Increased Risk of Hypertension in Low aged-carriers with rs2596542 Risk-allele


1. Department of Cell and Molecular Biology and Microbiology, Faculty of Biological Science and Technology, University of Isfahan, Isfahan, Iran
2. Assistant Professor, Department of Cell and Molecular Biology and Microbiology, Faculty of Biological Science and Technology, University of Isfahan, Isfahan, Iran (Corresponding author: Email: javadirad@yahoo.com)

Received: 31 August, 2020 Accepted: 9 September, 2020

ARTICLE INFO

Article type: Original Article

Keywords: Hypertension rs2596542 PASA

Abstract

Background: Hypertension with its related disorders is one of the most common health problems among the Iranian population. Hypertension can be developed by chronic stress and a positive association between stress and rs2596542 has been confirmed.

Methods: A Total number of 112 hypertensive patients and 97 healthy individuals were involved in the study. Total blood genomic-DNA was extracted and PASA (PCR amplification of specific alleles) method was used to amplify MICA-rs2596542 polymorphic site. Different genotypes were visualized. The normality of the data was assessed and the binary logistic regression was used for OR and 95%CI calculations.

Results: A-risk allele of rs2596542 increased the risk of hypertension development significantly (OR=1.734, p=0.006). Females were significantly more potent to hypertension development than males (OR=2.015, p=0.013). Risk-allele homozygotes (AA) showed a higher risk of hypertension development than GG (OR=2.132, p=0.020) and AG individuals (OR=3.206, p=0.006). Age adjustments at 70 years old, further increased the risk of hypertension development in GG (OR=3.772, p=0.011) and AG (OR=6.531, p=0.009) individuals.

Conclusion: A-risk allele of rs2596542 could increase the risk of hypertension up to 3.2 folds and this risk could be upraised after sex and age adjustments.

Introduction

Hypertension (HT) is a condition that can strain the heart because of the excessive force of blood flow throughout the blood vessels. According to the 2017 report of the global burden of hypertension, the number of individuals with HT and its associated deaths is on the rise (1). A systematic review from 1980-2012 was declared HT as one of the most common health problems among the Iranian population (2). It has been clarified that ethnicity could be a factor with high impacts on the occurrence of HT and its related disorders such as heart attack, stroke, kidney problems, and finally death (3, 4). Despite the numerous loci that have been identified and the linkage analysis that has been performed, there is a lot of uncertainty about the genetics of HT (5-9). Nevertheless, genetic polymorphic sites are one of the significances that could be differentially associated with HT.

MHC class I polypeptide-related sequence A (MICA, 6p21.33) gene is transcribed especially in the epitheliums of the blood vessels (10, 11). As a self-antigen and a responder to stress, MICA expression was also reported...
in the epithelium of intestinal gamma-delta T cells and in
tumor cells (12, 13). Our previous study also showed that
MICA polymorphisms could increase the risk of breast
cancer development in the Iranian population (14).

It has been revealed that chronic psychological stress
can increase blood pressure (12, 15). Genome-wide
association studies have showed a strong linkage of
rs2596542 polymorphic site (in the 5' flanking region of
MICA gene, MAF: 0.42) and chronic psychological
stresses such as hepatocellular carcinoma (16-19).
Therefore, in the present study, we tried to explore the
possible role of MICA-rs2596542 in the Iranian
population tackling HT.

Materials and Methods

Subjects

A case-control observational study was performed in
the Isfahan province of Iran. A Total number of 112 HT
patients and 97 healthy individuals were involved in the
study. HT was confirmed at least after three times reading
of blood pressure by the same expert physician. Written
informed consent forms were obtained before total blood
collection.

