The association of an adenine insertion variant in the 5' UTR of the endothelin-1 gene with hypertension and orthostatic hypotension

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Abstract

Introduction: An adenine insertion polymorphism in the 5' untranslated region of the endothelin-1 gene is functional and increases the expression of endothelin mRNA and protein in the insertion homozygote. In the present study we hypothesized that this functional polymorphism might be associated with hypertension and/or orthostatic hypotension.

Material and methods: The adenine insertion polymorphism was genotyped in 381 untreated hypertensive patients and 298 normotensive subjects, all of whom underwent an upright posture study for orthostatic blood pressure measurements. Orthostatic hypotension was defined as a drop in blood pressure of 20/10 mm Hg or more within 3 min of assuming the upright posture.

Results: The allele frequency of the adenine insertion was similar in hypertensive and normotensive subjects (0.152% vs. 0.153%, \( p > 0.05 \)). After adjustment for age, sex and body mass index, blood pressure levels did not differ significantly among the genotypes in both hypertensives and normotensives. No associations were found between the distribution of the adenine insertion genotypes and the risk of orthostatic hypotension in both hypertensive patients and normotensive subjects even after adjustment for demographic parameters and supine systolic or diastolic blood pressure. Neither hypertensive nor normotensive subjects showed significant differences in orthostatic systolic or diastolic blood pressure changes among the genotype groups (all \( p > 0.05 \)).

Conclusions: We concluded that the functional adenine insertion polymorphism in the endothelin-1 gene is not associated with either hypertension or orthostatic hypotension risk in Chinese.

Key words: polymorphism, genotype, blood pressure.

Introduction

Endothelin-1 (ET-1) is one of the most important vasoconstrictor peptides in the human vascular system [1], where it appears to play a fundamental role in the maintenance of basal vasomotor tone [2, 3]. The biological effects of ET-1 are mediated through the activation of two known ET-1 receptors, ET-A and ET-B [4, 5]. The ET-1 is thought to be involved in blood pressure (BP) regulation because of the elevated plasma ET-1 levels...
that have been observed in some hypertensive patients [6] and because of the reported elevated BP in mice heterozygous for a knockout of the ET-1 gene (Edn1) [7]. In mice, the knockout of collecting duct ET-1 or the combined ET-A and ET-B receptors also caused hypertension on both a normal and high sodium diet [8, 9]. In normal human subjects, upright tilt did not change arterial pressure but it did increase the plasma concentrations of ET-1 and vasopressin [10, 11]. The increase in plasma ET-1 concentration was impaired in patients with autonomic failure [11] as well as in patients with tilt-induced syncope [12]. Therefore, the Edn1 gene is a logical candidate to play a role in hypertension and orthostatic hypotension (OH).

The ET-1 is produced in endothelial cells and is predominantly secreted toward the adjacent vascular smooth muscle cells, supporting the notion that endothelins (ETs) are autocrine/paracrine agents rather than circulating hormones [13]. Elevated ET-1 levels have been found in some but not all studies of patients with essential hypertension [14, 15]. Furthermore, increased ET-1 levels are neither related to BP in hypertensive patients nor do they induce hypertension in patients with diabetes [15]. Edn1 mRNA is upregulated by inflammatory factors and downregulated by nitric oxide (NO), prostacyclin (PGI2), hypoxia and shear stress [16]. An adenine insertion, located in the 5' untranslated region (UTR) 138 bp downstream of the transcription start site, is functional and regulates the expression of Edn1 causing increased mRNA expression and endothelin protein expression in insertion homozygotes [17]. This polymorphism has been associated with orthostatic intolerance [18] and diastolic BP level in white people [19]. However, previous studies have produced inconsistent results for the association of hypertension with Edn1 polymorphisms [20, 21] and few data are available on the association of the adenine insertion polymorphism with OH. We therefore hypothesized that the adenine insertion variant of Edn1 may be associated with increased risk of hypertension and OH in Chinese people. We tested our hypothesis in 381 untreated hypertensive patients and 298 normotensive subjects.

