Expression of STK39 in peripheral blood of hypertension patients and the relationship between its genetic polymorphism and blood pressure

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ABSTRACT. This study investigated the *STK39* expression in peripheral blood of hypertension patients and the relation between its genetic polymorphism and blood pressure. The observation group comprised of 42 primary hypertension patients admitted to our hospital, and the control group comprised of 30 healthy individuals who underwent physical examination in our hospital during the same period. Fasting venous blood was collected from both groups in the morning to determine the STK39 mRNA and protein levels in peripheral blood using quantitative real-time PCR and western blot. *STK39* gene SNP (rs6433027) was sequenced using PCR and its genetic variation was analyzed. The relationship between STK39 protein level, genetic variation, and diastolic and systolic blood pressure was also analyzed. The observation group showed increased STK39 mRNA and protein levels in peripheral blood compared to the control group, and the difference was statistically significant ($P < 0.05$), suggesting C/T mutation

in *STK39* gene SNP (rs6433027). Correlation analysis showed positive association between STK39 protein level and diastolic and systolic blood pressure ($P < 0.05$), indicating a positive association between C/T genetic mutation and diastolic and systolic blood pressure ($P < 0.05$). In conclusion, STK39 mRNA and protein express abnormally in primary hypertension patients with genetic variation, which is related to the blood pressure.

Key words: STK39; Primary hypertension; Genetic variation; Diastolic blood pressure; Systolic blood pressure

INTRODUCTION

Hypertension is a common life-threatening cardiovascular disease that greatly affects human health and has become a public health issue requiring attention around the world. Worldwide, around 25% of adults had been diagnosed with hypertension; and the number is estimated to rise by 60% in 2025, i.e., 1560 million people will suffer from it (Su, 2010). Primary hypertension is an independent risk factor for the development of myocardial infarction, cardiac failure, cerebrovascular diseases, and chronic renal failure. However, the cause and pathogenesis of hypertension are not yet clear. Hypertension results from the interaction of multiple genetic and environmental factors. Genetic factors affecting blood pressure account for 30 to 50%; however, related researches on the role of gene in hypertension are rare (Keamey et al., 2005; Kato et al., 2011). Tremendous progression has been seen in the field of whole genome sequencing and restriction endonuclease length polymorphism. The advent of Genome-wide Association Study (GWAS) based on SNP chip technology has revolutionized the treatment of primary hypertension (Pan et al., 2015). Recently, the GWAS on diseases such as tumors, cardiovascular diseases, diabetes, hypertension, and schizophrenia has developed rapidly all around the world. GWAS reports on several susceptibility genes of primary hypertension in Europe, China, Japan, and South Korea lay a foundation for studying the pathogenesis of hypertension (Pearson and Manolio, 2008; Levy et al., 2009). Serine-threonine kinase 39 (STK 39) was reported to be a susceptibility gene locus for hypertension in Amish individuals. Studies showed that two common genetic variations of *STK39*, rs3754777, and rs6433027, are the susceptibility gene loci of hypertension in Han Chinese males (Duarte et al., 2010; Xu et al., 2013). Changes in *STK39* expression in the serum of hypertension patients and the genetic variation of *STK39* gene SNP (rs6433027) needs to be further explored. Hence, this study analyzes the expression and genetic variation of *STK39* in primary hypertension patients, aiming to provide a theoretical basis for studying the genetic factors of hypertension.

MATERIAL AND METHODS

Clinical data

The observation group comprised of 42 primary hypertension patients, admitted into our hospital from September 2012 to September 2014. The inclusion criteria are: 1) all patients conformed to the clinical diagnosis criteria in A Guide to Chinese Hypertension Prevention and Treatment; 2) patients aged from 50 to 75 were included; 3) patients were male; 4) the patients had signed the informed consent; 5) the research was approved and supervised by Medical Ethics

Committee of the hospital. The exclusion criteria are: 1) secondary hypertension patients; 2) patients diagnosed with coronary heart disease by coronary angiography; 3) patients with diabetes, hyperlipemia, or thrombosis; 4) patients with liver and kidney diseases, autoimmune diseases, and tumors; 5) patients who underwent surgery or were injured recently. The average age of the 42 patients was 63.12 ± 11.36. The control group comprised of 30 healthy individuals, aged from 50 to 76 with an average age of 62.26 ± 12.03, who underwent physical examination during the same period. The comparison of the indexes like age was not statistically significant ($P > 0.05$). This study was conducted in accordance with the declaration of Helsinki with the approval from the Ethics Committee of the Third Affiliated Hospital of Xinxiang Medical University. Written informed consent was obtained from all participants.

