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Z. Zhai[1,2] · Z. Wang[2] · L. Wang[2] · S. Chen[2] · H. Ren[2] · D. Wang[1]

[1] Department of Cardiology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

[2] Department of Cardiology, First Affiliated Hospital, Shihezi Medical College, Shihezi University, Shihezi, China

Relationship between inducible NOS single-nucleotide polymorphisms and hypertension in Han Chinese

Hypertension is a critical risk factor for cardiovascular disease [1], which accounts for 20–50% of all deaths [2]. In 2000, it affected 20–30% of the population worldwide, and it is estimated to rise to an alarming 60% by 2025 [3]. In China, the prevalence of hypertension varied from 18.8% in 2002 to 25.2% in 2012. The underlying pathogenetic mechanisms of hypertension are complex because it is affected by the interplay of multiple genetic and environmental factors [4]. The heritability of hypertension is reported to be around 30–60% [5].

As an important vasodilator in the cardiovascular system, nitric oxide (NO) is synthesized by specific NO synthase (NOS) enzymes with three isoforms: neuronal (nNOS, NOS1 gene), inducible (iNOS, NOS2 gene), and endothelial synthases (eNOS, NOS3 gene) [6]. Although iNOS is not constitutive, it plays a key role in the progression of inflammatory conditions [7]. There is evidence that the expression of iNOS can be induced in tissues, especially in inflammatory conditions [8]. There is also strong evidence that hypertension could have an inflammatory background [9]. Thus, iNOS might be linked to hypertension [10].

The iNOS promoter region has been widely studied and genetic polymorphisms in this region have been associated with hypertension [11]. One putative mechanism underlying hypertension-related microvascular dysfunction is through iNOS [12], but no clear consensus has been reached. Considering that different populations vary widely, the present study aimed to investigate for the first time whether iNOS genetic variants are associated with hypertension in a Chinese population.

Patients and methods

Study population

This case-control study comprised 1172 hypertensive patients and 1172 age– (3 years) and sex-matched controls from the First Affliated Hospital, Shihezi Medical College. All subjects in the study were unrelated, of Han origin, and with no history of intermarriage. Hypertensive patients included in the study were defined as having an elevated systolic blood pressure (SBP) of ≥ 140 mm Hg or sustained diastolic blood pressure (DBP) of ≥ 90 mm Hg, or who were already receiving antihypertensive medication. Any patients with secondary hypertension and diabetes were excluded. Control subjects were defined as those with a blood pressure of less than 140/90 mm Hg. All subjects gave written consent to participate in this study, which was conducted according to the Declaration of Helsinki principles and approved by the local ethics committee.

Collection of anthropometric and biochemical data

Blood pressure was measured in the seated position after 10 min of rest by well-trained examiners using a HEM-907 device. Body weight and height were measured with patients dressed in light indoor clothing and without any footwear. Plasma total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) concentrations were determined enzymatically using a commercially available kit and an autoanalyzer (Olympus AU600).

DNA extraction and genotyping analysis

Venous blood was collected from each patient in tubes containing EDTA. The serum was simultaneously isolated and frozen for biochemical assay. Genomic DNA was extracted using a blood extraction kit. Genotyping was undertaken using the TaqMan SNP Genotyping Assay. The primers and probes used in the TaqMan SNP Genotyping Assay (ABI) were chosen based on information available from the ABI website. Thermal cycling was done using Applied Biosystems 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, Calif.).

Table 1 Comparison of demographic and clinical characteristics between hypertensive and normotensive subjects

Characteristics	Hypertensive (n = 1172)	Normotensive (n = 1172)	P
Male (%)	520 (44.4)	520 (44.4)	1.000
Age (year)	55.5 ± 11.2	54.8 ± 11.2	0.1826
SBP mm Hg	141.4 ± 16.7	116.2 ± 11.2	<0.0001
DBP mm Hg	85.8 ± 11.9	71.0 ± 8.2	<0.0001
TC (mmol/l)	5.1 ± 1.0	4.9 ± 1.0	<0.0001
TG (mmol/l)	2.2 ± 2.1	1.7 ± 1.6	<0.0001
HDL-C (mmol/l)	1.4 ± 0.3	1.4 ± 0.3	<0.0001
LDL-C (mmol/l)	3.1 ± 0.8	2.9 ± 0.8	0.0005
BMI (kg/m^2)	27.4 ± 4.1	24.4 ± 3.6	<0.0001
Smoking (%)	406 (34.7)	384 (32.8)	0.3290
Drinking (%)	464 (40.0)	444 (38.2)	0.4046
Family of history of hypertension (%)	639 (54.8)	408 (34.8)	<0.0001

