Is there an Association between Angiotensinogen M235T Gene Polymorphism and Hypertension?

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Abstract

Background and Objective: The association between angiotensinogen (M235T) gene polymorphism with hypertension was reported, but the results were inconsistent. The aim of our study is to get our own results of this genetic polymorphism in our Egyptian patients paving way for more larger studies in the future applying more correlations with more cardiovascular risks associated with hypertension.

Material and Methods: In the present study, we recruited 50 normal controls and 100 patients with essential hypertension from Internal Medicine Department for the investigation. The angiotensinogen (M235T) genotypes were determined by polymerase chain reaction followed by digestion of the products with restriction endonuclease, PsyI.

Results: There is statistically significant associations with essential hypertension that identified for TT genotype of angiotensinogen M235T polymorphism (odds ratio at 95% CI, 3.1 (1.6-5.9), \( p = 0.0001 \)). Also, as regards gender, male subjects were at 2.7 times higher odds to develop essential hypertension.

Conclusions: This study shows that angiotensinogen (M235T) polymorphism is significantly associated with the essential hypertension. These results may provide a better understanding of the pathophysiology of essential hypertension and offer possibilities for identifying patients at risk. Larger association or linkage studies are needed for a more detailed risk assessment especially if applied in large section of patients with a diversity of cardiovascular risks related to hypertension. Such studies constitute the basic nucleus for pharmacogenetic customization therapy of hypertension and associated cardiovascular diseases.

Key Words: Angiotensinogen – Coronary artery disease – Essential hypertension – Gene polymorphism – Pharmacogenetic therapy.

Introduction

HYPERTENSION is a major public health problem all over the world. Essential hypertension is likely to be the consequence of an interaction between environmental and genetic factors. And genes contribute 20-40% of the pathogenesis of essential hypertension [1]. The renin-angiotensin system (RAS) contributes importantly to the regulation of vascular tone, cardiovascular remodeling, and electrolyte and volume homeostasis [2], and their role in blood pressure regulation has been well established [8]. Genes encoding components of RAS, including angiotensinogen, have been extensively investigated as genetic determinants of essential hypertension through genetic linkage studies, and by allelic association [4-6]. Fifteen molecular variants have been identified and only three have so far been reported to have a possible genetic association with hypertension [8]. The gene that encodes angiotensinogen is found on chromosome 1q42 to 43 where a tyrosine for cytosine substitution in the second exon results in the substitution of threonine for methionine at amino acid position 235 (M235T) in the translated protein. This missense mutation in exon 2 of the angiotensinogen (M235T) gene has been associated with elevated levels of angiotensinogen, with 235 TT homozygotes having between 10% and 20% more plasma angiotensinogen than 235 MM individuals [5,7]. Association (case-control) studies of the M235T polymorphism in essential hypertension have yielded conflicting results. Some found linkage or association in Nigerian [8], French [9], Han Chinese [10] and Malaysian populations [11], while others have not [12,13]. There is no study available in Indian context except one in which there was no gene - diseases association found. Our aim of this study is to confirm if there is any relationship
of angiotensinogen (M235T) gene polymorphism and essential hypertension in our Egyptian patients paving way for more larger studies in the future applying more correlations with more cardiovascular risks associated with hypertension.

**Material and Methods**

**Subjects:**

An informed consent was taken from all studied groups and the study was approved by the ethical committee of the Faculty. Patients were recruited from Internal Medicine Kasr El Aini Hospital (N=150, Age 28 - 61 years) with essential hypertension (systolic pressure >140mmHg and/or diastolic pressure >90 and secondary causes of hypertension ruled out). Patients were not on antihypertensive treatment, had no metabolic or endocrine disorder or any acute illness.

Another group which is the group of controls (n=50), the age and sex were matched from the similar population group which was enrolled in our study.

The study was carried out during the period of time from January 2011 till May 2012.

**Molecular biology techniques:**

The peripheral venous blood was drawn from the study subjects and DNA was extracted from lymphocytes using the QIAamp® DNA Blood Mini Kit by QIAGEN®.

