Case Report

An 18-year-old man was admitted to hospital due to malaise and jaundice. He had never been a smoker and had no previous medical history other than childhood asthma. Since age 15 years, he had been diagnosed with difficult-to-control asthma and had to use inhaled beclomethasone, 1200 μg daily; inhaled fenoterol, 400 μg daily; and oral theophylline, 400 mg daily to control his asthma symptoms. Despite this treatment, he was symptomatic with an impaired exercise tolerance, nocturnal symptoms, and frequent use of rescue medication. Skin allergy tests were positive for house dust mite. On admission, he had clinical hepatomegaly. The serum aspartate transaminase level was 802 IU/liter, serum alanine transaminase 914 IU/liter, total bilirubin 3.2 mg/100 ml (conjugated bilirubin 79%) and phosphatase alkaline 667 IU/liter. The prothrombin time was 28 seconds. Virological markers indicated active HB virus infection with positive HBsAg and high titers of anti-HBc IgM antibody. The RNA or DNA assays by polymerase chain reaction for hepatitis C virus, cytomegalovirus, herpes simplex virus, and Epstein Barr virus were all negative. Acute hepatitis B was diagnosed on clinical and biological criteria and he received conservative treatment. The asthma medication was continued. After admission, the serum level of bilirubin gradually increased (maximum value 14.6 mg/100 ml). From 5 days until 24 days after admission, the mean morning peak expiratory flow rate and asthma symptom score (Nakasato et al. 1999) were significantly improved compared with those measured at day 1, day 2 and day 3 after admission (432 [S.E. 27] liter/min. vs. 346 [18] liter/min., $p<0.05$ and 1.1 [0.2] vs. 8.2 [2.6], $p<0.05$, respectively), which is quite consistent with an elevation of serum bilirubin levels (Fig. 1). The mean exhaled carbon monoxide (CO) concentration, which is a marker for oxidative stress (Horvath et al. 1998), was significantly reduced during jaundice (measured at 11 days, 12 days and 13 days after admission) compared with baseline values (measured at 1 day, 2 days and 3 days after admission) (1.2 [0.2] vs. 6.2 [0.9] ppm, $p<0.01$, respectively). Serum theophylline concentrations were similar between day 2 and day 9 after admission (11.1 vs. 10.8 μg/ml), when he took the same dose of a sustained-

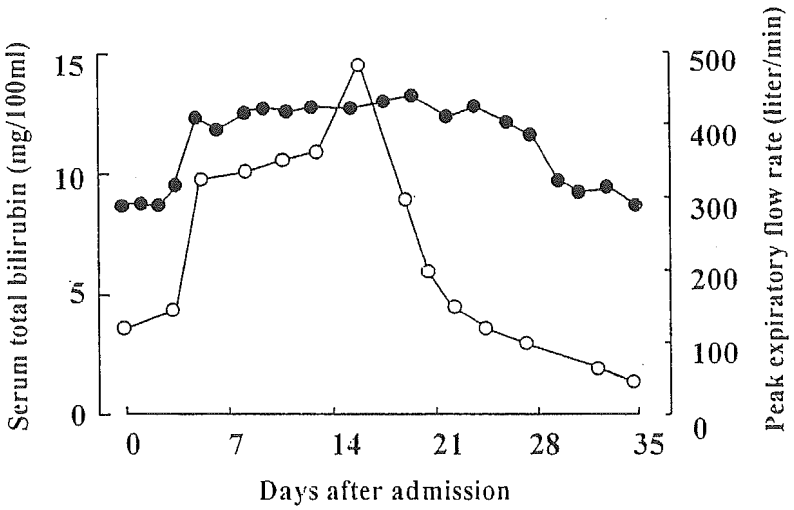

Fig. 1. Time course of serum total bilirubin and morning peak expiratory flow rate after admission. The morning peak expiratory flow rates (closed circles) were significantly improved compared with those at baseline condition in consistent with an elevation of serum bilirubin levels (open circles).

release of theophylline. He was relieved of asthma symptoms and asthma therapy was completely stopped at day 10 after admission, because drug induced liver damage had not been completely denied. However, 4 weeks after admission, when the serum level of bilirubin decreased below 4 mg/100 ml, his asthma symptoms recurred and he had to resume anti-asthma medication to control his asthma symptoms. Finally, 5 weeks after admission, he recovered completely and was discharged without any complications.

Discussion

The present case appears to be the first report describing a case of complete remission of difficult-to-control asthma during jaundice. The precise mechanism for this effect is unclear, but one possible mechanism is through hyperbilirubinemia and its antioxidant activity. Stocker et al. (1987) noted that bilirubin possesses strong antioxidant potential against peroxyl radicals and it is proposed that bile pigments function as natural antioxidants (Baranamo et al. 2002). In the present case, the asthma symptoms were relieved and an exhaled CO concentration, which is known as a marker for oxidative stress, was decreased to a normal range in association with an elevation of serum conjugated bilirubin. No other precipitating factors for asthma were identified during the observation period. Thus, the symptom-free interval during jaundice might be possibly explained by an antioxidant effect of bilirubin. The beneficial effect of bilirubin on asthma might be achieved at a concentration higher than 4 mg/100 ml, which is comparable to the effective concentration reported in experimental research (Stocker et al. 1987; Wang et al. 2002). Likewise, oxidative stress plays a critical role in the development of vascular disease. Several epidemiological studies have found that bilirubin levels are inversely associated with coronary artery disease and mortality from myocardial infarction. Gilbert's Syndrome is a genetic disorder of bilirubin conjugation leading to a mild unconjugated hyperbilirubinemia (Djousse et al. 2001). The incidence of ischemic heart disease in middle-aged individuals with Gilbert's Syndrome is reduced >5-fold compared with the general population (Vitek et al. 2002). In the general population, high plasma bilirubin levels correlate with a reduced risk of coronary heart disease (Schwertner et al. 1994; Hopkins et al. 1996). Premature babies treated with supplemental oxygen suffer retinopathy of prematurity because of increased oxidative stress. In these patients, a higher serum bilirubin level is associated with diminished incidence of retinal damage (Heyman et al. 1989). A 10-year retrospective study of the Belgian population found serum bilirubin levels inversely related to cancer mortality (Temme et al. 2001).

We previously reported that bilirubin attenuated the disease progression in bleomycin-induced pulmonary fibrosis in rats (Wang et al. 2002) and in a patient with exacerbations of idiopathic pulmonary fibrosis (Ohrui et al. 2001). Toward and Broadley (2002) reported that ozone caused an early-phase bronchoconstriction followed by a late-phase bronchoconstriction and increased respiratory rate. Furthermore, an increase in mucosal mast cells and increased histamine in guinea-pig airways has been reported immediately after ozone exposure (Murlas and Roum 1985). Bilirubin might give protection against oxidant-induced airway constriction through its anti-oxidant effect.