MICA genotyping

Genomic DNA extraction was performed using the
miller salting-out method (20). Polymerase chain
reaction (PCR) amplification of specific alleles (PASA)
was used for MICA rs2596542 polymorphic site
amplification. PASA primers were as follows:
GGTTATCTGCCTGCCATAG as the common reverse
primer, TCCCAAAGAACAGCTACAAG for detecting
ancestral-G allele and TCCCAAAGAACAGCTACAAAA
for amplifying mutated-A allele. PCR amplification
condition was as follow: 1) one step of initial
denaturation at 95°C for 5 min, 2) 35 cycles of
denaturation at 95°C for 1 min, annealing at 60°C for 1
min and extension at 72°C for 1 min, 3) final extension at
72°C for 10 min. Different genotypes were visualized on
1% agarose gel electrophoresis.

Statistical analysis

Genotype distributions and frequencies were
calculated using IBM SPSS statistics v21.0. The normality
of the data was assessed using the Kolmogorov-Smirnov
test. Binary logistic regression was used for odd ratio
(OR) and 95% confidence interval (95%CI) calculation.
p-value less than 0.05 was considered as significant.

Results

Population information

The total population consisted of 112 (53.6%)
patients and 97 (46.4%) control individuals. The mean
age of the patients was 74.38±1.17 years, and the mean
age of our control individuals was 71.68±1.40 years.
Among 209 individuals included in this study, 97 (46.4%)
were males and 112 (53.6%) were females with the mean
age of 71.46±1.37 and 74.57±1.18 respectively (Table 1).
Hypertension and rs2596542

Monsef, et al

Table 1. Population information of the studied individuals

<table>
<thead>
<tr>
<th>Gender</th>
<th>Frequency No (%)</th>
<th>Mean age ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>97 (46.4%)</td>
<td>71.46±1.37</td>
</tr>
<tr>
<td>Female</td>
<td>112 (53.6%)</td>
<td>74.57±1.18</td>
</tr>
<tr>
<td>Total</td>
<td>209 (100%)</td>
<td>73.13±0.90</td>
</tr>
<tr>
<td>Patients</td>
<td>112 (53.6%)</td>
<td>74.38±1.17</td>
</tr>
<tr>
<td>Control</td>
<td>97 (46.4%)</td>
<td>71.68±1.40</td>
</tr>
</tbody>
</table>

SEM: standard error of mean

MICA rs2596542 allele amplification and genotyping

Two separate amplifications were performed separately in two tubes and genotypes were visualized (Figure 1). Samples 1 and 4 were heterozygote as both G allele and A allele were amplified simultaneously. Sample 2 was GG homozygous and sample 3 was AA homozygous as the former was shown amplification for only G allele and the later for A allele only.

![Figure 1. PASA driven genotypes](image)

Genotype distribution of HT patients and control individuals

Three genotypes were observed in our total population designated as GG, AA, and AG (Table 2). The frequencies of the genotypes were 49.3% for GG, 32.5% for AA and 18.2% for AG. Homozygous AA genotype was the predominant genotype in patients with 67.6% frequency. In control individuals, AG genotype with 60.5% frequency was the most frequent genotype followed by GG genotype with 50.5% frequency. The
lowest frequent genotype in control individuals was AA genotype with 32.4% frequency. GG genotype was selected as the reference genotype because it showed a similar frequency between HT patients and control individuals.

Table 2. Genotype distribution of the studied individuals

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Frequency No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total population</strong></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>68 (32.5)</td>
</tr>
<tr>
<td>AG</td>
<td>38 (18.2)</td>
</tr>
<tr>
<td>GG</td>
<td>103 (49.3)</td>
</tr>
<tr>
<td>total</td>
<td>209 (100)</td>
</tr>
<tr>
<td><strong>Patients</strong></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>46 (67.6)</td>
</tr>
<tr>
<td>AG</td>
<td>15 (39.5)</td>
</tr>
<tr>
<td>GG</td>
<td>51 (49.5)</td>
</tr>
<tr>
<td>total</td>
<td>112 (100)</td>
</tr>
<tr>
<td><strong>Control individuals</strong></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>22 (32.4)</td>
</tr>
<tr>
<td>AG</td>
<td>23 (60.5)</td>
</tr>
<tr>
<td>GG</td>
<td>52 (50.5)</td>
</tr>
<tr>
<td>total</td>
<td>97 (100)</td>
</tr>
</tbody>
</table>

