Original Article

Association of a Mast Cell Chymase Gene Variant with HDL Cholesterol, but not with Blood Pressure in the Ohasama Study

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Two enzymes, chymase and angiotensin converting enzyme (ACE), are involved in the production of angiotensin II. Our previous study revealed the male-specific effect of the *ACE DD* genotype on the risk for hypertension, but the genetic role of chymase remains unclear. In the present study, we report the results of an association study involving 1,046 subjects recruited from a general population in Ohasama, a rural community in the northern part of Japan. In addition to casual blood pressure (casual BP) measurement, home BP measurements were obtained from all participants. There were no differences in either home or casual BP values according to G3255A polymorphism of the mast cell chymase gene (*MCC*). HDL cholesterol level was significantly higher among carriers of the A3255 allele ($p < 0.04$). After adjustment for confounding factors, the A3255 allele was still shown to have an effect on HDL cholesterol metabolism ($p < 0.03$). Multiple regression analysis showed that *MCC* polymorphism was significantly and independently related to serum HDL cholesterol level. In conclusion, G3255A polymorphism of *MCC* is not directly associated with blood pressure but may modulate the prevalence of hypertensive complications *via* alteration of lipid metabolism. (*Hypertens Res* 2002; 25: 179–184)

Key Words: chymase, hypertension, genetics, polymorphism, HDL cholesterol

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Introduction

The renin-angiotensin system plays an important role in the control of blood pressure and vascular remodeling (1). Two enzymes, chymase and angiotensin converting enzyme (ACE), are involved in the production of angiotensin II in humans (2) and have been considered candidates for genetic predisposing factors for essential hypertension (3) and cardiovascular diseases (4). Two recent papers (5, 6) revealed a male-specific effect of the *ACE DD* genotype on the risk for hypertension, but the genetic role of chymase remains unclear.

Most of the previous studies concerning the association between hypertension and several polymorphisms have been performed based on the measurement of conventional casual blood pressure (BP). However, casual BP measurement is known to be vulnerable to a number of biases, including observer bias, regression dilution bias, and the white-coat effect (7). In contrast, BP measured by the subject at home (home BP), which makes it possible to obtain multiple measurements over a long observation period under well-controlled conditions, has been reported to be more reliable than casual (screening) BP measurement because it avoids these biases (8–10). We previously initiated home BP measurements in the general population in a rural Japanese community, Ohasama (11–13), and have shown that home BP had a stronger predictive power for mortality than casual BP in this population (14, 15).

On the other hand, many of the mast cells present in human atherosclerotic lesions contain chymase, which may be involved in the pathogenesis of atherosclerosis *via* lipid metabolism. Several reports concerning the interaction between mast cell chymase and reverse cholesterol transport have been published (16–18).

The current study was undertaken to examine the genetic involvement of *G3255A* polymorphism of the mast cell chymase gene (*MCC*), designated *CMA1* in OMIM (Online Mendelian Inheritance in Man; No. 11898), in home BP and serum lipid profiles in a general Japanese population.

Methods

Study Design

This report is based on data from subjects who participated in our home BP measurement project in the rural community of Ohasama, Iwate Prefecture, Japan. The characteristics of this area and the details of the study project have been described previously (11–15). The study protocol was approved by the Institutional Review Board of Tohoku University School of Medicine and by the Department of Health of the Ohasama Town Government.

Study Population

DNA samples were obtained from 1,301 of the 1,789 participants aged 40 years or over who participated in the home BP measurement (14, 19). Details of the selection and representativeness of these study subjects have been reported previously (19, 20). All study subjects gave written informed consent. Of these 1,301 individuals, 1,046 were confirmed to have the *G3255A* polymorphism of *MCC* and no history of cardiovascular disease.

Detection of *MCC G3255A* Polymorphism

Chymase gene polymorphisms were genotyped by the TaqMan chemistry PCR-method. To design TaqMan probes and a primer set, we referred to the complete cds sequence data registered for the human mast cell chymase gene (NCBI accession number 64269). The polymorphic site (G to A) was located at nucleotide No. 3255, which was 1,873 bp upstream of the transcriptional initiation site. We used the following primers and probes: sense primer, 5'-CCTATTCCATTTCTCACC-3'; antisense primer, 5'-CTCCACAGCATCAAGATTCAG-3'; *G3255*-allele specific probe, 5'-Fam-CCAGGCAGGTGGA GCAAAAACCTT-Tamra-3'; A-allele specific probe, 5'-Tet-CCAGGCAGGTGAAGCAAAAACCTT-Tamra-3'. PCR was carried out using a Gene Amp 9700 thermocycler (Applied Biosystems, Inc., Foster City, 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 dyes separate 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, Inc.).

Plasma Lipid Measurements

We extracted blood samples by venopuncture in the morning. Plasma concentrations of total cholesterol and HDL cholesterol were determined enzymatically using the CE-CO-POD method and CHER-CHOD method, respectively. Plasma Lp(a) level was determined by Latex immuno-assay (LIA).