Pretreatment of blood samples

Fasting venous blood (5 mL) was collected from both groups in the morning. The blood sample (2.5 mL) was kept at room temperature for 30 min and then centrifuged for 5 min at 3000 rpm/min and the serum was collected. The other blood sample (2.5 mL) was separated with density gradient centrifugation to get peripheral blood mononuclear cell (PBMC) for total RNA extraction.

Quantitative real-time PCR

TRIzol (TaKaRa, Dalian, China) (1 mL) was added to the obtained PBMC and then mixed by shaking. Chloroform (200 µL) was then added and mixed, and placed on ice for segregation. It was then centrifuged for 15 min at 12,000 rpm/min. Equal volume of isopropanol was added to the supernatant (500 µL) and was agitated. It was placed on ice for 30 min and then centrifuged for 15 min at 15,000 rpm/min. The supernatant was discarded, and the sediment was washed with pre-cooled 70% alcohol once and centrifuged for 6 min at 8000 rpm/min. The sediment was dissolved in double-distilled water treated by DEPC, and the RNA concentration was detected and RNA extraction mass was measured. Reverse transcription was performed on extracted RNA to obtain cDNA with a reverse transcription kit (TaKaRa) following manufacturer instructions; the obtained cDNA was taken as the template of RT-PCR.

Based on STK39 mRNA sequence, STK39 primers were designed: STK39-F1, 5'-CGGCAGTGGAGCTACTGCTG-3' and STK39-R1, 5'-CACTACGTTGGGATGGCTGC-3'. First, the specificity and annealing temperature of primers were optimized with conventional PCR. Second, the following reaction systems were prepared: 25 µL 2X SYBR Green RT-PCR mix (Vazyme Biotech Co., Ltd., Nanjing, China), 1 µL primers, 1 µL cDNA, and enough water to make up the volume to 50 µL. A given volume of reactants was prepared according to the sample number, and then added into quantitative real time PCR 96-hole plate. Centrifugation at 1500 rpm/min for 1 min flung the reaction liquid to the bottom of the tube. Third, the following reaction conditions were set on the RT-PCR: pre-denaturation at 95°C for 30 s; denaturation at 95°C for 3 s; and annealing and extension at 60°C for 30 s. The results were recorded in the end (ABI 7500; Applied Biosystems, Foster City, CA, USA).

Western blot

Peripheral blood samples were collected from the two groups. Erythrocytes in the blood samples were lysed using erythrocyte lysate. White blood cells were collected after centrifugation

for 30 min and were mixed with lysate on ice and centrifuged for 5 min at 12,000 rpm/min. The supernatant was collected and added to boiling loading buffer and boiled for 10 min, and later SDS-PAGE was performed. When the bromophenol blue was out of the lower edge of the gel, the protein was moved onto nitrocellulose (NC) membrane. After 30 min of sealing with 5% of skimmed milk powder, it was incubated with mouse anti-human STK39 monoclonal antibody (Abcam, Cambridge, UK) diluted at 1:2000 overnight. Next day, it was washed three times with phosphate-buffered saline with Tween 20 (PBST), and then incubated at 37°C with horseradish peroxidase (HRP)-labeled goat anti-mouse secondary antibody (ZSJQ-BIO, Beijing, China) for 1 h, and luminescent liquid was used for development. Meanwhile, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was adopted as internal reference. The gradation of western blot bandings of STK39 and GAPDH protein was analyzed with software, and the ratio of STK39 to GAPDH banding gradation was used as the relative expression level of STK39 protein.

