G2736A polymorphism of thiazide-sensitive Na-Cl cotransporter gene predisposes to hypertension in young women

Akiko Matsuo, Tomohiro Katsuya, Kazuhiko Ishikawa, Ken Sugimoto, Yoshio Iwashima, Koichi Yamamoto, Mitsuru Ohishi, Hiromi Rakugi and Toshio Ogihara

Objective **The thiazide-sensitive Na-Cl cotransporter (TSC) is located in the distal renal tubules. Several mutations of the TSC gene cause Gitelman's syndrome, which is an autosomal recessive disease characterized by low blood pressure and hypokalemia. Recently, an association between TSC gene polymorphisms (Arg904Gln, G2736A; Thr465Thr, C1420T; Gly264Ala, G816C) and essential hypertension has been reported in Sweden. We examined the genetic involvement of the TSC gene in essential hypertension in Japanese.**

Design **Participants were recruited from outpatients of Osaka University Hospital. We investigated 386 hypertensive and 371 normotensive subjects.**

Methods **Genotypes of TSC polymorphisms (G2736A, C1420T, G816C) were determined by the TaqMan polymerase chain reaction (PCR) method, and statistical significance was examined using JMP 5.0.1J (SAS Institute Inc., Cary, North Carolina, USA). The allele frequency of A2736 and T1420 was 6.0 and 3.0%, respectively, whereas we could not detect the G816C polymorphism in this study. Only the G2736A polymorphism was significantly associated with the prevalence of hypertension ($P < 0.04$), and the estimated odds ratio was 1.8 (95% confidence interval, 1.1–3.0) in A2736 allele carriers. The odds ratio for hypertension in A2736 carriers was increased to 2.2 (1.1–4.9) in women ($n = 413$), and further to 3.3 (1.4–8.0) in women with early onset of hypertension (≤ 50 years old). In addition, all subjects with the homozygous A2736 allele in this study ($n = 2$) and the Swedish study ($n = 5$) were hypertensive.**

Conclusion **G2736A polymorphism of the TSC gene is a genetic predisposing factor for essential hypertension in Japanese women.** *J Hypertens* **22:2123–2127 © 2004 Lippincott Williams & Wilkins.**

Journal of Hypertension 2004, 22:2123–2127

Keywords: hypertension, thiazide-sensitive Na-Cl cotransporter gene, polymorphism

Department of Geriatric Medicine, Osaka University Graduate School of Medicine, Suita, Japan.

Sponsorship: The present study was supported by a Grant-in-Aid from the Japanese Ministry of Health, Labor, and Welfare, Grants-in-Aid for Scientific Research (12557063, 14207035, 15590342, 13204050) from the Ministry of Education, Science, Sports and Culture of Japan, and by research grants from the Salt Science Research Foundation, Ono Medical Research Foundation, the Tokyo Biochemical Research Foundation, the Osakagas-Group Welfare Foundation, Chiyoda Mutual Life Foundation and the Preventive Arteriosclerosis Research Association.

Correspondence and requests for reprints to Tomohiro Katsuya, MD, PhD, Assistant Professor of Medicine, Department of Geriatric Medicine, Osaka University Graduate School of Medicine, 2-2, Yamadaoka, #B6, Suita 565-0871, Japan.
Tel: +81 6 6879 3852; fax: +81 6 6879 3859;
e-mail: katsuya@geriat.med.osaka-u.ac.jp

Received 9 February 2004 **Revised** 10 June 2004
Accepted 30 June 2004

Introduction

Essential hypertension is a common disease that is affected by both environmental and genetic factors. Classically, aging, obesity, excess salt intake, ethnicity (e.g. African-American) and sex (male) are known to be risk factors for hypertension [1], and recent genetic investigations have revealed several genetic variants that are candidates in the pathogenesis of hypertension [2–4]. Genetic variants of the epithelial sodium channel or mineral corticoid receptor have not only been shown to cause monogenic hypertension [5], but also to be candidates for genetic predisposing factors for essential hypertension. For example, the T594M polymorphism in the beta-subunit of the epithelial sodium channel (ENaC) gene is associated with hypertension in black populations [6].

Gitelman's syndrome [7–10] is an autosomal recessive disease that shows similar clinical characteristics to Bartter's syndrome [11,12] and is characterized by low blood pressure due to renal sodium wasting, hypokalemia, hypomagnesemia, and hypocalciuria from a young age. It is reported that Gitelman's syndrome is caused by novel homozygous mutations of the thiazide-sensitive Na-Cl cotransporter (TSC) gene, which leads to loss of TSC function and thus reduced renal sodium reabsorption [10]. TSC is a member of the electroneutral cation-chloride-coupled cotransporter gene family

(SLC12: solute carrier family 12), which encompasses two major branches, one of which includes two bumetanide-sensitive Na^+-K^+-$2Cl^-$ cotransporters and TSC (SLC12A3) [13]. TSC, which is located in the distal renal tubules [14], is a target of the diuretic effect of thiazides [5], which are known to be useful for patients with hypertension who are salt sensitive, such as African-Americans [15]. Melander *et al.* [16] recently investigated some polymorphisms of the TSC gene, and showed that homozygous A2736 and T1420 alleles were significantly associated with hypertension in the Swedish population. In the present study, we investigated the association of these polymorphisms with essential hypertension in the Japanese population.

Methods

Study population

Patients with essential hypertension and control subjects were recruited from in- and outpatients of Osaka University Hospital. All cases and controls were Japanese and gave informed consent before participating in the research protocol, which was approved by the Hospital Ethics Committee. All cases ($n = 386$) had a family history of hypertension in first-degree relatives, and were diagnosed as having primary hypertension (those with secondary hypertension or apparent ischemic heart disease were excluded). The criteria for hypertension were defined as systolic blood pressure higher than 140 mmHg and/or diastolic blood pressure higher than 90 mmHg, or receiving antihypertensive therapy. Controls ($n = 371$) without a history of hypertension were recruited from the same population. To enhance detection of the genetic effect on hypertension, we excluded subjects under 50 years old from the control group, because blood pressure generally tends to increase as people get older. Subjects completed a standard questionnaire on their personal medical history and family history of hypertension. Blood pressure was measured twice with the subjects seated after 5 min of rest.

Genotype determination of TSC gene polymorphisms

To determine TSC genotype, we employed the TaqMan polymerase chain reaction (PCR) method. A fluorescent reporter dye, such as 6-carboxy-fluorescein (FAM) or (VIC), was linked covalently to the 5′ end of the nucleotide. For the present investigation, we prepared two probes and two primers for each fragment, as follows: 5′-VIC-CCA CCC TCT CCT CT-MGB-3′, 5′-FAM-CCA CTC TCT CCT CTG-MGB-3′, CCA AAT CCC CAC AGA CCA T (forward primer), GTC ATC TCG ACC CCT TTC TGC (reverse primer) for C1420T; 5′-VIC-CCC TCG GGC TGA G-MGB-3′, 5′-FAM-AGA ACC CTC AGG CTG-MGB-3′, CCA CAT CCT CCC TGA CAT CAA (forward primer), GAA GCC CCA AAA CAG AAC TTA CTG (reverse primer) for G2736A; 5′-VIC-ATC ATT GGC GTG GTC-MGB-3′, 5′-FAM-ATC ATT GCC GTG GTC-MGB-3′, TCG TGG ACC CCA TTA ACG A (forward primer), TGG CCA GCA GCA CAG TGA (reverse primer) for G816C.

PCR was carried out using a Gene Amp 9700 (Applied Biosystems, Foster City, California, USA) under the following conditions: initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 62°C for 60 s. During the PCR cycles, two TaqMan probes hybridize competitively to a specific sequence of the target DNA, and the reporter dye separates from the quencher dye, resulting in an increase of fluorescence of the reporter. The fluorescence level of PCR products was measured using an ABI PRISM 7200 or 7900 Sequence Detector (Applied Biosystems), resulting in clear identification of two polymorphisms (C1420T and G2736A) of TSC. Genotyping data of all minor allele carriers and representative subjects ($n = 15$) with homozygous major allele were confirmed by sequencing.

Thiazide-loading test

To examine the gain of function of TSC due to the G2736A polymorphism, we carried out the thiazide-loading test in healthy volunteers with homozygous GG ($n = 3$) or heterozygous GA ($n = 3$), with their informed consent. Before and after administration of 50 mg hydrochlorothiazide (HCT), we collected urine and peripheral blood using a Tempus Blood RNA Tube (Applied Biosystems), calculated the sodium excretion rate and quantified the expression level of the TSC gene by real-time reverse transcription PCR (RT-PCR) in comparison with 18sRNA. Urinary sodium excretion rate (UNaV) (net; mmol/150 min) was calculated by the following formula: UNaV (cumulative) (total sodium excretion from 30 to 180 min after HCT administration, mmol/150 min) $-$ 150 $\times$ UNaV (basal) (sodium excretion per minute for 1 h before administration of HCT, μmol/min).

Statistical analysis

All statistical analyses were conducted using JMP 5.0.1J (SAS Institute Inc., Cary, North Carolina, USA). Difference in genotype or allele frequency between normotensives and hypertensive subjects were examined by chi-squared analysis. The association between TSC polymorphisms and clinical variables was examined by one-way ANOVA. We assessed the quantitative effects of covariates by multiple logistic regression analysis with JMP.

Results

Clinical features of participants

There were significant differences in sex, age, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP) and triglyceride level (TG), but not in total cholesterol level and prevalence

of diabetes between hypertensives and normotensive subjects (Table 1).

Association between TSC polymorphisms and hypertension

The genotype distributions of the C1420T and G2736A variants were not significantly deviated from Hardy–Weinberg's expectation. In this Japanese population, there was no C816 allele of the G816C polymorphism. Neither the allele or genotype frequency of C1420T were significantly different between hypertensive and normotensive subjects (Table 2). In contrast, the G2736A polymorphism was significantly associated with the prevalence of hypertension ($P < 0.04$) (Table 2). The calculated odds ratio for hypertension in A2736 allele carriers was 1.8 [95% confidence interval (CI): 1.1–3.0] after adjustment for confounding factors of sex, age, BMI and TG. Although no significant association between the A2736 allele and hypertension was observed in men, female A2736 allele carriers were significantly ($P < 0.04$) predisposed to hypertension (odds ratio: 2.2, 95% CI: 1.1–4.9) (Table 3). Furthermore, the significance of the association with hypertension was enhanced in female subjects with early-onset (≤ 50 years old) hypertension ($P < 0.01$, odds ratio: 3.3, 95% CI: 1.4–8.0) (Table 4).

Table 1 Clinical features of study participants

	HT ($n = 386$)	NT ($n = 371$)	*P* value
Male/female	225/161	188/183	0.04
Age (years)	61 ± 11	67 ± 7	< 0.0001
BMI (kg/m^2)	24 ± 3	22 ± 3	< 0.0001
Systolic BP (mmHg)	170 ± 26	111 ± 10	< 0.0001
Diastolic BP (mmHg)	101 ± 17	69 ± 7	< 0.0001
Total cholesterol (mg/dl)	201 ± 36	202 ± 35	NS
TG (mg/dl)	147 ± 95	114 ± 67	< 0.0001
Diabetes (%)	15.8	19.5	NS

Variables are mean ± SD. HT, hypertension; NT, normotension; BMI, body mass index; TG, triglyceride; NS, not significant.

