



Association between peroxisome proliferator-activated receptor gamma coactivator-1 alpha polymorphism and hypertension in Mongolians in Inner Mongolia

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ABSTRACT. We investigated a possible association of peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1 α) Gly482Ser polymorphism with hypertension in Mongolians in Inner Mongolia. A total of 787 subjects were enrolled randomly, including 390 hypertension patients and 397 healthy controls. Triglycerides, cholesterol, and fasting plasma glucose were measured, and body mass index was calculated. PCR-RFLP was used to analyze Gly482Ser polymorphisms. There were significant differences in triglycerides, fasting plasma glucose, and body mass index between hypertension patients and healthy controls. Cholesterol levels did not differ significantly. The PGC-1 α gene GG, GA and AA genotype distributions were 37.2, 48.5 and 14.4%, respectively, in patients and 48.6, 37.3 and 14.1% in healthy controls. The frequencies of PGC-1 α genotype GA and allele A were significantly different between hypertension patients

and healthy Mongolians. We concluded that PGC-1 α Gly482Ser polymorphism is associated with hypertension in Mongolians in Inner Mongolia.

Key words: Mongolian; Genetic polymorphism; Hypertension; Peroxisome proliferator-activated receptor gamma coactivator-1 alpha

INTRODUCTION

Hypertension is a multifactorial disorder in which genetic and environmental factors are involved. Clinical and experimental studies have indicated that insulin resistance and hyperinsulinemia are important factors contributing to hypertension. Therefore, genetic factors affecting insulin resistance may be involved as a common genetic basis of susceptibility to hypertension.

Peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1 α) was found to be transcriptional coactivator of peroxisome proliferator-activated receptor gamma (PPAR- γ) in mouse (Puigserver and Spiegelman, 2003). Human PGC-1 α is located on chromosome 4p15.1 and encoded by the gene *PPARGCIA* (Esterbauer et al., 1999). PGC-1 α contains one RNA binding domain, two serine/arginine rich domains that can interaction with the C terminal domain of RNA polymerase II, three protein kinase A phosphorylation sites, and one LxxLL domain (lysine-rich domain) that can interact with PPAR, estrogen receptor (ER) and glucocorticoid receptor (GR) (Puigserver et al., 1998; Esterbauer et al., 1999; Tcherepanova et al., 2000; Vega et al., 2000; Michael et al., 2001; Yoon et al., 2001; Puigserver and Spiegelman, 2003). PGC-1 α is involved in the regulation of energy metabolism by playing an important role in adaptive thermogenesis, production of mitochondria, fatty acid β -oxidation, gluconeogenesis (Puigserver et al., 1998; Yoon et al., 2001; Puigserver and Spiegelman, 2003; Estall et al., 2009; Kong et al., 2010), and participates in different types of physiological processes by interaction with several transcriptional factors such as PPAR, ER, GR, and nuclear respiratory factor (NRF) (Tcherepanova et al., 2000; Vega et al., 2000; Jang et al., 2007; Yu and Yang, 2010).

Several polymorphisms of PGC-1 α have been found, including IVS42 11T>C, Thr394Thr and Gly482Ser. Association between PGC-1 α Gly482Ser polymorphism and diabetes has been widely studied. The Gly482Ser polymorphism has been found to be associated with type 2 diabetes (Ek et al., 2001; Bhat et al., 2007), reduced mRNA level of *PPARGCIA*, reduced insulin secretion (Ling et al., 2008) and lower plasma adiponectin level (Okauchi et al., 2008) in patients with type 2 diabetes. However, contrary results have been reported (Vimalaswaran et al., 2005; Nelson et al., 2007). Studies also found that polymorphisms of PGC-1 α were correlated to metabolic syndrome, a disease characterized by clinical symptoms of sugar, fat and energy metabolic disorders inducing diabetes, insulin resistance, abdominal obesity, hypertension and coronary heart disease in Danes, Japanese, Caucasians in France, Pima Indian, British, Austrians, Germans and Dutch (Lacquemant et al., 2002; Franks et al., 2003; Muller et al., 2003; Oberkofler et al., 2003; Stumvoll et al., 2004). However, few studies have been conducted on the association of PGC-1 α Gly482Ser polymorphism and hypertension. Previously, our group found that PPAR γ 2 Pro12Ala polymorphism is involved in genetic susceptibility to hypertension and metabolic lipid disorders in a population in Inner Mongo-

lia (Gao et al., 2010). Therefore, in the present study, we hypothesized that the Gly482Ser polymorphism of PGC-1 α , the transcriptional coactivator of PPAR- γ , is also associated with hypertension in a population in Inner Mongolia.