Material and methods

Study population

For this study, we recruited 381 unrelated hypertensive patients and 298 normotensive subjects from Xinyang in Henan Province. To exclude the effect of antihypertensive drugs on orthostatic BP regulation, only untreated patients were included in the study. Hypertensive patients were defined as untreated if they were newly diagnosed with hypertension (systolic BP (SBP)/diastolic BP (DBP) ≥ 160/95 mm Hg on 3 occasions within 2 months) and/or if they had been diagnosed as hypertensive (SBP/DBP ≥ 140/90 mm Hg) in the past but had not received any antihypertensive drugs for at least 8 weeks prior to the study. Subjects with systolic and/or diastolic BP levels < 130/85 mm Hg and no family history of hypertension were recruited as normotensive subjects. Subjects were excluded from the study if they had any known diseases, including heart failure, secondary hypertension, Parkinson disease, diabetes mellitus, severe debilitating chronic illness (cancer, renal or hepatic diseases), and any history of coronary heart disease and stroke, and/or if they were currently receiving antidepressant medications. All subjects underwent an upright posture study for orthostatic BP measurements. This study was reviewed and approved by the ethical committees of FuWai Hospital and local hospitals, and informed consent was obtained from each subject before they were recruited.

Data collection

Each eligible participant was interviewed in a community clinic. Anthropometric measurements, height (m) and weight (kg), and waist and hip circumference (measured at the umbilicus and the widest point, respectively), were obtained by trained researchers. All subjects underwent a standard 12-lead ECG. Overnight fasting blood was drawn for assays of fasting blood glucose, blood lipids [total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C)], serum uric acid and creatinine levels by the core laboratory at FuWai Hospital. Medical history and cigarette smoking and alcohol consumption status were obtained using a standardized questionnaire. Obesity was defined according to World Health Organization criteria as body mass index (BMI) ≥ 30.0 kg/m². Metabolic syndrome (MS) was determined according to the International Diabetes Federation (IDF) criteria of 2005. Dyslipidemia was diagnosed as any one of the following: TG > 200 mg/dl; TC ≥ 240 mg/dl; LDL-C ≥ 160 mg/dl; or HDL-C < 40 mg/dl, according to the Adult Treatment Panel III guideline [22].

Sitting BP was measured by a trained nurse or physician with a standardized mercury sphygmomanometer and appropriate cuff sizes (regular, large, or thigh) fitted to the subject’s right arm. Three readings were recorded in a sitting position at least 30 s apart after more than 5 min of rest, and three readings on average were analyzed. All BP investigators had to complete a training program on the preparation of study subjects for measuring BP, selection of correct cuff size, and standard BP measurement technique according to a common protocol adapted from procedures recommended by the American Heart Association [23].
Supine and standing BP were recorded with a mercury sphygmomanometer following a standardized protocol by a trained physician or nurse. After a 15-min ECG examination with the participant lying on an examination table, three supine measurements of BP and heart rate were recorded at approximately > 30 s intervals by a trained professional. Participants were then asked to rise from the supine position with the entire forearm relaxed and supported at heart level on an adjustable table, and standing measurements were taken at 30 s and 2 min. OH was defined as a decline in SBP of at least 20 mm Hg and/or a decline in DBP of at least 10 mm Hg either 30 s or 2 min after shifting from a supine to an upright posture [24].