The analysis of amplification and variation of STK39 gene SNP (rs6433027)

Based on *STK39* gene sequence, STK395-F2 and STK39-R2 were designed: STK395-F2, CAATCTTAATCTGAAGAAAGTG and STK39-R2: GTCTTATAGGACTACTCATCT. The following PCR system was prepared: 25 µL 2X Taq DNA polymerase mix (TaKaRa), 1 µL STK395-F2, 1 µL STK39-R2, 1 µL cDNA, and water was added to make up the volume to 50 µL. PCR was carried out according to the following reaction conditions: pre-denaturation at 94°C for 5 min, denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 2 min for 30 cycles, and then the temperature was lowered to 4°C. The PCR products were connected to T vector (TaKaRa) according to the T vector instruction manual. *Escherichia coli* DH5a (TaKaRa) was transformed and seeded on solid LB plates and cultured at 37°C overnight. On the next day, monoclonal colonies were selected for sequencing analysis.

Statistical analysis

All data were analyzed with SPSS 17.0 statistical software (SPSS Inc., Chicago, IL, USA). The measured data was expressed by means ± s, and the comparison of measured data was checked by Student *t*-test. The comparison of numeration data was checked with chi-square test; the correlation analysis was checked by Pearson's test. The difference $P < 0.05$ was considered statistically significant.

RESULTS

The comparison of general data

In both groups, the comparison of indexes such as age, gender, TC, HDL-C, LDL-C, WHR, fasting blood sugar, fasting insulin was of no statistical significance ($P > 0.05$). The observation group had a higher SBP and DBP level than the control group, and the difference was statistically significant ($P < 0.05$) (Table 1).

Comparison of STK39 mRNA levels in peripheral blood cells

In the experiment, quantitative real time PCR detected the level of STK39 mRNA, as

shown in Figure 1. Quantitative analysis indicated that the observation group has a higher level of STK39 mRNA in peripheral blood cells than the control group, and the difference was statistically significant ($P < 0.05$).

Table 1. Comparison of general information in both groups.

Items	Control group	Observation group	P value
Sex (Male/Female)	28/14	17/13	0.43
Age	63.1 ± 11.3	62.2 ± 12.0	0.79
TC (mM)	4.03 ± 0.49	4.31 ± 0.60	0.59
TG (mM)	1.22 ± 0.31	1.26 ± 0.33	0.78
HDL-C (mM)	1.61 ± 0.31	1.67 ± 0.31	0.32
LDL-C (mM)	2.69 ± 0.46	2.58 ± 0.51	0.79
Fasting blood sugar (mM)	5.11 ± 0.62	5.20 ± 0.49	0.58
SBP	127.3 ± 9.4	175.3 ± 10.5	0.02*
DBP	75.9 ± 8.5	96.55 ± 9.7	0.03*

*Compared with the control group, $P < 0.05$.

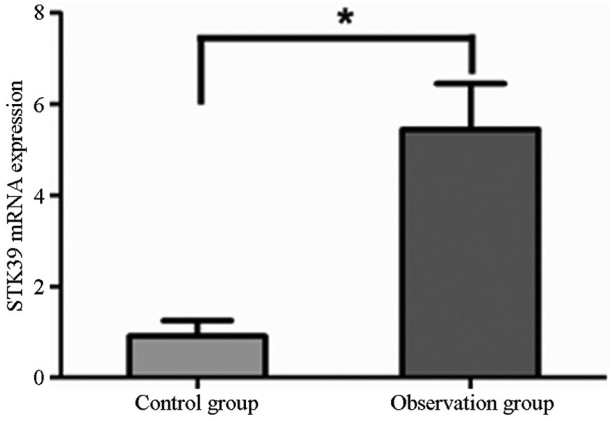

Figure 1. Comparison of STK39 mRNA levels in peripheral blood cells in both groups.

Comparison of STK39 protein level in peripheral blood cells

In this research, western blotting technique was used to analyze the expression of STK39 protein in peripheral blood cells of both groups; the results are shown in Figure 2A. Under similar GAPDH levels, STK39 protein banding in peripheral blood cells of the observation group was more distinct compared to the control group. The quantitative analysis results are shown in Figure 2B. The observation group has a more marked ratio of STK39 banding gradation/GAPDH banding gradation than the control group, and the difference was of statistical significance ($P < 0.05$).

