Original Article

Association between Hepatocyte Growth Factor Gene Polymorphism and Essential Hypertension

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Hepatocyte growth factor (HGF) is a growth factor which contributes to protection and/or repair of vascular endothelial cells. Serum HGF level is elevated in response to hypertensive organ damage, which suggests that blood pressure regulation may be affected by HGF gene polymorphisms *via* serum HGF. To examine the interaction between a HGF gene polymorphism and hypertension, we carried out a case-control study. The present study was conducted in outpatients of Osaka University Hospital. Subjects ($n=654$) who gave informed consent to the study protocol and genetic analysis were recruited. A C to A nucleotide substitution in intron 13 of the HGF gene was determined by the TaqMan polymerase chain reaction (PCR) method using an MGB (Minor Groove Binder) probe. The genotype distribution of the C/A polymorphism of the HGF gene in total subjects was as follows: CC, 83%; CA, 16%; and AA 1%. This distribution was not significantly different from the predicted by Hardy-Weinberg's equilibrium. The prevalence of hypertension was significantly higher in subjects with the CC genotype than in those with an A allele, and the positive association remained after adjustment for confounding factors, with the estimated odds ratio for hypertension (CC vs. CA + AA) being 1.71 (95% confidence interval: 1.02–2.93). A significant association with hypertension was observed in lean or female subjects but not in obese or male subjects. In conclusion, our data suggested that C/A polymorphism in intron 13 of the HGF gene is associated with susceptibility to essential hypertension in lean or female subjects. (*Hypertens Res* 2004; 27: 247–251)

Key Words: hepatocyte growth factor, genetics, essential hypertension, TaqMan polymerase chain reaction, single nucleotide polymorphism

Introduction

The presence of endothelial dysfunction is common in hypertensive patients and plays an important role in the pathogenesis of essential hypertension (1–3). Endothelial cells are known to secrete various antiproliferative and vasodilating

factors, such as nitric oxide (NO), vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF). HGF is a mesenchyme-derived pleiotropic factor which regulates cell growth, motility and morphogenesis of various types of cells. Serum HGF concentration is elevated in response to several disease states, such as hepatitis, nephritis, diabetic retinopathy and hypertension (4–9). It has been re-

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ported that serum HGF concentration is positively correlated with the prevalence of hypertension, severity of hypertension, systolic blood pressure (SBP) and carotid artery remodeling (10–15). Recent papers have also reported that serum HGF concentration was associated with night-time blood pressure, especially for non-dipper type patients (16), and with the vasodilator response to reactive hyperemia as an index of endothelial function measured by strain-gauge plethysmography, but not with pulse wave velocity (PWV) as an index of arterial stiffness (17).

On the other hand, the presence of a local HGF production system has been described in various tissues, including blood vessels (18–20) and cardiac myocytes (21, 22). The local HGF production system is regulated *via* several growth factors, such as transforming growth factor- β (TGF- β), angiotensin-II, fibroblast growth factor-2 (FGF-2) and HGF itself. Whereas TGF- β and angiotensin-II strongly suppress the local HGF production, FGF-2 and HGF itself upregulate it (23–28). In addition, endothelin-1 production is downregulated by serum HGF *via* the tyrosine kinase activity of the HGF receptor, c-met (29). Therefore, HGF may play a key role in endothelial dysfunction in the pathogenesis of hypertension. In order to clarify the effects of a HGF gene polymorphism on hypertension, we carried out a hospital-based case-control study.

Methods

Study Population

The study protocol was approved by the Ethical Committee for Human Genome Analysis of Osaka University, and subjects who gave informed consent for genetic analysis were recruited from among the outpatients and inpatients of Osaka University Hospital. According to the criteria of the sixth report of the Joint National Committee on Prevention, Detection, and Treatment of High Blood Pressure (JNC/VI), hypertension was defined as a mean SBP of ≥ 140 mmHg, a mean diastolic blood pressure (DBP) of ≥ 90 mmHg, or current administration of antihypertensive medication. After exclusion of the subjects with secondary hypertension or heart failure, we recruited 278 hypertensive subjects in this study. Normotensives ($n = 376$) were defined as those with an SBP < 140 mmHg, a DBP < 90 mmHg, and no history of antihypertensive medication.

