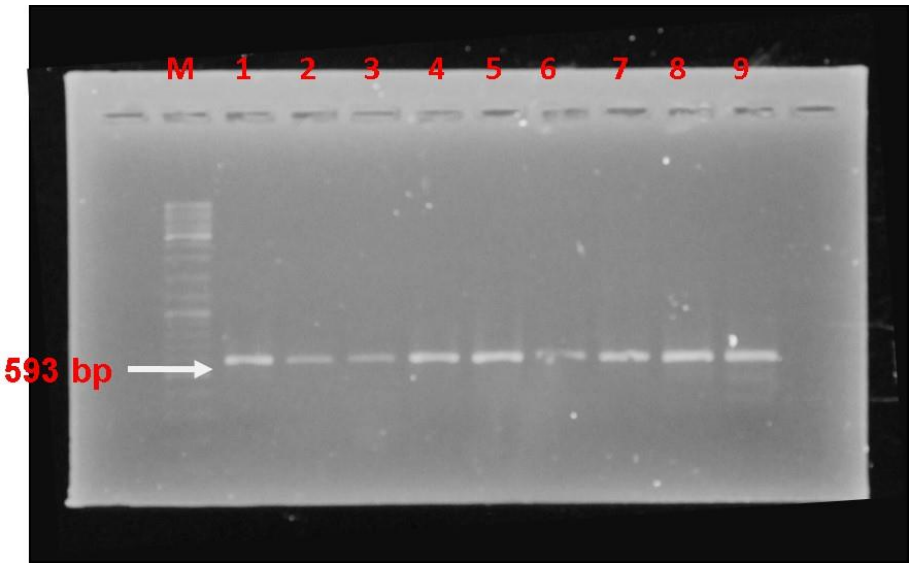

Figure 1: PCR Products. Gel electrophoresis 1.5% showed 593 bp of PCR product after amplification of *AGT* gene; presented *AGT* in the promoter area.

```
                118       128       138       148       158       168

....|....|....|....|....|....|....|....|....|....|....|....|....|.
cap_1595566_25_hAGT
AGTGAAACTCTGCATCGATCACTAAGACTTCCTGGAAGAGGTCCCAGCGTGAGTGTCGCTTCTGGC
cap_1595559_18_hAGT
AGTGAAACTCTGCATCGATCACTAAGACTTCCTGGAAGAGGTCCCAGCGTGAGTGTCGCTTCTGGC
cap_1595558_17_hAGT
AGTGAAACTCTGCATCGATCACTAAGACTTCCTGGAAGAGGTCCCAGCATGAGTGTCGCTTCTGGC
cap_1595560_19_hAGT
AGTGAAACTCTGCATCGATCACTAAGACTTCCTGGAAGAGGTCCCAGCGTGAGTGTCGCTTCTGGC
cap_1595557_16_hAGT
AGTGAAACTCTGCATCGATCACTAAGACTTCCTGGAAGAGGTCCCAGCATGAGTGTCGCTTCTGGC
Contig-0
AGTGAAACTCTGCATCGATCACTAAGACTTCCTGGAAGAGGTCCCAGCGTGAGTGTCGCTTCTGGC
```

Figure 2: Alignment analysis using *Bioedit Sequence Alignment Editor software.* We found 2 alleles at -152 site, G allele and A allele.

(Geneaid™). Each DNA sample was amplified by polymerase chain reaction (PCR) using forward primer 5'–TTC CAG AAG CGA CTT TTC AC AC–3' and reverse primer 5'–TAG TAC CCA GAA GAA CGG CA–3'[8]. Then, the PCR product were sequenced by automatic sequencing method (Macrogen) and the SNP -152 of *AGT* were analyzed using Genescane software (Applied Biosystem).

Serum Angiotensinogen Levels

Serum samples of all patients were screened using sandwich ELISA for their angiotensinogen levels. Measurement of serum angiotensinogen was conducted according to Human angiotensinogen (aGT) ELISA Kit-CUSABIO.