Thiazide-loading test

Baseline TSC gene expression level in the peripheral blood in heterozygous patients (GA) (0.17 ± 0.10) was slightly but not significantly higher than that in those homozygous for GG (0.12 ± 0.03). The sodium excretion rate after HCT administration [UNaV (net)] was higher in heterozygous subjects (26.6 ± 15.9) than in those homozygous for GG (11.7 ± 19.6), but there was no significant difference between them.

Discussion

The current study suggested a positive association between the TSC G2736A polymorphism and essential hypertension in younger women. G2736A (Arg904Gln) is located in the intracellular region close to the C terminus, and the Arg to Gln amino acid substitution leads to a change in the protein from electronically positive to negative. However, the role of this site is not known, and the amino acid substitution is not conserved between rats and humans. Loss of function in TSC causes a decrease of sodium reabsorption and leads to Gitelman's syndrome [10,17], so we speculate that G2736A is a gain of function polymorphism. As an example, gain of function in the amiloride-sensitive epithelial sodium-channel gene causes hypertension (Liddle's syndrome) [18,19], whereas loss of function in the same gene causes hypotension [20] (pseudohypoaldosteronism type I). Furthermore, subjects with heterozygous mutations that cause Gitelman's syndrome in the homozygous state have lower blood pressure than subjects without mutations [21]. Even though we could not clearly show proof of gain of function due to G2736A polymorphism, our preliminary investigation using thiazide loading suggested an increase of TSC gene expression and sodium excretion after HCT administration in the subjects with the A2736 allele.

In the present study, the G2736A polymorphism was positively associated with hypertension in younger

Table 2 Genotype and allele distribution of C1420T and G2736A polymorphisms in all subjects ($n = 757$)

C1420T	C allele	T allele	*P* value	Odds ratio (95%CI)	CC	CT	TT	*P** value	Odds ratio (95% CI)
HT	753	19	NS	0.6	367	19	0	NS	0.5***
%	97.5	2.5		(0.3–1.1)	95.1	4.9	0		(0.6–1.6)***
NT	713	29			344	25	2		
%	96.1	3.9			92.7	6.7	0.5		
G2736A	**G allele**	**A allele**	***P* value**	**Odds ratio (95%CI)**	**GG**	**GA**	**AA**	***P*** value**	**Odds ratio (95%CI)**
HT	716	56	0.02	1.7	332	52	2	< 0.04***	1.8***
%	92.8	7.2		(1.1–2.6)	86.0	13.5	0.5		(1.1–3.0)***
NT	709	33			338	33	0		
%	95.6	4.4			91.1	8.9	0		

*CC versus CT + TT. **GG versus GA + AA. ***Adjusted by sex, age, body mass index (BMI) and triglyceride level (TG). HT, hypertension; NT, normotension; NS; not significant; 95% CI, 95% confidence interval.

Table 3 Genotype and allele distribution of G2736A polymorphism in male ($n = 413$) and female ($n = 344$) subjects

	G allele	A allele	*P* value	Odds ratio (95% CI)	GG	GA + AA	*P* value	Odds ratio (95% CI)
Male								
HT	425	25	NS	1.1	200	25	NS	1.5*
(%)	(94.4)	(5.6)		(0.6–2.0)	(88.9)	(11.1)		(0.7–3.1)*
NT	357	19			169	19		
(%)	(94.9)	(5.1)			(89.9)	(10.1)		
Female								
HT	291	31	< 0.01	2.7	132	29	< 0.04*	2.2*
(%)	(90.4)	(9.6)		(1.4–5.1)	(82.0)	(18.0)		(1.1–4.9)*
NT	352	14			169	14		
(%)	(96.2)	(3.8)			(92.4)	(7.6)		

*Adjusted by age, body mass index (BMI) and triglyceride level (TG). HT, hypertension; NT, normotension; NS, not significant; 95% CI, 95% confidence interval.

Table 4 Distribution of G2736A polymorphism in females with onset of hypertension at ≤ 50 years of age ($n = 256$)

	G allele	A allele	*P* value	Odds ratio (95% CI)	GG	GA + AA	*P* value	Odds ratio (95% CI)
HT	131	15	< 0.01	2.9 (1.4–6.1)	59	14	< 0.01*	3.3* (1.4–8.0)*
(%)	(89.8)	(10.2)			(80.8)	(19.2)		
NT	352	14			169	14		
(%)	(96.2)	(3.8)			(92.3)	(7.7)		

*Adjusted by body mass index (BMI) and triglyceride level (TG). HT, hypertension; NT, normotension; 95% CI, 95% confidence interval.

women. This could be explained as follows. First, female hormones, such as estrogen, accelerate sodium and water retention in young premenopausal women. Hurwitz *et al.* [22] reported that the highest systolic salt sensitivity (SSS) was observed in premenopausal women with low renin activity, and Verlander *et al.* [23] reported that estrogen enhances TSC density in the distal convoluted tubule. In addition, it has been known that estrogen reduces renal sodium excretion [24]. Another feasible explanation of 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 TSC polymorphism in men and/or postmenopausal women, whereas the effect of the polymorphism on hypertension was significant and relatively major in young women who were less exposed to environmental risks.

Previously, a Swedish group reported a borderline association of the G2736A polymorphism with hypertension ($P = 0.05$) [16], but their analysis did not divide the subjects by sex. Furthermore, Asian populations, such as the Japanese, are known to be more salt sensitive than Caucasian people [25]. So these results of two genetically different populations seem to be reasonable. Interestingly, there were two A2736 homozygous subjects in our population, who were both hypertensive, and five AA homozygous subjects in the Swedish study, were also hypertensive [16]. In contrast, another polymorphism, the T1420 allele, was positive in Caucasians but not in Japanese. Even though the allele frequency of T1420 was too low to discuss the significance of the difference between Japanese and Caucasian populations, the frequency of T1420 was lower in hypertensive than normotensive subjects in the present study. Furthermore, C1420T was a synonymous polymorphism and not in linkage disequilibrium with G2736A, suggesting that genetic determination of G2736A is worthwhile in the risk estimation for hypertension rather than C1420T.

There were some study limitations. We do not show plasma potassium and renin activity, because the lack of data in many subjects could lead to ambiguous results. We analyzed the available data of renin activity and potassium, but they were approximately the same in subjects with G2736 and A2736. It is known that salt-sensitive patients with hypertension have low renin activity [26]. However, some patients had a moderate level of renin activity in a previous report [27].

Even though we examined TSC function using the thiazide-loading test, the examined number was too small to discuss the significance of the association, and the results of TSC expression were obtained from peripheral blood and not from distal tubules. Furthermore, we only examined heterozygotes (GA subjects) but not homozygotes (AA subjects), so we could not exclude the possibility that subjects with AA clearly show a gain of function of TSC.

Salt-sensitive hypertension is a relatively clear category of essential hypertension, and administration of diuretics is reasonable and effective therapy; however, it takes much effort to distinguish these subjects in the present clinical situation. In the future, determination of the TSC gene polymorphism may contribute to identifying patients with salt-sensitive hypertension and the choice of antihypertensive medication. In conclusion, the G2736A genotype of the TSC gene may be a risk factor for essential hypertension in younger Japanese woman.

Acknowledgements

We would like to express our extreme gratitude to Miss Sayaka Ohashi, Mr Masafumi Kuremura and Miss Shiori Takase for their continuous support of our investigations.

References

1 Stanton JL, Braitman LE, Riley AM Jr, Khoo CS, Smith JL. Demographic, dietary, life style, and anthropometric correlates of blood pressure. *Hypertension* 1982; 4:III135–142.
2 Asai T, Ohkubo T, Katsuya T, Higaki J, Fu Y, Fukuda M, *et al.* Endothelin-1 gene variant associates with blood pressure in obese Japanese subjects: the Ohasama Study. *Hypertension* 2001; **38**:1321–1324.
3 Sugimoto K, Hozawa A, Katsuya T, Matsubara M, Ohkubo T, Tsuji I, *et al.* Alpha-adducin Gly460Trp polymorphism is associated with low renin hypertension in younger subjects in the Ohasama study. *J Hypertens* 2002; **20**:1779–1784.
4 Shintani M, Ikegami H, Fujisawa T, Kawaguchi Y, Ohishi M, Katsuya T, *et al.* Leptin gene polymorphism is associated with hypertension independent of obesity. *J Clin Endocrinol Metab* 2002; **87**:2909–2912.
5 Kunau RT Jr, Weller DR, Webb HL. Clarification of the site of action of chlorothiazide in the rat nephron. *J Clin Invest* 1975; **56**:401–407.
6 Baker EH, Dong YB, Sagnella GA, Rothwell M, Onipinla AK, Markandu ND, *et al.* Association of hypertension with T594M mutation in beta subunit of epithelial sodium channels in black people resident in London. *Lancet* 1998; **351**:1388–1392.
7 Gitelman HJ, Graham JB, Welt LG. A new familial disorder characterized by hypokalemia and hypomagnesemia. *Trans Assoc Am Physicians* 1966; **79**:221–235.
8 Rudin A. Bartter's syndrome. A review of 28 patients followed for 10 years. *Acta Med Scand* 1988; **224**:165–171.
9 Bettinelli A, Bianchetti MG, Girardin E, Caringella A, Cecconi M, Appiani AC, *et al.* Use of calcium excretion values to distinguish two forms of primary renal tubular hypokalemic alkalosis: Bartter and Gitelman syndromes. *J Pediatr* 1992; **120**:38–43.
10 Simon DB, Nelson-Williams C, Bia MJ, Ellison D, Karet FE, Molina AM, *et al.* Gitelman's variant of Bartter's syndrome, inherited hypokalaemic alkalosis, is caused by mutations in the thiazide-sensitive Na-Cl cotransporter. *Nat Genet* 1996; **12**:24–30.
11 Bauer FM, Glasson P, Vallotton MB, Courvoisier B. [Bartter's syndrome, chondrocalcinosis and hypomagnesemia]. *Schweiz Med Wochenschr* 1979; **109**:1251–1256.
12 de Heide LJ, Birkenhager JC. Bartter's syndrome, hypomagnesaemia and chondrocalcinosis. *Neth J Med* 1991; **39**:148–152.
13 Hebert SC, Mount DB, Gamba G. Molecular physiology of cation-coupled Cl^- cotransport: the SLC12 family. *Pflugers Arch* 2004; **447**:580–593.
14 Plotkin MD, Kaplan MR, Verlander JW, Lee WS, Brown D, Poch E, *et al.* Localization of the thiazide sensitive Na–Cl cotransporter, rTSC1 in the rat kidney. *Kidney Int* 1996; **50**:174–183.
15 Peters RM, Flack JM. Salt sensitivity and hypertension in African Americans: implications for cardiovascular nurses. *Prog Cardiovasc Nurs* 2000; **15**:138–144.
16 Melander O, Orho-Melander M, Bengtsson K, Lindblad U, Rastam L, Groop L, *et al.* Genetic variants of thiazide-sensitive NaCl-cotransporter in Gitelman's syndrome and primary hypertension. *Hypertension* 2000; **36**:389–394.
17 Mastroianni N, De Fusco M, Zollo M, Arrigo G, Zuffardi O, Bettinelli A, *et al.* Molecular cloning, expression pattern, and chromosomal localization of the human Na–Cl thiazide-sensitive cotransporter (SLC12A3). *Genomics* 1996; **35**:486–493.
18 Shimkets RA, Warnock DG, Bositis CM, Nelson-Williams C, Hansson JH, Schambelan M, *et al.* Liddle's syndrome: heritable human hypertension caused by mutations in the beta subunit of the epithelial sodium channel. *Cell* 1994; **79**:407–414.
19 Schild L, Canessa CM, Shimkets RA, Gautschi I, Lifton RP, Rossier BC. A mutation in the epithelial sodium channel causing Liddle disease increases channel activity in the *Xenopus laevis* oocyte expression system. *Proc Natl Acad Sci USA* 1995; **92**:5699–5703.
20 Chang SS, Grunder S, Hanukoglu A, Rosler A, Mathew PM, Hanukoglu I, *et al.* Mutations in subunits of the epithelial sodium channel cause salt wasting with hyperkalaemic acidosis, pseudohypoaldosteronism type 1. *Nat Genet* 1996; **12**:248–253.
21 Cruz DN, Simon DB, Nelson-Williams C, Farhi A, Finberg K, Burleson L, *et al.* Mutations in the Na–Cl cotransporter reduce blood pressure in humans. *Hypertension* 2001; **37**:1458–1464.
22 Hurwitz S, Fisher ND, Ferri C, Hopkins PN, Williams GH, Hollenberg NK. Controlled analysis of blood pressure sensitivity to sodium intake: interactions with hypertension type. *J Hypertens* 2003; **21**:951–959.
23 Verlander JW, Tran TM, Zhang L, Kaplan MR, Hebert SC. Estradiol enhances thiazide-sensitive NaCl cotransporter density in the apical plasma membrane of the distal convoluted tubule in ovariectomized rats. *J Clin Invest* 1998; **101**:1661–1669.
24 Christy NP, Shaver JC. Estrogens and the kidney. *Kidney Int* 1974; **6**:366–376.
25 Katsuya T, Ishikawa K, Sugimoto K, Rakugi H, Ogihara T. Salt sensitivity of Japanese from the viewpoint of gene polymorphism. *Hypertens Res* 2003; **26**:521–525.
26 White RP, Sealey J, Reidenberg M, Stenzel KH, Sullivan JF, David DS, *et al.* Mechanisms of blood pressure control in anephrics: plasma renin and dopamine beta hydroxylase activity. *Trans Am Soc Artif Intern Organs* 1976; **22**:420–424.
27 Fujita T, Henry WL, Bartter FC, Lake CR, Delea CS. Factors influencing blood pressure in salt-sensitive patients with hypertension. *Am J Med* 1980; **69**:334–344.