Determination of a Single Nucleotide Polymorphism (SNP) in the HGF Gene

Three SNPs (C/A substitution in intron 13, T/C substitution in intron 14 and T/A substitution in intron 8) were cited in a public database of Japanese Single Nucleotide Polymorphisms (JSNPs, <http://snp.ims.u-tokyo.ac.jp/>) as a result of sequencing for the general population, including 24 unrelated Japanese. The C/A substitution in intron 13 and the T/A

substitution in intron 8 were thought to be in linkage disequilibrium because the allele frequency of both polymorphisms was almost equal. The T/C substitution in intron 14 had a very low allele frequency. In consideration of the allele frequency and linkage disequilibrium, we chose the SNP (C57488A, IMS-JST001765) in intron 13 of the HGF gene (*HGF*) as a genetic marker for the present association study. This SNP has also been reported as rs207425 in the dbSNP database (<http://www.ncbi.nlm.nih.gov/SNP/index.html>).

Genomic DNA was extracted from the buffy coat using a QIAamp DNA Blood Kit (Qiagen Inc., Stanford, USA). To determine the C/A polymorphism of the HGF gene using the TaqMan polymerase chain reaction (PCR) method, we prepared two Minor Groove Binder (MGB) probes: an A allele-specific probe, 5'-Fam-TCCAGAGCTTACaGTCTGGCAA GCA-Tamra-3', and a C allele-specific probe, 5'-Vic-TCCA GAGCTTACcGTCTGGCAAGC-Tamra-3'. Each of the reporters was quenched by Tamra, which was typically located at the 3' end. The design of primers for PCR of the flanking region of the C/A polymorphism in *HGF* was as follows: forward, 5'-TAAAAAGGCACTACCTCTGGAG-3'; reverse, 5'-ACCTGGGTGAGGCAGTAAA-3'. PCR was carried out using a thermal cycler GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, USA). During PCR cycles (initial denaturation at 95°C for 10 min after 50°C for 2 min, followed by 40 cycles of 92°C for 15 s and 60°C for 60 s), the fluorescence level of PCR products was measured using an ABI PRISM 7900 Sequence Detector (Applied Biosystems), resulting in the clear identification of three genotypes of *HGF*.

Statistical Analysis

The associations between genotypes or alleles of *HGF* and blood pressure or clinical variables were analyzed using one-way analysis of variance (ANOVA). Differences in the genotype or allele distribution of *HGF* were examined by χ^2 analysis. To assess the contribution of confounding factors, multiple logistic regression analysis was performed using the computer software application, JMP 3.2.2 (SAS Institute Inc., Cary, USA). A p value less than 0.05 was considered to be statistically significant. When all subjects were divided into two categories, a p value less than 0.025 was considered to be statistically significant using Bonferroni/Dunn Method.

Results

The allele frequencies of the total subjects were not significantly different from that predicted by Hardy-Weinberg equilibrium ($\chi^2 = 0.05$; $p = 0.8$; C:A allele frequency = 0.91:0.09). The distribution of the three genotypes in total subjects was as follows: CC, 82.6%; CA, 16.5%; AA, 0.9%. Table 1 shows the baseline characteristics of total subjects classified as hypertensives ($n = 278$) and normotensives ($n = 376$). While the baseline characteristics of sex (% male) and

Table 1. Clinical Characteristics of Hypertensive and Normotensive Subjects

	Hypertensives (n = 278)	Controls (n = 376)	p
Age (years)	61.7 ± 0.5	68.2 ± 0.4	<0.0001
Sex (% male)	50.0%	48.6%	0.7
SBP (mmHg)	178.8 ± 0.8	111.1 ± 0.7	<0.0001
DBP (mmHg)	103.7 ± 0.6	69.3 ± 0.5	<0.0001
BMI (kg/m ²)	23.9 ± 0.2	21.9 ± 0.2	<0.0001
T-chol (mg/dl)	210.9 ± 2.2	209.7 ± 1.8	NS
TG (mg/dl)	143.2 ± 4.6	115.5 ± 3.8	<0.0001
HDL-chol(mg/dl)	56.4 ± 0.8	59.1 ± 1.0	0.04
FPG (mg/dl)	101.1 ± 1.4	97.4 ± 1.1	0.04
Creatinine (mg/dl)	0.9 ± 0.01	0.7 ± 0.01	<0.0001

SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; T-chol, total cholesterol; TG, triglyceride; HDL-chol, high density lipoprotein cholesterol; FPG, fasting plasma glucose.