Analysis

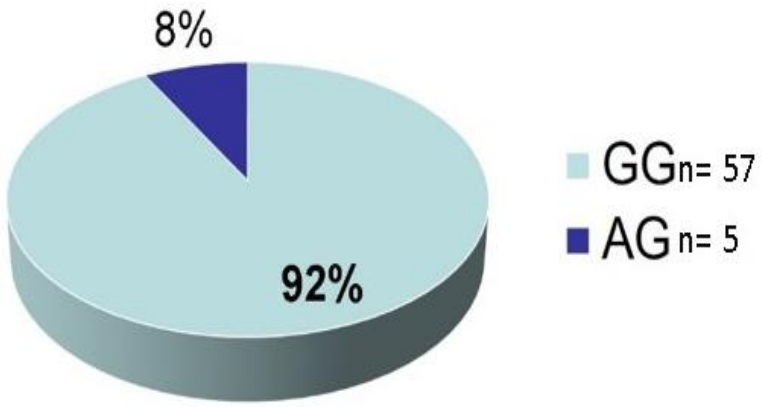

Figure 3: **Frequency of SNP G-152A.** The data showed that the *AGT* promoter in our *patients* had genetic variants -152G (n=57) and -152A (n=5) with a frequency of 0.92 : 0.08, respectively.

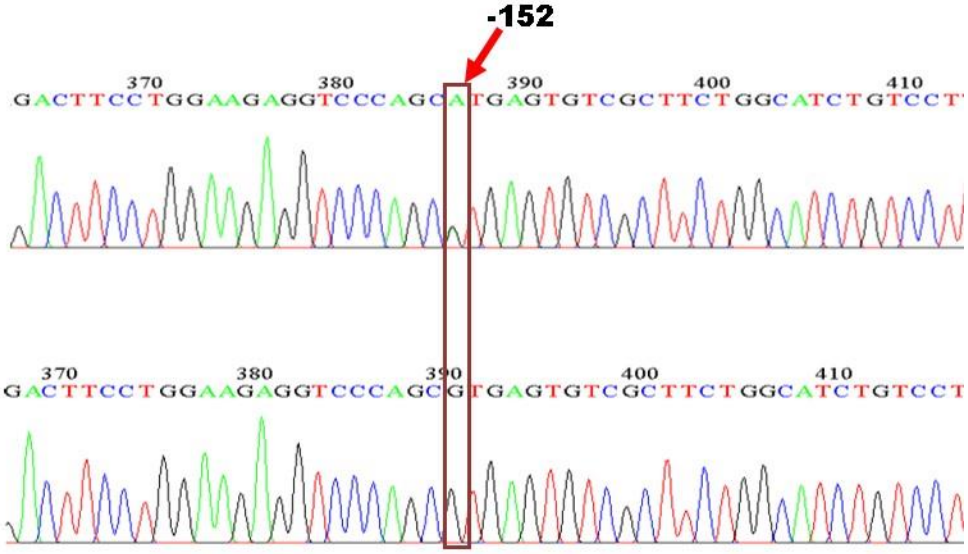

Figure 4: **Electropherogram analysis.** The arrows indicate the polymorphic site of AG (heterozygote) & GG (homozygote). No patient with AA genotype.

We analyzed the frequency of each genotype and allele of SNP -152. Baseline characteristics were compared by *Independen sample t-test* for parametric and *Chi-Square Test* for non- parametric analysis. Serum angiotensinogen levels was compared by *Independen sample t-test*. P-value ≤0.05 was considered statistically significant. Statistical analysis was performed under the SPSS (Statistical Package for the Social Sciences) 14.0 (SPSS Inc. Chicago, IL, USA).

Ethics

The study was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in humans and approved by the Ethics Committee on Faculty of Medicine, Universitas Brawijaya and Saiful Anwar General Hospital. Written informed consent was obtained from all study participants.

RESULTS

Baseline characteristics

The clinical features of hypertensive patients with G allele and A allele (*see analysis of the SNP result)* are summarized in Table 1. There were no significant differences in age, gender, weight, height (was used to calculate Body mass index-BMI), ureum, creatinin, blood glucose, cholesterol and percentage of patients with smoker.

Analysis of SNP -152

For the genotyping of the angiotensinogen polymorphism, each DNA sample was amplified by polymerase chain reaction (PCR) (figure 1). Based on the alignment results using the Bioedit Sequence Alignment Editor software (http://www.mbio.ncsu.edu/bioedit/bioedit.html) and confirmed on GeneBank, all sequencing results found 2 different alleles at position -152, G and A (Figure 2). All patients have been identified and showed that the *AGT* promoter had genetic variants -152G (G allele/GG genotype) and -152A (A allele/AG genotype) with a frequency of 0.92: 0.08, respectively (57:5) (Figure 3). Sequence analysis in the electropherogram results indicated that there were no patients with AA genotype (Figure 4).

SNP G-152A and serum angiotensinogen levels

Sixty two patients were divided into 2 groups according to genotyping results, 57 patients (GG genotype/G allele) and 5 patients (AG genotype/A allele). In the analysis of serum angiotensinogen levels (mean ± SD), it was found that G

Tabel 2: Serum Angiotensinogen Levels between 2 groups (G and A).