Original Article

Association between Hepatocyte Growth Factor Gene Polymorphism and Essential Hypertension

Masaharu MOTONE, Tomohiro KATSUYA, Kazuhiko ISHIKAWA, Yoshio IWASHIMA, Ken SUGIMOTO, Koichi YAMAMOTO, Yuxiao FU, Akiko MATSUO, Mitsuru OHISHI, Hiromi RAKUGI, and Toshio OGIHARA

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-

From the Department of Geriatric Medicine, Osaka University Graduate School of Medicine, Suita, Japan.

The present study was supported by a Grant-in-Aid from the Japanese Ministry of Health, Labour, and Welfare, by Grants-in-Aid for Scientific Research (12557063, 13204050, 14207035, 15590342) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and by research grants from the Uehara Memorial Foundation, Takeda Medical Foundation, Salt Science Research Foundation, Yokoyama Foundation of Clinical Pharmacology, Ono Medical Research Foundation, Tokyo Biochemical Research Foundation, Kurozumi Medical Foundation, Osakagas-Group Welfare Foundation, Osaka Kidney Foundation of Japan and Preventive Arteriosclerosis Research Association.

Address for Reprints: Tomohiro Katsuya, M.D., Ph.D., Department of Geriatric Medicine, Osaka University Medical School, 2-2 # B6, Yamadaoka, Suita 565-0871, Japan. E-mail: katsuya@geriat.med.osaka-u.ac.jp

Received December 1, 2003; Accepted in revised form January 13, 2004.

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 $\geq$140 mmHg, a mean diastolic blood pressure (DBP) of $\geq$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 (χ^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)
	χ^2=4.0, p=0.14	
Recessive model		
CC	239 (86.0)	301 (80.0)
CA, AA	39 (14.0)	75 (20.0)
	χ^2=4.0, p=0.047	
	Odds ratio=1.53 (95% CI: 1.00–2.33)	

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

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
HGF 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.

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 factors (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/m^2, 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 (%)		p	Odds ratio (95% CI)
	CC	CA, AA		
Male (n=323)	42.4	39.0	0.63	1.42* (0.73–2.86)
Female (n=331)	46.0	29.1	0.019	2.63* (1.32–5.50)
Lean (n=335)	31.2	14.6	0.013	2.79** (1.23–7.25)
Obese (n=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 detection 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, $\alpha 2/\delta$ subunit gene (*CACN2*) and $\beta 2$ 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.

Acknowledgements

We would like to express our heartfelt appreciation to Ms. Sayaka Ohashi, Ms. Shiori Takase and Mr. Masafumi Kuremura for their continuous support of our investigations.

References

1. Cohn JN, Finkelstein SM: Abnormalities of vascular compliance in hypertension, aging and heart failure. *J Hypertens* 1992; **10** (Suppl): S61–S64.
2. Lockette W, Otsuka Y, Carretero O: The loss of endothelium-dependent vascular relaxation in hypertension. *Hypertension* 1986; **8**: II61–II66.
3. Panza JA, Quyyumi AA, Brush JE Jr, Epstein SE: Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N Engl J Med* 1990; **323**:

22–27.
4. Hamanoue M, Kawaida K, Takao S, *et al*: Rapid and marked induction of hepatocyte growth factor during liver regeneration after ischemic or crush injury. *Hepatology* 1992; **16**: 1485–1492.
5. Nagaike M, Hirao S, Tajima H, *et al*: Renotropic functions of hepatocyte growth factor in renal regeneration after unilateral nephrectomy. *J Biol Chem* 1991; **266**: 22781–22784.
6. Igawa T, Matsumoto K, Kanda S, Saito Y, Nakamura T: Hepatocyte growth factor may function as a renotropic factor for regeneration in rats with acute renal injury. *Am J Physiol* 1993; **265**: F61–F69.
7. Nishimura M, Nakano K, Ushiyama M, *et al*: Increased serum concentrations of human hepatocyte growth factor in proliferative diabetic retinopathy. *J Clin Endocrinol Metab* 1998; **83**: 195–198.
8. Shinoda K, Ishida S, Kawashima S, *et al*: Clinical factors related to the aqueous levels of vascular endothelial growth factor and hepatocyte growth factor in proliferative diabetic retinopathy. *Curr Eye Res* 2000; **21**: 655–661.
9. Szprynger K, Szczepanska M, Dyduch A: Hepatocyte growth factor in kidney diseases. *Przegl Lek* 2000; **57**: 757–760.
10. Nakamura S, Moriguchi A, Morishita R, *et al*: A novel vascular modulator, hepatocyte growth factor (HGF), as a potential index of the severity of hypertension. *Biochem Biophys Res Commun* 1998; **242**: 238–243.
11. Nakamura Y, Morishita R, Nakamura S, *et al*: A vascular modulator, hepatocyte growth factor, is associated with systolic pressure. *Hypertension* 1996; **28**: 409–413.
12. Morishita R, Moriguchi A, Higaki J, Ogihara T: Hepatocyte growth factor (HGF) as a potential index of severity of hypertension. *Hypertens Res* 1999; **22**: 161–167.
13. Yamamoto Y, Kohara K, Tabara Y, Miki T: Association between carotid arterial remodeling and plasma concentration of circulating hepatocyte growth factor. *J Hypertens* 2001; **19**: 1975–1979.
14. Yamamoto Y, Kohara K, Tabara Y, Igase M, Nakura J, Miki T: Plasma hepatocyte growth factor and the relationship between risk factors and carotid atherosclerosis. *Hypertens Res* 2002; **25**: 661–667.
15. Morishita R, Aoki M, Yo Y, Ogihara T: Hepatocyte growth factor as cardiovascular hormone: role of HGF in the pathogenesis of cardiovascular disease. *Endocr J* 2002; **49**: 273–284.
16. Hayashi Y, Saitoh S, Takagi S, Tuchihashi K, Miura T, Shimamoto K: Hepatocyte growth factor and 24-hour ambulatory blood pressure monitoring. *Hypertens Res* 2002; **25**: 655–660.
17. Komai N, Ohishi M, Morishita R, *et al*: Serum hepatocyte growth factor concentration is correlated with the forearm vasodilator response in hypertensive patients. *Am J Hypertens* 2002; **15**: 499–506.
18. Nakamura T: Structure and function of hepatocyte growth factor. *Prog Growth Factor Res* 1991; **3**: 67–85.
19. Boros P, Miller CM: Hepatocyte growth factor: a multifunctional cytokine. *Lancet* 1995; **345**: 293–295.
20. Nakamura Y, Morishita R, Higaki J, *et al*: Expression of local hepatocyte growth factor system in vascular tissues. *Biochem Biophys Res Commun* 1995; **215**: 483–488.
21. Morishita R, Nakamura S, Hayashi S, *et al*: Contribution of a vascular modulator, hepatocyte growth factor (HGF), to the pathogenesis of cardiovascular disease. *J Atheroscler Thromb* 1998; **4**: 128–134.
22. Morishita R, Aoki M, Nakamura S, *et al*: Potential role of a novel vascular modulator, hepatocyte growth factor (HGF), in cardiovascular disease: characterization and regulation of local HGF system. *J Atheroscler Thromb* 1997; **4**: 12–19.
23. Matsumoto K, Tajima H, Okazaki H, Nakamura T: Negative regulation of hepatocyte growth factor gene expression in human lung fibroblasts and leukemic cells by transforming growth factor-beta 1 and glucocorticoids. *J Biol Chem* 1992; **267**: 24917–24920.
24. Okajima A, Miyazawa K, Kitamura N: Characterization of the promoter region of the rat hepatocyte-growth-factor/scatter-factor gene. *Eur J Biochem* 1993; **213**: 113–119.
25. Liu Y, Michalopoulos GK, Zarnegar R: Structural and functional characterization of the mouse hepatocyte growth factor gene promoter. *J Biol Chem* 1994; **269**: 4152–4160.
26. Gohda E, Matsunaga T, Kataoka H, Yamamoto I: TGF-beta is a potent inhibitor of hepatocyte growth factor secretion by human fibroblasts. *Cell Biol Int Rep* 1992; **16**: 917–926.
27. Nakamura Y, Morishita R, Higaki J, *et al*: Hepatocyte growth factor is a novel member of the endothelium-specific growth factors: additive stimulatory effect of hepatocyte growth factor with basic fibroblast growth factor but not with vascular endothelial growth factor. *J Hypertens* 1996; **14**: 1067–1072.
28. Onimaru M, Yonemitsu Y, Tanii M, *et al*: Fibroblast growth factor-2 gene transfer can stimulate hepatocyte growth factor expression irrespective of hypoxia-mediated downregulation in ischemic limbs. *Circ Res* 2002; **91**: 923–930.
29. Haug C, Schmid-Kotsas A, Zorn U, *et al*: Hepatocyte growth factor is upregulated by low-density lipoproteins and inhibits endothelin-1 release. *Am J Physiol Heart Circ Physiol* 2000; **279**: H2865–H2871.
30. Makondo K, Kimura K, Kitamura N, *et al*: Hepatocyte growth factor activates endothelial nitric oxide synthase by Ca^{2+}- and phosphoinositide 3-kinase/Akt-dependent phosphorylation in aortic endothelial cells. *Biochem J* 2003; **374**: 63–69.

