Study of Association between PGC-1α Gly482Ser Polymorphism and Hypertension

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ABSTRACT: Blood pressure is one of the main factors in global mortality and was diagnosed according to the WHO criteria. If high blood pressure was not treated, leads to death in most cases. Genetics play a major role blood pressure. For example, 70 percent of high blood pressure in a certain family generally has its roots in genetics rather than environmental issues. Clinical studies have found that insulin resistance and hyperinsulinemia in hypertension are important co-factors. Thus, genetic factors influencing insulin resistance and hyperinsulinemia a genetic basis are for prone to high blood pressure. The gene coding for PGC-1α in tissues with high metabolic activity, such as heart, liver and kidneys are produced and Interaction with estrogen and glucocorticoid receptors or detoxification of reactive oxygen leads to the regulation of blood pressure. PGC-1α contains of several polymorphisms: including IVS42 11T>C, Thr394Thra and Gly482Ser. This study aimed to investigate the frequency PGC-1α Gly482Ser polymorphism in blood pressure in both patients and control group. In this study, Triglycerides, blood sugar and cholesterol and measured their body mass index was calculated. PCR-RFLP for Gly482Ser polymorphism analysis was used. Genotype frequencies of GG, GA and AA, respectively, 32%, 52% and 16% in hypertensive patients, 32%, 48% and 20% in the control group. Allele frequency of G and A, respectively, were 58% and 42% of hypertensive patients and 56% and 44% in the control group. In this study, was not found Difference between the frequencies and this result was that the gene PGC-1α Gly482Ser polymorphism is not associated with blood pressure.

Keywords: polymorphism, PGC-1α gene, Gly482Ser, hypertension

INTRODUCTION

Because of the high prevalence of hypertension among individuals and genetic influences on the development of numerous studies and researches on the relationship of genes with this disease has been made. There are multiple strands of evidence showing that genetic factors contribute to blood pressure and hypertension. Firstly, the normal distribution of blood pressure in the general population indicates the presence of multiple environmental and genetic factors and thus a polygenic aetiology. Secondly, rare monogenic forms of hypertension associated with major defects in renal salt handling prove that gene mutations can cause hypertension leading to a hypothesis that minor variations in these genes may contribute to the common essential hypertension (Hastie et al., 2010). Using genome scans areas where of chromosomes that are involved in blood pressure regulation have been identified. One of these loci is on chromosome 4p15, where Human PGC-1α is located on and encoded by the gene PPARGC1A (Allayee et al., 2001). PGC-1α is a co-activator of numerous transcription factors such as PPAR, ER, GR, and nuclear respiratory factor (NRF)(Anderson et al., 2005). PGC-1α gene consists of 13 exons has consisted of 6261 bp. a total of 11 polymorphic loci in this gene have been identified. PPARGC1A gene is a significant task in Lipid metabolism, glucose and energy balance and in humans are able to coordinate metabolic processes in the liver and adipose tissue and muscle (kumar et al., 2012). Most significant of polymorphisms associated with PGC-1α gene can be noted Gly482Ser, Thr528Thr, Thr612Met, Asp457Asp and Ser74Leu (Ek et al., 2001). The most common SNP G1444A (rs8192678) and C1835T (rs3736265) have been in exon 8 and 9, and Causing change the amino acid Gly482Ser and Thr612Met (Nitz et al., 2007). However, studies have been conducted on the association of PGC-1α Gly482Ser polymorphism and hypertension in Japanese, Caucasians in France, Chinese, Austrians, Danes, Argentine and Mongolian (X.L. Su et al., 2011).
In this study, we hypothesized that PGC-1α gene Gly482Ser polymorphism as a genetic factor is involved in the increased risk of high blood pressure.