SBP systolic blood pressure, *DBP* diastolic blood pressure, *TC* total cholesterol, *TG* triglycerides, *LDL-C* low-density lipoprotein cholesterol, *HLD*-C high-density lipoprotein cholesterol, *BMI* body mass index

Statistical analysis

The SAS software (version 9.2) was used for data analysis. Hardy–Weinberg equilibrium was assessed by chi-square analysis. Data on the characteristics of all subjects are expressed as means ± SD. The differences between groups were assessed by using a two-tailed Student's t test. Allele frequencies were calculated from genotype frequencies and were compared using chi-squared statistics. Multiple logistic regression analysis was used to estimate the odds ratio (OR) and its 95% confidence interval (CI).

A p value < 0.05 was considered statistically significant.

Results

Characteristics of the study population

In all, 1172 hypertensive patients and 1172 normotensive subjects were included in this analysis. The demographic and clinical characteristics of the study population are shown in **Table 1**. There was a significant difference between the hypertensive and normotensive groups with respect to SBP, DBP, TC, TG, HDL-C, LDL-C, BMI, and family of history of hypertension (p < 0.01). No differences were found in sex, age, smoking, and drinking between the two groups.

Distribution of allele frequencies of the iNOS gene

All the genotype distributions were in accordance with Hardy–Weinberg equilibrium. The analyses of the allelic distribution of rs2779249 and rs2297518 are shown in **Table 2**. There were significant differences in the distribution of genotype and allele frequencies of rs2779249 and rs2297518 between hypertensive and normotensive subjects (p < 0.01). The frequency of the A allele of rs2779249 (21.0% vs. 18.6%) and rs2297518 (12.1% vs. 7.3%) was higher in the hypertensive than in the normotensive group (p < 0.01).

Association of iNOS gene with hypertension under different genetic models

Logistic regression analyses were performed with different genetic models (additive, dominant, recessive) adjusting for confounding risk covariates, including age, sex, BMI, TC, TG, HDL-C, LDL-C, smoking, drinking, and family history of hypertension. The results of the logistic regression analysis are shown in **Table 3**. The analysis showed that rs2779249 and rs2297518 were significantly associated with hypertension in both the additive – rs2779249:1.27 (1.12, 1.44); rs2297518:1.26 (1.06, 1.50) – and dominant models –rs2779249:1.31 (1.09, 1.59); rs2297518:1.46 (1.13, 1.89). There was a significant difference between rs2779249 and hypertension in the recessive model: 1.68 (1.28, 2.19). No significant association was found between rs2297518 and hypertension in the recessive model.

Discussion

Hypertension results from the interplay of a variety of complex pathogenetic mechanisms. It is suggested that increased oxidative stress plays an important role in the development of hypertension [13]. Reactive oxygen species (ROS) can generate peroxynitrite, which promotes endothelial dysfunction [14]. This reaction is followed by excessive amounts of iNOS-derived NO that is usually accompanied by increased iNOS expression [15]. iNOS polymorphism in exon 16 results in an amino acid substitution from serine to leucine, which increases iNOS activity and promotes excessive NO formation and inflammation [16].

Increased iNOS expression was evidenced in the cutaneous microvasculature of hypertensive patients compared with normotensive subjects, i.e., this process may be related to iNOS upregulation [17]. Moreover, another study showed increased macrophage iNOS activity in hypertensive patients compared with normotensive subjects, thus further supporting the idea that iNOS plays an important role in the pathogenesis of hypertension [18].

To the best our knowledge, this is the first study to investigate the association of iNOS with hypertension in a sample of Chinese Han individuals. The distributions of the rs2779249 and rs2297518 genotypes were in accordance with Hardy–Weinberg equilibrium in both groups, and the genotype distribution was significantly different between the two groups. The frequency of the A allele of rs2779249 was higher in the hypertensive group than in the nor-

motensive group, while the C allele was higher in the normotensive group than in the hypertensive group. The frequency of the A allele of rs2297518 was higher in the hypertensive group than in the normotensive group, while the G allele was higher in the normotensive group than in the hypertensive group.