Hypoadiponectinemia Is an Independent Risk Factor for Hypertension

Yoshio Iwashima, Tomohiro Katsuya, Kazuhiko Ishikawa, Noriyuki Ouchi, Mitsuru Ohishi, Ken Sugimoto, Yuxiao Fu, Masaharu Motone, Kouichi Yamamoto, Akiko Matsuo, Koji Ohashi, Shinji Kihara, Tohru Funahashi, Hiromi Rakugi, Yuji Matsuzawa, Toshio Ogihara

Abstract—Adiponectin is one of the key molecules in the metabolic syndrome, and its concentration is decreased in obesity, type-2 diabetes, and coronary artery disease. Genetic investigation has revealed that 2 polymorphisms (I164T and G276T) are related to adiponectin concentration and diabetes. To examine whether adiponectin affects hypertension genetically or biologically, we performed a case-control study. A total of 446 diagnosed cases of hypertension (HT) in men and 312 normotensive (NT) men were enrolled in this study. Plasma adiponectin concentration was measured using an enzyme-linked immunosorbent assay system. Single nucleotide polymorphisms were determined by TaqMan polymerase chain reaction method. After adjustment for confounding factors, adiponectin concentration was significantly lower in HT (HT: 5.2±0.2 μg/mL; NT: 6.1±0.2 μg/mL; $P<0.001$). Furthermore, multiple regression analysis indicated that hypoadiponectinemia was an independent risk factor for hypertension ($P<0.001$). Blood pressure was inversely associated with adiponectin concentration in normotensives regardless of insulin resistance. In subjects carrying the TC genotype of the I164T polymorphism, adiponectin concentration was significantly lower (TC: 2.6±0.9 μg/mL; TT: 5.5±0.1 μg/mL; $P<0.01$), and most of them had hypertension. In contrast, the G276T polymorphism was not associated with adiponectin concentration or hypertension. In conclusion, hypoadiponectinemia is a marker for predisposition to hypertension in men. (***Hypertension.*** **2004;43:1318-1323.)**

Key Words: blood pressure ■ genetics ■ hypertension, genetic ■ men ■ mutation

Adipose tissue participates in the regulation of a variety of homeostatic processes as an endocrine organ that secretes many biologically active molecules such as leptin, tumor necrosis factor-α, and plasminogen-activator inhibitor type 1, which contribute to the development of cardiovascular disease.[1–5] Furthermore, some of these molecules, such as leptin and plasminogen-activator inhibitor type 1, are known to contribute to the development of hypertension.[6–8] Adiponectin is an adipose tissue-specific collagen-like factor, which is abundant in plasma, and a decrease of adiponectin is associated with obesity[9] and type-2 diabetes.[10] Adiponectin modulates the endothelial inflammatory response in vitro, and its concentration is decreased in patients with coronary artery disease.[10–12] Furthermore, adiponectin has been reported to be associated with lipid metabolism,[13,14] glucose metabolism,[15] and insulin resistance.[13,14,16] It was recently reported that treatment of diabetic animals with adiponectin markedly improved insulin sensitivity via reducing triglyceride accumulation in skeletal muscle.[17] These results suggest that adiponectin is one of the key molecules in the metabolic syndrome.

Hypertension is a common disease that increases the risk for cardiovascular disease, and it is also a component of the metabolic syndrome, which is defined as the combination of obesity, insulin resistance, glucose intolerance, and hyperlipidemia. Hypertensive patients are known to have higher body mass index (BMI), triglyceride level, and insulin resistance compared with normotensive subjects.[18] Even though an association between hypertension and serum adiponectin concentration has been reported by several groups using a small number of subjects,[19–22] the obtained results were not identical. Mallamaci et al[19] reported an increased plasma adiponectin concentration in hypertensive patients with renal dysfunction, but Adamczak et al[20] reported decreased adiponectin in hypertensive subjects. Kazumi et al[21] reported that young Japanese men with high-normal blood pressure had lower adiponectin. Recently, Furuhashi et al[22] reported that only hypertensive patients with insulin resistance showed lower adiponectin concentration. Furthermore, in these studies, the association between plasma adiponectin and hypertension was evaluated without adjusting for confounding factors or without dividing the subjects by sex. It is well known that normal women have a higher adiponectin concentration than men,[23] so sex is a potential confounding factor. Thus, the clinical importance of hypoadiponectinemia in hypertension has not been fully elucidated.

Received February 24, 2004; first decision March 9, 2004; revision accepted March 30, 2004.

From the Departments of Geriatric Medicine (Y.I., T.K., K.I., M.O., K.S., Y.F., M.M., K.Y., A.M., H.R., T.O.) and Internal Medicine and Molecular Science (N.O., K.O., S.K., T.F., Y.M.), Osaka University Graduate School of Medicine, Japan.

Correspondence to Dr Tomohiro Katsuya, Department of Geriatric Medicine, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita 565-0871, Japan. E-mail katsuya@geriat.med.osaka-u.ac.jp

Hypertension **is available at http://www.hypertensionaha.org** **DOI: 10.1161/01.HYP.0000129281.03801.4b**

On the other hand, a genetic investigation revealed that subjects with the I164T polymorphism (T-to-C substitution at nucleotide 517 leading to amino acid substitution from isoleucine to threonine at position 164) more frequently had diabetes and had lower concentrations of adiponectin. It was interesting that all 9 patients with the I164T polymorphism had hypertension.[16] In addition, another report showed that the G276T polymorphism in intron 2 was also associated with type-2 diabetes, partially through affecting plasma adiponectin concentration.[24]

To examine whether adiponectin affects blood pressure genetically or biologically, we performed a case-control study using a large number of subjects. In addition, we confirmed the hypothesis that hypoadiponectinemia is correlated with increased insulin resistance.

Methods

Subjects

A total of 758 male subjects (mean age 58.4±0.4 years, BMI 23.9±0.1 kg/m²) were selected from people who were admitted and underwent medical investigation at Osaka University Hospital or its affiliated hospitals. The numbers of normotensive subjects and hypertensive subjects were 312 and 446, respectively. Hypertension was defined as a systolic blood pressure of ≥140 mm Hg and/or a diastolic blood pressure of ≥90 mm Hg on repeated measurements, or receiving antihypertensive treatment. Diabetes was defined as fasting plasma glucose of ≥7.0 mmol/L or receiving treatment for diabetes. All subjects enrolled were Japanese, and subjects with ischemic heart disease including myocardial infarction, congestive heart failure, abnormal electrocardiogram results, valvular heart disease, atrial fibrillation, arteriosclerosis obliterans, or renal failure were excluded. The study protocol was approved by the Ethical Committee of Osaka University, and subjects gave informed consent to participate in the present study, including genetic analysis.

Clinical Features

Blood pressure was measured with an appropriate arm cuff and a mercury column sphygmomanometer on the left arm after a resting period of at least 10 minutes in the supine position. Blood pressure was measured by well-trained physicians who were blinded during the study, and 3 measurements at 1 visit were averaged to evaluate the systolic and diastolic blood pressures. After blood pressure measurements, venous blood sampling from all subjects was performed after fasting overnight. Height and body weight were measured, and BMI was calculated. Plasma samples for subsequent assay were stored at −80°C. Insulin sensitivity was estimated using the homeostatic model assessment (HOMA) index (ie, plasma glucose level×[plasma insulin level/22.5]). Insulin resistance was defined as HOMA ≥3. Plasma concentration of adiponectin was determined by a sandwich enzyme-linked immunosorbent assay system (adiponectin ELISA kit; Otsuka Pharmaceutical Co. Ltd.) as previously reported.[9]

The following parameters were also determined: total cholesterol (T-chol), triglyceride (TG), high-density lipoprotein cholesterol (HDL-chol), and serum creatinine (Cr) levels.

Genotype Determination of Adiponectin Polymorphisms

To investigate the association between adiponectin polymorphisms and hypertension, we selected 2 polymorphisms (I164T and G276T) that were previously reported to be related to plasma adiponectin concentration.[16,24] Genomic DNA was prepared from the buffy coat using a QIAmp DNA blood kit (QIAGEN, Valencia, Calif). The genotypes of the I164T and G276T polymorphisms were determined by the TaqMan polymerase chain reaction (PCR) method.[25] The following primers and probes were included in the reactions: I164T, forward primer, 5′-AAC ATT CCT GGG CTG TAC TAC TTT G-3′; reverse primer, 5′-GGC TGA CCT TCA CAT CCT TCA TA-3′; probes, 5′-FAM-CCA CAC CAC AGT CT-3′, 5′-VIC-ACC ACA TCA CAG TCT A-3′; G276T, forward primer, 5′-AGA ATG TTT CTG GCC TCT TTC ATC-3′; reverse primer, 5′-TTC TCC CTG TGT CTA GGC CTT AGT-3′; probes, 5′-FAM-AAA CTA TAT GAA GTC ATT CAT TA-3′, 5′-VIC-CTA TAT GAA GGC ATT CAT TA-3′. The fluorescence level of PCR products was measured using an ABI PRISM 7900 HT Sequence Detector (Applied Biosystems).

TABLE 1. Clinical Characteristics of Study Subjects

Characteristics	HT (n=446)	NT (n=312)
Age, y	59.4±0.5	57.1±0.6*
BMI, kg/m²	24.4±0.1	23.1±0.2*
Systolic BP, mm Hg	138±1	119±1*
Diastolic BP, mm Hg	83±1	72±1*
Adiponectin, μg/mL	5.2±0.2	6.4±0.2*
T-chol, mmol/L	5.34±0.06	5.17±0.05†
TG, mmol/L	1.77±0.05	1.65±0.07
HDL-chol, mmol/L	1.32±0.02	1.32±0.03
FPG, mmol/L	6.24±0.11	5.98±0.13
HbA1c, %	5.7±0.1	5.7±0.1
HOMA	2.4±0.2	2.1±0.2
Cr, μmol/L	84.6±3.2	90.6±4.2

Values are given as mean±SE. FPG, indicates fasting plasma glucose; other definitions are provided in the text.