In the present case, the limitation of the interpretation of the effect of hyperbilirubinemia on the clinical course of asthma should be discussed. First, there is no information on the likely effects of hepatitis infection on the metabolism of all of the controller drugs, except theophylline. Although serum theophylline concentrations were not affected by HB virus infection, it is likely that hepatic metabolism of these drugs was considerably reduced during infection, and thus there would

have been considerable accumulation of these drugs. This mechanism may well have played a role in the dramatic improvement. Second, there is no information about the induction or suppression of relevant cytokines, which could have been measured in serum specimens; such information would add considerably to mechanistic information. Finally, we did not evaluate serum levels of stress endocrines such as cortisol or epinephrine, which might affect the clinical course of asthma in this patient.

Acknowledgments

We thank Mr. Grant Crittenden for the English correction.

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LETTERS TO THE EDITOR

BIOMARKERS IN SUBJECTS WITH AMNESTIC MILD COGNITIVE IMPAIRMENT

To the Editor: Alzheimer's disease (AD) most commonly begins with insidious memory failure and later progresses to more-generalized cognitive deficits and dementia, but memory complaints also are quite common even in normal elderly people. Therefore, it is of particular importance to establish an accurate and objective laboratory test to distinguish normal elderly people from subjects with mild cognitive impairment (MCI) who are likely to develop AD so that clinicians can make decisions on instituting therapies.

Fifty consecutive individuals complaining of memory dysfunction were evaluated by the Japanese version of the Wechsler Memory Scale—Revised (WMS-R).[1] As described previously,[2] the WMS-R has been validated for clinical use in the Japanese language. Subjects were functioning fairly well in the community and were neither psychoactive drug users nor excessive alcohol drinkers. Depression also was excluded by using the Geriatric Depression Scale. Thyroid function tests and vitamin B_{12} and folic acid levels were all within normal range. Serological test for syphilis was negative in all subjects. Global cognitive function was assessed using the Mini-Mental State Examination (MMSE). Because delayed episodic memory was found to be most severely impaired in MCI,[3] scores of logical memory II, visual reproduction II, verbal paired associates II, and visual paired associates II in the WMS-R subscales were summed and expressed as "delayed recall score" after adjustment for age. The diagnosis of amnestic MCI was made based essentially on the following published criteria:[4] complaint of defective memory function by patient or informant, normal activities of daily living, normal general cognitive function, abnormal memory function in the WMS-R as revealed by delayed recall score of 1.5 standard deviations (SDs) or more below age-matched control subjects, and absence of dementia. All values were expressed as mean $\pm$SD. As a result, 28 individuals (mean age = 71.1 ± 5.5) fulfilled the criteria, and the other 22 subjects (mean age = 68.5 ± 8.5) were judged as having no cognitive impairment (NCI). Education did not differ significantly between the MCI (mean = $11.7 \pm$ 2.0 years) and the NCI (mean = 10.6 ± 1.7 years) groups. The MMSE scores were 26.1 ± 1.5 in the MCI group and 28.0 ± 2.0 in the NCI group ($P = .003$). Cerebrospinal fluid (CSF) tau protein (CSF-tau) and CSF amyloid-β_{1-42} peptide (CSF-Aβ_{1-42}) were quantitated as described previously.[5] Regional cerebral blood flow in the posterior cingulate cortex (rCBFpc) was measured on (^{123}I)iodoamphetamine–single positron emission computed tomography (IMP-SPECT) images according to previously described methods.[6]

Figure 1 shows receiver operating characteristic curves with an area under the curve (AUC) of 0.93 for the CSF-tau, AUC of 0.72 for the rCBFpc, and AUC of 0.72 for the CSF-Aβ_{1-42} ($P < .05$). The CSF-tau levels were significantly higher in the amnestic MCI group than in the NCI group (524.7 ± 238.6 vs 201.5 ± 89.6 pg/mL, $P < .0001$). The rCBFpc was significantly lower in the amnestic MCI group than in the NCI group (0.91 ± 0.12 vs 1.00 ± 0.08, $P = .005$), but there was no significant difference in CSF-Aβ_{1-42} levels between the two comparison groups. For the distinction between amnestic MCI and NCI, the cutoff level of 341.0 pg/mL of the CSF-tau yielded a sensitivity of 83.3% and a specificity of 95.0%.

To our knowledge, no study has ever described a relationship between delayed memory function and biomarkers such as CSF-tau and IMP-SPECT images in the same patient population of amnestic MCI. Despite a moderately invasive technique, CSF-tau appears to be the most appropriate indicator to support a differential diagnosis between MCI and normal elderly people. This study also demonstrates that amnestic MCI is not merely an age-associated condition, but appears to be a more pathological state that is associated with abundant neuron death, as revealed by elevated CSF-tau levels. In previous studies, it was demonstrated that CSF-tau alone or in combination with IMP-SPECT was highly predictive of developing AD in MCI subjects.[5,6] Taking these results into consideration, we argue for clinical trials for early and

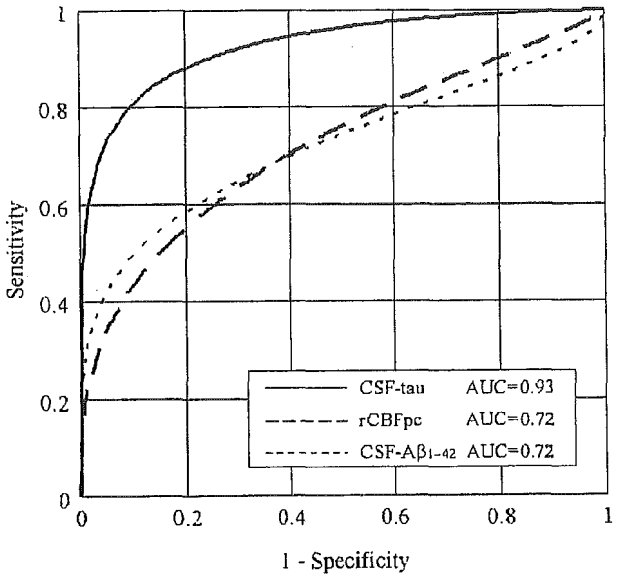

Figure 1. Receiver operating characteristic curves for cerebrospinal fluid (CSF)-tau, CSF amyloid-β_{1-42} peptide (CSF-Aβ_{1-42}), and regional cerebral blood flow in the posterior cingulate cortex (rCBFpc) in the differential diagnosis between mild cognitive impairment (MCI) and no cognitive impairment (NCI). There was a statistically significant difference in the area under curve (AUC) between the CSF-tau and the other two indices.

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effective intervention of MCI subjects using currently available AD therapies.

