Practical Aspect of Monitoring Hypertension Based on Self-measured Blood Pressure at Home

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Abstract

Devices for home blood pressure (BP) measurement are produced worldwide at a rate of more than 10 million a year and 30 million such devices have already been distributed in Japan. The clinical significance of home BP measurement is obvious; patients can recognize the effects of antihypertensive treatment. Home BP measurements encourage medication compliance, follow-up clinic visits, and active participation in the medical treatment, thus resulting in improved management of hypertension. Home BP measurements more accurately reflect damage to target organs and the prognosis of cardiovascular diseases. The purpose of home BP measurements is to obtain information on the patient's inherent BP pattern using long-term, repetitive measurement under controlled conditions. Since home BP is measured under controlled condition, values are reproducible, and thus, useful in the diagnosis and treatment of hypertension. Blood pressures measured under standardized condition are indispensable when comparing data among individuals, among groups and among institutes. Working Group of Japanese Society of Hypertension (JSH) established JSH Guidelines for Self-Monitoring of Blood Pressure at Home in 2003. Standardization of the measurement procedure may elevate the position of home BP measurements for the purpose of diagnosing and treating hypertension. As a result, home BP measurements may improve the accuracy of screening for hypertension and assessment of BP control during treatment and encourage drug compliance. Home BP measurements, under such controlled conditions, should have a beneficial effect on the economics of diagnosing and treating hypertension.
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Key words: blood pressure, home measurements, drug effect, compliance, guidelines

Introduction

Devices for home blood pressure (BP) measurement are produced worldwide at a rate of more than 10 million a year and 30 million such devices have already been distributed in Japan. The clinical significance of home BP measurement is obvious; patients can monitor the effects of antihypertensive treatment and obtain objective information on medication response. Patients can also recognize elevations of BP when they discontinue or fail to take routine doses of medication. The immediate feedback of home BP measurements encourages medication compliance, follow-up clinic visits, and active participation in medical treatment, thus resulting in improved management of hypertension.

Recent guidelines for the treatment of hypertension such as the Sixth and Seventh Reports of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC-VI and 7) (1, 2), the 1999 World Health Organization-International Society of Hypertension (WHO-ISH) Guidelines for the Management of Hypertension (3), the 2003 European Society of Hypertension—European Society of Cardiology (ESH-ESC) Guidelines for the Management of Arterial Hypertension (4), and the Japanese Society of Hypertension (JSH) Guidelines for the Management of Hypertension (5) have all emphasized the importance of home BP measurements in clinical applications of practice, research, and epidemiology. Home BP measurements more accurately and reliably reflect target organ damage and the prognosis of cardiovascular disease. These guidelines include the reference values of hypertension and normotension for home BP measurements. However, none of the guidelines have defined measurement procedure for home BP.

The purpose of home BP measurements is to obtain information on the patient's inherent BP pattern using long-term, repetitive measurement under controlled conditions. Since home BP is measured under controlled conditions, values are

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reproducible, and thus, useful in the diagnosis and treatment of hypertension. Blood pressures measured under standardized conditions are indispensable for comparing data among individuals, among groups and among institutes. However, such standards for the measurement of home BP have not been fully established. The Working Group of JSH established the JSH Guidelines for Self-Monitoring of Blood Pressure at Home in 2003 (6). This review referred to the practice aspect of monitoring hypertension based on self-measured BP at home and also to the JSH Guideline for home BP measurements.

History of Home Blood Pressure Measurements

In 1896, Riva-Rocci developed the indirect arm-cuff method for the measurement of BP (7), and in 1905 Korotkoff introduced the use of auscultation in conjunction with the indirect method (8). Since then, the indirect method for BP measurement has remained essentially unchanged for 100 years. Over the past 100 years, BP has been measured in the clinic or other medically oriented settings and has been called casual-clinic BP (CBP). Since the development of indirect BP measurement, hypertension research and treatment methodologies have substantially advanced. The gold standard of BP measurement for practice and research has been CBP. However, an alternative to the CBP was proposed soon after the introduction of indirect BP measurements. The rationale for BP measurement outside the clinical setting is based on the acknowledged and marked variability of BP. Time-dependent and incidental BP variations are well known phenomenon since the 18th century when Stephen Hale observed such variabilities. Clinically, Bevan et al initially demonstrated marked and time-dependent variability of BP in an unrestricted human male subject using direct, continuous BP monitoring for 24 hours (9). In 1940, Ayman and Goldshine reported the concept of "self-BP measurement" and demonstrated an apparent difference between the CBP and the self-measured BP (10). Initially, self-measurement was done using the auscultation method. In the 1970s, an electric device based on the microphone method was marketed, but not widely distributed because of high price, mechanical difficulties, and the issue of auscultation gap. Explosive distribution of home measurement devices since the 1980s is mediated by the development of devices based on the cuff-oscillometric principle.