*P<0.01 and †P<0.05 compared with hypertensive subjects for each parameter.

Statistical Analysis

Values are expressed as mean±SE. Associations between hypertension and all other parameters were first analyzed by simple logistic regression and then by multivariate analysis. Differences in genotypes and alleles were examined by χ^2 analysis. The association between polymorphisms and clinical variables was examined by multivariate analysis. The quantitative effects of covariates were assessed by multiple regression analysis. P<0.05 was considered statistically significant. All calculations were performed using a standard statistical package (JMP 4.0; SAS Institute Inc).

Results

Plasma Adiponectin Concentration and Hypertension

The average length of time since the first diagnosis of hypertension was 12.5±0.6 years. Furthermore, 342 of 758 hypertensive subjects also had close relatives (parents, brothers, and sisters) who were hypertensive. To assess whether adiponectin was related to hypertension, we compared the clinical characteristics of hypertensive male subjects (HT) and normotensive male subjects (NT) (Table 1). Plasma adiponectin concentration was significantly lower in hypertensive subjects than in normotensive subjects. Age, BMI, and T-chol were also significantly higher in hypertensive men than in normotensive men. Consequently, we selected these parameters as confounding factors. After adjustment for confounding factors (age, BMI, and T-chol), adiponectin concentration was significantly lower in HT (HT: 5.2±0.2 μg/mL; NT: 6.1±0.2 μg/mL; P<0.001). Multiple regression analysis revealed that each confounding factor, age, BMI,

TABLE 2. Multiple Logistic Regression Analysis for Hypertension

Term	Estimate	SE	*P*
Age	−0.0497	0.0086	<0.0001
BMI	−0.1144	0.0293	<0.0001
Adiponectin	0.1017	0.0278	0.0003
T-chol	−0.0048	0.0023	0.0374
Intercept	3.7284	1.0341	0.0003

R^2=0.0754 (n=758).

T-chol, and adiponectin concentration, independently affected the risk for hypertension (Table 2).

We examined simple correlations between plasma adiponectin concentration and clinical variables. The hypertensive subjects were divided into 2 groups: with and without antihypertensive medication; the normotensive subjects were divided into 3 subgroups: with diabetes, with insulin resistance (HOMA≥3) but without diabetes, and without insulin resistance or diabetes. Thus, we compared the clinical variables among 5 subgroups (Table 3). Adiponectin concentration significantly increased with age (in hypertensives using medication and normotensives without diabetes or insulin resistance, $P<0.01$, respectively) and HDL-chol (in hypertensives using medication and normotensives without diabetes, $P<0.01$, respectively), and decreased with BMI (in hypertensives using medication and normotensives, $P<0.01$, respectively) and TG (in hypertensives using medication and normotensives with diabetes, $P<0.01$, respectively). Systolic blood pressure was inversely associated with adiponectin concentration in normotensive subjects without diabetes ($P<0.01$). Diastolic blood pressure was inversely associated with adiponectin concentration in normotensive subjects ($P<0.01$). The association between plasma adiponectin concentration and blood pressure in normotensive subjects without diabetes is shown in Figure 1. However, adiponectin concentration was not associated with blood pressure in hypertensives without medication (Table 3).

TABLE 3. Simple Correlations Between Plasma Adiponectin Concentration and Clinical Characteristics

	Hypertensives		Normotensive		
	Medication		Diabetes	Insulin Resistance	
Characteristics	(+) (n=367)	(−) (n=79)	(+) (n=67)	(+) (n=93)	(−) (n=152)
Age	0.21*	0.26†	0.22	0.17	0.44*
BMI	−0.19*	−0.12	−0.36*	−0.36*	−0.37*
T-chol	−0.05	−0.09	−0.20	−0.11	−0.09
TG	−0.21*	−0.18	−0.43*	−0.19	−0.20†
HDL-chol	0.18*	0.29†	0.11	0.27*	0.34*
FPG	−0.06	−0.15	−0.15	−0.32*	−0.10
HbA1C	−0.03	−0.04	−0.18	−0.04	−0.03
HOMA	−0.21†	−0.13	−0.18	−0.25†	−0.25†
Cr	0.15†	0.17	0.48†	0.03	0.07
SBP		−0.02	−0.32†	−0.35*	−0.31*
DBP		−0.05	−0.44*	−0.38*	−0.38*

Data indicates correlation coefficient. FPG indicates fasting plasma glucose; other definitions are defined in the text.

*$P<0.01$ and †$P<0.05$.

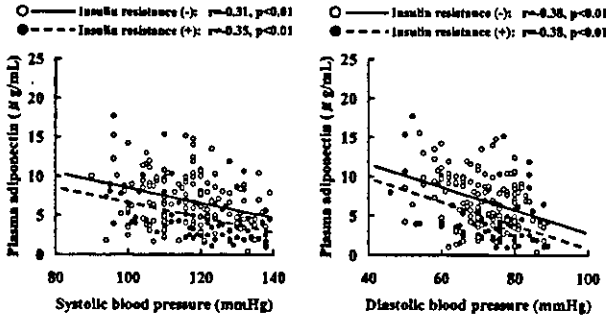

Figure 1. Correlation between plasma adiponectin concentration and blood pressure in normotensives without diabetes. ● indicates subjects with insulin resistance (n=93); ○, subjects without insulin resistance (n=152).

Polymorphisms of Adiponectin and Hypertension

We examined the association between the I164T and G276T polymorphisms and plasma adiponectin concentration. After adjustment for confounding factors (age, BMI, TG, HDL-chol, and HOMA), plasma adiponectin concentration was significantly lower in subjects with the TC genotype of the I164T polymorphism compared with those with the TT genotype (TC: 2.6±0.9 μg/mL; TT: 5.5±0.1 μg/mL; $P<0.01$). No subject with the CC genotype was found in this study. The G276T polymorphism was not significantly related to plasma adiponectin concentration (GG: 5.4±0.2 μg/mL; GT: 5.8±0.2 μg/mL; TT: 4.9±0.4 μg/mL; NS) (Figure 2). We also examined the influence of these polymorphisms on the prevalence of hypertension by case-control study. The G276T polymorphism showed no association with hypertension. Table 4 shows that the TC genotype of the I164T polymorphism was significantly associated with hypertension.

Discussion

The initial finding of the present study was that plasma adiponectin concentration was significantly lower in men

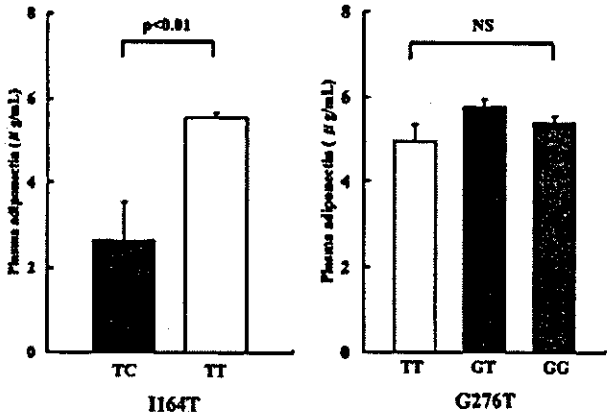

Figure 2. Plasma adiponectin concentration in subjects with I164T and G276T polymorphisms, after adjustment for confounding factors (age, BMI, triglyceride, HDL cholesterol, and homeostatic model assessment index). Data represent mean±SEM.

TABLE 4. Frequencies of Genotypes of Adiponectin Polymorphisms

Polymorphisms		HT	NT	χ^2	P
I164T, n	TT	433	311	6.815	0.009
	TC	13	1		
	GG	225	165		
G276T, n	GT	180	124	0.950	0.622
	TT	41	23		

with hypertension than in normotensive men and was negatively correlated with blood pressure in subjects without hypertension. Furthermore, multiple regression analysis clearly showed that hypoadiponectinemia is an independent risk factor for hypertension. Even though several studies have examined plasma adiponectin level, most of them focused on insulin resistance or diabetes and not on hypertension.

Our results were in accordance with the previous report that HOMA was significantly related to adiponectin concentration.[24] Recently, Furuhashi et al[22] reported that only hypertensive patients with insulin resistance showed a decreased adiponectin concentration. However, the cause–effect relationship among hypoadiponectinemia, insulin resistance, and hypertension has not been clearly elucidated. Even though the consensus has been that insulin resistance is correlated with hypertension,[26,27] the association between insulin and hypertension is controversial.[28–31] In fact, HOMA was not significantly different between hypertensive and normotensive subjects in the present study. As a specific finding of this study, plasma adiponectin level significantly decreased with an increase in blood pressure, even in the normotensives without insulin resistance or diabetes. These results indicate that hypoadiponectinemia may affect the pathogenesis of hypertension at a very early stage without involving insulin resistance. Recently, Lindsay et al[32] reported that there were loci on chromosomes 2, 3, 9, and 10 affecting the circulating adiponectin concentration in the Pima population, suggesting the possibility of an unknown modulator of adiponectin level. However, further investigation is required to examine this hypothesis.

There are 4 possible reasons for the negative correlation between hypertension and plasma adiponectin concentration. First, as Ouchi et al[33] recently reported that plasma adiponectin concentration was independently correlated with the vasodilator response to reactive hyperemia, adiponectin concentration could be an independent parameter of endothelial function. Endothelial dysfunction is an important feature of the early stage of atherosclerosis, which is related to pathogenic conditions including hypertension.[34,35] Furthermore, in adiponectin-knockout mice, hypoadiponectinemia causes diet-induced hypertension. Second, an increase in sympathetic nerve activity, which is common in hypertensives,[36] may inhibit adiponectin gene expression via β-adrenergic stimulation.[37] Third, the reciprocal association of adiponectin and high-sensitive C-reactive protein or increased risk of arteriosclerosis suggests that a low adiponectin concentration might enhance the predisposition to hypertension via vascular injury.[10,11] Fourth, activation of the renin-angiotensin system may be induced in adipose tissue by hypoadiponectinemia, resulting in an increase in fat mass and blood pressure.[38,39] However, further investigation is required to examine these hypotheses.

Another important finding of this study was the positive association between plasma adiponectin concentration and age. There is a supportive report that adiponectin was decreased by sex hormones like androgens, which are suppressed with aging.[23] A reduction in adiponectin clearance in older men is another possible reason for the age-related increase in adiponectin concentration. Furthermore, a previous report also suggested that age is an independent regulating factor for adiponectin concentration.[40] However, it is well known that the prevalence of hypertension, insulin resistance, and diabetes increases with age. There may appear to be a discrepancy, but these results lead to the hypothesis that the implication of hypoadiponectinemia in youth is different from that in old age, and adiponectin may exert an insufficient effect without increasing sufficiently with age. The finding of a lower adiponectin concentration in elderly subjects may indicate the existence of a metabolic disorder like "adiponectin resistance." Further investigation is required to examine these hypotheses.