Masahiro Maruyama, MD
Hiroyuki Arai, MD, PhD
Mari Ootsuki, BS
Nobuyuki Okamura, MD
Toshifumi Matsui, MD
Hidetada Sasaki, MD, PhD
Department of Geriatric Medicine

Tetsuro Yamazaki, MD
Tomohiro Kaneta, MD
Department of Radiology
Tohoku University School of Medicine
Miyagi, Japan

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THE PHENOMENON OF PREMONITION OF DEATH IN OLDER PATIENT

To the Editor: Phenomena surrounding the subjects of dying and death are intriguing. Although premonition of death (POD) is a recognized phenomenon, specific case report on older patients expressing POD shortly before its occurrence is rare. I would like to report such a case encountered during my recent on-call as a medical registrar.

I was called at 3 a.m. to resuscitate an 81-year-old man, admitted 2 days previously with infective exacerbation of chronic obstructive pulmonary disease, who had a cardiopulmonary arrest. He received standard cardiopulmonary resuscitation (CPR). Cardiac output and respiratory effort were restored 15 minutes later. His family members were promptly informed.

The patient re-arrested at 3:45 a.m. and aspirated during subsequent CPR. Resuscitation was stopped 15 minutes later because he remained in pulseless electrical activity, and further resuscitation was thought futile. He was certified dead at 4:02 a.m.

The patient's family had arrived by then. Although distraught, they showed no surprise at hearing about the patient's sudden death. During our conversation, I sensed that they had expected this to happen. Remarkably, the daughter-in-law volunteered that, when they visited the patient at 9 p.m., earlier that night—a mere 6 hours before the patient's first cardiopulmonary arrest—the patient had held her hand and mentioned that he would "die tonight."

The premonition of death or an adverse clinical event is a curious phenomenon that the patient may express,[1–5] or relatives and carers may experience.[6–8] In 1945, Huessy reported several cases of POD based on his clinical experience.[1] Exton-Smith, in 1961, observed that seven (3%) of a series of 220 dying patients in his geriatric unit experienced premonition of death within a few hours of its occurrence.[2] In contrast, the patient in this case was not dying when he verbally expressed POD.

The premonition in this case should be differentiated from the ancient Chinese phenomenon of Hui Guang Fan Zhao (HGFZ).[9] HGFZ was recently termed as Lazarus premonition or Witżel-Ngeh phenomenon.[9] It refers to a clinically observed premonition characterized by a transient revival of the dying person before death. Although the patient in this case was not terminally ill on admission, a dying patient may express POD with or without the observed occurrence of HGFZ. Conceivably, the two distinct yet related phenomena or premonitions may coexist in an individual patient.[9]

Several theories of premonition have been proposed. The chance-coincidence hypothesis refers to the fact that highly unlikely events do happen occasionally by mere chance and that some apparently amazing associations are not substantiated when analyzed by statistical method.[7] The death wish or psychobolie concept is another theory of premonition,[7] but a patient who had a POD might not necessarily wish for death. From talking to the relatives, the patient in my case did not wish for his death. Indeed, Exton-Smith differentiated this wish for death as a separate entity, and reported that 11 (5%) of his 220 dying patients had repeatedly expressed a wish to die.[2] Finally, the concept of telepathy[7] or even prophecy may be used to explain premonition. Examples of prophecies or premonitions concerning persecution and death are plentiful in the Bible. A famous one relates to the persecution surrounding the birth and crucifixion of Jesus Christ, but the patient in my case was not known to be telepathic or prophetic.

The occurrence of the phenomenon of POD may have a psychical[7,10] or spiritual[8] basis that is not easily understood. In the clinical setting, it is difficult to determine the sensitivity and specificity of older patients' expressions of POD, because a significant number of older patients would die during their hospital admissions. Nevertheless, premonitions have been regarded as an evolutionary development that might provide functional forewarnings of disasters.[7] Premonition experienced by nursing staff, if acted upon, has proved life-saving to patients.[8] Similarly, carers and healthcare professionals should take a patient's POD seriously, because the occurrence of death may be sudden or imminent. If such a patient is not terminally ill or dying, more vigilant nursing and medical care may avert sudden deterioration and death. Opportunities should be offered tactfully to such patients and their relatives to discuss the matter of CPR in the event of a cardiopulmonary arrest and to explore and resolve any social, psychological, and spiritual concerns. If the patient concerned is terminally ill and dying, high-quality palliative care should be assured.

Angelo Bianchetti, MD
Giuseppe Bellelli, MD
Geriatric Research Group
Brescia, Italy
'Ancelle della Carità' Hospital
Cremona, Italy

Marco Trabucchi, MD
Geriatric Research Group
Brescia, Italy
'Tor Vergata' University of Rome, Italy

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ROLE OF CHRONIC SINUSITIS IN COGNITIVE FUNCTIONING IN THE ELDERLY

To the Editor: Chronic sinusitis is a significant cause of morbidity and a substantial source of medical expenses.[1] Patients with chronic sinusitis complain of nasal obstruction, rhinorrhea, and postnasal drip. Rhinitis and disturbed normal ventilation of the sinus involved may predispose to the condition.[2] Recently, a published study reported that allergic rhinitis could cause significant cognitive difficulties in subjects ranging from age 23 to 50.[3] Because a large part of the population aged 65 and older is cognitively impaired,[4] chronic sinusitis also may contribute to cognitive impairment such as slowed thinking, memory problems, and difficulty sustaining attention in elderly people.

One hundred seventy-two community-dwelling people aged 65 (male/female: 76/96) living in the rural community of Onagawa town, Miyagi prefecture in Japan were examined between June and August 2001, which was not during the allergy season. All participants were healthy volunteers and were living independently at home without apparent history of dementia. All participants underwent a baseline evaluation of nasal symptoms and standardized physical and neuropsychological examinations. Those who had acute disorders, including upper respiratory tract infections, stroke, or heart failure requiring special treatments, were excluded. The presence or absence of chronic sinusitis was determined as high-intensity fluid in the paranasal sinuses on the T2-weighted images using magnetic resonance imaging (MRI). Cognitive function was assessed using the Mini-Mental State Examination (MMSE).[5] Brain infarctions and white-matter lesions were also identified using MRI. Two independent radiologists who were blinded to the identity of the study subjects evaluated MRI findings.

Chronic sinusitis was found in 32 (18.8%) of the 170 eligible subjects. Although the MMSE scores were not significantly different in participants with chronic sinusitis than in those without chronic sinusitis (mean score ± standard deviation = 27.5 ± 1.8 vs 27.9 ± 2.0, $P = .28$), the frequency distribution of MMSE scores between the two groups was different (Figure 1). The frequency of participants with an MMSE score of 30 points was significantly lower in participants with chronic sinusitis than in those without chronic sinusitis (9.4% vs 26.8%, $P = .04$). Moreover, logistic regression analysis after adjusting for sex and education confirmed that chronic sinusitis was significantly associated with MMSE score (30/30 points or not: odds ratio (OR) = 3.9, 95% confidence interval (CI) = 1.1–13.8, $P = .04$) independent of the presence or absence of brain infarction (OR = 1.9, 95% CI = 0.7–5.2, $P = .18$) and white matter lesions (OR = 2.3, 95% CI = 1.1–4.8, $P = .03$).