Genotyping

Genomic DNA was isolated from peripheral leukocytes. The adenine insertion/deletion polymorphism (rs10478694, also described as 4A/3A polymorphism) 138 bp downstream of the transcription start site in the 5' UTR of EDN1 was detected by standard polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) analysis, gel electrophoresis, and ethidium bromide staining. All aspects of the DNA source, preparation and genotyping were controlled using the paradigms of blindness and randomization. The reproducibility of the genotyping was confirmed by bidirectional sequencing in 100 randomly selected samples, and was found to be 100%. The primer sequences used were as follows: forward, 5'-AGCTCCTTGGCAAGCCACAAACAGCA-3'; and reverse, 5'-AGGCGCTGCCTTTTCTCCCCGTTTAA-3'. The resultant PCR products were digested using the restriction enzyme DraI (New England Biolabs). The genotypes were 3A/3A, 221 bp; 4A/3A, 197 bp, 221 bp and 24 bp; and 4A/4A, 197 bp and 24 bp.

Statistical analysis

All of the data were analyzed with SPSS statistical software (version 13.0; SPSS USA Inc). Quantitative variables were compared using one-way analysis of variance (ANOVA), and Tukey’s test or t-test was used for comparison of the mean values for pairs of groups. The \( \chi^2 \) test was used for qualitative variables, genotype/allele frequencies, and for the Hardy-Weinberg equilibrium of polymorphisms. Stepwise multiple logistic regression analysis was used to assess the contribution of genotypes to hypertension or OH with adjustment for age, BMI, supine BP levels, heart rates, fasting blood glucose and dyslipidemia. The orthostatic BP changes among genotypes were compared first by ANOVA or t test, and then by a general linear model with adjustment for age, sex, BMI, and supine BP levels. A two-tailed \( p \) value of < 0.05 was considered significant. Assuming an additive model, the study had more than 80% statistical power to detect an association (at \( p = 0.05 \)) with an odds ratio (OR) of 1.5-1.75 for alleles at 10-20% frequency, which indicated a low probability of obtaining a false negative result.

Results

The association of the EDN1 3A/4A polymorphism with hypertension risk

Table I summarizes the characteristics of hypertensive patients and normotensive subjects. As expected, SBP, DBP and most of the other characteristics were higher in hypertensive patients than in normotensive subjects. The EDN1 3A/4A genotypes were in Hardy-Weinberg equilibrium in the hypertensive patients (\( \chi^2 = 2.74, p = 0.10 \)) and in the normotensive subjects (\( \chi^2 = 3.29, p = 0.07 \)). However, no significant differences were found in the genotype/allele frequencies of the 3A/4A polymorphism between hypertensive and normotensive subjects in either the additive, dominant or recessive models (Table II). After adjustment for age, BMI, heart rates, fasting blood glucose, plasma lipids and other conventional risk factors, the association of hypertension with BMI and heart rates remained statistically significant but the adenine insertion polymorphism was still not found to be associated with hypertension risk.

When the systolic and diastolic BP levels were analyzed separately in hypertensive and normotensive controls.

Table I. Clinical characteristics of hypertensive patients and normotensive controls

| Characteristic | Normotensive controls (n = 298) | Hypertensive patients (n = 381) | Value of p |
|---------------|---------------------------------|-------------------------------|------------|
| Gender, M/F   | 107/191                         | 133/248                       | NS         |
| Age [year]    | 53.1 ±10.5                      | 55.3 ±8.2                     | < 0.05     |
| BMI [kg/m²]   | 24.3 ±3.3                       | 25.9 ±3.5                     | < 0.05     |
| SBP [mm Hg]   | 124.0 ±11.8                     | 160.2 ±22.8                   | < 0.05     |
| DBP [mm Hg]   | 80.5 ±6.9                       | 98.5 ±11.2                    | < 0.05     |
| Heart rate [bpm] | 73.8 ±12.2                    | 69.7 ±11.2                    | < 0.05     |
| HDL-C [mmol/l] | 1.50 ±0.31                      | 1.57 ±0.34                    | < 0.05     |
| LDL-C [mmol/l] | 2.65 ±0.81                      | 3.10 ±0.88                    | < 0.05     |
| TC [mmol/l]   | 4.97 ±1.02                      | 5.49 ±1.15                    | < 0.05     |
| FBG [mmol/l]  | 4.97 ±1.69                      | 5.50 ±1.62                    | < 0.05     |
| Smoking [%]   | 20.1                            | 16.5                          | NS         |
| Drinking [%]  | 21.8                            | 21.0                          | NS         |

