

A Novel 5'-Uncoding Region -1248 A>G Variation of Mitofusin-2 Gene Is Associated with Hypertension in Chinese

Zuoguang Wang,¹ Ya Liu,¹ Jieling Liu,¹ Qiuli Niu,¹ Jie Wen,¹ Shaojun Wen,¹ and Zhaosu Wu²

Departments of ¹Hypertension and ²Epidemiology, Beijing Institute of Heart, Lung, Blood Vessel Diseases, Beijing Anzhen Hospital, Capital Medical University, Beijing, China.

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Co-corresponding authors:

Dr. Zuoguang Wang,

Department of Hypertension Beijing Institute of Heart, Lung, Blood Vessel Diseases, Beijing Anzhen Hospital, Capital Medical University, No. 2, Anzhenlu, Andingmenwai, Chaoyang District, Beijing, 100029, P.R.China.

Tel: 86-10-64456348, Fax: 86-10-64446095,

E-mail: wangzg70@gmail.com and

Dr. Shaojun Wen,

Department of Hypertension Beijing Institute of Heart, Lung, Blood Vessel Diseases, Beijing Anzhen Hospital, Capital Medical University, No. 2, Anzhenlu, Andingmenwai, Chaoyang District, Beijing, 100029, P.R.China.

Tel: 86-10-64456462, Fax: 86-10-65725770,

E-mail: wzg70@hotmail.com

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Purpose: Mitofusin2 gene (Mfn2, also named Hyperplasia suppressive gene, HSG) is very important in the origin and development of hypertension. However, the mechanism of Mfn2/HSG expression regulation was not uncovered. This study was designed to explore the association of a novel 5'-uncoding region (UCR) -1248 A>G variation of HSG/Mfn2 gene and hypertension. **Materials and Methods:** 472 healthy, normotensive subjects [normotension (NT) group], 454 prehypertensive subjects [prehypertension (PH) group] and 978 hypertensive patients [essential hypertension (EH) group] were screened for an association study between 5'-UCR -1248 A>G of Mfn2/HSG and hypertension by polymerase chain reaction and DNA sequencing after venous blood was drawn and DNA was extracted. **Results:** When comparing the A and G frequency in EH, PH and NT groups, in total, NT group significantly had higher A frequency than in PH group [odds ratio (OR)=1.605, confidence interval (CI) 95%=1.063-2.242, $p=0.025$] and EH group (OR=5.395, CI 95%=3.783-7.695, $p<0.01$). When subgrouped by gender, A frequency in NT group was still significantly higher than in EH group (male: OR=4.264, CI 95%=2.780-6.543, $p<0.01$; female: OR=8.897, CI 95%=4.686-16.891, $p<0.01$), but not from PH group, either in male group or in female group. Ordinal Logistic Regression analysis showed that A>G variation was significantly related with blood pressure level ($B=-1.271$, Wald=40.914, CI 95%=-1.660 - -0.881, $p<0.01$). **Conclusion:** 5'-UCR -1248 A>G variation of Mfn2/HSG gene was a novel variation and may be associated with hypertension in Chinese.

Key Words: Hyperplasia suppressor gene, Mitofusin-2, single nucleotide polymorphism, essential hypertension, transcription regulation

INTRODUCTION

Mitofusin2 gene (Mfn2, NG_007945; also named Hyperplasia suppressive gene, HSG) is a novel gene related with essential hypertension (EH). It belongs to the family of large GTP-binding proteins and located on the short (p) arm of chromosome 1 at position 36.22.¹⁻³

In previous studies, we found it played an key role in the down regulation of vascular smooth muscle cells (VSMCs) hyperplasia in experimental animal mod-

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els as well as in essential hypertensive patients.^{3,4} The expression product of Mfn2/HSG is a mitochondrial membrane protein that participates in mitochondrial fusion and contributes to the maintenance and operation of the mitochondrial network.⁵ If an equilibrium between fusion and fission of mitochondria could not be maintained, energy metabolism, oxidation, calcium signaling and apoptosis disorder might be induced.^{6,7}

Mfn2/HSG also is a negative regulator of ras-raf-ERK signal transduction pathway in the proliferation of VSMCs.^{5,8} Since EH is a disease characteristic of hyperplasia of VSMCs, and is affected by oxidative factors, much attention is paid to the expression regulation of HSG/Mfn2 gradually. A genetically related disease, single nucleotide polymorphisms (SNPs) of EH related genes are very important in the uncovering of nosogenesis of EH. We have found several SNPs are closely related with EH in Chinese.⁹ In this study, we focus on the SNP of 5'-uncoding region of Mfn2/HSG which may provide clues to the understanding of the potential mechanism of Mfn2/HSG down regulation in hypertension.