Home BP Measurements

Physicians and/or public health nurses instructed the subjects on how to perform home BP measurements. The subjects were asked to measure their BP every morning, within 1 h of waking, in the sitting position after more than 2 min of rest and to record the results for 4 weeks. The home BP of an individual was defined as the mean of all measurements obtained for that person. The mean number of home BP measurements was 20.8 ± 8.3 (mean $\pm$ SD), with a range of 3 to 38.

Table 1. Clinical Features According to MCC G3255A Polymorphism

	GG	GA	AA	p value
Number of subjects	684	315	47	
Age (years)	59.9 ± 0.3	59.4 ± 0.5	60.8 ± 1.1	0.55
Men (%)	32.2	35.9	21.3	0.12
Body mass index (kg/m ²)	23.7 ± 0.1	23.4 ± 0.2	24.4 ± 0.5	0.064
Smoking (%)	21.4	23.2	25.5	0.68
Alcohol consumption (%)	38.5	41.0	36.2	0.69
History of diabetes (%)	18.0	15.2	12.8	0.42
Antihypertensive medication (%)	30.3	29.2	34.0	0.79

Table 2. Home and Casual Blood Pressure Values According to MCC G3255A Polymorphism

		GG	GA	AA	p value
Home BP (mmHg)					
Systolic	Crude	122.4 ± 0.6	123.0 ± 0.8	124.8 ± 2.3	0.47
	Adjusted*	122.3 ± 0.4	123.3 ± 0.6	124.1 ± 1.7	0.29
Diastolic	Crude	74.2 ± 0.4	75.0 ± 0.6	75.4 ± 1.6	0.42
	Adjusted*	74.1 ± 0.3	75.0 ± 0.5	75.5 ± 1.2	0.27
Casual BP (mmHg)					
Systolic	Crude	131.8 ± 0.5	132.9 ± 0.8	130.5 ± 1.5	0.35
	Adjusted*	131.7 ± 0.5	133.1 ± 0.7	130.0 ± 1.9	0.30
Diastolic	Crude	73.8 ± 0.3	75.1 ± 0.5	75.0 ± 1.4	0.085
	Adjusted*	73.8 ± 0.3	75.2 ± 0.5	74.8 ± 1.2	0.061

Data are shown as the mean ± SEM. *Adjusted for age, gender, body mass index, and use of antihypertensive medication (ANCOVA).

Casual BP Measurements

Annual health check-ups, including BP measurements, are available to all Japanese citizens aged 40 years or older. BP was measured twice consecutively with subjects in the sitting position after at least 2 min of rest, by nurses or technicians using a semiautomatic device. Casual BP was defined as the mean of the two readings.

BP Measuring Device

Home BP was measured with a semiautomatic HEM 401C monitor (Omron Life Science Co. Ltd., Tokyo, Japan) based on the cuff-oscillometric method (11), which generates a digital display of both systolic BP (SBP) and diastolic BP (DBP). Casual BP was measured with a fully automatic USM-700F monitor (UEDA Electronic Works Co., Ltd., Tokyo, Japan) based on the Korotkoff sound technique (microphone method). The circumference of the arm was less than 34 cm in most cases, so we used a standard arm cuff for both BP measurements. The devices used to measure home BP and casual BP have been validated previously (15) and meet the criteria of the Association for the Advancement of Medical Instrumentation.

Statistical Analysis

SAS software, Version 6.12 (SAS Institute Inc., Cary, USA) was used for all statistical calculations. Fisher's exact test, Student's *t*-test, analysis of variance (ANOVA) or analysis of covariance (ANCOVA) were used as appropriate. Data are shown as the mean (SEM). Values of *p* < 0.05 were considered to indicate statistical significance.

Results

The TaqMan PCR method clearly determined the genotype of the MCC G3255A polymorphism. There were no significant differences in age, body mass index, or distributions according to gender, smoking, alcohol intake, use of antihypertensive medication or diabetes among the AA, GA, and GG genotype groups (Table 1).

There were no differences in home or casual BP values according to G3255A polymorphism (Table 2). The significance of associations was not altered when the data were adjusted for possible confounding factors such as age, gender, body mass index, and the use of antihypertensive medication (Table 2). The frequency of patients with hypertension (home BP ≤ 137/84 mmHg (19) or treated with antihypertensive medication) was 20/47 (42.6%) in homozygous subjects

Table 3. Serum Lipid Levels According to *MCC* G3255A Polymorphism

	<i>GG</i>	<i>GA</i>	<i>AA</i>	<i>p</i> value
Total cholesterol (mg/dl)				
Crude	197.1 ± 1.2	194.6 ± 1.9	191.3 ± 4.9	0.33
Adjusted*	196.9 ± 1.2	195.7 ± 1.8	187.6 ± 4.6	0.29
HDL-cholesterol (mg/dl)				
Crude	54.4 ± 0.6	56.9 ± 0.9	56.2 ± 2.1	0.038
Adjusted*	54.4 ± 0.5	56.7 ± 0.8	57.0 ± 2.1	0.027
Lipoprotein (a) (mg/dl)				
Crude	25.0 ± 0.9	24.6 ± 1.4	22.1 ± 2.9	0.74
Adjusted*	25.0 ± 0.9	24.6 ± 1.4	22.5 ± 3.6	0.74

Data are shown as the mean ± SEM. *Adjusted for age, gender, body mass index, and smoking and alcohol consumption status (ANCOVA).