Our results show that there is an association between the SNPs rs2779249 and rs2297518and hypertension. Previous reports indicated that SNP rs2779249 was significantly associated with hypertension, which we also found in our study [19]. High NO levels produced by iNOS can impair cell respiration and induce cytotoxic mechanisms in the cardiovascular system [20]. This results in cardiac depression and has several other detrimental effects such as endothelial injury and multiorgan failure [21]. In view of the studies mentioned here, it is highly likely that iNOS upregulation leads to cardiovascular disorders and therefore iNOS inhibition could have protective effects against clinical events [21].

Increasing vascular iNOS expression and activity contributes to the pathogenesis of hypertension [22], which was also supported by experimental evidence in spontaneously hypertensive rats, and thus it was inferred that iNOS could be used as a potential therapeutic target in the treatment of hypertension.

SNP rs2297518 was significantly associated with hypertension, which was not consistent with other studies [19]. This discrepancy between findings may be due to the epigenetic mechanisms [23] that are involved in gene expression, influenced by environmental conditions such as lifestyle and diet [24].

Limitations

Several potential limitations of the present study should be acknowledged. First, the study had a small sample size. Indeed, a small sample with limited participants might lack sufficient power to support any association between the parameters studied [25]. Further large-scale and population-based studies are warranted to confirm the findings. Second, as a case-control study, potential bias – including information bias and

Abstract · Zusammenfassung

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Relationship between inducible NOS single-nucleotide polymorphisms and hypertension in Han Chinese

Abstract

Background. Inducible nitric oxide synthase (iNOS) single-nucleotide polymorphisms have been reported to confer susceptibility to hypertension, but no consensus has been reached. The aim of this study was to investigate the association between iNOS and hypertension in a Chinese population.
Methods. This was a case-control study including 1172 hypertensive and 1172 control subjects to investigate the association between iNOS and hypertension.
Results. There were significant differences in the distribution of genotype and allele frequencies of rs2779249 and rs2297518 between hypertensive and normotensive subjects. Logistic regression analyses were performed with different genetic models (additive, dominant, recessive) adjusting for confounding risk covariates, including age, sex, body mass index, total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, smoking, drinking, and family history of hypertension. The odds ratio (OR) was 1.27 (1.12, 1.44) in the additive model, 1.31 (1.09, 1.59) in the dominant, and 1.68 (1.28, 2.19) in the recessive model of rs2779249; the OR was 1.26 (1.06, 1.50) in the additive model and 1.46 (1.13, 1.89) in the dominant model of rs2297518.
Conclusion. The current study provides evidence that iNOS is strongly associated with hypertension.

Keywords
Nitric oxide synthetase · Blood pressure, high · Chinese · Inflammation · SNPs

Zusammenhang zwischen iNOS-Einzelnukleotidpolymorphismen und Hypertonie bei Han-Chinesen

Zusammenfassung

Hintergrund. Von Einzelnukleotidpolymorphismen der induzierbaren Stickstoffmonoxidsynthase (iNOS) wird berichtet, dass sie mit der Neigung zur Hypertonie einhergehen, darüber herrscht jedoch noch kein Konsens. Ziel der vorliegenden Studie war es, den Zusammenhang zwischen iNOS und Hypertonie in einer chinesischen Population zu untersuchen.
Methoden. Es handelt sich um eine Fall-Kontroll-Studie zur Untersuchung des Zusammenhangs zwischen iNOS und Hypertonie an 1172 Teilnehmern mit Hypertonie und 1172 Kontrollen.
Ergebnisse. Zwischen Teilnehmern mit Hypertonie und Kontrollen mit Normotonie bestanden signifikante Unterschiede in Bezug auf die Verteilung der Genotyp- und Allelhäufigkeiten von rs2779249 und rs2297518. Logistische Regressionsanalysen wurden mit verschiedenen genetischen Modellen (additiv, dominant, rezessiv) durchgeführt, unter Berücksichtigung von störenden Risikokovariaten wie Alter, Geschlecht, Body-Mass-Index, Gesamtcholesterin, Triglyzeride, High-Density-Lipoprotein-Cholesterin, Low-Density-Lipoprotein-Cholesterin, Rauchen, Alkohol und Hypertonie in der Familienanamnese. Die Odds Ratio (OR) betrug 1,27 (1,12; 1,44) im additiven Modell, 1,31 (1,09; 1,59) im dominanten und 1,68 (1,28; 2,19) im rezessiven Modell von rs2779249; die OR lag bei 1,26 (1,06; 1,50) im additiven Modell und bei 1,46 (1,13; 1,89) im dominanten Modell von rs2297518.
Schlussfolgerung. Die vorliegende Studie liefert Hinweise darauf, dass ein deutlicher Zusammenhang zwischen iNOS und Hypertonie besteht.