MATERIALS AND METHODS

Mfn2/HSG DNA sequence was analyzed by the Proscan software (Version 1.7; program developed by Dan Prestridge and supplied online by the Advanced Biosciences Computing Center, University of Minnesota, Minneapolis, MN, USA) for transcription factors and promoters.¹⁰ Based on genetic analysis, literature review, and predictive analyses with an emphasis on gene expression regulation, 5'-uncoding region (UCR) of Mfn2/HSG which included some critical promoters was selected a priori for polymerase chain reaction and genotypes analysis. We screened normotensive, prehypertensive and hypertensive participants and then investigated the difference of genotype frequency of Mfn2/HSG in the studied participants.

Study population

All the normotensive, prehypertensive and hypertensive participants were screened at the physical examination center and hypertension clinic at Anzhen hospital. A total of 472 normotensive participants [normotensive subjects (NT) group], 454 prehypertensive participants [prehypertension (PH) group] and 978 hypertensive patients (EH group) were screened. BP was measured according to the seventh report of the joint national committee on prevention, detection,

evaluation, and treatment of high blood pressure (JNC-VII). The definition of normotension [systolic blood pressure (SBP)<120 mm Hg and diastolic blood pressure (DBP)<80 mm Hg], prehypertension (120 mm Hg≤SBP<140 mm Hg, and 80 mm Hg≤DBP<90 mm Hg) and hypertension (140 mm Hg≤SBP, and or 90 mm Hg≤DBP) were based on the classification of the BP recommended by JNC-VII standards.¹¹ All the patients were diagnosed for the first time as having essential hypertension and were never treated with any antihypertensive drugs before the screening. The normotensive subjects were screened from physical examination people and having not suffered from any diseases. BP was accurately measured three times on different days by experienced internists in the office with a mercury sphygmomanometer. The measurements were taken after the patients had been seated on a chair with their feet on the floor and their arms supported at heart level for 10 min.¹² The mean value of BP (SBP and DBP) for three measurements was used for the study.

All the hypertensive patients were not subjected to any known diseases, including secondary hypertension, diabetic disease and kidney diseases, which might affect BP. This study complied with the Declaration of Helsinki. All participants signed a consent form, and the Hospital Ethics Committee approved the study.

DNA preparation

A 5-mL peripheral venous blood sample was drawn with the subject in the sitting position and with minimal use of a tourniquet. The blood was collected in an EDTA-Na anticoagulated vacutainer tube. DNA was extracted using the PURGENE kit from Gentra Systems (Minneapolis, MN, USA) and stored at -20°C in aliquots until required.¹³

Detection of genotype

Five microliters of DNA extract was amplified in a 50-μL total reaction volume with 1 U Taq polymerase (Truestart Taq; Fermentas, UK), using a conventional thermal cycler (iCycler, Bio-Rad, Hertfordshire, UK). The upstream primer was TGACCCACAAATGCAAGCTA; the downstream primer was GACATGGGGAAGCATTTCAC. The software was Primer 3 online designing system (simgene.com). Thermal cycling conditions were as follows: 1 cycle of 95°C for 15 min, followed by 40 cycles of 94°C for 30 s, 57°C for 60 s, and 72°C for 60 s and then 1 cycle of 72°C for 5 min and 94°C for 30 s. The product was 261 bp. The product was then sequenced by ABI 3730XL sequencer (Applied Biosys-

tem, Foster City, CA, USA) to detect the genotype.

Statistics

Values were expressed as means±SD. The association of genotypes with hypertension was analyzed using the chi-squared test. To compare some indexes, including SBP, DBP and body mass index (BMI) between the EH and NT groups, a Student's t-test was used. To test for an association between related risk factors and blood pressure, Ordinal Logistic Regression analysis was used for the three groups (which BP was ordinal). All analyses were performed using SPSS v.17.0 (SPSS Inc., Chicago, IL, USA) statistical analysis software. The Hardy-Weinberg equilibrium was analyzed using SNPalyze, version 7.0 Pro (Dynacom Co., Ltd, Mobarra, Japan). A two-tailed p -value <0.05 was considered statistically significant.