Although it should be addressed whether such a subtle decline of cognitive function influences daily living in

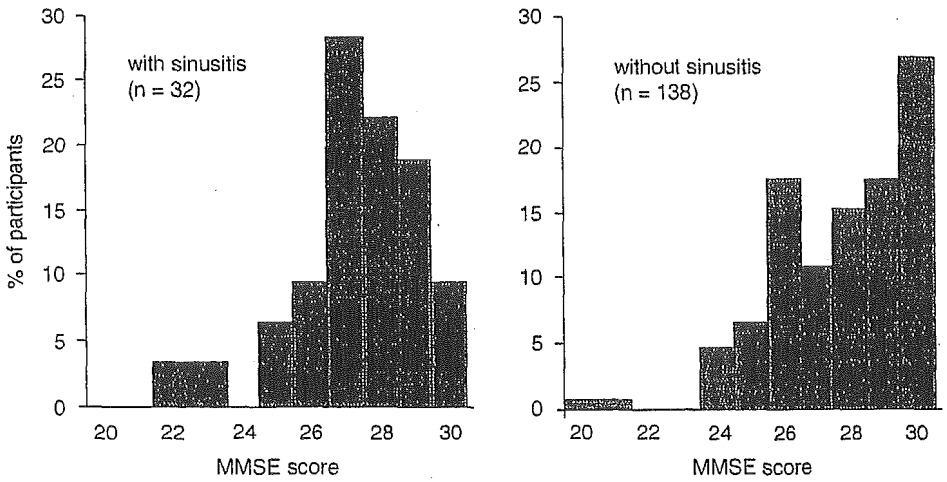

Figure 1. Frequency distribution of Mini-Mental State Examination (MMSE) scores in elderly participants aged 65 with and without chronic sinusitis. The frequency of 30/30 points on MMSE was 3/32 (9.4%) in participants with sinusitis (left) and 37/138 (26.8%) in participants without sinusitis (right).

elderly people, the present study suggests that chronic sinusitis may not only exacerbate chest disease[6] but also affect cognitive function either by decreasing the power of concentration or affecting specific cognitive functions. Therefore, early medical intervention for neglected chronic sinusitis should be taken into account to sustain cognitive function in the elderly.

Toshifumi Matsui, MD
Hiroyuki Arai, MD, PhD
Mizue Nakajo, MD
Masahiro Maruyama, MD
Satoru Ebihara, MD, PhD
Hidetada Sasaki, MD, PhD
Department of Geriatric and Respiratory Medicine
Tohoku University School of Medicine
Sendai, Japan

Mizue Nakajo, MD
Yo-ichi Yoshida, MD
Department of Internal Medicine
Onagawa Municipal Hospital
Miyagi, Japan

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USE OF ANTIBACTERIAL DRUGS IN COMMUNITY-DWELLING OLDER PERSONS

To the Editor: The most common infections in community-dwelling elders include urinary tract, respiratory tract, and skin and soft tissue infections. The use of antibacterial drugs has remarkably reduced the morbidity and mortality of these infections. Although there have been studies of antibacterial drug use in elders in the hospital and long-term care settings, to our knowledge, none have focused on their use in community-dwelling elders.[1–4] Responding to this lack of information, analyses were conducted to describe the prevalence of antibacterial drug use over a 10-year period and to determine whether there were racial differences in their use after controlling for demographic, health-status, and access-to-care factors in older, community-dwelling African Americans and whites.

Ten classes[5] of oral and parenteral antibacterial agents were examined from four in-person interviews (Wave 1 in 1986/87, Wave 4 in 1989/90, Wave 7 in 1992/93, Wave 10 in 1996/97) of the Duke Established Populations for Epidemiologic Studies of the Elderly (EPESE). Details of the Duke EPESE study design and methodology are presented elsewhere.[6] Complete medication use information during the 2 weeks before each interview was available for 4,136, 3,234, 2,508, and 1,633 participants from Waves 1, 4, 7, and 10, respectively. The outcome variable was to determine oral or parenteral antibacterial use (yes, no). Demographic (age, race, sex, education), health-status (functional status, presence or absence of diabetes mellitus, chronic obstructive pulmonary disease, cancer), and access-to-care (health insurance, primary physician, number of physician visits in the previous year) characteristics from the accompanying in-person interviews were evaluated as possible independent factors associated with antibacterial use. All data were weighted to adjust for the sampling design. Multivariate regressions for Waves 1 and 10 were performed. Details regarding statistical model construction and data fitting are available from the authors.

African Americans represented approximately one-third of the study population at each wave. Women constituted two-thirds of the sample populations at Waves 1 and 10. Table 1 depicts antibacterial use at all four waves. As shown in Table 1, in Wave 1, 6.3% of participants used one or more antibacterial agent(s) within the previous 2 weeks, falling to 5.1% at Wave 10 (test of trends Mantel-Haenszel $\chi^2 = 0.22$, $df = 1$, $P = .64$). The top two therapeutic classes at Waves 1 and 10 were sulfonamides (including trimethoprim-sulfamethoxazole) and penicillins. Fluoroquinolone use after the introduction of ciprofloxacin (post-Wave 1) remained relatively stable. Sulfonamide and tetracycline use decreased by half from Wave 1 to Wave 10.

Cross-sectional multivariate results at Wave 1 reveal that antibacterial drug use was significantly less prevalent in the previous 2 weeks in African Americans (adjusted odds ratio (AOR) = 0.70, 95% confidence interval (CI) = 0.50–0.98, $P = .037$) after controlling for demographic, health, and access-to-care factors. At Wave 10, a similar trend was seen, with antibacterial use being less prevalent in African Americans (AOR = 0.79, 95% CI = 0.47–1.32, $P = .362$) after controlling for the same factors, but this was not statistically significant.