Table 4. Multiple Regression Analysis of Confounding Factors for Serum HDL-Cholesterol Level

Variables	Coefficient	SEM	<i>p</i> value
Age (per year)	- 0.15	0.05	0.0033
Gender (men = 1, women = 0)	- 3.69	1.45	0.011
Body mass index (per kg/m ²)	- 1.09	0.14	< 0.001
Smoking status (current or ex-smoker = 1, never smoked = 0)	- 6.09	1.48	< 0.001
Alcohol intake (current or ex-drinker = 1, never drank = 0)	5.29	1.12	< 0.001
<i>MCC</i> polymorphism (<i>GG</i> = 0, <i>GA</i> or <i>AA</i> = 1)	1.82	0.77	0.018

with *AA*, whereas it was 122/315 (38.7%) in subjects with the *GA* genotype and 260/684 (38.0%) in subjects with the *GG* genotype; these differences were not statistically significant ($p = 0.82$).

HDL-cholesterol level was significantly higher in carriers of the A3225 allele (Table 3). This association became more apparent when the data were adjusted for confounding factors such as age, gender, body mass index, and smoking and alcohol consumption status, showing the highest HDL-cholesterol level in homozygous subjects with *AA* (Table 3). There were no significant differences in total cholesterol or lipoprotein (a) levels according to *G3255A* polymorphism, and these levels did not change when the data were adjusted for possible confounding factors (Table 3).

Multiple regression analysis showed that the *MCC* polymorphism was significantly and independently related to serum HDL-cholesterol level: carriers of the A3225 allele had a 1.8 mg/dl higher HDL-cholesterol level than subjects with the *GG* genotype, independent of established risk factors for HDL-cholesterol level such as age, gender, body mass index, and smoking and alcohol consumption status (Table 4).

Discussion

In the treatment of hypertension, the introduction of angiotensin converting enzyme (ACE) inhibitors has altered

the concept of the prevention of cardiovascular complications and increased the understanding of the renin-angiotensin system. Chymase is a major angiotensin II-forming enzyme, and also a mast cell-derived chymotrypsin-like serine protease (21) that may play important roles in the process of immunoglobulin E-mediated degranulation and in pathological alterations in tissues (22). Since another angiotensin II-forming enzyme, ACE, is now being examined with interest in the genetic investigation of hypertension (5, 6, 23–26), we hypothesized that *MCC* may be genetically associated with the susceptibility to hypertension.

In the present study, *MCC* G3255A polymorphism significantly altered the serum lipid profile, suggesting that chymase may be involved in the pathogenesis of atherosclerosis. Lee *et al.* showed that the reduced ability of granule remnant-treated HDL3 and plasma to induce cholesterol efflux from macrophage foam cells is caused by selective depletion by mast cell chymase of quantitatively minor A1- and A4-containing subpopulations of HDL (17). Since these particles are efficient acceptors of cholesterol from the cell surface, their depletion by mast cells may block the initiation of reverse cholesterol transport *in vivo* and thereby favor foam cell formation in the arterial intima, the site of atherogenesis. Although the direct effect of *G3255A* polymorphism in the 5' flanking region of *MCC* on transcriptional regulation is uncertain (27), it is a feasible hypothesis that subjects with the A3255 allele have a higher potential for reverse chole-

terol transport and might have resistance to atherogenesis. Furthermore, mast cell chymase is predominant at the site of atheromatous erosion or rupture in the events preceding the vast majority of acute coronary syndromes, suggesting that the genetic difference in *MCC* might modulate the risk for coronary heart disease (28). In addition, a recent paper revealed that subjects with the *ACE/DD* genotype had a greater reduction in total cholesterol, LDL-C and apo B in response to fluvastatin therapy than did those with the *ID* and *II* genotypes (29), suggesting the possibility of genetic involvement of *MCC* in the lipid profiles for coronary heart disease.

Several case-control studies using small numbers of subjects have examined the association between the *MCC* polymorphism and cardiovascular disease (30–34), and have failed to detect any association between them. Although we also could not detect a direct association between hypertension and *MCC G3255A*, our observation of a small but definite effect of the *MCC* polymorphism on HDL-cholesterol metabolism should be important to the future investigation of atherosclerosis.

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