Allele	Mean *(ng/mL)*±SD	N	*P**
G	343.53 ± 74.95	57	0.437
A	371.30 ± 69.92	5	
Total	345.77 ± 74.40	62	

N: Total sample

**p* value ≤ 0.05: significantly different between groups

allele had an angiotensinogen level of 371.30 ± 69.92 (ng/mL) and A allele was 343.53 ± 74.95 (ng/mL). The A allele had slightly higher angiotensinogen level than G allele with no significant difference (P=0.437).

DISCUSSION

Sato *et al* reported that the SNP G-152A of *AGT* in the Japanese population was found in essential hypertensive subjects. A total 180 patients with hypertension were identified and there were three kinds of genotypes in -152 site, GG, GA and AA with the frequency of genotypes was 78.9%, 17.2% and 3.9%, respectively. The percentage of G dan A alleles were 87.5%: 12.5%. Thus, G allele had much higher than A allele[9]. The background of genetic differences among two ethnic groups may lead to different conclusions[10,11]. Sato *et al* used nine *AGT* gene variants which included new polymorphisms (G-152A and T 131C), and examined the association between hypertension and plasma concentration by a case-control study. No significant association was observed between G-152A and angiotensinogen levels at single analysis[9] similar to our study. On other hand suggested that G-152A, A-20C, G-6A and M235T polymorphisms of *AGT* gene might play an important role in the occurrence of essential hypertension in Chinese Han population. This study evaluated the relationship of six single nucleotide polymorphisms (SNPs) and their haplotypes of angiotensinogen gene (multilocus study) to essential hypertension in Chinese Han population[7]. Thus SNP -152 site has linkage disequilibrium with other polymorphisms in generating a particular phenotype. For further study recommends that the analysis of G-152A *AGT* has to be more comprehensive by using multilocus study approach to analyse the correlation with serum angotensinogen.

Depending on where a SNP occurs, it might have different consequences at the phenotypic level such as the transcription levels or protein structure. SNPs in the coding regions of genes that alter the function or structure of the encoded proteins are a necessary and sufficient cause of most of the known recessively or dominantly inherited monogenic disorders. The SNPs are routinely analysed for medical purposes[12]. However, SNPs in the promoter affect transcriptional activity of genes. The promoter is the center for regulation of gene transcription due to containing numerous transcription factor binding sites[13], and in the end it affects the level of protein produced.

Variation of *AGT* prometers were found and affected the regulation of plasma angiotensinogen concentrations associated with blood pressure. Some variation within *AGT* gene is located at position -6, -20, -152 promoter area[7]. In study of the promoter activity for the presence of SNPs and DNA binding properties indicate that the nucleotide substitution in the promoter region of the 5 'upstream of *AGT* gene affected its basal transcription levels. Thus, the binding and transcriptional response will be loss if there was a mutation of *transcription factor binding sites* in the nucleotide sequences (G→A allele). For example, in other study with hypertensive patients analyzed the mechanism of interaction between SNP -5434 renin gene and its transcription factor (STAT3). Focused examination was to identify the influence of C and T alleles with STAT3 protein. STAT3 was more favorable contact with T allele than C allele. The T to C alteration at the position -5434 leads to decrease of STAT3 activity to induce gene transcription[14]. Thus, in this study it is possible that the G allele is more functional to affect elevated levels of serum angiotensinogen than the A allele. However, the transcription factor and the mechanism associated with the SNP -152 of *AGT* remains unknown[6].

In this study the higher frequency of G allele contributed by its transcription factor may be as a genetic susceptibility factor in the proceeding of hypertension. Previous study in Taiwan concluded that the GG genotype at the G-152A is positively associated with the incidence of Systolic Heart Failure than genotypes GA/AA[15]. But we still found no significant difference of angiotensinogen levels between G and A alleles in this study. It was probably because the number of our samples was too small and no patients with homozygous genotype of A allele (AA genotype). Further research is still needed to enlarge our sample size and to confirm the homozygous AA genotype, analyze by multilocus study and determine the transcription factor directly regulate the SNP -152 of *AGT* and its impact on the occurrence of essential hypertension (in silico/in vitro study).

CONCLUSIONS

The higher frequency of G allele in the *AGT* gene (-152) site in Indonesian hypertensive patients suggests that this allele may be more functional in the influencing of angiotensinogen levels and as a genetic susceptibility factor in the proceeding of essential hypertension in the Indonesian population. However no significant difference of serum angiotensinogen levels was observed between G and A alleles. The protein angiotensinogen products by regulation of related transcription activity needs further research.