Table 2. Genotype Distribution of *HGF* C/A Polymorphism in Hypertensives and Normotensives

	Hypertensives (n (%))	Normotensives (n (%))
Additive model		
CC	239 (86.0)	301 (80.1)
CA	37 (13.3)	71 (18.9)
AA	2 (0.7)	4 (1.1)
	$\chi^2 = 4.0, p = 0.14$	
Recessive model		
CC	239 (86.0)	301 (80.0)
CA, AA	39 (14.0)	75 (20.0)
	$\chi^2 = 4.0, p = 0.047$	
	Odds ratio = 1.53 (95% CI: 1.00–2.33)	

HGF, hepatocyte growth factor gene; CI, confidence interval.

total cholesterol (T-chol) were not significantly different between the two groups, age, body mass index (BMI), high density lipoprotein cholesterol (HDL-chol), fasting plasma glucose (FPG), creatinine and triglyceride (TG) were significantly different between hypertensives and normotensives. The genotype distribution of the *HGF* C/A polymorphism was compared in a contingency table using two types of model: an additive and a recessive model. The results showed that there was a significant difference ($p = 0.047$) between hypertensives and normotensives in the recessive model, but not in the additive model (Table 2). The dominant model is not shown in this table, because there were very few AA carriers. There was no significant association between prevalence of hypertension and allele distribution ($p = 0.052$), and the calculated odds ratio (C allele vs. A allele) was 0.68 (95% confidence interval: 0.46–1.01). The calculated odds ratio (CC vs. CA + AA) for hypertension in the recessive model was 1.53 (95% confidence interval: 1.00–2.33). Multiple logistic regression analysis revealed that the significant association of *HGF* CC with hypertension ($p = 0.047$) remained after adjustment for confounding fac-

Table 3. Effect of Confounding Factors on the Prevalence of Hypertension by Multiple Logistic Regression Analysis

	df	Wald χ^2	p
Age	1	52.8	<0.0001
Creatinine	1	29.5	<0.0001
BMI	1	18.6	<0.0001
Sex	1	8.8	0.003
FPG	1	4.9	0.027
<i>HGF</i> CC genotype	1	4.0	0.047
TG	1	1.3	0.26
HDL-chol	1	0.31	0.58

df, degree of freedom; BMI, body mass index; *HGF*, hepatocyte growth factor gene; TG, triglyceride; HDL-chol, high density lipoprotein cholesterol.

tors (Table 3), and the calculated odds ratio was 1.71 (95% confidence interval: 1.02–2.93).

In order to investigate the effects of sex or obesity on the prevalence of hypertension, total subjects were divided into two groups: male or female, and lean or obese. We used the median BMI distribution, BMI = 22.5 kg/m², as the boundary between lean and obese. A significant predisposition to hypertension in subjects with the CC genotype (vs. those with CA or AA) was observed in female ($p = 0.019$) or lean subjects ($p = 0.013$) but not in male or obese subjects (Table 4). After full adjustment for confounding factors, the calculated odds ratio for hypertension was 2.63 (1.32–5.50) in female and 2.79 (1.23–7.25) in lean subjects.