The final finding of our study was related to adiponectin gene polymorphism. We examined 2 polymorphisms that were previously reported to be related to plasma adiponectin concentration in the Japanese population. Subjects with the TC genotype of the I164T polymorphism showed a significantly lower plasma adiponectin concentration, and most of the C allele carriers had hypertension. Furthermore, we also found a significant association between the TC genotype of the I164T polymorphism and hypertension. It seems to be a novel finding that >80% of C164 carriers were hypertensive in a previous study[16] and in the present study. In contrast, we could not find an association between the G276T polymorphism and adiponectin concentration or hypertension. A previous study has shown an association between theG276T polymorphism and adiponectin concentration only in obese subjects (BMI ≥26.7 kg/m^2).[24] Because few obese subjects were included in the present study, we could not conclude a lack of association between the G276T polymorphism and adiponectin.

Study Limitations

This study was designed to be cross-sectional and case-controlled, but not prospective. Several important determinants of plasma adiponectin level, such as body fat content and waist circumference, were not measured in our study. Instead of these measurements, we used HOMA to evaluate insulin resistance. In addition, verification of the cause–effect relationship between hypertension and hypoadiponectinemia would require a study design with a cohort base.

It has been reported that renal function, as indicated by creatinine clearance (Ccr), is an independent regulator of adiponectin concentration in hypertensive subjects.[19] In our study, also, adiponectin concentration was significantly associated with Ccr ($r=-0.38$, $P<0.01$). However, the number of subjects whose Ccr was measured was small (n=102) compared with the total number of study subjects (n=758). The mean Ccr was almost the same in normotensive and hypertensive subjects. Therefore, Ccr was not included in the

discussion of the association between adiponectin and hypertension in this study. However, it was revealed that adiponectin concentration was significantly associated with creatinine in hypertensives using medication and normotensives with diabetes (Table 3), suggesting that hyperadiponectinemia is also involved in the progression of renal damage.

In conclusion, the present findings suggest that a lower plasma adiponectin concentration is significantly associated with hypertension. Interestingly, hypoadiponectinemia is one of the risk factors for hypertension and could be a possible target for antihypertensive treatment.

Acknowledgments

The present study was supported by a grant-in-aid from the Japanese Ministry of Health, Labor, and Welfare, grants-in-aid for Scientific Research (12557063, 14207035, 15590342, 13204050) from the Ministry of Education, Science, Sports, and Culture of Japan, and research grants from Takeda Medical Foundation, the Tokyo Biochemical Research Foundation, Ono Medical Foundation, the Salt Science Research Foundation, the Osaka Medical Research Foundation for Incurable Diseases, the Osaka Gas Group Welfare Foundation, the Osaka Kidney Foundation of Japan, and the Preventive Arteriosclerosis Research Association. We are indebted to Sayaka Ohashi and Sachiyo Tanaka for their excellent technical assistance.

References

1. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature.* 1994;372:425–432.
2. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science.* 1993;259:87–91.
3. Shimomura I, Funahashi T, Takahashi M, Maeda K, Kotani K, Nakamura T, Yamashita S, Miura M, Fukuda Y, Takemura K, Tokunaga K, Matsuzawa Y. Enhanced expression of PAI-1 in visceral fat: possible contributor to vascular disease in obesity. *Nat Med.* 1996;2:800–803.
4. Wallace AM, McMahon AD, Packard CJ, Kelly A, Shepherd J, Gaw A, Sattar N. Plasma leptin and the risk of cardiovascular disease in the West of Scotland Coronary Prevention Study (WOSCOPS). *Circulation.* 2001; 104:3052–3056.
5. Ridker PM, Rifai N, Pfeffer M, Sacks F, Lepage S, Braunwald E. Elevation of tumor necrosis factor-alpha and increased risk of recurrent coronary events after myocardial infarction. *Circulation.* 2000;101: 2149–2153.
6. Agata J, Masuda A, Takada M, Higashiura K, Murakami H, Miyazaki Y, Shimamoto K. High plasma immunoreactive-leptin level in essential hypertension. *Am J Hypertens.* 1997;10:1171–1174.
7. Wall U, Jern C, Bergbrant A, Jern S. Enhanced levels of tissue-type plasminogen activator in borderline hypertension. *Hypertension.* 1995; 26:796–800.
8. Eliasson M, Jansson JH, Nilsson P, Asplund K. Increased levels of tissue plasminogen activator antigen in essential hypertension. A population-based study in Sweden. *J Hypertens.* 1997;15:349–356.
9. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun.* 1999; 257:79–83.
10. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, Iwahashi H, Kuriyama H, Ouchi N, Maeda K, Nishida M, Kihara S, Sakai N, Nakajima T, Hasegawa K, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Hanafusa T, Matsuzawa Y. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol.* 2000;20:1595–1599.
11. Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, Hotta K, Nishida M, Takahashi M, Nakamura T, Yamashita S, Funahashi T, Matsuzawa Y. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation.* 1999;100: 2473–2476.
12. Kumada M, Kihara S, Sumitsuji S, Kawamoto T, Matsumoto S, Ouchi N, Arita Y, Okamoto Y, Shimomura I, Hiraoka H, Nakamura T, Funahashi T, Matsuzawa Y; Osaka CAD Study Group. Coronary artery disease. Association of hypoadiponectinemia with coronary artery disease in men. *Arterioscler Thromb Vasc Biol.* 2003;23:85–89.
13. Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med.* 2001;7:941–946.
14. Berg AH, Combs TP, Du X, Brownlee M, Scherer PE. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat Med.* 2001; 7:947–953.
15. Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, Yamashita S, Noda M, Kita S, Ueki K, Eto K, Akanuma Y, Froguel P, Foufelle F, Ferre P, Carling D, Kimura S, Nagai R, Kahn BB, Kadowaki T. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med.* 2002;8:1288–1295.
16. Kondo H, Shimomura I, Matsukawa Y, Kumada M, Takahashi M, Matsuda M, Ouchi N, Kihara S, Kawamoto T, Sumitsuji S, Funahashi T, Matsuzawa Y. Association of adiponectin mutation with type 2 diabetes: a candidate gene for the insulin resistance syndrome. *Diabetes.* 2002;51: 2325–2328.
17. Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, Tataranni PA. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab.* 2001;86:1930–1935.
18. Mikhail N, Golub MS, Tuck ML. Obesity and hypertension. *Prog Cardiovasc Dis.* 1999;42:39–58.
19. Mallamaci F, Zoccali C, Cuzzola F, Tripepi G, Cutrupi S, Parlongo S, Tanaka S, Ouchi N, Kihara S, Funahashi T, Matsuzawa Y. Adiponectin in essential hypertension. *J Nephrol.* 2002;15:507–511.
20. Adamczak M, Wiecek A, Funahashi T, Chudek J, Kokot F, Matsuzawa Y. Decreased plasma adiponectin concentration in patients with essential hypertension. *Am J Hypertens.* 2003;16:72–75.
21. Kazumi T, Kawaguchi A, Sakai K, Hirano T, Yoshino G. Young men with high-normal blood pressure have lower serum adiponectin, smaller LDL size, and higher elevated heart rate than those with optimal blood pressure. *Diabetes Care.* 2002;25:971–976.
22. Furuhashi M, Ura N, Hishiura K, Murakami H, Tanaka M, Moniwa N, Yoshida D, Shimamoto K. Blockade of renin-angiotensin system increases adiponectin concentration in patients with essential hypertension. *Hypertension.* 2003;42:76–81.
23. Nishizawa H, Shimomura I, Kishida K, Maeda N, Kuriyama H, Nagaretani H, Matsuda M, Kondo H, Furuyama N, Kihara S, Nakamura T, Tochino Y, Funahashi T, Matsuzawa Y. Androgens decrease plasma adiponectin, an insulin-sensitizing adipocyte-derived protein. *Diabetes.* 2002;51:2734–2741.
24. Hara K, Boutin P, Mori Y, Tobe K, Dina C, Yasuda K, Yamauchi T, Otabe S, Okada T, Eto K, Kadowaki H, Hagura R, Akanuma Y, Yazaki Y, Nagai R, Taniyama M, Matsubara K, Yoda M, Nakano Y, Tomita M, Kimura S, Ito C, Froguel P, Kadowaki T. Genetic variation in the gene encoding adiponectin is associated with an increased risk of type 2 diabetes in the Japanese population. *Diabetes.* 2002;51:536–540.
25. Ishikawa K, Baba S, Katsuya T, Iwai N, Asai T, Fukuda M, Takiuchi S, Fu Y, Mannami T, Ogata J, Higaki J, Ogihara T. T+31C polymorphism of angiotensinogen gene and essential hypertension. *Hypertension.* 2001; 37:281–285.
26. Zavaroni I, Bonora E, Pagliara M, Dall'Aglio E, Luchetti L, Buonanno G, Bonati PA, Bergonzani M, Gnudi L, Passeri M. Risk factors for coronary artery disease in healthy persons with hyperinsulinemia and normal glucose tolerance. *N Engl J Med.* 1989;320:702–706.
27. Ferrannini E, Natali A, Capaldo B, Lehtovirta M, Jacob S, Yki-Jarvinen H. Insulin resistance, hyperinsulinemia, and blood pressure. *Hypertension.* 1997;30:1144–1449.
28. Haffner SM. Insulin and blood pressure: fact or fantasy? *J Clin Endocrinol Metab.* 1993;76:541–543.
29. Reaven PD, Barrett-Connor EL, Browner DK. Abnormal glucose tolerance and hypertension. *Diabetes Care.* 1990;13:119–125.
30. Mbanya JC, Thomas TH, Wilkinson R, Alberti KG, Taylor R. Hypertension and hyperinsulinaemia: a relation in diabetes but not essential hypertension. *Lancet.* 1988;1:733–734.

31. Raji A, Williams GH, Jeunemaitre X, Hopkins PN, Hunt SC, Hollenberg NK, Seely EW. Insulin resistance in hypertensives: effect of salt sensitivity, renin status and sodium intake. *J Hypertens.* 2001;19:99–105.
32. Lindsay RS, Funahashi T, Krakoff J, Matsuzawa Y, Tanaka S, Kobes S, Bennett PH, Tataranni PA, Knowler WC, Hanson RL. Genome-wide linkage analysis of serum adiponectin in the Pima Indian population. *Diabetes.* 2003;52:2419–2425.
33. Ouchi N, Ohishi M, Kihara S, Funahashi T, Nakamura T, Nagaretani H, Kumada M, Ohashi K, Okamoto Y, Nishizawa H, Kishida K, Maeda N, Nagasawa A, Kobayashi H, Hiraoka H, Komai N, Kaibe M, Rakugi H, Ogihara T, Matsuzawa Y. Association of hypoadiponectinemia with impaired vasoreactivity. *Hypertension.* 2003;42:231–234.
34. Luscher TF. The endothelium and cardiovascular disease: a complex relation. *N Engl J Med.* 1994;330:1081–1083.
35. Vita JA, Keaney JF Jr. Endothelial function: a barometer for cardiovascular risk? *Circulation.* 2002;106:640–642.
36. Trimarco B, Volpe M, Ricciardelli B, Picotti GB, Galva MD, Petracca R, Condorelli M. Studies of the mechanisms underlying impairment of beta-adrenoceptor-mediated effects in human hypertension. *Hypertension.* 1983;5:584–590.
37. Fasshauer M, Klein J, Neumann S, Eszlinger M, Paschke R. Adiponectin gene expression is inhibited by beta-adrenergic stimulation via protein kinase A in 3T3–L1 adipocytes. *FEBS Lett.* 2001;507:142–146.
38. Jones BH, Standridge MK, Taylor JW, Moustaid N. Angiotensinogen gene expression in adipose tissue: analysis of obese models and hormonal and nutritional control. *Am J Physiol.* 1997;273:R236–R242.
39. Massiera F, Bloch-Faure M, Ceiler D, Murakami K, Fukamizu A, Gasc JM, Quignard-Boulange A, Negrel R, Ailhaud G, Seydoux J, Meneton P, Teboul M. Adipose angiotensinogen is involved in adipose tissue growth and blood pressure regulation. *FASEB J.* 2001;15:2727–2729.
40. Cnop M, Havel PJ, Utzschneider KM, Carr DB, Sinha MK, Boyko EJ, Retzlaff BM, Knopp RH, Brunzell JD, Kahn SE. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. *Diabetologia.* 2003;46:459–469.