**AGT M235T gene polymorphism:**

M235T variants were amplified by polymerase chain reaction to amplify part of the exon 2 gene with primers designed to insert a half restriction endonuclease site into the product. Primers used were: Sense-primer 5’ GAT GCG CAC AAG GTC CTG TC 3’ and antisense primer 5’ CAG GGT GCT GTC CAC ACT GGA CCC C 3’ designed to amplify 303 bp fragment of the angiotensinogen gene. The PCR products were digested by the PsyI (Fermentas life sciences) and the fragments produced were electrophoresed on 3% agarose gel. PsyI digested fragments were 279 bp and 24 bp. Homozygous allele MM, showed a 303-bp band on the gel, homozygous allele TT (showed only 279-bp band) and heterozygous MT (showed 303-bp band and a 279-bp band on the gel).

**Statistical analysis:**

All computations were carried out with STATA program, version 8. Chi-square goodness of fit was used to verify the agreement of observed genotype frequencies with those expected (Hardy-Weinberg equilibrium). Allele and genotype frequencies in patients and controls were compared by multivariate logistic regression analysis. Odds ratios (95% confidence intervals (CI)) were calculated as an index of the association of the angiotensinogen (M235T) genotypes (MM,MT,TT) with the disease. Statistical significance was defined as a p-value <0.05.

**Results**

Average age of the controls and patients was comparable. Male volunteers number was higher than female number in both the study subjects. There was no history of smoking in our groups. The mean values of lipid profile tests in patients were comparable in both the study subjects. Systolic and diastolic blood pressures in patients were significantly higher than controls (Table 1).

The frequency of MT and TT genotype in controls were statistically significant higher than the patients with essential hypertension. We found significant association of (MT+TT) with essential hypertension ($\chi^2=14.5, p=0.0001$, Odds ratio=3.1 (1.6-5.9) at 95% CI) (Table 2), Figs. (1-4). As regards the gender, the male subjects with MT+TT genotypes were at 3 times higher odds (Odds ratio at 95% CI=3 [1.3-6.8], Yates corrected chi=7.05, $p=0.005$) to develop essential hypertension (Table 3), Figs. (5-12).

**Table (1): Baseline characteristics of the study subjects.**

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Number (N)</td>
<td>150</td>
<td>50</td>
</tr>
<tr>
<td>2- Sex (M/F)</td>
<td>101/49</td>
<td>34/16</td>
</tr>
<tr>
<td>3- Age (years)</td>
<td>54.1±6.8</td>
<td>51.9±11.3</td>
</tr>
<tr>
<td>4- LDL cholesterol (mg/dl)</td>
<td>96.3±21.5</td>
<td>82.3±27.3</td>
</tr>
<tr>
<td>5- HDL cholesterol (mg/dl)</td>
<td>47.4±6.4</td>
<td>48.3±6.6</td>
</tr>
<tr>
<td>6- Total cholesterol (mg/dl)</td>
<td>167.1±30.3</td>
<td>129.9±20</td>
</tr>
<tr>
<td>7- Triglyceride (mg/dl)</td>
<td>119±45</td>
<td>108±44</td>
</tr>
<tr>
<td>8- Systolic blood pressure (SBP) mm Hg</td>
<td>145.9±13.0</td>
<td>110±3.3*</td>
</tr>
<tr>
<td>9- Diastolic blood pressure (DBP) mm Hg</td>
<td>94.6±8.7</td>
<td>75.6±2.8*</td>
</tr>
</tbody>
</table>

* $p$-value <0.01, significant.
Table (2): Genotype and allele frequencies of M235T variant of the angiotensinogen gene in the study subjects.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Genotypes*</th>
<th>Allele frequency**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MM (%)</td>
<td>MT (%)</td>
</tr>
<tr>
<td>Controls (n=50)</td>
<td>12 (24.0)</td>
<td>37 (74.0)</td>
</tr>
<tr>
<td>Patients (n=150)</td>
<td>13 (8.7)</td>
<td>128 (85.3)</td>
</tr>
</tbody>
</table>

*Odds Ratio at 95% CI 3.1 (1.6-5.9), $X^2=14.6, p=0.0001,$

**Odds Ratio at 95% CI 1.4 (1.07-1.91), $X^2=6.2, p=0.014,$

Adjusted for age and sex; Patients groups were compared with controls with chi-square ($X^2$) test.

Table (3): Distribution of genotype and allele frequencies of angiotensinogen M235T gene in the study subjects according to gender.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Groups</th>
<th>Genotypes*</th>
<th>Alleles*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MM</td>
<td>MT</td>
</tr>
<tr>
<td>Controls</td>
<td>Male</td>
<td>8</td>
<td>25</td>
</tr>
<tr>
<td>(n=50)</td>
<td>(n=34)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>(n=16)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>Male</td>
<td>8</td>
<td>88</td>
</tr>
<tr>
<td>(n=150)</td>
<td>(n=101)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>4</td>
<td>42</td>
</tr>
<tr>
<td>(n=49)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Adjusted for age and sex; Patients groups were compared with controls with chi-square ($X^2$) test.