ACKNOWLEDGMENTS

We would like to thank hypertension project team (Prof. Widodo, Bu Tika, Arina M., Mas Dian, Adit, Mas Didik) for supporting this work. We also thank our parents and *Fastrack* Program, *Master of Biomedical Science* (Shila, Fraztiqa, Musthika, Nurona, Ika Arum, Imama, Amelia and Aprillia) to advise and assist this research.

REFERENCES

1. Kong LZ and Hu SS. Report on cardiovascular disease in China. Encyclopedia of China Publishing House, Beijing, 2005, 86–88.

2. Juan J. Bolívar. Essential Hypertension: An Approach to Its Etiology and Neurogenic Pathophysiology. International Journal of Hypertension 2013; Article ID 547809, 11 pages. doi:10.1155/2013/547809.
3. Perazella MA & Setaro JF. Renin-Angiotensin Aldosterone System: Fundamental Aspects and Clinical Implications in Renal and Cardiovascular Disorders. Journal of Nuclear Cardiology 2003; 10: 184–196.
4. Corvol P and Jeunemaitre X. Molecular genetics of human hypertension: role of angiotensinogen. Endocrin Reviews 1997; 18: 662–677.
5. Wu C, Lu H, Cassis LA, Daugherty A. Molecular and Pathophysiological Features of Angiotensinogen: A Mini Review. North American Journal of Medical Sciences 2011; 4(4):183-190.
6. Dickson ME, Sigmund CD. Genetic basis of hypertension: revisiting angiotensinogen. Hypertension 2006; 48(1): 14-20.
7. Liu Y, Jin W, Jiang ZW, Zhang KX, Sheng HH, Jin L, Sheng YY, Huang W, Yu JD, Zhonghua Yi Xue Yi Chuan Xue Za Zhi. Relationship between six single nucleotide polymorphisms of angiotensinogen gene and essential hypertension. Chinese Journal of Medical Genetics 2004; 21(2):116-119.
8. Woodiwiss AJ, Nkeh B, Samani NJ, Badenhorst D, Maseko M, Tiago AD, Candy GP, Libhaber E, Sareli P, Brooksbank R, Norton GR. Functional variants of the angiotensinogen gene determine antihypertensive responses to angiotensin-converting enzyme inhibitors in subjects of African origin. Journal of Hypertension 2006; 24(6): 1057-1064.
9. Sato N, Katsuya T, Nakagawa T, Ishikawa K, Fu Y, Asai T, Masayuki Fukuda M, Suzuki F, Nakamura Y, Higaki J, Ogihara T. Nine polymorphisms of angiotensinogen gene in the susceptibility to essential hypertension. Life Sciences 2000; 68: 259–272.
10. Yanna Li, Sudhir Jain, Sai Patil, Ashok Kumar. A haplotype of angiotensinogen gene that is associated with essential hypertension increases its promoter activity in adiposit. Vascular Pharmacology 2005; 44: 29–33.
11. Watkins WS, Hunt SC, Williams GH, Tolpinrud W, Jeunemaitre X, Lalouel JM, Jorde LB. Genotype-phenotype analysis of angiotensinogen polymorphisms and essential hypertension: the importance of haplotypes. Journal of Hypertension 2011; 28(1): 65–75.
12. Evans WE, Relling MV. Pharmacogenomics: translating functional genomics into rational therapeutics. Science 1999; 286: 487–91.
13. Chen J, Luo X, Xie G, Chen K, Jiang H, Pan F, Li J, Ruan Z, Pang X, Liang H. Functional analysis of SNPs in the ERCC5 promoter in advanced colorectal cancer patients treated with oxaliplatin–based chemotherapy. Medicine 2016; 95(19): e3656.
14. Maslahah I, Rohman MS, Widodo N, Tri Endharti A , Utomo DH. The Difference of STAT3 Binding Pattern on -5434C and -5434T REN and Its Consequence on Serum Renin Levels. International Journal of Hypertension 2015; 33: e43.
15. Nan Chang, Jou-Wie Lin, Jyh-Ming Juang, Chai-Ti Tsai, Juey-Jen Hwang, Fu-Tien Chiang. Association between Genetic Polymorphisms in the Renin Angiotensin System and Systolic Heart Failure Revised by a Propensity Score-Based Analysis. Cardiology 2010;116: 279–285.