ATP2B1 rs2681472 and STK39 rs35929607 polymorphisms and risk of Hypertension in Iranian Population

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Abstract

Background: ATP2B1 and STK39 have been introduced as essential hypertension candidate genes. The association of these genes’ variations have not been studied in Iranian population yet. Here we aimed to investigate the association of ATP2B1 rs2681472 and STK39 rs35929607 polymorphisms with the risk of hypertension in an Iranian population.

Methods: We included 400 individuals in our case-control study: 200 cases with essential hypertension and 200 healthy sex and age matched controls. All subjects were genotyped for rs2681472 and rs35929607 using a PCR-RFLP method. Genotype and allele frequencies were compared between the two groups using chi-squared test. The association was further assessed under log-additive, dominant and recessive genetic models.

Results: There was no association between rs2681472 and rs35929607 polymorphisms and risk of essential hypertension in our population (p>0.05). There was also no association between the studied polymorphisms and hypertension under different genetic models.

Conclusion: Our study indicated that rs2681472 of ATP2B1 and rs35929607 of STK39 may not have a significant effect on the risk of essential hypertension in Iranian population. More studies are still needed to validate our results.

Keywords: Essential hypertension, ATP2B1, STK39, Polymorphism, Association study

Introduction

Blood pressure (BP) is a quantitative and highly variable trait in humans (1). There is a strong positive association between BP and cardiovascular disorders (CVD) such as stroke, heart failure and myocardial infarction (2). High BP or hypertension is a predominant risk factor for CVD which is preventable. It is estimated that about 31% of world’s adults had hypertension in 2010, although this is a mean and hypertension prevalence can be very variable among different countries and races (3). The age-standardized prevalence of hypertension have been reported to be about 41.8% in a large cohort of Iranian population (4). Essential or primary hypertension is defined as high BP with the absence of secondary causes such as renal failure, pheochromocytoma, aldosteronism, or other causes which accounts for 95% of all cases (5). Both environmental and genetic factors are involved in the development of essential hypertension. The heritability of BP is estimated to be between 30-60% which shows a significant contribution of genetics to this trait (6), and because of that many researches have been dedicated to find genetic components of hypertension.

ATPase plasma membrane Ca2+ transporting 1 (ATP2B1) plays a critical role in intracellular calcium homeostasis (7). This gene was shown to be related to sys-

What is “already known” in this topic:

There is evidence that ATP2B1 and STK39 polymorphism are associated with the risk of essential hypertension. The majority of evidences are from European and East Asian populations and the results are not all consistent. There was no data available considering the relation of these genes with essential hypertension in Iranian population.

What this article adds:

Our data indicated that variants in ATP2B1 and STK39 genes probably do not have a significant effect on the risk of essential hypertension in Iranian population.

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Table 1. Demographic and laboratory data of case and control groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Case (N=200)</th>
<th>Control (N=200)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>53.72 ± 7.94</td>
<td>56.59 ± 12.98</td>
<td>0.57</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>24.67 ± 4.17</td>
<td>24.51 ± 3.66</td>
<td>0.68</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>112/88</td>
<td>119/81</td>
<td>0.41</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>22 (11)</td>
<td>14 (7)</td>
<td>0.16</td>
</tr>
<tr>
<td>DM (%)</td>
<td>15 (7)</td>
<td>8 (4)</td>
<td>0.13</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>140.59 ± 33.42</td>
<td>113.22 ± 9.96</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>84.86 ± 11.89</td>
<td>72.12 ± 8.55</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL (g/dL)</td>
<td>12.81 ± 1.78</td>
<td>12.49 ± 2.07</td>
<td>0.10</td>
</tr>
<tr>
<td>Cr (mg/dL)</td>
<td>1.14 ± 1.38</td>
<td>1.15 ± 0.32</td>
<td>0.69</td>
</tr>
<tr>
<td>FBS (mg/dL)</td>
<td>125.15 ± 62.68</td>
<td>120.52 ± 58.41</td>
<td>0.44</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>128.50 ± 80.60</td>
<td>118.66 ± 25.84</td>
<td>0.10</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>106.57 ± 54.12</td>
<td>100.53 ± 47.23</td>
<td>0.19</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>43.95 ± 8.43</td>
<td>45.12 ± 5.87</td>
<td>0.10</td>
</tr>
</tbody>
</table>