Given these results, if these use patterns hold for other regions of the country, approximately 1.75 million community-dwelling elders use systemic antibacterials during any given 2-week period. Although the introduction of fluoroquinolones did not result in their expanded use between 1986 and 1997 in a five-county region of North Carolina, new guidelines for community-acquired pneumonia regarding the use of second-generation fluoroquinolones may lead to expanded use of these agents.[7] The decline in sulfonamide use (including the combination with trimethoprim) over the study period was an encouraging trend, given that trimethoprim's use has been associated with urinary coliform resistance.[8] The disparity between antibacterial use in whites and African Americans is consistent with findings from two previous studies.[9,10] Because diagnostic data were not available, it is unknown whether this underuse of antibacterials was good or bad for African Americans. It is also possible that this disparity may be due to differences in provider characteristics or cultural beliefs between races. Health-status risk factors for infections (e.g., physical disabilities, diabetes mellitus, pulmonary disease, cancer) and certain access-to-care factors

Original Article

Association between Angiotensin II Type 1 Receptor Gene Polymorphism and Essential Hypertension: the Ohasama Study

Ken SUGIMOTO, Tomohiro KATSUYA, Takayoshi OHKUBO*, Atsushi HOZAWA**, Koichi YAMAMOTO, Akiko MATSUO, Hiromi RAKUGI, Ichiro TSUJI**, Yutaka IMAI***, and Toshio OGIHARA

Gene targeting approaches have suggested that the angiotensin II type 1 receptor (AT1R) is involved in blood pressure (BP) regulation and modulation of the effect of angiotensin II. The A1166C polymorphism of the AT1 receptor gene (*AT1R/A1166C*) is associated with hypertension in Caucasians, but not in Japanese. The goal of this study, the Ohasama Study, was to examine the association between *AT1R/A1166C* and hypertension, especially home BP, in the Japanese general population. The Ohasama Study was a cohort study based on Japanese rural residents of Ohasama Town in the northern part of Japan. Subjects who gave informed consent to the study protocol and genetic analysis were recruited. Home BP was measured twice in the morning within 1 h of waking up and in the evening just before going to bed. The TaqMan polimerase chain reaction (PCR) method clearly determined *AT1R/A1166C* genotypes (n=1,207). The genotype distribution of *AT1R/A1166C* was as follows: *AA* 84%; *AC* 15%; *CC* 1%. There was almost no difference in baseline characteristics among the AT1R genotypes (*AA*, *AC*, *CC*). In the subjects not receiving antihypertensive medication (n=817), both casual BP and home BP were not different among the AT1R genotypes after adjusting for confounding factors (age, sex, body mass index, current smoking habit and current alcohol consumption). The frequency of hypertension showed no difference among AT1R genotypes after adjusting for confounding factors, though the *AC* and *CC* genotypes were more frequent in hypertensives than in normotensives. Our data suggested that the *AT1R/A1166C* polymorphism is not a major genetic predisposing factor for hypertension in Japanese. (*Hypertens Res* 2004; 27: 551–556)

Key Words: genetics, hypertension, TaqMan polimerase chain reaction, single nucleotide polymorphism, angiotensin II type 1 recepter gene

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From the Department of Geriatric Medicine, Osaka University Graduate School of Medicine, Suita, Japan, and *Department of Planning for Drug Development and Clinical Evaluation, **Department of Public Health, and ***Department of Clinical Pharmacology and Therapeutics, Tohoku University Graduate School of Medicine and Pharmaceutical Science, Sendai, Japan.

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

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Introduction

Most cases of human hypertension are classified as essential hypertension of unknown cause, because secondary or monogenic hypertension is relatively rare (*1*). The renin-angiotensin system (RAS) plays an important role in blood pressure (BP) regulation, and recent advances in molecular biology have highlighted the genetic importance of some components of RAS, such as angiotensin converting enzyme (ACE) (*2–4*) and angiotensinogen (AGT) (*5*), in the pathogenesis of cardiovascular disease. Gene targeting approaches have suggested that the angiotensin II type 1 receptor (AT1R) and angiotensin II type 2 receptor are involved in BP regulation and modulation of the effect of angiotensin in different manners (*6–9*). An A-to-C nucleotide substitution in the 3′ untranslated region of the human AT1R gene (on 3q21–25, *AT1R/A1166C*) has been shown to be associated with the prevalence of hypertension in a Caucasian population (*10*).

On the other hand, several reports have shown a positive association between cardiovascular features such as aortic stiffness, left ventricular hypertrophy (LVH), increased carotid intima-media thickness or atheromatous plaque formation and *AT1R/A1166C* (*11*, *12*). In addition, up-regulation of the AT1R gene has been observed in the ventricles of cardiomyopathic hamsters (*11*, *13*, *14*). We also previously reported a positive relationship of *AT1R/A1166C* with LVH and risk for lacunar infarction (*15*, *16*), but not with blood pressure. An association between precise blood pressure (*e.g.*, ambulatory blood pressure (ABP)) and *AT1R/A1166C* was reported by Castellano, but did not reach statistical significance (*12*). However, the association between *AT1R/A1166C* and home BP has not yet been examined. To examine the precise interaction between *AT1R/A1166C* and cardiovascular risk, we carried out a large genetic epidemiological study in Japanese, the Ohasama Study, with a large number of measurements of home BP in a Japanese general population.

Methods

Population

Ohasama Town is a rural community located 100 km north of Sendai, the central city of north-eastern Japan. The Ohasama Study was started in 1987 with a cohort base, whose design was described precisely by Imai *et al.* (*17*). The study protocol was approved by the Institutional Review Board of the Tohoku University School of Medicine. DNA samples were obtained from 1,301 of the 1,789 study participants aged 40 years or over who participated in home BP measurement (*18*). Details of the selection and representativeness of these study subjects have been reported previously (*18*, *19*). All study subjects gave written informed consent to participate in the study.

BP Measurements

Detailed medical histories and risk factors for cardiovascular disease were ascertained for each subject. Casual BP was measured by nurses or technicians twice consecutively with the individuals seated after at least 2 min of rest. An automatic microphone-based BP measuring device (USM-700F; UEDA Electronic Works Co., Ltd., Tokyo, Japan) was used for the measurements. The average of two measurements of systolic BP (SBP) and diastolic BP (DBP) was used for the analysis. Home BP was measured for 4 weeks with a semi-automatic device (HEM-401C; Omron Life Science Co., Ltd., Tokyo, Japan) every morning within 1 h of waking and every evening within 1 h of going to bed, while the participants were seated and after they had rested for more than 2 min. These devices used to measure casual BP and home BP had been previously validated (*20*) and the devices met the criteria set by the Association for the Advancement of Medical Instrumentation (AAMI) (*21*). We defined hypertension by the following criteria: mean SBP of casual BP $\geq$140 mmHg, mean DBP of casual BP $\geq$90 mmHg, or taking antihypertensive medication when the subjects were first enrolled in the Ohasama study. The remaining population was defined as normotensive, according to the criteria of the sixth report of the Joint National Committee on Prevention, Detection, and Treatment of High Blood Pressure (JNC/VI) (*22*).

AT1R/A1166C Genotype Determination

AT1R genotypes were determined using the TaqMan polymerase chain reaction (PCR) method, which we modified in order to accommodate the large number of samples (n=1,301). In the current investigation, we prepared two minor groove binder (MGB) probes: an A allele-specific probe, 5′-Fam-CAAATGAGCATTAGCTAC-3′, and a C allele-specific probe, 5′-Vic-CAAATGAGCCTTAGCTACT-3′. Neither of the reporters was quenched. The following primers were designed for PCR of the flanking region of the A/C polymorphism in AT1R: forward, 5′-CATTCCTCTGC AGCACTTCACT-3′; reverse, 5′-CGGTTCAGTCCACATA ATGCAT-3′. PCR was carried out using a thermal cycler GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, USA). The PCR conditions were as follows: an initial cycle of 50°C for 2 min, followed by a single cycle of denaturation at 95°C for 10 min, and then 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 clear identification of the three genotypes of AT1R.