MATERIAL AND METHODS

Subjects
All subjects gave informed consent. Fifty hypertension patients (25 males and 25 females) with 45- to 55-year-old were enrolled. Blood pressure was measured 3 times by sphygmomanometer, taking the average. Hypertension was defined as: (i) systolic blood pressure greater than 140 mm Hg and/or diastolic blood pressure greater than 90 mm Hg; and (ii) blood pressure below these values in the presence of antihypertensive medication and history of hypertension (Churfa et al., 2004). Subjects were excluded if they had one of the following: secondary hypertension, diabetes mellitus, or severe liver, kidney and thyroid dysfunction. Fifty control subjects (25 males and 25 females) with 45- to 55-year-old had normal blood pressure and no history of hypertension (SBP <140 mmHg and/or DBP <90 mmHg). Body mass index (BMI) was calculated by the measurements of weight and height. Triglycerides (TG), cholesterol (CHO), fasting plasma glucose (FPG) were measured according to standardized methods.

DNA Isolation and Genotyping
Genomic DNA for analyses was isolated from blood leukocytes by the standard salt precipitation method. PCR-RFLP was used to analyze Gly482Ser polymorphisms. Primers to detect the Gly482Ser variant were 5’-TGAGAGAGACTTTGGAGGCA-3’, and 5’-GGAATATGGTGATCGGGACC-3’. PCR was performed in a volume of 25 μL including 100 ng DNA, 7 μL Taqman PCR master mix, 2 μL primers, and 11 μL deionized water. PCR was carried out with the following program: initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing and extension at 54 °C for 30 s, and extension at 72 °C for 45 s with a final extension at 72 °C for 10 min. PCR products were digested overnight at 37 °C with Hap II (TaKaRa Biotechnology) for this polymorphism followed by detection on 1% ethidium bromide-stained agarose gels.

Statistical Analysis
Data were expressed as mean±standard deviation. Basic continuous characteristics between case and control subjects were analyzed by Student’s t-test. The chi-square test was used to compare genotype and allele frequencies between groups and to determine whether individual variants were in Hardy-Weinberg equilibrium. Logistic regression was used to investigate the risk factor of hypertension including age, body mass index (BMI), Triglycerides (TG), cholesterol (CHO), fasting plasma glucose (FPG) and PGC-1α Gly482Ser polymorphism, using SPSS 13.0 software. A p-value less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION
The clinical characteristics of hypertensive patients and healthy controls are shown in Table 1. Average values for systolic and diastolic blood pressure, BMI, Triglycerides and cholesterol except for age and fasting plasma glucose were higher in hypertensive than in normotensive subjects. The DNA fragment of the PGC-1α gene was 452 bp after PCR amplification. Homozygous wild type (AA) without a restriction site only had a fragment of 452 bp. Homozygotic variants (GG) containing a restriction site in each DNA chain yielded two fragments of 310 and 142 bp after digestion with Hap II. Heterozygote (GA) containing restriction sites in one of the DNA chains yielded 3 fragments of 452, 310 and 142 bp.

Genotypic distributions at this polymorphic site were in agreement with Hardy-Weinberg equilibrium in all subjects. The PGC-1α gene GG, GA and AA genotype distributions were 32, 52, and 16% in patients and 32, 48 and 20% in healthy controls, respectively. The G allele frequency was 58 and 56% in patients and healthy controls.
and the A allele frequency was 42 and 44%, respectively (Table 2). Distributions of genotypic and allelic frequencies of this polymorphism in the patients were not statistically different from this in the control group. Logistic regression analysis showed that, in the additive model, PGC-1α Gly482Ser polymorphism was not associated with hypertension (OR 0.985, CI 0.832-1.167, p value 0.863). The findings of logistic regression analysis indicated that age, MBI, TG, CHO and FPG were risk factors of hypertension (Table 3).

### Table 2. Distribution of PGC-1α Gly482Ser genotype and allele frequency in hypertensive patients and healthy controls.