Circ J 2004; **68:** 650–655

Insulin Resistance in Patients With Hypertrophic Cardiomyopthy

Kazuo Murakami, MD; Yuji Shigematsu, MD*; Mareomi Hamada, MD**; Jitsuo Higaki, MD*

Background The aim of the present study was to investigate whether or not insulin resistance is present in hypertrophic cardiomyopathy (HCM), and also whether it is related to the clinical manifestations of HCM.
Methods and Results The study group comprised 55 patients with HCM, 35 with essential hypertension (EHT) and 15 normotensive control subjects (NC). An insulin resistance index was estimated using the homeostasis model assessment (HOMA-IR) of fasting insulin–glucose interactions. In patients with HCM, prognosis and cardiovascular events were also checked. The HOMA-IR values in the HCM group (2.90±1.22) were significantly higher than those in the EHT (1.69±0.77) and NC groups (0.91±0.24). The HOMA-IR values in the EHT group were significantly higher than those in the NC group. Multiple regression analyses determined that left ventricular pressure gradient without provocation ($p<0.0001$), interventricular septal thickness ($p=0.0143$) and body mass index ($p=0.0412$) were independent determinants of insulin resistance in patients with HCM. During a mean follow-up of 105±50 months, 4 patients with HCM died suddenly and all of them had high HOMA-IR values.
Conclusions The results suggest that patients with HCM without apparent diabetes mellitus or hypertension have insulin resistance and that insulin resistance may be related to the manifestations of HCM. (*Circ J* 2004; **68:** 650–655)

Key Words: Hypertrophic cardiomyopathy; Insulin resistance; Left ventricular hypertrophy; Prognosis

Echocardiographically determined left ventricular (LV) mass is a potent independent predictor of cardiovascular morbidity and mortality in essential hypertension (EHT)[1,2] and furthermore, there is increasing evidence of a link between hyperinsulinemia and cardiovascular risk[3] Insulin may exert a direct growth-promoting effect on cardiac myocytes and could be involved in the pathogenesis of EHT[4] Not only insulin, but insulin-like growth factor-1 (IGF-1) is also important in the hypertrophic response of cardiac myocytes, by binding to the IGF-1 receptors because of its structural similarity to insulin[4,5] In the clinical setting, an inverse association is reported between insulin sensitivity and left ventricular wall thickness in EHT[6]

It is well established that cardiac myocyte hypertrophy and disarray, increased interstitial collagen synthesis and medial thickening in the intramural coronary arteries are the clinical and pathological manifestations of hypertrophic cardiomyopathy (HCM). However, the factors that account for the variability in the phenotypic expression of the disorder remain largely unknown. According to the hypothesis of Marian, increased expression of trophic and mitotic factors such as IGF-1, transforming growth factor β_1 and angiotensin II is a major contributor to the pathological manifestations of HCM[7] In fact, several reports have shown that the expression of both the mRNA and protein for IGF-1 are elevated in HCM cardiac myocytes[8,9] In addition, Saeki et al showed that the level of circulating IGF-1 is related to the clinical condition of HCM, particularly congestive heart failure associated with HCM[10] However, despite its structural similarity to IGF-1, pathological role of insulin in HCM is still incompletely known.

Accordingly, the present study was undertaken to examine whether hyperinsulinemia caused by insulin resistance is present in HCM and whether insulin resistance (IR) is related to clinical manifestations of HCM.

Methods

Study Population

We enrolled 55 nondiabetic patients with HCM (46 men, 9 women; mean age: 57±10 years), 35 nondiabetic patients with EHT (20 men, 15 women; mean age: 56±9 years) and 15 normotensive control subjects (NC) (10 men, 5 women; mean age: 53±15 years) after they gave informed consent. They had normal findings on chemical screening battery and were nondiabetic by the criteria of the American Diabetes Association[11] All of them underwent a coronary angiographic study and none had coronary artery disease. The study was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association.

HCM was defined as the presence of a hypertrophied, nondilated ventricle in the absence of underlying cardiac or systemic secondary causes and was based on the World Health Organization/International Society and Federation of Cardiology definition of cardiomyopathies[12] Patients with HCM were subdivided into 2 groups: hypertrophic obstructive cardiomyopathy (HOCM) and hypertrophic nonobstructive cardiomyopathy (HNCM). HOCM was diagnosed when a patient had a left ventricular pressure gradient (LVPG) greater than 30 mmHg without provocation in the left ventricular outflow tract and/or midventricle[13]

(Received February 9, 2004; revised manuscript received April 5, 2004; accepted April 16, 2004)
*Department of Internal Medicine, Matsuyama Red Cross Hospital, Matsuyama, *The Second Department of Internal Medicine, Ehime University School of Medicine, Shigenobu and **Department of Internal Medicine, Uwajima City Hospital, Uwajima, Japan*
Mailing address: Yuji Shigematsu, MD, The Second Department of Internal Medicine, Ehime University School of Medicine, Shigenobu-cho, Onsen-gun, Ehime 791-0295, Japan. E-mail: yujis@m.ehime-u.ac.jp

Table 1 Comparison of the Biochemical and Hemodynamic Characteristics Between Hypertrophic Cardiomyopathy (HCM) Patients With and Without Obstruction

	HCM with obstruction (n=20)	*HCM without obstruction (n=35)*	*p value*
Age (years)	57.3±9.2	56.7±10.3	*NS*
M/F	16/4	30/5	*NS*
BMI (kg/m²)	24.4±4.0	24.3±2.7	*NS*
FPG (mmol/L)	5.35±0.75	5.17±0.47	*NS*
FIRI (μU/ml)	15.2±6.9	11.0±3.7	*0.005*
HOMA-IR	3.57±1.50	2.52±0.83	*0.0015*
Blood pressure (mmHg)			
Systole	132±26	135±20	*NS*
Diastole	77±13	82±12	*NS*
IVST (mm)	17.3±3.5	14.0±3.8	*0.0029*
LVPWT (mm)	11.2±3.3	10.1±1.5	*0.1006*

Data are the mean value±SD. BMI, body mass index; FPG, fasting plasma glucose; FIRI, fasting immunoreactive insulin; IVST, interventricular septal thickness; LVPWT, left ventricular posterior wall thickness; NS, not significant.

Table 2 Comparison of the Biochemical, Hemodynamic Characteristics and Prognosis Between Hypertrophic Cardiomyopathy (HCM) Patients With High HOMA-IR (≥2.6) and Low HOMA-IR (<2.6) Values

	HCM with high HOMA-IR (n=28)	*HCM with low HOMA-IR (n=27)*	*p value*
Age (years)	53.5±9.0	60.4±9.5	*0.0082*
M/F	23/5	23/4	
BMI (kg/m²)	24.4±4.0	24.3±2.7	*NS*
FPG (mmol/L)	5.21±0.64	5.27±0.54	*NS*
FIRI (μU/ml)	16.5±4.9	8.5±1.6	*<0.0001*
HOMA-IR	3.79±1.09	1.98±0.37	*<0.0001*
Blood pressure (mmHg)			
Systole	133±21	135±22	*NS*
Diastole	79±12	81±12	*NS*
IVST (mm)	15.5±3.3	15.0±4.7	*NS*
LVPWT (mm)	10.8±2.5	10.1±2.2	*NS*
Left ventricular pressure gradient (mmHg)	48.9±35.2	24.6±25.3	*0.0434*
Prognosis			
Follow-up period (months)	98±50	113±47	*NS*
Sudden cardiac death	4	0	*0.0414*
Heart failure death	2	1	*NS*
Cancer death	0	3	*NS*
Heart failure event	5	4	*NS*

Data are the mean value±SD. BMI, body mass index; FPG, fasting plasma glucose; FIRI, fasting immunoreactive insulin; IVST, interventricular septal thickness; LVPWT, left ventricular posterior wall thickness; NS, not significant.

Thirty-eight (69%) patients had been taking β-blockers and 42 patients (76%) had been taking calcium antagonists after the evaluation of IR.

In never treated and nondiabetic hypertensive patients, a complete medical history and physical examination and appropriate laboratory evaluation failed to reveal a secondary cause for the hypertension![14] Blood pressure was measured in triplicate by a single physician who was expert in the evaluation of hypertension, with an appropriate arm cuff and a mercury sphygmomanometer after a 5-min rest while seated. The arithmetic mean of the last 2 measurements was calculated. Korotkoff phase V was taken for diastolic blood pressure. Hypertension was defined as systolic blood pressure equal to or greater than 140 mmHg and/or diastolic blood pressure equal to or greater than 90 mmHg![15]

Assessment of Insulin Resistance

After an overnight fast, a venous blood sample was taken from each subject in the morning in the outpatient clinic. Plasma glucose was immediately determined by the glucose oxidase method, and plasma insulin was determined in duplicate by highly specific and sensitive immunoradiometric assay (Abbott, Japan; intra-assay coefficient of variation (CV) 1.6%, interassay CV 2.2%).

Insulin resistance was assessed from the values for fasting immunoreactive insulin (FIRI) and fasting plasma glucose (FPG) and the previously validated homeostasis model assessment (HOMA-IR);[16] thus, HOMA-IR=FIRI (μU/ml)×FPG (mmol/L)/22.5. Low HOMA-IR values mean normal insulin sensitivity and high values mean the presence of resistance. The upper limit of HOMA-IR of juveniles is reported as 1.0![16]

Echocardiography

Two-dimensionally guided M-mode echocardiography was performed by standard methods, as previously described![14,17] using an SSD-870 or SSD-5500 echocardiograph with a 3.5 MHz transducer (Aloka Inc, Tokyo, Japan). LV internal dimension (LVID), interventricular septal thickness (IVST) and left ventricular posterior wall thickness (LVPWT) were measured at end-diastole and end-systole, according to the American Society of Echocardiography guidelines![18] and used for all purposes except determination of left ventricular mass (LVM, which was calculated at end-diastole using the Penn convention![19] The LVM index was measured as follows: LVMI=LVM/body

Table 3 Comparisons of the Biochemical and Hemodynamic Characteristics of the Normotensive Control Subjects (NC) and the Patients With Hypertrophic Cardiomyopathy (HCM) and Essential Hypertension (EHT)

	n	Age (years)	M/F	BMI (kg/m²)	SBP/DBP (mmHg)	IVST (mm)	LVPWT (mm)	FPG (mmol/L)	FIRI (μU/ml)
NC	15	53±15	10/5	23.3±3.2	129±5 /80±6	8.3±0.7	8.6±0.5	4.86±0.51	4.2±1.0
HCM	55	57±10	46/9	24.4±3.2	134±22/80±12	15.2±2.4	10.5±2.4	5.24±0.59	12.6±5.4
EHT	35	56±9	20/15	24.2±2.7	163±17/95±12	10.2±1.3	10.0±1.2	5.37±0.75	7.1±3.1
p value									
NC vs HCM		NS		NS	NS/NS	<0.0001	0.0014	0.0430	<0.0001
NC vs EHT		NS		NS	<0.0001/0.0141	0.0464	0.0226	0.0103	<0.0001
HCM vs EHT		NS		NS	<0.0001/0.0013	<0.0001	NS	NS	0.0343

Data are the mean value±SD.