BMI – body mass index, SBP – systolic blood pressure, DBP – diastolic blood pressure, HDL-C – high-density lipoprotein cholesterol, LDL-C – low-density lipoprotein cholesterol, TC – total cholesterol, FBG – fasting blood glucose, smoking, current and past cigarette smoking, drinking, current and past alcohol drinking.
motensive subjects, the results showed that the hypertensive and normotensive subjects carrying different 3A/4A genotypes had similar systolic and diastolic BP levels even after adjustment for age, sex, and body mass index (Figure 1).

The association of the EDN1 3A/4A polymorphism with OH in hypertensive and normotensive subjects

Most of the measured characteristics were comparable between the patients with and without OH except that normotensive subjects with OH had significantly lower SBP and DBP than those without OH (Table III). Both hypertensives and normotensives exhibited a similar pattern of BP response to posture change: higher supine SBP or DBP levels and lower standing SBP or DBP levels in all subjects with OH compared to the levels in subjects without OH.

The results in Table IV show that no significant differences were found in the genotype/allele frequencies of the 3A/4A polymorphism between subjects with and without OH in both hypertensives and normotensives in the additive, dominant and recessive models. After adjustment for supine SBP or DBP levels, heart rates, and conventional risk factors using a stepwise multiple logistic regression model, the contribution of the EDN1 adenine insertion allele to the presence of OH remained statistically not significant in the hypertensive and normotensive subjects. A statistically significant association was found between OH and supine systolic BP in hypertensive patients (OR 1.03, 95% CI 1.00-1.06, \(p = 0.049\)) and in normotensive subjects (OR 1.04, 95% CI 1.02-1.06, \(p < 0.001\)).

We then analyzed the associations between the EDN1 3A/4A polymorphism and orthostatic SBP/DBP changes at 30 s and 2 min after standing as well as supine SBP/DBP in both hypertensive and normotensive subjects; no association was found even after adjustment for age, sex, BMI and supine SBP or DBP using a general linear model (Table V).

Discussion

In the present study, we investigated the association of the adenine insertion polymorphism in the 5' UTR of the EDN1 gene with hypertension and OH risk. After adjustment for age, sex, BMI, and the other conventional risk factors that were measured, the EDN1 adenine insertion polymorphism was found not to be associated with hypertension risk or with systolic and diastolic BP levels. In both hypertensive and normotensive subjects, no asso-

**Table II. ET1 3A/4A genotypes in hypertensive and normotensive subjects**

| Subject       | N    | ET1 genotype frequencies n (%) |Allele frequencies n (%) | Adjusted OR (95% CI) |
|---------------|------|--------------------------------|-------------------------|----------------------|
|               |      | 3A/3A 3A/4A 4A/4A p (d/r)* | 3A 4A p | Dominant model (3A ↔ 4A) | 3A/4A + + 4A/4A ↔ ↔ 3A/3A |
| Hypertensives | 381  | 278 (73.0) 90 (23.6) 13 (3.4) | 0.97 646 (84.8) 116 (15.2) | 0.98 1.00 0.99 | (0.75-1.35) (0.78-1.29) |
| Normotensives | 298  | 218 (73.2) 69 (23.2) 11 (3.7) | (0.95/0.84) 505 (84.7) 91 (15.3) | (0.78-1.29) | |

*(d/r) p values under dominant/recessive models

![Figure 1](image_url) Mean SBP and DBP levels in individuals carrying EDN1 3A/4A genotypes. Values were adjusted for age, sex and body mass index using a general linear model. A – Hypertensive patients. B – Normotensive subjects
The association of an adenine insertion variant in the 5' UTR of the endothelin-1 gene with hypertension and orthostatic hypotension

Association was found between the EDN1 3A/4A polymorphism and OH or between the EDN1 3A/4A polymorphism and orthostatic SBP/DBP changes at 30 s and at 2 min after standing.