The Problems of Home Blood Pressure Measurements

Although the mercury column sphygmomanometer with auscultation is becoming obsolete, we should remember that the gold standard for clinical practice is the Korotkoff sound method using a mercury column sphygmomanometer. The differential properties of the Korotkoff sounds and cuff-oscillation lead to an unavoidable difference in BP values between the two methods. The basic algorithm of cuff-oscillometric principle has been improved by including procedures to correctly approximate the characteristic changes in cuff-oscillation during phase I and phase V Korotkoff sounds. Furthermore, the accuracy of the automatic device is determined by comparison with the auscultation method, and no other standard method is currently available for this purpose. The issue here is the subjectivity and the possible inaccuracy of auscultation when the auscultation method is used as a standard.

Since BP measurements in clinical settings are now primarily obtained by cuff-oscillometric devices, it is inevitable that cuff-oscillometric devices be used in home BP measuring systems. The accumulation of clinical and epidemiological data obtained by authorized cuff-oscillometric devices may finally validate the efficacy of these tools for clinical decision making.

At present, three types of electrical devices for home BP measurements are commercially available: the arm-cuff device, the wrist-cuff device, and the finger-cuff device. Ten million such electrical devices are produced each year in the Far East (including Japan, Korea, Taiwan and China), which represents 85% of the world production (11). Of those, 35% are wrist-cuff devices (11). Previously, finger-cuff devices commanded a considerable portion of the market share due to their convenience and ease-of-use. However, it is now apparent that finger BP is physiologically different from brachial BP, and issues of vasospasm in the winter season as well as hydrostatic difference are inevitable. Therefore, manufacturers have now decreased production of finger-cuff devices and extensively increased production of wrist-cuff devices. In Japan, wrist-cuff devices possess 30% of the market share (12). Wrist-cuff devices are much easier to handle and more portable, but include serious shortcomings. The most important issue is the necessity for correction of the hydrostatic pressure. The reference level for BP measurement is the right atrium. When the measurement site is 10 cm below (above) the right atrium, systolic BP (SBP) and diastolic BP (DBP) are measured 7 mmHg higher (lower) than those at the level of the right atrium. Even after appropriate correction of the hydrostatic pressure, another issue remains concerning the anatomy of the wrist (11). At the wrist, the radial and ulnar arteries are surrounded by the radial bone, the ulnar bone and several long tendons, including the palmaris longus tendon. Therefore, even a sufficient amount of cuff pressure over the arterial area does not necessarily occlude these arteries completely. As a result, wrist-cuff devices sometime provide erroneous readings, especially for SBP (11). Therefore, arm-cuff devices based on the cuff-oscillometric method are recommended for home BP measurement (1–6).

Practical Aspect of Monitoring Hypertension based on Home Blood Pressure Measurements

Home BP measurements and ambulatory BP monitoring are characterized by increased measurement frequency, and

Table 1. Characteristics of Casual, Ambulatory, and Home Blood Pressure Measurements

	Casual BP (office, clinic, screening)	Ambulatory BP	Home BP
Characteristic	including reactive pressor response	measurements under several psychological and physical conditions	measurements under relatively stable condition
Measurement bias	+	−	−~±
Measurement frequency	few	many	many
Estimation of circadian BP variation	impossible	possible	partially possible
Estimation of night-time BP	impossible	possible	possible
Estimation of short-term BP variation	impossible	adequate	inadequate
Estimation of long-term BP variation	inadequate	inadequate	adequate
Reproducibility	poor	poor fair	good
Estimation of drug effect	insufficient due to placebo effect	occasionally insufficient due to regression to the mean	adequate
Estimation of duration of drug action	impossible	possible	adequate
Estimation of drug resistance	inadequate	adequate	adequate
Estimation and diagnosis of white-coat effect (hypertension)	impossible	adequate	adequate
Estimation of paroxysmal hypertension or episodic hypotension	impossible	adequate	occasionally possible

thus, increased information on BP. In addition, these methods provide BP information in relation to time. Such characteristics of home BP measurements provide advantages and superiority when compared to CBP (Table 1).

Home blood pressure measurement and diagnosis of hypertension

References value of home blood pressure

Although home BP measurement devices are distributed widely throughout the world, the practice of monitoring hypertension using these devices has not been established because of the deficiency of reference values for hypertension and normotension using this home equipment. Another issue is that standardization of home BP measurements has not been established.