<table>
<thead>
<tr>
<th>Group</th>
<th>Case</th>
<th>Genotype</th>
<th>Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td>GA</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>50</td>
<td>16(32)</td>
<td>26(52)</td>
</tr>
<tr>
<td>Control</td>
<td>50</td>
<td>16(32)</td>
<td>24(48)</td>
</tr>
<tr>
<td>P value</td>
<td>0.860</td>
<td>0.775</td>
<td></td>
</tr>
</tbody>
</table>

Data are reported as number with percent in parentheses.

### Table 3. Logistic regression analysis of parameters associated with hypertension.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>1.022</td>
<td>1.012-1.032</td>
<td>0.000</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>1.098</td>
<td>1.097-1.106</td>
<td>0.000</td>
</tr>
<tr>
<td>FPG (mM)</td>
<td>1.005</td>
<td>1.002-1.009</td>
<td>0.003</td>
</tr>
<tr>
<td>CHO (mM)</td>
<td>1.005</td>
<td>1.004-1.005</td>
<td>0.000</td>
</tr>
<tr>
<td>TG (mM)</td>
<td>1.019</td>
<td>1.018-1.020</td>
<td>0.000</td>
</tr>
<tr>
<td>PGC-1α Gly482Ser</td>
<td>0.985</td>
<td>0.832-1.167</td>
<td>0.863</td>
</tr>
</tbody>
</table>

PGC-1α reacts estrogen receptors (ER) α and β, which are also involved in blood pressure control. Interestingly, estrogen receptor β deficient in mice expands arterial pressure (Zhu et al., 2002). Gly482Ser polymorphism in exon 8 of the PGC-1α gene was studied between hypertensive and healthy groups in our present study. Also, clinical characteristics of population was studied and it was shown that cholesterol, triglycerides, and BMI was significantly different between hypertensive and healthy individuals and people with high blood pressure have higher cholesterol, triglycerides, and BMI. Logistic regression analysis revealed that age (p value: 0.000 OR: 1.022 CI: 1.012-1.032), cholesterol (p value: 0.000 OR: 1.005 CI: 1.004-1.005), fasting plasma glucose (p value: 0.003 OR: 1.005 CI: 1.002-1.009), triglycerides (p value: 0.000 OR: 1.019 CI: 1.018-1.020) and BMI (p value: 0.000 OR: 1.098 CI: 1.097-1.106) as a risk factor in the increased risk of high blood pressure in this study are considered. Each of these features increase the likely to develop hypertension increases.

Using the results of the chi-square test, were found genotype frequencies of the GA, GG and AA (Gly / Ser, Gly / Gly, Ser / Ser) and two alleles A and G in patients with hypertension compared with healthy no significant difference. So we conclude that PGC-1α gene Gly482Ser polymorphism does not influence the development hypertension. Logistic regression analysis revealed that Gly482Ser polymorphism of PGC-1α gene is not considered a risk factor in hypertension and has no effect on development hypertension which was similar to that in Caucasians (Lacquemant et al., 2002), Chinese (Chen et al., 2004) and Japanese (Hara et al., 2002). Regarding the association of the PGC-1α polymorphisms with hypertension, controversial data have been published.

Andersen was reported that subjects with Ser/Ser have lower SBP and DBP, and that subjects with Ser/Ser have a much lower risk of developing hypertension than with Gly/Gly (Anderson et al., 2005) and Oberkofler (Oberkofler et al., 2003) was reported that PGC-1α 482Ser was considered to correlate with early onset of hypertension in male Europeans. But Churfa, Kunej, Sookoian and X.L. Su reported that subjects with Ser/Ser have higher SBP and DBP, and that subjects with Ser/Ser have a much higher risk of developing hypertension than with Gly/Gly (Churfa et al., 2004, Kunej et al., 2004, Sookoian et al., 2005, X.L. Su et al., 2011).

In summary, we did not confirm the effects of the G482S polymorphism in the PGC-1α gene on the risk of hypertension and additional studies should be performed to clarify the contribution of variations of the PGC-1α gene to the pathogenesis of hypertension in a variety of ethnic groups.
ACKNOWLEDGEMENTS

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REFERENCES