The analysis of *STK39* gene SNP genetic variation

PCR was conducted to amplify *STK39* gene SNP（rs6433027）segment, which was connected to T carrier, and monoclonal ones were taken out for sequencing. The sequencing results are shown in Figure 3. C/T mutation existed in *STK39* gene SNP (rs6433027). There were

three cases of variation in the control group accounting for 10% (3/30). Whereas, there were 24 cases of variation in the observation group accounting for 57.15% (24/42). The patients of the observation group had significantly higher genetic variation rate of *STK39* gene SNP (rs6433027) than the control group, and the difference was of statistical significance ($P < 0.05$). The patients of the observation group were divided into two groups based on the variation of *STK39* gene SNP (rs6433027): variation group and wild-type group. The variation group had significantly higher diastolic and systolic blood pressure than the wild-type group, and the difference was statistically significant ($P < 0.05$) (Figure 4).

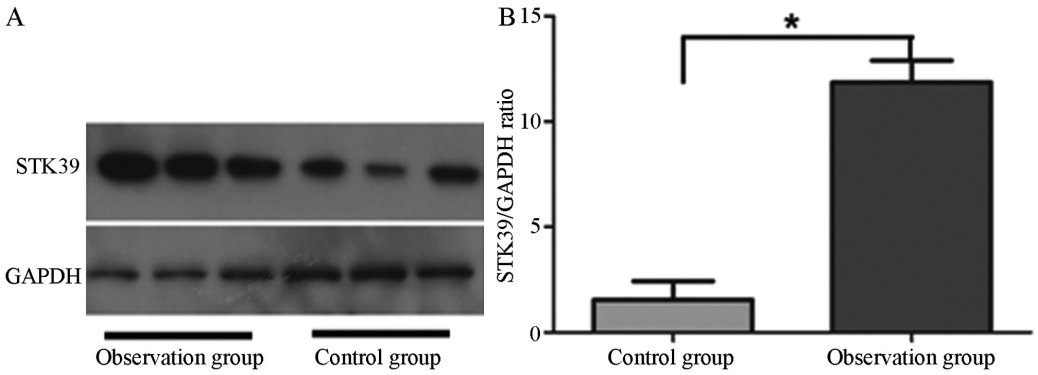

Figure 2. Comparison of STK39 protein level in peripheral blood cells in both groups. **A.** Results of western blot. **B.** Quantitative analysis results.

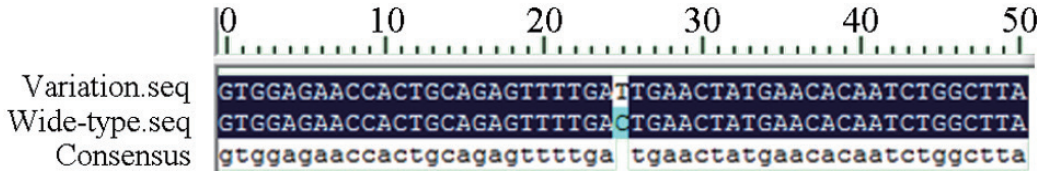

Figure 3. Analysis of *STK39* gene SNP genetic variation.

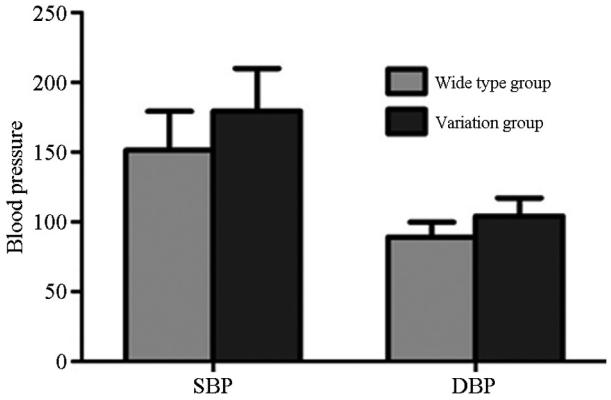

Figure 4. Comparison of blood pressure in patients with different *STK39* gene SNP genetic variation.