Table 2. The Primer sequences and digestion conditions for studied polymorphisms

<table>
<thead>
<tr>
<th>Polymorphisms</th>
<th>Primer sequences (5'-3')</th>
<th>PCR conditions (°C/s)</th>
<th>Restriction enzyme digestion</th>
<th>Alleles</th>
<th>DNA fragment size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2681472</td>
<td>F: TCTGAGGATGAGGCATTGGA R: CCGTAAAGGCAGGAGATCA</td>
<td>95/35</td>
<td>58/30</td>
<td>72/30</td>
<td>PvuII at 37°C for 3 hours</td>
</tr>
<tr>
<td>rs35929607</td>
<td>F: AACACTCTCAAGAAGAGATCCCGAGTG R: CCTCCAGGTCGTTTTGCAAAATAA</td>
<td>95/35</td>
<td>61/40</td>
<td>72/45</td>
<td>MspI at 37°C for 3 hours</td>
</tr>
</tbody>
</table>

DNA extraction and genotyping

About 3ml of peripheral blood was taken from every participant and DNA was extracted using a standard salting out method. PCR-RFLP method was used for genotyping of rs2681472 and rs3592607. The PCRs were performed using 20 µl of reaction mixture containing 0.4 µM of each primer, 200 mM of dNTPs, 2 mM MgCl₂ and 1 unit of Taq DNA polymerase (Amplicon, Denmark). The products were digested using appropriate restriction enzymes in 37 °C for 3 hours. The details of PCR programs and digestions are described in Table 2. The digested PCR products were separated using 3% agarose gel electrophoresis staining with Power load (Kawsar Biotech, Iran).

Statistical Analysis

Quantitative variables are presented as mean ± standard deviation, and difference between groups was determined using student’s t-test. The Hardy-Weinberg equilibrium was estimated in the population (whole study population and also control group) using chi-squared test. Chi-square test was also used to compare the genotype and allele frequencies between the case and the control groups. Addi-
tionally, the association of genotypes with essential hypertension was assessed under log-additive, dominant and recessive genetic models using SNPassoc package of R program (14). All statistical analysis was carried out using R version 3.2.3.

**Results**

Four hundred subjects, 200 with hypertension (mean age ± standard deviation: 57.22±9.34 years) and 200 healthy sex and age matched controls (mean age ± standard deviation: 56.59±12.98 years) were included in this study. Except for SBP and DBP (which was expected) group analysis revealed no significant difference in demographic and laboratory data of the case and the control groups (Table 1).

The studied population was in Hardy-Weinberg equilibrium for both SNPs (p>0.05). The distribution of genotype and allele frequencies were not different between the case and the control groups for any of rs2681472 and rs35929607 polymorphisms (Table 3). There was also no association between essential hypertension and studied polymorphisms when analyzing under different genetic models (Table 4).

**Discussion**

In the current study, we demonstrated that there is no association between essential hypertension and ATP2B1 rs2681472 and STK39 rs35929607 in a cohort of Iranian population. The genotypes were evenly distributed among patients with hypertension and control group and still allele frequencies were not different.

**ATP2B1** have been reported as a candidate gene for blood pressure in several GWAS studies in people of European, and east Asian origin (Japanese, Chinese and Koreans) (8, 15-17). The gene is involved in calcium homeostasis which is critical for normal vascular contraction (18), the function that could be related to BP regulation. **ATP2B1** variants have been widely studied in east Asian populations through replication studies, and the results are in favor of **ATP2B1** involvement in hypertension in these populations (19). Despite this, there are only a few studies reporting the association of **ATP2B1** variations and risk of hypertension in other populations. Nonetheless, **ATP2B1** was not associated with blood pressure in an African American cohort (20), which is consistent with our study findings. Based on our findings we assume that **ATP2B1** is not a significant determinant in the pathophysiology of hypertension in Iranian population or there may be a protective gene functioning in this population.

Since the introduction of STK39 as a candidate gene for essential hypertension in a GWAS, there has been interest toward (8) delineation of its function in the pathophysiology of hypertension. Results of the replication studies were also mixed and some support the association of **STK39** variations with hypertension, like those being conducted in Belgian (rs3754777) (21), Chinese (rs6433027 and rs3754777) (11) and Swedish populations (rs35929607) (22) while others failed to prove any association, such as studies in Chinese (rs6749447, rs3754777, rs35929607, rs4667569, rs4977950) (23), Korean (rs3754777 and rs6749447) (24) and British Caucasian populations(rs6749447, rs3754777, rs35929607) (25). Our study is in agreement with the latter studies and supports a lack of association in Iranian population. Along with the studies with conflicting results in Chinese population (11, 23), two meta-analyses about **STK39** and risk of hypertension are also demonstrative of inconclusive findings (13, 26).