HT development, alleles, genotypes, sex and age adjustments

The association of HT development with alleles, genotypes, age and sex of the individuals were presented in Table 3. A-allele of MICA-rs2596542 increased the risk of HT development 1.7 times more than G-allele and it was significant (OR=1.7, p=0.006). The risk of HT development was 2.132 times higher (p=0.020) when AA genotype was compared with the GG reference genotype. Homozygous AA genotype also increased the risk of HT more than 3 times (OR =3.206, p=0.006), when it was compared to AG heterozygotes.

Table 3. The association of HT risk with sex and genotypes

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Unadjusted Odd ratio (OR)</th>
<th>Adjusted OR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (p-value) 95% CI</td>
<td>OR (p-value) 95% CI</td>
</tr>
<tr>
<td>Female\male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A \ G</td>
<td>2.015 (0.013) 1.152-3.502</td>
<td></td>
</tr>
<tr>
<td>AA \ GG</td>
<td>2.132 (0.020) 1.126-4.036 3.772 (0.011)’ 1.353-10.513</td>
<td></td>
</tr>
<tr>
<td>AA \ AG</td>
<td>2.783 (0.034)* 1.079-7.176</td>
<td></td>
</tr>
<tr>
<td>AA \ AG</td>
<td>3.206 (0.006) 1.404-7.319 6.531 (0.009)’ 1.592-26.788</td>
<td></td>
</tr>
<tr>
<td>AA \ AG</td>
<td>4.030 (0.024)* 1.201-13.526</td>
<td></td>
</tr>
</tbody>
</table>

*Age cut-off of 70 was used for HT risk assessment

†Female were compared with men for HT risk assessment
The risk of HT development in females was 2 folds greater than that in males and the difference was significant (OR=2.015, p=0.013). Comparison of AA patients respectively with GG and AG ones, showed OR=3.772 (p=0.011) and OR=6.531 (p=0.009) after age adjustment. Sex adjusted-ORs for AA patients were 2.783 (p=0.034) and 4.030 (p=0.024) when they were compared with GG and AG ones respectively.

Discussion

Hypertension as a traditional cause of cardiovascular diseases has been refocused because of its new embedded roles in dementia, physical disability, and falls/fractures (21-23). Common biological aging as the foremost cause of HT could induce the condition by several mechanisms such as inflammation, oxidative stress induction, and endothelial dysfunction (24). However the influence of genetics on this classical complex trait is just explored via genome-wide association studies, but a lot of uncertainties remain to be clarified (25).