The ET-1 is a powerful controller of vasomotor tone. A recent study reported normal BP in mice systemically heterozygous for the ET-1 null allele and decreased BP in endothelial cell-specific ET-1 knockout mice [25]. It has been found that increased ET-1 activity could impair endothelium-dependent vasodilator function in hypertensive patients [26]. These results indicated that ET-1 played an important role in the regulation of BP and suggested that the functional EDN1 genetic variation may contribute to the development and variation of BP. The EDN1 3A/4A polymorphism was found to be of functional importance for ET-1 expression [17]. It has also been associated with smoking in hypertensive patients and with less risk for chronic heart failure [27, 28]. However, the association of the 3A/4A polymorphism with hypertension needs to be investigated further because the results from the present study and from previous studies found no significant difference in the allele or genotype frequency between the hypertensive patients and the normotensive controls [21, 28, 29].

In a genome-wide association study, a few loci (chromosome regions) associated with BP at a genome-wide level of statistical significance have been reported [30, 31]. However, no genetic vari-

### Table II. Clinical characteristics of hypertensive and normotensive subjects with and without OH

| Characteristics          | Hypertensive patients | Normotensive subjects |
|--------------------------|-----------------------|-----------------------|
|                         | With OH | Without OH | With OH | Without OH |
| N                       | 78      | 303         | 72      | 226         |
| Age [years]             | 55.6 ±7.9 | 55.0 ±8.7 | 51.3 ±10.3 | 53.5 ±10.9 |
| > 60 years, % (n)       | 31.0 (24) | 27.1 (82)  | 20.8 (15) | 27.9 (63)   |
| Male, % (n)             | 32.1 (25) | 36.0 (109) | 37.5 (27) | 41.2 (93)   |
| BMI [kg/m²]             | 26.0 ±3.6 | 25.9 ±3.4 | 24.6 ±3.5 | 24.2 ±3.2   |
| Seated SBP [mm Hg]      | 162.3 ±20.0 | 160.6 ±23.1 | 121.4 ±12.7 | 124.8 ±11.3^* |
| Seated DBP [mm Hg]      | 96.8 ±10.9 | 98.4 ±11.7 | 78.4 ±7.8  | 81.1 ±7.5^*  |
| HR [bpm]                | 75.4 ±12.5 | 73.0 ±12.0 | 69.1 ±9.3  | 69.8 ±11.4   |
| Dyslipidemia, % (n)     | 26.9 (21) | 32.0 (97)  | 23.6 (17) | 27.0 (61)   |
| MS, % (n)               | 21.8 (17) | 26.7 (81)  | 12.5 (9)   | 12.4 (28)   |
| Obesity, % (n)          | 16.7 (13) | 11.0 (33)  | 5.6 (4)    | 4.9 (11)    |
| Supine SBP [mm Hg]      | 162.9 ±24.8 | 153.6 ±24.7^* | 130.0 ±15.3 | 124.5 ±14.8^* |
| Supine DBP [mm Hg]      | 99.2 ±15.6 | 91.9 ±12.3^* | 83.4 ±10.0  | 79.2 ±8.7^*   |
| Standing SBP at 30 s [mm Hg] | 146.5 ±22.3 | 153.2 ±24.8^* | 113.4 ±15.3 | 124.2 ±14.7^* |
| Standing DBP at 30 s [mm Hg] | 91.2 ±12.9 | 96.9 ±12.7^* | 73.7 ±10.1  | 81.8 ±9.8^*   |
| Standing SBP at 2 min [mm Hg] | 146.3 ±22.1 | 155.4 ±24.6^* | 114.3 ±15.7 | 125.3 ±14.8^* |
| Standing DBP at 2 min [mm Hg] | 93.7 ±12.7 | 98.5 ±13.9^* | 74.6 ±8.6  | 83.1 ±9.9^*   |