Genotypes:
Male odds ratio=3 (1.3-6.8)
Female odds ratio=3.4 (1.2-10.3)

Alleles:
Male odds ratio=1.36 (0.94-1.9)
Female odds ratio=1.41 (0.87-2.31)
Fig. (3): Patients genotypes.

Fig. (4): Patients genotypes.

Fig. (5): Genotype frequency in control group males.

Fig. (6): Allele frequency in control group males.

Fig. (7): Genotype frequency in control group females.

Fig. (8): Allele frequency in control group females.

Fig. (9): Genotype frequency in patients group males.

Fig. (10): Allele frequency in patients group males.
Discussion

Several studies have reported association between essential hypertension and angiotensinogen (M235T) gene polymorphism. These associations were reported in the French [9], Han Chinese [10], and Malaysian populations [11], whereas others have found no association [12,13]. In German population, Ortlepp et al. [15] reported that the effect of angiotensinogen M235T gene polymorphism on blood pressure regulation is detectable very early in life far before the onset of arterial hypertension. In Japanese population [15,16], some studies found a positive association of TT genotype with essential hypertension. A couple of studies are available from India, in which reported sample size is comparatively less than our study as well as lacking any association of this gene polymorphism with essential hypertension [17]. Results from our study found that the distribution of M235T genotypes among both the study groups was in the order of MT>MM>TT, which was similar to French [9] and East Anglians [13] and quite different from the Asian populations reported i.e. Han Chinese and Japanese [10,12], however, in our study, the T allele frequency was 0.49, quite similar to what was reported in Malaysians, where the allele frequency was in the order of 0.44 [11]. In our study, the heterozygote genotype is predominately present in both the study subjects, however the percentage is more in patients as compared to controls. Co dominant pattern of inheritance was also not seen in both the study subjects. This is a very peculiar phenomena existing in our study. Population admixture could be the reason for this account. On applying multivariate logistic regression analysis to our data, we have found the significance difference in the distribution of genotypes between patients and controls ($\chi^2=14.49, p=0.00014$, Odds ratio=3.11 (1.64-5.96) at 95% CI), suggesting an association of T allele of the angiotensinogen (M235T) gene polymorphism with the essential hypertension. Further analysis on gender basis, has shown male subjects were at 2.9 times higher odds to develop essential hypertension, whereas this association was restricted to females with MT+TT genotypes only and not with alleles. This study provides the normal distribution of the genotypes and alleles in this polymorphism among Asian Indians from Northern region of India which can be used as the baseline data to elucidate the contribution of this polymorphism in the disease state.

One of the most promising studies for the application of pharmocogenetic customized therapies based upon the genetic polymorphism of angiotensinogen gene was carried out by Erwan et al in France in 2003. In this study a significant association between the M235T polymorphism of the AGT gene and carotid intima media thickness (IMT) was observed not only in a cross-sectional study but also during a longitudinal trial. In a multivariate analysis, carotid IMT was higher in previously untreated hypertensive patients homozygous for the T allele than in MM patients, and was reduced to a larger extent after treatment with angiotensin converting enzyme inhibitor (enalapril) in TT than in MM patients [18].

Recently, the results of nine studies that were carried out in China including 2281 subjects correlating the M 235T gene polymorphism & coronary artery disease (CAD), were published in October 2012. The results showed a significant association between M 235T gene polymorphism & CAD in the studied population. The estimates (OR) of CAD were calculated in a homozygote comparison, a heterozygote comparison, a dominant model & a recessive model & confirmed the above mentioned correlation [19].
**Conclusion:** Our study shows that the angiotensinogen (M235T) polymorphism is significantly associated with the essential hypertension and male subjects were at 3 times higher odds to develop essential hypertension. These results may provide a better understanding of the pathophysiology of essential hypertension and offer possibilities for identifying patients at risk. Larger association or linkage studies are needed for a more detailed risk assessment especially if applied in large section of patients with a diversity of cardiovascular risks related to hypertension. Such studies constitute the basic nucleus for pharmacogenetic customisation therapy of hypertension and associated cardiovascular diseases.

**References**