RESULTS

Basic character of the studied subjects

Table 1 showed the basic character of all the studied subjects, including normotensive participants (NT group), prehypertensive participants (PH group), and hypertensive patients (EH group). The EH group had higher body weight, BMI, uric acid, and high density lipoprotein-cholesterol (HDL-C) compared with PH and NT groups ($p<0.05$). Some indexes, such as triglyceride (TG), low density lipoprotein-cholesterol (LDL-C) and blood urea nitrogen (BUN), were also higher in EH group than in PH and NT groups, but were not statistically significant ($p>0.05$).

Genotype frequency of Mfn2/HSG gene 5'-UCR -1248 A/G variation

When we analyzed the 5'-UCR of Mfn2/HSG, we found five promoters. After sequencing, an A>G variation was found contained in one promoter (promoter sequence: TG TAGTCAGCATATAAATAGTAATGGGCTAATAGCCCT GCACTGTGGCAG; value: 0.89; software: http://www.fruitfly.org/cgi-bin/seq_tools/promoter.html). As shown in Fig. 1, A to G variation was found in the 5-UCR of Mfn2/HSG gene. In Table 2, we compared the A and G frequency in EH, PH and NT groups. In total, the NT group had a significantly higher A frequency than the PH and EH groups ($p=0.025$, $p<0.01$ respectively). When sub grouped by gender, A frequency in the NT group was still significantly higher than in EH group ($p<0.01$), but not in PH group ($p>0.05$)

in the male group and female group.

Transcript factor analysis showed that ATGTAGTCA of 5'-UCR (-1249 - -1241) was an activator protein 1 (AP-1) combing site. The 5'-UCR -1248 A>G variation of Mfn2/HSG gene was just located in the middle of the AP-1 receptor sequence (Fig. 1).

Association of A/G and hypertension

For further study of A>G variation and hypertension, we performed Ordinal Logistic Regression analysis of the three groups and related risk factors (Table 3). The results showed that age, BMI, LDL-C, total cholesterol (TC) and A>G variation were significantly related with blood pressure level. Interestingly, A frequency was negatively related with blood pressure level.

DISCUSSION

SNP is very important in the genetic study of diseases. It can affect how humans develop diseases and respond to pathogens, chemicals, drugs, vaccines, and other agents. SNPs are also thought to be key enablers in realizing the concept of personalized medicine.¹⁴ Therefore, it is reasonable that SNPs can affect the expression of gene. In the previous studies, we found several SNPs of Mfn2/HSG gene were related with hypertension and there had been 387 SNPs reported presently.¹⁴

In this study, we found a novel SNP in 5'-UCR -1248 A>G of Mfn2/HSG gene. As already uncovered, TATA box and promoter played very important roles in the origination and regulation of gene transcription.¹⁵ Since this SNP was close to the TATA box and located in the promoter region noted above, which was closely related with the expression of Mfn2/HSG, this SNP was very important. The mutated A was in the middle of AP-1 binding site sequence. AP-1 was a very important transcript regulator. The A/G variation may have an impact on the transcription of Mfn2/HSG gene by obstruct combination of transcript factors,^{16,17} but the detailed mechanism needs to be studied further.

In this study, we screened hypertensive patients, prehypertensive and normotensive participants and compared the frequency of 5'-UCR -1248 A>G of Mfn2/HSG gene among them. As it was shown in Table 2, the EH group had a significantly lower AT frequency than the NT group ($p<0.01$). When the NT and PH participants were treated as one group and compared with the EH group, we got the same results

Table 1. Clinical Characteristics of Normotensive, Prehypertension and Essential Hypertensive Participants