Statistical Analysis

The associations between the *AT1R/A1166C* polymorphism

Table 1. Baseline Characteristics of Hypertensive and Normotensive Groups in Total Subjects

Characteristics	Hypertensive (n=576)	Normotensive (n=631)	p value
Age (years)	62.6±0.36	58.1±0.34	<0.0001
Male (%)	38.5	33.8	0.08
Na^+ (mEq/l)	142.0±0.08	142.2±0.08	0.27
K^+ (mEq/l)	4.37±0.08	4.37±0.01	0.93
PRA (ng/ml/h)	1.54±0.07	1.42±0.06	0.18
BMI (kg/m^2)	24.1±0.13	23.3±0.13	<0.0001
SBP (mmHg)	141.3±0.46	124.2±0.44	<0.0001
DBP (mmHg)	79.4±0.33	70.4±0.32	<0.0001
Diabetes (%)	21.2	14.4	0.002
Hyperlipidemia (%)	17.5	11.4	0.002
Smoking (%)	21.9	26.8	<0.05
Drinking (%)	41.5	39.7	0.53
AT1R genotypes			
AA	476 (82.6)	538 (85.3)	
AC	100 (17.4)	89 (14.1)	
CC	0 (0)	4 (0.6)	<0.03*
AC+*CC*	100 (17.4)	93 (14.7)	0.21**

Values are mean±SEM. PRA, plasma renin activity; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; AT1R, angiotensin II type 1 receptor. * p value by ANOVA between hypertension and AT1R genotypes (*AA*, *AC*, *CC*). ** p value by ANOVA between hypertension and *AA* genotype *vs.* *AC* and *CC* genotypes.

Table 2. Baseline Characteristics of AT1R Genotypes in Total Subjects

Characteristics	*AA* (n=1,021)	*AC* (n=190)	CC (n=4)	p value
Age (years)	60.0±0.3	61.1±0.6	56.8±4.5	0.23
Male (%)	35.3	40.5	25.0	0.34
BMI (kg/m^2)	23.6±0.1	23.9±0.2	22.5±1.6	0.51
Diabetes (%)	17.1	20.5	0.0	0.25
Hyperlipidemia (%)	15.5	8.4	0.0	<0.02
Smoking (%)	24.8	22.1	25.0	0.73
Drinking (%)	40.4	41.4	50.0	0.90
Antihypertensive agent(%)	31.4	36.3	0.0	0.09

Values are mean or mean±SEM. AT1R, angiotensin II type 1 receptor; BMI, body mass index.

and BP or clinical variables were analyzed using one-way analysis of variance (ANOVA). The difference in AT1R genotype or allele distribution was examined by χ^2 analysis. To assess the contribution of confounding factors, we performed multiple logistic regression analysis using the computer software application, JMP 3.2.2 (SAS Institute Inc., Cary, USA). A p value less than 0.05 was considered statistically significant.

Results

Of the 1,301 representative individuals who gave DNA samples, *AT1R/A1166C* genotyping was successful in 1,207 individuals (93%). The genotype frequencies were not significantly different from the expected value by Hardy-Weinberg equilibrium (χ_1^2=0.19, p=0.17, *A* allele: *C* allele=0.92: 0.08). The distribution of the three genotypes in the Ohasama Study was as follows: *AA* 84%; *AC* 15%; *CC* 1%. Because the number of subjects with the *CC* genotype was very small (n=4) and was insufficient for statistical analysis, we divided all subjects into an *AA* group or a combined *AC* or *CC* group for the following analysis.

The ratio of hypertensives by casual BP in the current study was 47.7%. We divided total subjects into two groups: hypertensives (HT, n=576) and normotensives (NT, n=631). Table 1 shows the baseline characteristics of all subjects in the hypertensive and normotensive groups. Age, body mass index (BMI; kg/m^2), prevalence of diabetes and hyperlipidemia, and frequency of current smoking were significantly higher in the subjects with hypertension than in normotensives. In sex and frequency of current drinking, there were no differences between the two groups. In order

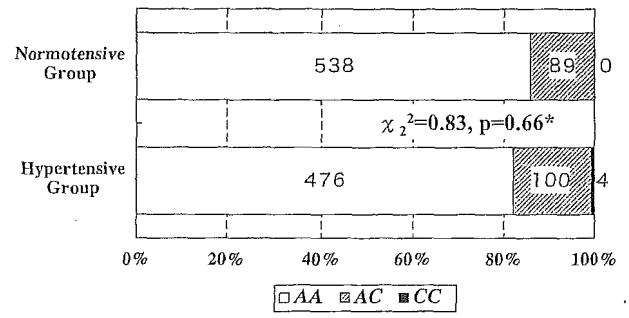

Fig. 1. *Frequencies of AT1R genotypes. The frequency of subjects with* AC+CC *genotypes was higher in hypertensives than in normotensives.* * p *value was after adjusted for age, sex, BMI, current smoking habit and current drinking. Odds ratio for hypertension* (AA vs. AC+CC) *after adjusted for sex and BMI=1.33 (95% CI: 1.00–1.78),* p<*0.05. Odds ratio for hypertension* (AA vs. AC+CC) *after adjusted for sex, BMI and age=1.23 (95% CI: 0.92–1.66),* p=*0.17.*

to compensate for environmental factors, we selected age, sex, BMI, and frequency of current smoking and drinking as confounding factors for the following multivariate analysis.

Table 2 shows the baseline characteristics of AT1R genotypes in the total subjects. There were no differences in age, sex, BMI, frequency of current smoking or drinking, or use of antihypertensive medication. None of the subjects with the *CC* genotype (n=4) had diabetes, hyperlipidemia or hypertension.

Figure 1 shows the distribution of AT1R genotypes in hypertensive and normotensive subjects. Subjects with the *AA* genotype were all in the normotensive group, but the difference in the distribution of AT1R genotypes between the hypertensive and normotensive groups did not reach statistical significance. We also analyzed the association between hypertension and AT1R genotypes (*AA vs. AC* or *CC*); however, the difference was not significant after adjusting for age, sex, BMI, and frequency of current smoking and drinking (odds ratio=1.10; 95% CI=0.79–1.54; p=0.56).

Table 3 shows a comparison between the genotypes of AT1R and BP in the subjects not taking antihypertensive medication. The systolic and diastolic blood pressure of home BP in both the morning and evening were lower in subjects with the *CC* genotype than in those with the *AA* or *AC* genotype by ANOVA, but the difference was not significant (*AA vs. AC* or *CC*: SBP of home BP, 118.3±0.5 *vs.* 119.2±1.1 mmHg (p=0.41) in the morning, and 116.5±0.5 *vs.* 117.4±1.1 mmHg (p=0.47) in the evening; DBP of home BP, 71.9±0.3 *vs.* 72.8±0.8 mmHg (p=0.30) in the morning, and 70.1±0.3 *vs.* 71.0±0.8 mmHg (p=0.29) in the evening respectively). After adjusting for age, sex, BMI, and frequency of current smoking and drinking, there were no significant differences between casual or home BP and AT1R genotypes (Table 4).