Since 1986, we have been conducting an epidemiological survey of hypertension using home BP in Ohasama, in the northern part of Japan. Ohasama initially had a population of 9,400, but this has now dropped to 6,800. Over the past 18 years, we have obtained home BP data from 5,000 subjects aged over 7 years, as well as long-term clinical outcomes and information on risk factors and predictors. One of the initial purposes of the study was to define reference values for home BP measurements with respect to prognosis in a long-term prospective study.

Several methods are available for obtaining these reference values. The first involves the distribution criteria, for example mean +SD, mean +2SD or 95th percentile value of the reference population. These values provided us with the distribution of home BP level in the population, but clinical significance of these values is still uncertain.

Another method uses correspondence criteria, which derives home BP levels corresponding to CBP of 140/90 mmHg. However, the relationship between CBP and home BP has not been defined well enough to obtain accurate corresponding values; the correlation coefficient of the relationship between CBP and home BP has been calculated to be approximately 0.5 (13). However, the linear regression analysis deduced that 140/90 mmHg for CBP corresponds to 125/80 mmHg for home BP, suggesting that the normative value of home BP is less than 125/80 mmHg.

The most meaningful reference values would be provided by a long-term prospective study based on the resultant cardiovascular morbidity and mortality. Several observational and interventional studies are currently ongoing worldwide. The Ohasama study was initiated first and is the only study aiming to provide such reference values. Subjects from the Ohasama population aged 40 years and over were followed up for an average of 10.6 years. Home BP and CBP values were classified equally into quintiles on the basis of BP level. The relationship between BP level and stroke incidence being analyzed by a Cox regression model was adjusted for age, sex, and drug treatment. No specific tendency was observed in CBP. In subjects in the highest quintile of home BP (≧135/85 mmHg), a significant increase in relative hazard was observed, suggesting the higher predictability of home BP when compared with CBP (14) (Fig. 1).

These results obtained from Ohasama studies were cited in the JNC-VI (1) and 1999 WHO-ISH guidelines (3) and were the basis of reference values (Table 2) for home BP measurements given in these guidelines.