Index	Total			Male			Female		
	NT	PH	EH	NT	PH	EH	NT	PH	EH
Age (yrs)	49.51±7.16	51.04±8.40	52.26±11.13	48.95±6.91	50.70±8.78	50.91±11.42	50.26±7.44	51.57±7.77	53.57±9.61
Sample number (M/F)	472	454	978	290	260	694	182	194	284
SBP (mm Hg)	106.37±8.36	124.36±5.29 [†]	149.34±11.68 [†]	106.30±8.64	124.42±5.17 [†]	148.17±11.91 [†]	106.47±8.01	124.25±5.50 [†]	152.16±10.62 [†]
DBP (mm Hg)	68.58±6.65	81.44±2.56 [†]	97.42±9.32 [†]	69.30±7.42	81.80±2.77 [†]	99.32±8.77 [†]	67.62±5.33	80.87±2.08 [†]	92.77±9.01 [†]
Height (cm)	166.96±8.30	166.46±7.19	166.80±8.15	172.37±5.94	170.58±5.43*	170.49±5.84 [†]	160.04±5.16	159.95±4.20	157.95±5.70 [†]
Weight (kg)	67.56±11.26	71.52±11.78 [†]	76.22±12.81 [†]	73.27±9.86	76.00±11.08	79.98±11.98 [†]	60.19±8.32	64.45±9.14 [†]	67.13±9.90 [†]
BMI (kg/m ²)	24.16±3.09	25.71±3.23	27.84±5.72 [†]	24.66±3.01	26.06±3.15	28.24±4.95 [†]	23.51±3.11	25.17±3.29 [†]	26.87±3.52 [†]
TG (mmol/L)	1.87±1.56	1.72±1.13	2.23±1.88	1.83±1.11	2.06±1.27	2.33±2.09 [†]	1.93±0.35	1.18±0.48	1.97±1.20
TC (mmol/L)	5.94±1.86	5.03±0.84	5.55±3.46	5.10±1.05	5.07±0.78	5.43±0.73	7.09±0.23	4.98±0.93	5.83±2.69
LDL-C (mmol/L)	4.10±0.66	3.50±0.79	3.40±0.89	3.43±0.77	3.50±0.81	3.34±0.88	5.54±0.43	3.48±0.72	3.56±0.91
HDL-C (mmol/L)	1.59±0.74	1.18±0.27	1.19±0.63*	1.22±0.30	1.17±0.27	1.13±0.69	2.39±0.63	1.27±0.29	1.30±0.44
Glu (mmol/L)	4.43±0.99	5.04±0.76	5.53±1.13	5.22±0.53	5.09±0.87	5.54±1.11 [†]	5.73±0.66	4.96±0.53	5.47±1.17
Cr (μmol/L)	77.25±15.65	74.19±14.34	80.49±22.15*	85.43±12.11	82.39±15.60	85.84±23.62	66.25±12.90	69.11±10.25	67.71±9.99
BUN (mmol/L)	5.44±1.46	5.41±1.10	5.77±1.65	5.56±1.28	5.51±1.07	5.95±1.67*	5.17±1.76	4.74±1.09	5.34±1.50
UA (μmol/L)	86.03±6.96	83.87±9.38	95.24±5.10 [†]	386.24±73.07	407.62±78.29	421.76±88.49 [†]	287.74±72.94	317.20±82.41	332.69±80.35 [†]

EH, essential hypertensive patients; PH, prehypertension; NT, normotensive subjects; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; Glu, blood glucose; BUN, blood urea nitrogen; Cr, creatinine; UA, uric acid.

* $p<0.05$.

[†] $p<0.01$.

Table 2. Genotype Frequency of the Studied Groups

Index	Total			Male			Female		
	NT	PH	EH	NT	PH	EH	NT	PH	EH
n	472	454	978	290	260	694	182	194	284
A	364 (77.1%)	308 (67.8%)	374 (38.2%)	214 (73.8%)	166 (63.8%)	276 (39.8%)	150 (82.4%)	142 (73.2%)	98 (34.5%)
G	108 (22.9%)	146 (32.2%)	604 (61.8%)	76 (26.2%)	94 (36.2%)	418 (60.2%)	32 (17.6%)	52 (26.8%)	186 (65.5%)
OR	1.605	1.605	5.395	1.594	1.594	4.264	1.717	1.717	8.897
95% CI	1.063-2.242	1.063-2.242	3.783-7.695	0.953-2.668	0.953-2.668	2.780-6.543	0.850-3.465	0.850-3.465	4.686-16.891
p value	0.025	0.025	0.000	0.076	0.076	0.000	0.132	0.132	0.000

EH, essential hypertensive patients; PH, prehypertension; NT, normotensive subjects; OR, odds ratio; 95% CI, 95% confidence interval; n, subject number. All the groups were compared with NT group.