MATERIAL AND METHODS

Subjects

All subjects gave informed consent. Three hundred and ninety hypertension patients (196 males and 194 females) with average age of 53.31 ± 13.42 years were enrolled. Blood pressure was measured 3 times by sphygmomanometer, taking the average. Hypertension was diagnosed according to the WHO criteria (1999): systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg, including those who received pharmacologic treatment with antihypertensive drugs in the last two weeks. Individuals with secondary hypertension, diabetes mellitus, or severe liver, kidney and thyroid dysfunction were excluded from the study. Three hundred and ninety seven normotensives (NT, 168 males and 230 females) with an average age of 44.03 ± 12.87 years were enrolled as control. The NTs were selected based on SBP < 140 mmHg and/or DBP < 90 mmHg, excluding those with antihypertensive medication history, hypertension and other cardiovascular or cerebrovascular diseases. Body mass index (BMI) was calculated by weight (kg) divided by square height (m²). Blood samples were drawn after an overnight fast. Triglycerides (TG), cholesterol (CHO), fasting plasma glucose (FPG) were measured according to standardized methods. All subjects were Mongolians and from families who had been living in the pastoral area of Inner Mongolian (Siziwang Banner, Wulanchabu League and Dongwu Banner, Xilinguole League) for at least 3 generations. Our experiment was approved by Inner Mongolia Medical College Affiliated Hospital Ethics Committee.

DNA isolation and PCR-RFLP

Three milliliters of peripheral venous blood were drawn into sodium citrate solution, and genomic DNA was extracted using a kit (Genomic by DNA purification kit, TaKaRa Biotechnology, Dalian, China). Primers (sense 5'-TGAGAGAGACTTTGGAGGCA-3' and anti-sense 5'-GGAATATGGTGATCGGGAAC-3') were synthesized by TaKaRa Biotechnology. PCR was performed in a volume of 25 μ L including 5 μ L DNA, 12.5 μ L Taqman PCR master mix, 1.25 μ L primers, and 6.25 μ L deionized water. The reaction conditions were as follows: 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 with Hap II (TaKaRa Biotechnology) in 37°C water bath for 2 h. Digestion products (7 μ L) were electrophoresed using a 4% agarose gel at 120 V for 1 h and analyzed with a Gel-Pro imaging instrument.

Sequencing

To confirm that the detection of this G \rightarrow A nucleotide substitution by PCR-RFLP analysis was reproducible, we also performed PCR-based direct sequencing analysis. The

genotype of each study subject was determined blindly without knowledge of clinical status. GA and AA genotypes were selected and re-amplified, and the DNA sequences were verified by direct sequencing (United States ABI Prism 3700 DNA analyzer 377; Applied Biosystems, Foster City, California, USA).

Statistical analysis

Data were expressed as mean±standard deviation. 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. The comparison of frequencies of genotype and allele between the two groups was performed by the chi-square test. Logistic regression was used to investigate the risk factor of hypertension. Statistical analysis was performed by the SPSS 13.0 software. Statistical significance was established at $P < 0.05$.

RESULTS

The clinical characteristics of hypertensive patients and healthy controls are shown in Table 1. There was a significant difference in clinical characteristics between hypertensive patients and healthy controls except for CHO. The DNA fragment of the PGC-1 α gene was 452 bp after PCR amplification. Homozygous wild type (GG) without a restriction site only had a fragment of 452 bp. Homozygous variants (AA) containing a restriction site in each DNA chain yielded two fragments of 310 and 142 bp after digestion with Hap II. Heterozygotes (GA) containing restriction sites in one of the DNA chains yielded 3 fragments of 452, 310 and 142 bp.

Table 1. Comparison of clinical characteristics between hypertensive patients and healthy controls.

Clinical characteristic	Hypertensive patients (N = 390)	Healthy controls (N = 397)	P value
Age (years)	53.31 ± 13.42	44.03 ± 12.87	0.00*
BMI (kg/m ²)	25.67 ± 4.52	22.79 ± 3.54	0.00*
SBP (mmHg)	165.01 ± 28.78	114.80 ± 13.83	0.00*
DBP (mmHg)	105.15 ± 14.56	75.03 ± 7.63	0.00*
FPG (mM)	5.21 ± 1.24	5.20 ± 1.16	0.93
CHO (mM)	5.22 ± 1.25	4.49 ± 0.99	0.00*
TG (mM)	2.05 ± 1.62	1.27 ± 0.56	0.00*

The *t*-test was used to compare clinical characteristics between hypertensive patients and healthy controls. *Indicates significant difference.