It seems that despite many studies that have investigated the association of **ATP2B1** and **STK39** gene variations with essential hypertension, the results are still inconsistent. It could be concluded that these variations only have significant effect in specific populations and ethnic groups which can be explained by the different genetic backgrounds in various ethnicities. Furthermore, environmental exposures and life styles as hypertension risk factors have different implications and significance among various populations. Similarly, the observed discrepancy regarding this polymorphisms and hypertension in different populations can be attributed to gene-environment interactions which itself can be secondary to the modifier variations (26).

### Table 3. Genotype and allele frequencies in case and control groups

<table>
<thead>
<tr>
<th>Gene (SNP)</th>
<th>Subjects (n)</th>
<th>Genotype frequencies (%)</th>
<th>p</th>
<th>Allele frequencies (%)</th>
<th>p</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ATP2B1</strong> (rs2681472)</td>
<td>Case (200)</td>
<td>86 (43)</td>
<td>91 (45.5)</td>
<td>23 (11.5)</td>
<td>0.99</td>
<td>263 (66)</td>
<td>137 (34)</td>
</tr>
<tr>
<td></td>
<td>Control (200)</td>
<td>86 (43)</td>
<td>92 (46)</td>
<td>22 (11)</td>
<td></td>
<td>264 (66)</td>
<td>136 (34)</td>
</tr>
<tr>
<td></td>
<td>Total (400)</td>
<td>172 (43)</td>
<td>183 (45.7)</td>
<td>45 (11.3)</td>
<td></td>
<td>527 (65.8)</td>
<td>273 (34.2)</td>
</tr>
<tr>
<td><strong>STK39</strong> (rs35929607)</td>
<td>Case (200)</td>
<td>118 (59)</td>
<td>70 (35)</td>
<td>22 (11)</td>
<td>0.56</td>
<td>306 (76.5)</td>
<td>94 (23.5)</td>
</tr>
<tr>
<td></td>
<td>Control (200)</td>
<td>112 (56)</td>
<td>79 (39.5)</td>
<td>9 (4.5)</td>
<td></td>
<td>303 (76)</td>
<td>97 (24)</td>
</tr>
<tr>
<td></td>
<td>Total (400)</td>
<td>230 (58)</td>
<td>149 (37)</td>
<td>21 (5)</td>
<td></td>
<td>609 (76)</td>
<td>191 (24)</td>
</tr>
</tbody>
</table>

### Table 4. Analysis of Genotype distribution between the case and the control groups

<table>
<thead>
<tr>
<th>Gene (SNP)</th>
<th></th>
<th>log-Additive (TT=0, TC=1, CC=2)</th>
<th>Recessive (TT and TC vs CC)</th>
<th>Dominant (TC and CC vs TT)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ATP2B1</strong> (rs2681472)</td>
<td>p OR</td>
<td>0.98</td>
<td>0.87</td>
<td>1</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.05</td>
<td>1.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.54-2.02</td>
<td>0.57-1.96</td>
<td>0.67-1.49</td>
<td></td>
</tr>
<tr>
<td><strong>STK39</strong> (rs35929607)</td>
<td>p OR</td>
<td>0.96</td>
<td>1.35</td>
<td>0.88</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.69-1.33</td>
<td>0.59-1.32</td>
<td>0.59-1.32</td>
<td></td>
</tr>
</tbody>
</table>
effects like what has been proposed as the mechanism for the attenuation or blunting of the genetic factors. For instance, it has been demonstrated that smoking is a significant risk factor for the association of rs3754777 of STK39 with hypertension (13). Therefore, investigations in subgroups and taking multiple exposures into account as well would yield in a better understanding to the role of these polymorphisms in the susceptibility to hypertension.

Being a multifactorial disease with a handful of environmental, genetic and behavioral contributors, hypertension must be viewed as a complex outcome. Excluding secondary hypertension, the etiology of essential hypertension cannot be explained by a single determinant like a mutation. There are plenty of modifier and protective traits as well, which mostly are related to life style and genetic factors (27, 28). Polymorphisms that are proposed as candidates for hypertension development in GWASs, then must be studied separately in different populations with special considerations about the confounding effects of diet, life style and behavioral factors. A longitudinal cohort study of non-hypertensive population is the best fit for such association studies and can clearly account for major confounders. This study must be interpreted with its limitations including restricted number of cases, possible confounding factors and limited number of studied SNPs. A larger cohort of seemingly healthy individuals with long enough follow ups to get the hypertension as an outcome, will properly address the causality of these polymorphisms in hypertension.

Conclusion
Our study indicates that rs2681472 and rs35929607 and probably ATP2B1 and STK39 genes do not have a significant effect on susceptibility to hypertension in Iranian population. Functional in vitro studies may help to clarify the exact effect of these variations in essential hypertension. More studies are still required to validate our study results.

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Conflict of Interests
The authors declare that they have no competing interests.

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