BMI, body mass index; SBP and DBP, systolic and diastolic blood pressure; IVST, interventricular septal thickness; LVPWT, left ventricular posterior wall thickness; FPG, fasting plasma glucose; FIRI, fasting immunoreactive insulin; NS, not significant.

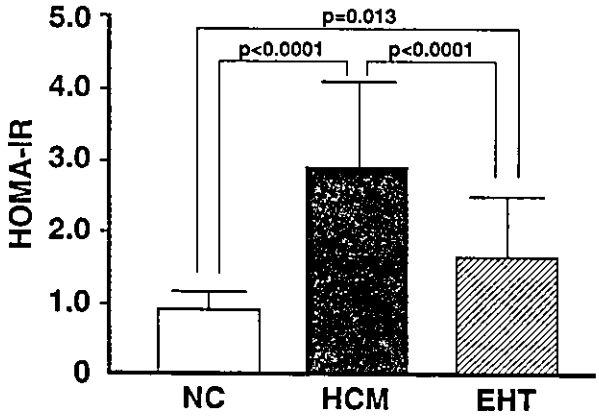

Fig 1. Comparison of the HOMA-IR values in normotensive control subjects (NC), hypertrophic cardiomyopathy (HCM) patients and patients with essential hypertension (EHT). Column height represents mean; bars indicate 95% confidence intervals.

surface area. Relative wall thickness (RWT) was also measured as follows:[20] RWT=2×(LVPWTd/LVIDd), where d is end-diastole. LVPG was measured on the continuous-wave Doppler recording of the left ventricular outflow tract and/or midventricle?[21]

Subgroups Analysis

Patients with HCM were distributed into the following 2 balanced subgroups: HOCM group (LVPG ≥30 mmHg) (n=20) and HNCM group (LVPG <30 mmHg) (n=35) (Table 1). On the basis of IR, patients with HCM were then subdivided into 2 different groups by median HOMA-IR value (Table 2): HOMA-IR ≥2.6 and HOMA-IR <2.6.

To evaluate whether the abnormality of IR is related to the prognosis and cardiovascular events in patients with HCM, the following events were prospectively confirmed. Total deaths, including sudden cardiac death, congestive heart failure and cancers, and congestive heart failure as a cardiovascular event were checked. Congestive heart failure was defined as the clinical symptoms were New York Heart Association Functional Class III or IV and lung congestion associated with left heart failure was confirmed on chest X-ray.

Statistical Analysis

All values are expressed as mean±SD. One-way analysis of variance (ANOVA) was used to evaluate difference among groups, with Scheffe's correction for multiple comparisons. Two tailed unpaired Student's t-test was used to compare study the response of variables between categories. Categorical variables were compared with Fisher's exact and chi-square tests. Correlation coefficients were calculated according to Pearson's method. A multiple regression analysis was also performed to select appropriate independent variables producing the highest partial correlation with HOMA-IR in HCM group. Probability values less than 0.05 were considered statistically significant in all analyses.

Results

Baseline Characteristics

The biochemical and hemodynamic characteristics of the 3 groups are shown in Table 3. There were no significant differences in age, sex distribution or body mass index (BMI) among the 3 groups. FPG in the HCM or EHT group was significantly higher than that in the NC; however, there was no significant difference in FPG between the HCM and EHT groups. FIRI in the HCM or EHT group was significantly higher than that in the NC group and FIRI was significantly higher in the HCM group than in the EHT group. Office blood pressure in the EHT group was significantly higher than that in either the HCM or NC group. There was no significant difference in office blood pressure between the HCM and NC groups. IVST was largest in the HCM group, followed by that in the EHT group and then the NC group. PWT in both the HCM and EHT groups was larger than that in the NC group, but there was no significant difference in PWT between the HCM and EHT groups.

The biochemical and hemodynamic characteristics of the HOCM and HNCM groups are shown in Table 1. There were no significant differences in age, sex distribution, BMI or office blood pressure. FIRI in the HOCM group was significantly higher than that in the HNCM group, but there was no significant difference in FPG between 2 the groups. IVST was larger in the HOCM than in the HNCM group, but there was no significant difference between them in LVPWT.

Determinants of IR

Fig 1 shows the HOMA-IR values in the NC, HCM and EHT groups. The HOMA-IR values in the HCM group (2.90±1.22) were significantly higher than those in the EHT (1.69±0.77) or NC group (0.91±0.24). The HOMA-IR values in the EHT group were significantly higher than those in the NC group. The HOMA-IR values showed a significant association with the LVMI (r=0.66, p<0.0001)

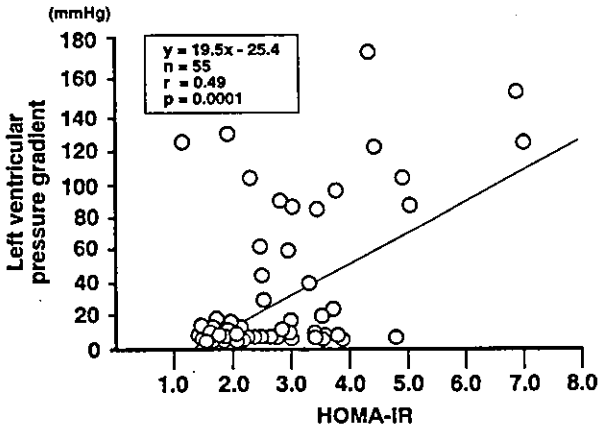

Fig 2. There was a statistically significant positive relationship between the HOMA-IR values and the left ventricular pressure gradient in patients with hypertrophic cardiomyopathy (HCM).

in the EHT group. In addition, the HOMA-IR values in the HOCM group were significantly higher than those in the HNCM group (Table 1).

There were no significant differences in sex distribution, office blood pressure, IVST and LVPWT between patients with HCM having high HOMA-IR values and those with low HOMA-IR values. However, HCM patients with high HOMA-IR values were significantly younger than those with low HOMA-IR values (Table 2).

As indicated in Fig 2, the HOMA-IR values showed a significant association with the LVPG in the HCM group. Table 4 shows the results of multiple regression analysis: independent determinants of the HOMA-IR values were LVPG, IVST and BMI in the HCM group. Age and systolic blood pressure did not achieve enough significance to enter the model.

Follow-up Results

Table 2 also shows the prognosis in patients with HCM during a mean follow-up of 105±50 months. Of these, 18 (64%) patients taking β-blockers and the 22 (79%) taking calcium antagonists had high HOMA-IR values compared with 20 (74%) and 20 (74%), respectively, who had low HOMA-IR values. There were no significant differences in medical treatment between the 2 HCM groups. The incidence of sudden cardiac death was significantly higher in patients with HCM and high HOMA-IR values than in patients with HCM and low HOMA-IR values. The HOMA-IR values in 4 patients with HCM who died suddenly were 2.73, 2.93, 3.29 and 4.79, respectively. There was, however, no significant difference in the incidence of congestive heart failure between the 2 HCM groups.

Discussion

The present study documents for the first time that patients with HCM without apparent diabetes mellitus or hypertension have IR. The LVPG without provocation, IVST and BMI were powerful independent determinants of IR in patients with HCM. HCM is a primary disease of the myocardium that is diagnosed by the presence of left ventricular hypertrophy without increased external load and a small left ventricular cavity. However, the results of our present study strongly suggest that HCM is also a systemic disease that involves systemic skeletal muscles and vascular endothelial cells.

Table 4 Independent Predictors of the HOMA-IR Values From Multiple Regression Analysis

	β	T value	p value
Age	*-0.007*	*-0.059*	*0.9529*
Body mass index	*0.263*	*2.093*	*0.0415*
Interventricular septal thickness	*-0.302*	*-2.420*	*0.0193*
Systolic blood pressure	*0.028*	*0.233*	*0.8169*
Left ventricular pressure gradient	*0.611*	*5.103*	*<0.0001*

Multiple R^2=0.402, p<0.0001.

Effects of Insulin on Left Ventricular Hypertrophy

It is well established that peripheral hyperinsulinemia in patients with hypertension is a marker of IR[6,22] Diminished insulin sensitivity with regard to glucose utilization causes a substantial increase of insulin production in an attempt to maintain normal glucose utilization, making it possible that cardiovascular trophic effects and other actions of insulin are exaggerated. We calculated the HOMA-IR values to obtain a better quantitative estimate of IR[23] and in the present study, we showed a significant association between the echocardiographically determined LVMI and the HOMA-IR values in hypertensive patients, thereby confirming previous positive reports. Furthermore, Paternostro et al have reported that IR is a feature of the hypertrophied heart even in the absence of hypertension, coronary artery disease and diabetes mellitus[24]

The direct effect of insulin on cardiac myocyte growth could be mediated at least in part, by the IGF-1 receptors[25] but unfortunately, we could not determine the IGF-1 binding protein in the present study. However, because the HOMA-IR values reflect fasting insulin levels, our data suggest that insulin is powerful determinant of LVM in subjects with untreated EHT and normal glucose tolerance. In addition, hypertensive patients with glucose intolerance have more severe left ventricular hypertrophy and left ventricular diastolic dysfunction than those with normal glucose tolerance[26]

Hyperinsulinemia Caused by IR in HCM

Verdecchia et al reported that insulin and IGF-1 were powerful independent determinants of LVM in nondiabetic patients with hypertension[27] On the other hand, Marian reported the possibility that IGF-1 was up-regulated in patients with HCM because of decreased cardiac contractility, resulting in the pathologic manifestations of HCM? Despite its structural similarity to IGF-1, the relationship between insulin and HCM, which is another cause of left ventricular hypertrophy and diastolic dysfunction, is incompletely understood. The present study disclosed that the LVPG without provocation, IVST and BMI are important determinants of IR, and that these relations are independent of age, sex distribution and office blood pressure in patients with HCM. In hypertensive patients, it has been reported that IR plays an important role in the initiation and development of hypertension[28] but insulin may not play a major independent role in the elevation of blood pressure in patients with HCM.

Almost half of the cases of HCM are reported to have autosomal-dominant type family history of this disease. At the present time, the gene abnormalities reported in patients