The PGC-1 α gene GG, GA and AA genotype distributions were 37.18, 48.46, and 14.36% in patients and 48.61, 37.28 and 14.11% in healthy controls, respectively. The G allele frequency was 61.41 and 67.25% in patients and healthy controls and the A allele frequency was 38.59 and 32.75%, respectively. We observed that the PGC-1 α Gly482Ser polymorphism genotype distribution was in accordance with Hardy-Weinberg expectations in the Mongolian population in Inner Mongolia ($P > 0.05$). There was a significant difference in the distribution of the PGC-1 α genotype GA and allele A frequency between hypertensive and healthy Mongolian ($P = 0.001$ and 0.02 , respectively; Table 2). The logistic regression showed that age, MBI, TG, CHO, FPG and PGC-1 α Gly482Ser polymorphism were not risk factors of hypertension (Table 3).

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

Group	Cases	Genotype			Allele	
		GG	GA	AA	G	A
Hypertensive	390	145 (37.18)	189 (48.46)	56 (14.36)	479 (61.41)	301 (38.59)
Control	397	193 (48.61)	148 (37.28)	56 (14.11)	534 (67.25)	260 (32.75)
P value			0.001			0.02

Data are reported as number with percent in parentheses.

Table 3. Logistic regression analysis of independent risk factors of hypertension.

Characteristic	OR	95%CI	P value
Age (years)	1.015	0.749-1.376	0.924
BMI (kg/m ²)	0.798	0.475-1.342	0.396
FPG (mM)	0.873	0.442-1.724	0.695
CHO (mM)	1.434	0.806-2.549	0.220
TG (mM)	0.888	0.536-1.472	0.646
PGC-1 α Gly482Ser polymorphism	0.513	0.283-2.499	0.755

DISCUSSION

PPAR γ is a transcription factor and specific for adipose tissue, where it plays a key role in regulating adipogenic differentiation. Our previous study showed that the PPAR γ 2 gene Pro12Ala polymorphism was associated with hypertension in a population in Inner Mongolia (Gao et al., 2010). Gly482Ser polymorphism of PGC-1 α , the transcriptional coactivator of PPAR γ and other nuclear transcriptional factors, was also found to correlate with hypertension in Mongolians in our present study. Our result indicated that PGC-1 α Gly482Ser polymorphism may contribute to the pathogenesis of hypertension.

Our study showed that the distribution of PGC-1 α genotype GA and allele A frequency was significantly different between hypertensive and healthy Mongolians. Individuals with PGC-1 α Ser482Ser had a higher level of blood pressure ($P < 0.05$), suggesting that Ser482Ser may increase the risk of developing hypertension in our subjects. The frequency of 482Ser in Mongolians was 35.67%, which was similar to that in Caucasians (30.8-38.1%) (Ek et al., 2001; Lacquemant et al., 2002; Oberkofler et al., 2003) but lower than that in Chinese (42.9%) (Chen et al., 2004) and Japanese (43.7%) (Hara et al., 2002). Regarding the association of the PGC-1 α polymorphisms with hypertension, controversial data have been published. It 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 (Andersen et al., 2005). PGC-1 α 482Ser was considered to correlate with early onset of hypertension in male Europeans (Oberkofler et al., 2003). Chen et al. found that +1302G>A and Gly482Ser polymorphisms of PGC-1 α do not play roles in the pathogenesis of hypertension in Chinese (Chen et al., 2004). Xie et al. reported that Gly482Ser and +2962A/G polymorphisms of PGC-1 α are associated with severe hypertension (Xie et al., 2007), which is consistent with the results of Oberkofler et al. (2003) and indicates that severe hypertension may be correlated to genetic background. All these controversial results may be due to differences in race, aims, sample size, observed variables, methods, and environmental factors.

In summary, our study found that there was a significant difference in the distribution of PGC-1 α genotype GA and allele A frequency between hypertensive and healthy Mongolians. PGC-1 α Ser482Ser carriers had a higher level of blood pressure, which suggests that Ser482Ser may increase the risk of developing hypertension in Mongolians in Inner Mongolia.

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