Sixteen genetic loci on chromosomes 1, 2, 3, 4, 5, 7, 12, 15, 17 and 20, have impacts on the development of HT. Striatin, adducin-1, beta 2 adrenergic receptor, cytochrome P450 family 11 subfamily B member 2 (CYP11B2), CYP17A1, Endothelin-1, Estrogen receptor beta, Lysine-specific demethylase 1, Serum- and glucocorticoid-inducible kinase 1, Bradykinin receptor B2, Electrogenic sodium bicarbonate cotransporter 4, Plasma membrane calcium-transporting ATPase 1, Serine/threonine 39, WNK lysine-deficient protein kinase 1, Angiotensin receptor type 1, Fibroblast growth factor 5, G protein beta 3 subunit, genes polymorphisms and also another 41 genes with insufficient evidences were also reported to be associated with HT (26). The most effective genes with strong correlation and with supportive cohort evidences are caveolin-1 (CAV1), angiotensinogen (AGT), renin enzyme (REN gene), angiotensin I (ANG I) and angiotensin II (ANG II). These genes are the members of renin-angiotensin system that directly control the vascular resistance and regulate blood pressure (27). CAV1 gene variants (rs926198 and rs3807989) were in linkage disequilibrium with HT and the prevalence of the disease in Japanese men was associated with CAV1-rs2255991 and rs1799998 (28, 29). Null mice experiments candidated CAV1 gene as the regulators of eNOS activity, the key developer of HT (30, 31). AGT gene variants were also in positive association with HT and an Iranian population study presented a relationship between an AGT-TT genotype and hypertension (32). AGT gene variant (rs699) was associated with HT development in Caucasian-Brazilians (33). Different AGT gene haplotypes and polymorphisms were shown to be associated with HT in Indians, but not with Hellenic population (34, 35). Finally, AGT-rs5051 was reported to act synergistically with β1-adrenergic receptor blocker and angiotensin-converting enzyme inhibitors, in order to fight HT (36, 37). Renin enzyme converts AGT to ANG I and ANG II and all these three related genes were considered with the risk of HT in women (38, 39). REN- rs5707 and two haplotypes were correlated with HT in women aged from 40-70 years old (38). Therefore it seems that gene polymorphisms behave in a sex dependent manner to influence blood pressure.
New genes with impacts on the development of HT could be beneficial to uncover the molecular dark side of blood pressure. MICA gene polymorphisms that have been extensively studied and mutated “rs2596542-A” allele, was introduced as a risk factor of hepatocellular carcinoma (16, 40, 41). Interestingly in the present study, we also showed MICA-A-allele as the risk allele of HT development. Accordingly, patients with AA genotypes showed an elevated risk of HT development. In accordance to the previous studies, we showed that being female could also increase the risk of HT development. According to our knowledge, it is the first time that females’ carriers of two “A” risk alleles of the MICA gene were shown to be at higher risk of HT development. Heterozygote advantage also observed in our studied population because AG heterozygotes showed higher OR than AA ones, but it is not clear why over-dominance is seen and why being AG heterozygote, could even increase the risk of HT. The highest risk of HT development achieved when age adjustment was considered; as being younger than 70 years old could dramatically upraise the risk of HT development up to 6.5 folds significantly.

MICA-A-allele is a membrane-bound anti-tumor ligand for natural killer cell receptors (NKG2D), while rs2596542-G allele produces a soluble form of MICA (42, 43). Owing to the vicinity of MICA locus to HLA-B gene, and because the HLA-B gene is linked with numerous autoimmune diseases, a lot of efforts have been done to discover the role of MICA in the development of autoimmune diseases (44, 45). It is postulated that MICA gene purposed its effects via T cells interaction with NKG2D (44). Moreover, a comprehensive study of patients carrying MICA-A-allele opened the eyes to the role of TGF-β in liver fibrosis (46). Consequently it seems that the MICA-A-allele is not only able to lower the level of its soluble form in serum, but also is able to reduce the mRNA of its membranous bound isoform. Therefore MICA-A-allele is a negative regulator of itself, meanwhile it could elevate TGF-β levels in cells and TGF-β instead, could decrease the expression of MICA-mRNA (47, 48). We proposed here that A-risk allele might elevate the cell stress because of i) the elevation of TGF-β mRNA that in turn diminishes cell death and ii) the loss of its protein content and therefore the loss of an stress response product in cells (41, 49). Elevation of cell stress alongside immune suppression, because of the lack of MICA/NKG2D interaction, could lead to the development of HT (50).

In conclusion, MICA-rs2596542 is composed of the ancestral “G” allele and the mutated “A” allele with an influence on HT development. HT patients were compared with control individuals and it was cleared that AA genotype significantly could increase the risk of HT more than 4 times in women, and even more elevated risk of HT could be achieved when age cut-off 70 was considered.

Acknowledgments

This study was performed at University of Isfahan and the authors sincerely thank the volunteers for their participation.
Competing Interests

The authors declared no competing interests.

References


14. Vallian S, Rad MJ, Tavallaei M, Tavassoli M. Correlation of major histocompatibility complex class I related A (MICA) polymorphism with the risk

Author statement

Each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in Journal of Kerman University of Medical Sciences.