*OH – orthostatic hypotension, BMI – body mass index, SBP – systolic blood pressure, DBP – diastolic blood pressure, MS – metabolic syndrome, |p < 0.05 patients with OH vs. patients without OH

### Table IV. Prevalence of OH among ET1 genotypes in hypertensive and normotensive subjects

| Subject | N          | Genotype frequencies n (%) | Allele frequencies n (%) |
|---------|------------|-----------------------------|--------------------------|
|         |            | 3A/3A | 3A/4A | 4A/4A | p (r/d)* | 3A  | 4A  | p  |
| Hypertensives |           |            |       |       |         |     |     |    |
| OH      | 78         | 59 (75.6) | 15 (19.2) | 4 (5.1) | 0.42 (0.55/0.35) | 133 (85.3) | 23 (14.7) | 0.85 |
| Non OH  | 303        | 219 (72.3) | 75 (24.8) | 9 (3.0) | 513 (84.7) | 93 (15.3) |       |    |
| Normotensives |         |            |       |       |         |     |     |    |
| OH      | 72         | 54 (75.0) | 16 (22.2) | 2 (2.8) | 0.86 (0.68/0.64) | 124 (86.1) | 20 (13.9) | 0.60 |
| Non OH  | 226        | 164 (72.6) | 53 (23.5) | 9 (4.0) | 381 (84.3) | 71 (15.7) |       |    |

*(d/r) p values under dominant/recessive models
The ET-1 induces multiple effects on vascular tone by binding to two kinds of receptors on endothelial or smooth muscle cells. The ET-A receptors are highly expressed in vascular smooth muscle cells and appear to be the major receptor subtype causing vasoconstriction in human arteries. The ET-B receptors on endothelial cells mediate vasodilation by releasing NO and prostacyclin and they in turn inhibit ET converting enzyme-1 (ECE-1) expression in endothelial cells and play an important role in ET-1 clearance [42, 43]. The infusion of ET-1 into intact rats caused transient hypotension related to an increase in the production of endothelium-derived relaxing factor and prostacyclin and their release from vascular endothelial cells [44, 45]. All this evidence suggested that, in addition to endothelial integrity, the balance between the distribution of ET-A and ET-B receptors may determine the ET-1-dependent regulation of vascular tone. Investigations of the association between another \textit{EDN1} polymorphism (Lys198Asn) and BP levels have also produced inconsistent results [46-48]. Clearly, further investigations into the association of the vascular endothelin system with BP regulation are needed.
Our study had several strengths that included the consistent results found in both hypertensive patients and normotensive subjects for the association of the 3A/4A polymorphism with OH and orthostatic BP changes, and the exclusion of any antihypertensive drug effect because only untreated hypertensive patients were enrolled. A limitation of our study was that the plasma or urine levels of ET-1 were not measured. Previous studies have shown that ETs are autocrine/paracrine agents rather than circulating hormones, and plasma levels of ETs have been found to be poorly correlated with the hypertensive state. Another limitation of the present study was the focus on only one functional polymorphism of the ET-1 gene. Further research is needed to investigate the association between OH and other polymorphisms of the genes that encode the vascular endothelin system components (ET-1, and the ET-A and ET-B receptors).

In conclusion, we found no evidence that the insertion variant in the 5’ UTR of the EDN1 was associated with a genetic predisposition to hypertension and OH risk. Our data suggested that the role of ET-1 in BP regulation needs to be evaluated further in future studies.

Acknowledgments

This work was financially supported by a grant from the National Natural Science Foundation of China (Grant No. 30871054) to Dr Xiao-han Fan, and by a grant from the Ministry of Science and Technology of China (Grant No. 2006CB503805) to Dr Hui Rutai.

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