Discussion

The current study revealed a significant association between hypertension and the C/A polymorphism in intron 13 of *HGF*. The main reason for the significance of the association was the lower prevalence of hypertension in female or lean subjects with the A allele of the *HGF* polymorphism. Multi-

Table 4. Effect of *HGF* C/A Polymorphism on the Prevalence of Hypertension and Estimated Odds Ratio

	Prevalence of hypertension (%)		<i>p</i>	Odds ratio (95% CI)
	CC	CA, AA		
Male (<i>n</i> = 323)	42.4	39.0	0.63	1.42* (0.73–2.86)
Female (<i>n</i> = 331)	46.0	29.1	0.019	2.63* (1.32–5.50)
Lean (<i>n</i> = 335)	31.2	14.6	0.013	2.79** (1.23–7.25)
Obese (<i>n</i> = 319)	54.3	46.7	0.25	0.84** (0.53–1.35)

The odds ratio was estimated after adjustment for age and *BMI or age and **sex. *HGF*, hepatocyte growth factor gene; BMI, body mass index; CI, confidence interval.

ple regression analysis suggested that the significant association of the *HGF* polymorphism with hypertension was attenuated in male or obese subjects. A feasible explanation for this result is that the effect of environmental risk factors, such as smoking, drinking or excessive eating, on hypertension was dominant and masked the genetic effect of the *HGF* polymorphism in male and/or obese subjects, whereas the effect of the polymorphism on hypertension was significant and relatively major in female and/or lean subjects who had had less exposure to environmental risks. In this study, the *HGF* polymorphism was not significantly associated with BMI ($p = 0.23$), drinking ($p = 0.19$), smoking ($p = 0.55$), TG ($p = 0.64$) or FPG levels ($p = 0.2$), suggesting that *HGF* may not be a thrifty gene, and may not be causal for lifestyle-related diseases such as hypertension or diabetes.

On the other hand, a unique characteristic of HGF is that high serum HGF concentration is attributed to compensation for downregulation of the local HGF production system, reflecting endothelial dysfunction. Our preliminary results in another population ($n = 129$) showed that serum HGF concentration was higher in subjects with the CC genotype (0.67 ± 0.05 ng/ml) than in those with CA (0.57 ± 0.12 ng/ml) or AA (0.39 ± 0.26 ng/ml), but there was no significant difference among genotypes. Therefore, local *HGF* expression in the cardiovascular system might be relatively increased in A allele carriers compared to those with the CC genotype, whereas compensatory HGF production in the liver may be enhanced in CC subjects. Since HGF suppresses the expression of the endothelin-1 (ET-1) gene in vessels (29), an increase of local HGF production would down-regulate the vascular ET-1 gene expression, with the result that endothelial function and blood pressure might be well maintained in A allele carriers, especially in female or lean subjects. A recent paper showed that HGF directly stimulates endothelial nitric oxide synthase (eNOS) activity by a phosphoinositide 3-kinase/Akt-dependent phosphorylation in a Ca^{2+} -sensitive manner in vascular endothelial cells (30).

There are several limitations of this study. Since the C/A polymorphism was located in intron 13 of *HGF*, we could not exclude the possible existence of another SNP which was tightly associated with hypertension *via* alteration of *HGF* expression. Unfortunately, all reported SNPs in *HGF* are located in introns, and there has been no reports of the detec-

tion of a polymorphism in the exon or promoter regions. Recent investigations using haplotype block analysis revealed that linkage disequilibrium was much more widely conserved than expected, suggesting that neighboring gene polymorphisms at the same 7q21 locus, such as those of the calcium channel, voltage-dependent, L type, α_2/δ subunit gene (*CACN2*) and β_2 guanine nucleotide binding protein (*GNB2*), might be associated with genetic susceptibility to hypertension. Although *GNB2* and *CACN2* have been reported to play important roles in signal transduction through G protein and to have some SNPs, there have been no reports on the association between hypertension and their polymorphisms. However, a conclusion regarding the locus-specific association is eagerly awaited, based on the results of genome-wide screening of hypertensive genes by the Japan Hypertension Genetics Consortium, which is currently in progress.

In conclusion, the C/A polymorphism in intron 13 of *HGF* was associated with susceptibility to essential hypertension only in the recessive model. The significance of the association was enhanced in female or lean subjects, suggesting that the A allele of the C/A polymorphism in intron 13 of *HGF* might protect against endothelial dysfunction and hypertension *via* local HGF production. Future studies will be needed to examine the biological relevance of *HGF* polymorphisms in hypertension using other SNPs of HGF and other races.

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