Schlüsselwörter
Stickstoffmonoxidsynthetase · Hoher Blutdruck · Chinesen · Entzündung · SNPs

Table 2 Distribution of iNOS polymorphisms in the hypertensive and normotensive groups

		Genotype (*N*, %)				Allele (*N*, %)		
SNP	Group	AA	AC	CC	*p*	A	C	*p*
rs2779249	Hypertensive	192 (16.4)	270 (23.0)	710 (60.6)	0.001	654 (27.9)	1690 (72.1)	<0.0001
	Normotensive	132 (11.3)	266 (22.7)	774 (66.0)	–	530 (22.6)	1814 (77.4)	–
–	–	AA	AG	GG	*p*	A	G	*p*
rs2297518	Hypertensive	93 (7.9)	118 (10.1)	961 (82.0)	0.0006	304 (13.0)	2040 (87.0)	<0.0001
	Normotensive	55 (4.7)	93 (7.9)	1024 (87.4)	–	203 (8.7)	2141 (91.3)	–

Table 3 Association of iNOS polymorphisms with hypertension in different genetic models

SNP	Model	Genotype	*N*	OR (95% CI)	OR (95% CI)[a]	OR (95% CI)[b]
rs2779249	Additive	AA/AC/CC	324/536/1484	1.22 (1.09, 1.37)	1.27 (1.12, 1.44)	1.26 (1.11, 1.43)
	Dominant	AA + AC/CC	860/1484	1.27 (1.07, 1.50)	1.31 (1.09, 1.59)	1.30 (1.08, 1.57)
	Recessive	AA/AC + CC	324/2020	1.54 (1.22, 1.96)	1.68 (1.28, 2.19)	1.65 (1.27, 2.15)
rs2297518	Additive	AA/AG/GG	148/211/1985	1.35 (1.15, 1.57)	1.26 (1.06, 1.50)	1.27 (1.07, 1.51)
	Dominant	AA + AG/GG	359/1985	1.52 (1.21, 1.91)	1.46 (1.13, 1.89)	1.46 (1.13, 1.89)
	Recessive	AA/AG + GG	148/2196	1.75 (1.24, 2.47)	1.37 (0.93, 2.02)	1.37 (0.93, 2.02)

[a]Denotes adjusting for age, sex, BMI, TC, TG, HDL-C, LDL-C, smoking, drinking, and family of history of hypertension
[b]Denotes adjusting for age, sex, BMI, smoking, drinking, and family of history of hypertension
OR odds ratio, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *TC* total cholesterol, *TG* triglycerides, *LDL-C* low-density lipoprotein cholesterol, *HLD*-C high-density lipoprotein cholesterol, *BMI* body mass index

selection bias – may have led to confounding. Third, there are potential gene–gene and gene–environment interactions between the polymorphisms and hypertension.

Conclusion

Our study found a significant association of SNP with hypertension even after adjusting for potential confounders such as age, sex, BMI, TC, TG, HDL-C, LDL-C, and a history of smoking and drinking.

Corresponding address

Z. Wang
Department of Cardiology, First Affiliated Hospital, Shihezi Medical College, Shihezi University
832000 Shihezi, Xinjiang, China
Wangzhong_1234@126.com

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Compliance with ethical guidelines

Conflict of interest. Z. Zhai, Z. Wang, L. Wang, S. Chen, H. Ren, and D. Wang declare that they have no competing interests.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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