Analysis of the relationship between STK39 protein level, genetic variation, and blood pressure

Linear correlation analysis indicated that STK39 protein level of peripheral blood was positively associated with the diastolic and systolic blood pressure ($r = 0.577$, $P < 0.05$; $r = 0.394$, $P < 0.05$). C/T mutation in *STK39* gene SNP (rs6433027) was positively associated with diastolic and systolic blood pressure ($r = 1.064$, $P < 0.05$; $r = 0.796$, $P < 0.05$).

DISCUSSION

Hypertension can be classified as primary (hereafter referred to as hypertension) and secondary hypertension, with more than 95% of cases categorized as primary hypertension. Hypertension results from a complex interaction of genes and environmental factors. The environmental factors lead to varied clinical types when affecting different genetic background, while genetic factors play a vital role in the occurrence and development of primary hypertension. It is considered that minor gene mode functions in the genetic mechanism of primary hypertension, i.e., most risk alleles from many loci interact with each other, reaching the critical threshold of disease, and they co-decide the genetic susceptibility of hypertension (Li and Liu, 2008; Brenner and Chung, 2011). The determination of hypertension susceptibility genes contributes to defining the pathophysiology, and provides theoretical basis for preventing and treating hypertension. The current strategies for the genetic study of hypertension are candidate genes and whole genome scanning. In the GWAS, many new genes relating to primary hypertension are found, such as *ATP2B1*, *CYP17A1*, *PLEKHA7*, *SH2B3*, *MTHFR*, *CASZ1*, *ULK4*, *HFE*, *EBF1*, and *STK39* (Germain et al., 2013; Xi et al., 2014).

STK39 was determined in Amish individuals by GWAS. However, its expression is not found in British Caucasian, which suggests the possible reason for the ethnic variation of primary hypertension (Cunnington et al., 2009). Wang et al. (2009) reported the first GWAS meta-analysis of hypertension. They first conducted an analysis on 542 subjects, and then combined the data of six flocks of people (N = 7 125) and conducted meta-analysis, and found that *STK39* gene is significantly associated with blood pressure level. Chen et al. (2012) found two common genetic mutation of *STK39*, rs3754777 and rs6433027, which are the susceptibility loci of hypertension of Han Chinese males, but are not related to female hypertension. Besides, the study also indicates that T allele of rs6433027 presents strong epistasis in A allele of rs3754777. The study results manifest that *STK39* is an independent risk factor of male hypertension, and its SNPs can interact with each other and control blood pressure.

Our control study analyzed the expression of *STK39* in peripheral blood of male primary hypertension patients and male healthy individuals. STK39 mRNA level in peripheral blood cells of hypertension patients is remarkably higher compared to healthy individuals; the result was further verified on protein level with ELISA, and STK39 protein level of peripheral blood was found to be higher in hypertension patients than in healthy individuals. It demonstrates that primary hypertension patients have abnormal expression of STK39 mRNA and protein levels, so they possibly participate in the occurrence and development of hypertension.

The report also indicated that genetic variation may exist in or near *STK39* in hypertension patients. It is related to the diastolic pressure and systolic pressure of the patient (Xi et al., 2013). We amplified *STK39* gene SNP (rs6433027) segment and analyzed the genetic variation of the

segment, and investigated the association between the variations and the blood pressure of hypertension patients. The study results manifest that C/T genetic mutation exists in *STK39* gene SNP (rs6433027), and correlation analysis indicates that C/T mutation is positively associated with diastolic pressure and systolic pressure. These results further verify previous study results, providing theoretical basis for figuring out the pathogenesis of genetic factors of hypertension.

Sampling size considered in this study is small. It possibly requires the application of diverse samples, centers, and ethnic groups. Together, this study offers a basis for understanding the pathogenesis of genetic factors of hypertension. It is believed that in the near future, the development of molecular genetics and molecular biology will help control the incidence of primary hypertension.

Conflicts of interest

The authors declare no conflict of interest.

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