Table 3. Ordinal Logistic Regression Analysis of Blood Pressure and Related Risk Factors

Risk factors	B value	Wald value	p value	95% CI
Age	0.643	5.014	0.025	0.080-1.206
BMI	0.271	29.883	0.000	0.026-0.056
TC	0.329	15.240	0.000	0.164-0.495
LDL-C	0.582	19.352	0.000	0.323-0.841
A/G	-1.271	40.914	0.000	-1.660 - -0.881

BMI, body mass index; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; 95% CI, 95% confidence interval.

($p < 0.01$, data were not shown). Since the expected and observed genotypic frequencies of this SNP was in good agreement with the predicted Hardy-Weinberg equilibrium values. By Ordinal Logistic Regression analysis of blood pressure and related risk factors, A/G was still closely related with blood pressure. Therefore, this SNP may be an independent risk factor and related with high blood pressure. When the subjects in this study were sub grouped by gender, the AT frequency in EH group was still significantly lower than NT group ($p < 0.01$), in both the male and female group. This result was consistent with the results from the total population. The genotypic frequencies of this SNP agreed to the Hardy-Weinberg equilibrium, ; therefore, SNP was independent to sex, or sex related risk factors.¹⁸ This further supported the notion that 5'-UCR -1248 A>G of Mfn2/HSG gene was an independent risk factor of high blood pressure.

Prehypertension was demonstrated as having an increased risk of hypertension, cardiovascular disease and early death from cardiovascular causes. The prehypertension classification of blood pressure was used by the JNC-VII to define a group of individuals at increased risk for cardiovascular events because of elevated blood pressure, an increased burden of other risk factors such as obesity, diabetes mellitus, dyslipidemia, and inflammatory markers. In this study, the frequencies of AT were 77.1%, 67.8%, 38.2%, which were consistent with the BP level in NT, PH and EH groups. Therefore, with higher BP values AT frequency was decreased, and vice versa. 5'-UCR -1248 A>G of Mfn2/HSG gene may be a useful SNP in the study of essential hypertension. However, further studies were needed to explore the association between 5'-UCR -1248 A>G variation and prehypertension in different races using a large sample size.¹⁹

By Ordinal Logistic Regression analysis, we found age, BMI, TC as well as LDL-C, to be closely related with blood pressure. This was consistent with the results in this study and previous studies.^{11,19,20} We also found A/G variation to be a promoter factor of high blood pressure and a detrimen-

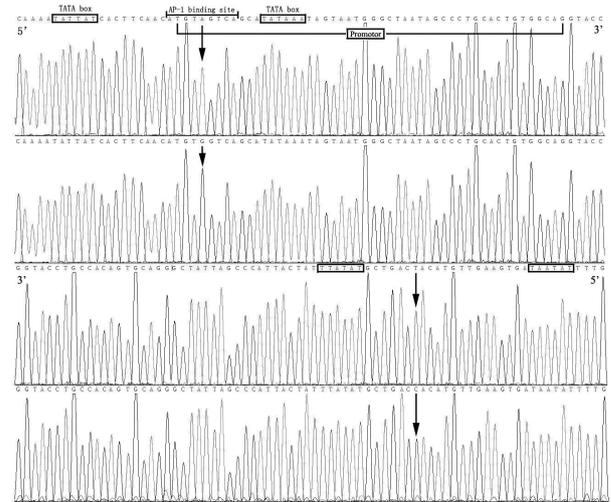


Fig. 1. The -1248 A>G variation of Mfn2 gene in 5'-uncoding region and the transcription factor binding site.

tal factor in healthy participants. Of course, certain limitations in the population under this study may lead to an increased risk of overestimating the statistical significance of minor allele frequency. Accordingly, to enhance the reliability of conclusions, future studies should strive to achieve a more representative population distribution, larger sample sizes, and a wider age distribution.

In conclusion, this study provides new evidence in support of an association between a novel 5'-UCR -1248 A>G variation of Mfn2/HSG gene and hypertension, showing that 5'-UCR -1248 A>G of Mfn2/HSG gene is a novel candidate essential hypertension-associated SNP. Future studies might explore these associations in different ethnic groups and investigate the mechanisms by which the 5'-UCR -1248 A>G of Mfn2/HSG gene might lead to hypertension.

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