Table 3. Comparison between Genotypes of AT1R and BP in Subjects without Antihypertensive Medication

	AT1R genotype		p value
	AA (n=693)	*AC* or *CC* (n=124)	
Casual BP (n=817)			
SBP (mmHg)	129.2±0.5	129.5±1.2	0.86
DBP (mmHg)	73.2±0.3	72.7±0.8	0.60
Home BP (n=817)			
Morning			
SBP (mmHg)	118.3±0.5	119.2±1.1	0.41
DBP (mmHg)	71.9±0.3	72.8±0.8	0.30
Evening			
SBP (mmHg)	116.5±0.5	117.4±1.1	0.47
DBP (mmHg)	70.1±0.3	71.0±0.8	0.29

Values are mean±SEM. AT1R, angiotensin II type 1 receptor; BP, blood pressure; SBP, systolic BP; DBP, diastolic BP.

Table 4. Comparison between Genotypes of AT1R and BP in Subjects without Antihypertensive Medication after Adjustment for Confounding Factors

	AT1R genotype		p value
	AA (n=693)	*AC* or *CC* (n=124)	
Casual BP (n=817)			
SBP (mmHg)	129.2±0.6	129.2±1.2	0.96
DBP (mmHg)	73.2±0.4	72.5±0.8	0.38
Home BP (n=817)			
Morning			
SBP (mmHg)	119.2±0.5	119.4±1.0	0.90
DBP (mmHg)	73.1±0.4	73.4±0.8	0.78
Evening			
SBP (mmHg)	117.3±0.5	117.4±1.0	0.96
DBP (mmHg)	71.0±0.4	71.4±0.7	0.66

Values are mean±SEM. All data are adjusted for age, sex, body mass index and frequency of current smoking and drinking. AT1R, angiotensin II type 1 receptor; BP, blood pressure; SBP, systolic BP; DBP, diastolic BP.

Discussion

The main finding of this study is that subjects with the *C1166* allele had a higher genetic predisposition to high blood pressure than *AA* homozygotes, but this association was not significant in a large rural Japanese population, the Ohasama Study cohort. The results of some previous association studies between gene polymorphisms and hypertension in the Ohasama cohort were positive, but some were negative. Positive results of gene polymorphisms were obtained for the angiotensinogen gene (*AGT*) *T+31C* (*23*), endothe-

lin-1 gene (*ET1*) *C677T* (*24*), aldosterone synthase gene (*CYP11B2*) (*25*), and α-adducin gene (*ADD1*) *Gly460Trp* (*26*), and negative results were obtained for the chymase gene (*27*), β sodium channel gene (*SCNN1B*) (*28*), guanine nucleotide-binding protein gene (*GNB3*) (*29*), and angiotensin-converting enzyme gene (*ACE*) insertion/deletion polymorphism (*30*). In this way, the Ohasama Study is suitable for studying the genetics of hypertension because it has the advantage of including a large number of subjects with home BP measurement. Bonnardeaux *et al.* (*10*) reported a positive association between the *C1166* allele and essential hypertension in a case-control study in Caucasians. On the other hand, Castellano *et al.* (*12*) reported that *CC* homozygotes showed lower BP values, a lower prevalence of hypertension and a less frequent positive family history of hypertension than heterozygotes and *AA* homozygotes in a case-control study, although their study population was small ($n=194$). Recently, Liu *et al.* (*31*) reported that the *A1166* allele of the AT1R gene is a predisposing factor for hypertension in Tibetan males (in China).

In the current study, the frequency of hypertension based on home BP measurements was higher in subjects with the *AC* or *CC* genotype than in those with the *AA* genotype. These results were similar to Bonnardeaux's study, but not to Castellano's study. Since most previous studies showed that the *C1166* allele was a risk factor for cardiovascular abnormalities, the conflicting results of Castellano's study were likely to have resulted from the small study population. The finding that the association between home BP measurements and *AT1R/A1166C* was not significant even after adjustment for confounding factors (age, sex, BMI, current smoking and alcohol use) suggested that the *AT1R/A1166C* polymorphism plays a particular but very weak role in regulating BP, because this effect on hypertension was strongly attenuated by environmental factors.

Subjects with the *CC* genotype of AT1R, although small in number ($n=4$), showed unique characteristics of an absence of cardiovascular risk factors, hypertension, diabetes, hyperlipidemia and obesity. The reason for this may be that these subjects were younger than those with the *AA* and *AC* genotypes, but we cannot completely exclude that the *AA* genotype of AT1R may have the effect of reducing cardiovascular risk, such as by improving insulin resistance *via* the RAS. Even though a positive association between the *C1166* allele and hypertension was observed in the present, additive model and in the dominant model of Liu *et al.* (*31*), the frequency of the *CC* genotype is so small in Asian people that we cannot rule out a possible significant effect of the *CC* genotype in hypertension among Caucasians.

Another important issue is the biological relevance of the AT1R gene polymorphism. The *A/C* mutation occurs in the 3′untranslated region of the AT1R gene and is not characterized by any functional diversity. Erdmann *et al.* (*32*) reported that *AT1R/A1166C* showed weak but significant linkage disequilibrium with a polymorphism (810AV) in the promoter region of the AT1R gene, and suggested that the *A1166C* polymorphism may be slightly associated with expression of the AT1R gene. Plumb *et al.* showed that a mutation at position −810T/A destroys a transcriptional factor-binding site for GATA-binding factors (*33*). Even though the *A1166C* polymorphism can be considered a possible marker, in linkage disequilibrium with other functionally relevant genetic variants affecting the structure or expression of the AT1R, we could not conclude that *AT1R/A1166C* plays a main role in the genetic predisposition to essential hypertension.

Although a recent report by Kikuya *et al.* (*34*) reached the same conclusion based on ABP measurements in the Ohasama cohort, our present results indicate that *AT1R/A1166C* is not strongly related to high BP. In conclusion, our results suggest the possibility that the *AT1R/A1166C* polymorphism is a genetic marker of increased BP, but this association may be weak.

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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.

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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

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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/m^2) 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/m^2	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 †$P<0.05$.

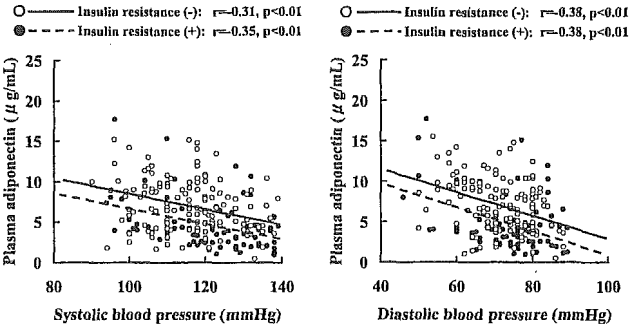

Figure 1. Correlation between plasma adiponectin concentration and blood pressure in normotensives without diabetes. ● indicates subjects with insulin resistance (n=93); ○, subjects without insulin resistance (n=152).

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

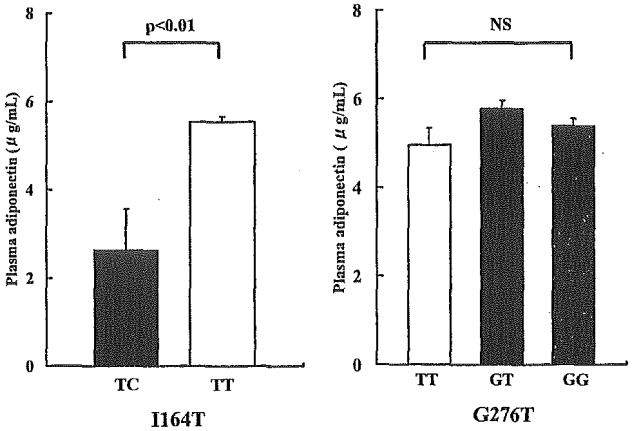

Figure 2. Plasma adiponectin concentration in subjects with I164T and G276T polymorphisms, after adjustment for confounding factors (age, BMI, triglyceride, HDL cholesterol, and homeostatic model assessment index). Data represent mean±SEM.

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.

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Adiponectin I164T Mutation Is Associated With the Metabolic Syndrome and Coronary Artery Disease

Koji Ohashi, MD,* Noriyuki Ouchi, MD, PHD,* Shinji Kihara, MD, PHD,* Tohru Funahashi, MD, PHD,* Tadashi Nakamura, MD, PHD,* Satoru Sumitsuji, MD,‡ Toshiharu Kawamoto, MD, PHD,§ Satoru Matsumoto, MD,‖ Hiroyuki Nagaretani, MD,* Masahiro Kumada, MD,* Yoshihisa Okamoto, MD,* Hitoshi Nishizawa, MD, PHD,* Ken Kishida, MD, PHD,* Norikazu Maeda, MD,* Hisatoyo Hiraoka, MD, PHD,* Yoshio Iwashima, MD,† Kazuhiko Ishikawa, MD, PHD,† Mitsuru Ohishi, MD, PHD,† Tomohiro Katsuya, MD, PHD,† Hiromi Rakugi, MD, PHD,† Toshio Ogihara, MD, PHD,† Yuji Matsuzawa, MD, PHD*

Suita, Izumisano, Kure, and Toyonaka, Japan

OBJECTIVES This study examined the association of mutations in adiponectin gene with the prevalence of coronary artery disease (CAD).

BACKGROUND Coronary artery disease is a major cause of mortality in the industrial countries. Adiponectin gene locus, chromosome 3q27, is the candidate site for CAD. We have reported that adiponectin has antiatherogenic and antidiabetic properties, and that the plasma levels negatively correlated with body mass index (BMI) are significantly low in patients with CAD or type 2 diabetes.

METHODS The study subjects were 383 consecutive patients with angiographically confirmed CAD and 368 non-CAD subjects adjusted for age and BMI in the Japanese population. Single nucleotide polymorphisms (SNPs) in the adiponectin gene were determined by Taqman polymerase chain reaction (PCR) method or a PCR-based assay for the analysis of restriction fragment length polymorphism. The plasma adiponectin concentration was measured by enzyme-linked immunosorbent assay.

RESULTS Among SNPs, the frequency of I164T mutation was significantly higher in CAD subjects (2.9%) than in the control (0.8%, $p < 0.05$). The plasma adiponectin levels in subjects carrying the I164T mutation were significantly lower than in those without the mutation, and were independent of BMI. In contrast, SNP94 and SNP276, which are reported to be associated with an increased risk of type 2 diabetes, were associated neither with CAD prevalence nor with plasma adiponectin level. Subjects with I164T mutation exhibited a clinical phenotype of the metabolic syndrome.

CONCLUSIONS The I164T mutation in the adiponectin gene was a common genetic background associated with the metabolic syndrome and CAD in the Japanese population. (J Am Coll Cardiol 2004;43:1195–200) © 2004 by the American College of Cardiology Foundation

Cardiovascular disease is a major cause of morbidity and mortality in industrial countries. Both environmental and genetic factors contribute to the development of cardiovascular disease (1). Among various adipocyte-derived bioactive substances, adipocytokines, dysregulated production of leptin, tumor necrosis factor (TNF)-α, and plasminogen activator inhibitor type 1 is closely associated with increased cardiovascular mortality and morbidity (2–6). Adiponectin is an adipocyte-specific adipocytokine, which we identified in the human adipose tissue complementary DNA library (7). The mouse homologue of adiponectin was identified as ACRP30 and AdipoQ (8,9). Hypoadiponectinemia (low plasma adiponectin level) has been identified in patients with coronary artery disease (CAD) (10) and type 2 diabetes, and is a predictor of cardiovascular outcome in patients with end-stage renal failure (11). Plasma adiponectin rapidly accumulates in the subendothelial space of an injured human artery (12). We have reported that human recombinant adiponectin suppresses endothelial adhesion molecule expression, vascular smooth muscle cell proliferation, and macrophage-to-foam cell transformation as well as TNF-α production by macrophages in vitro (13,14). Recently, we reported that the adiponectin-knockout mice exhibited enhanced neointimal thickening after vascular injury (15). In addition, we and others demonstrated that adiponectin treatment improved insulin resistance and glucose metabolism in diabetic mice model (16–18). These findings suggest that adiponectin has both antiatherogenic and antidiabetic properties and acts as an endogenous mediator of vascular and metabolic diseases.

We have previously identified several mutations of the adiponectin gene, including missense mutations (R112C, I164T, R221S, and H241P) in the globular domain and the G/T single nucleotide polymorphism at nucleotide 94

From the *Department of Internal Medicine and Molecular Science, Graduate School of Medicine, Osaka University, Suita, Japan; †Department of Geriatric Medicine, Graduate School of Medicine, Osaka University, Izumisano, Japan; ‡Department of Cardiology, Rinku General Medical Center, Rinku, Japan; §Department of Cardiology, National Hospital Kure Medical Center, Kure, Japan; and ‖Department of Cardiology, Toyonaka Municipal Hospital, Toyonaka, Japan. Supported by grants from the Japanese Ministry of Education, the Japan Society for Promotion of Science-Research for the Future Program, the Takeda Medical Research Foundation, and the Fuji Foundation for Protein Research. Drs. Ohashi and Ouchi contributed equally to this work.

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