Definition of white-coat hypertension and white-coat effect

White-coat hypertension - reproducible hypertension in

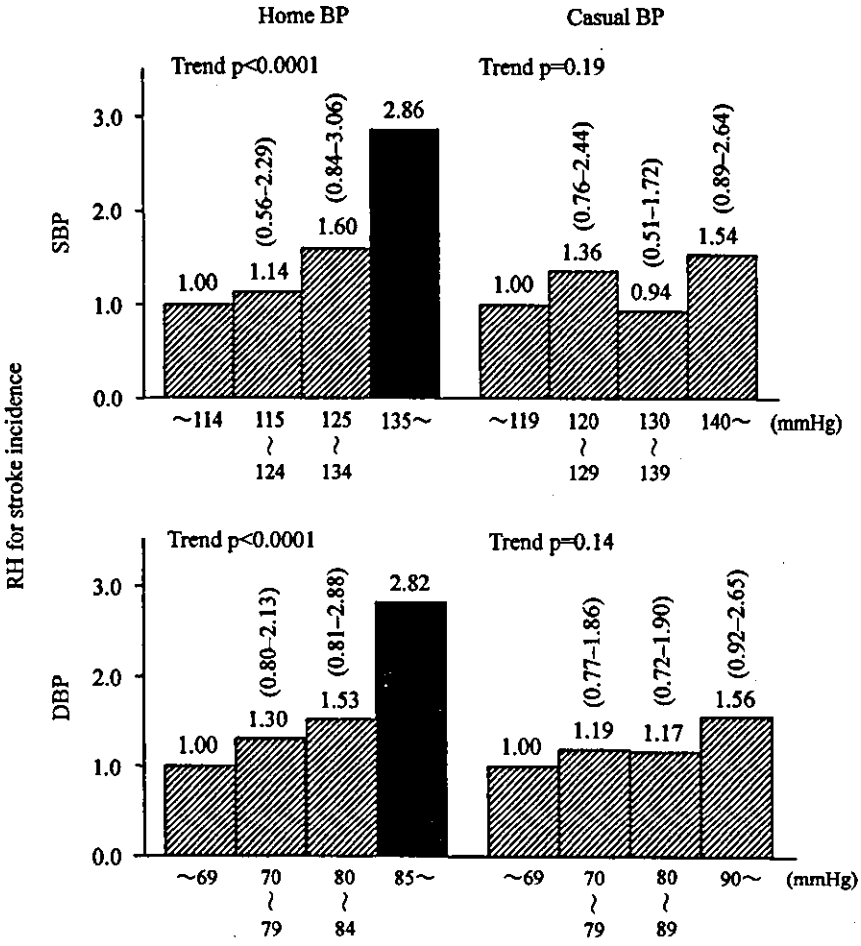

Figure 1. Association between home and casual-screening blood pressure (BP) values and stroke risk. Relative hazards (RH) and 95% confidence intervals (CI) of home and casual-screening systolic BP (SBP) and diastolic BP (DBP) level adjusted for age, gender, smoking status, the use of antihypertensive medication, history of heart disease, hypercholesterolemia, and diabetes for first symptomatic stroke. The group with the lowest risk was treated as the reference category (RH=1) (14).

Table 2. Reference Values of Home BP Value by JNC VI and WHO/ISH

	HBP value (Ohasama) (mmHg)	Reference value (mmHg)
Hypertension		
Cox model (non-parametric)	≥138/≥83	135/85 (JNC VI)
Normotension		
Cox model (non-parametric)	120–127/72–76	
Corresponding value of 140/90 (Clinic BP)	123/77	125/80 (WHO/ISH)
Mean home BP value + 1SD (with normal Clinic BP value)	125/77	

medical settings and normotension in non-medical settings is accurately defined using the normative value of home BP, i.e., CBP equal to or higher than 140/90 mmHg and home BP less than 125/80 mmHg. The Ohasama study examined the prognostic significance of white-coat hypertension (15). According to the Cox regression model, the relative hazard in white-coat hypertensive patients was similar to that seen in true normotensive subjects, whereas true hypertension and reversed white-coat hypertensive subjects (hypertension in the non-medical setting and normotension in the medical setting) carried a significantly higher relative hazard for cardiovascular mortality (Fig. 2) (15). However, recent analysis demonstrated that the development of sustained hypertension was more frequent in patients with white-coat hypertension than in those with true normotension during a 10-year observation period, suggesting that white-coat hypertension is a

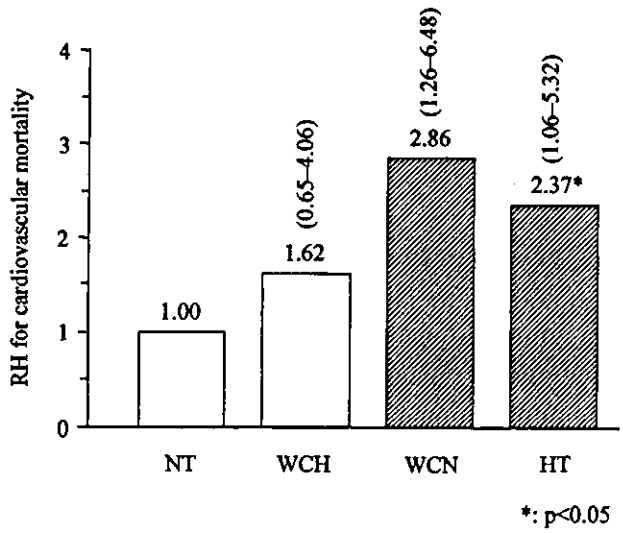

Figure 2. Risk of white-coat hypertension, white-coat normotension and sustained hypertension for cardiovascular mortality. Relative hazard (RH) for cardiovascular mortality and 95% confidential intervals (CI). NT: normotension, WCT: white-coat hypertension, WCN: white-coat normotension (masked hypertension), HT: sustained hypertension. Normotension was treated as the reference category (RH=1) (15).

benign condition during short-term observation periods but it becomes a cardiovascular risk during long-term observation periods.

Definition of the white-coat effect leads to the diagnosis of resistant hypertension or intractable hypertension. For example, in cases of essential hypertension, patients whose CBP was continuously higher than 160/100 mmHg and home BP was higher than 135/90 mmHg were treated with a calcium antagonist. The antihypertensive effect of the drug was never observed in CBP, while the drug sufficiently decreased home BP (Fig. 3). The clinical significance of home BP is apparent from this case; the white-coat effect is resistant to an antihypertensive regimen.

Circadian blood pressure variation and home blood pressure measurements

Recently, circadian BP variation has received attention as a risk factor in cardiovascular diseases. In the Ohasama study, non-dipper and inverted dipper circadian BP variations were apparent cardiovascular risk factors. However, the so-called extreme-dipper circadian BP variation was a benign condition (16). Such BP information was obtained only by ambulatory BP monitoring. Recently, we developed new equipment for self-BP measurements which allows monitoring of BP during sleep (17). Thus, the nocturnal BP level is

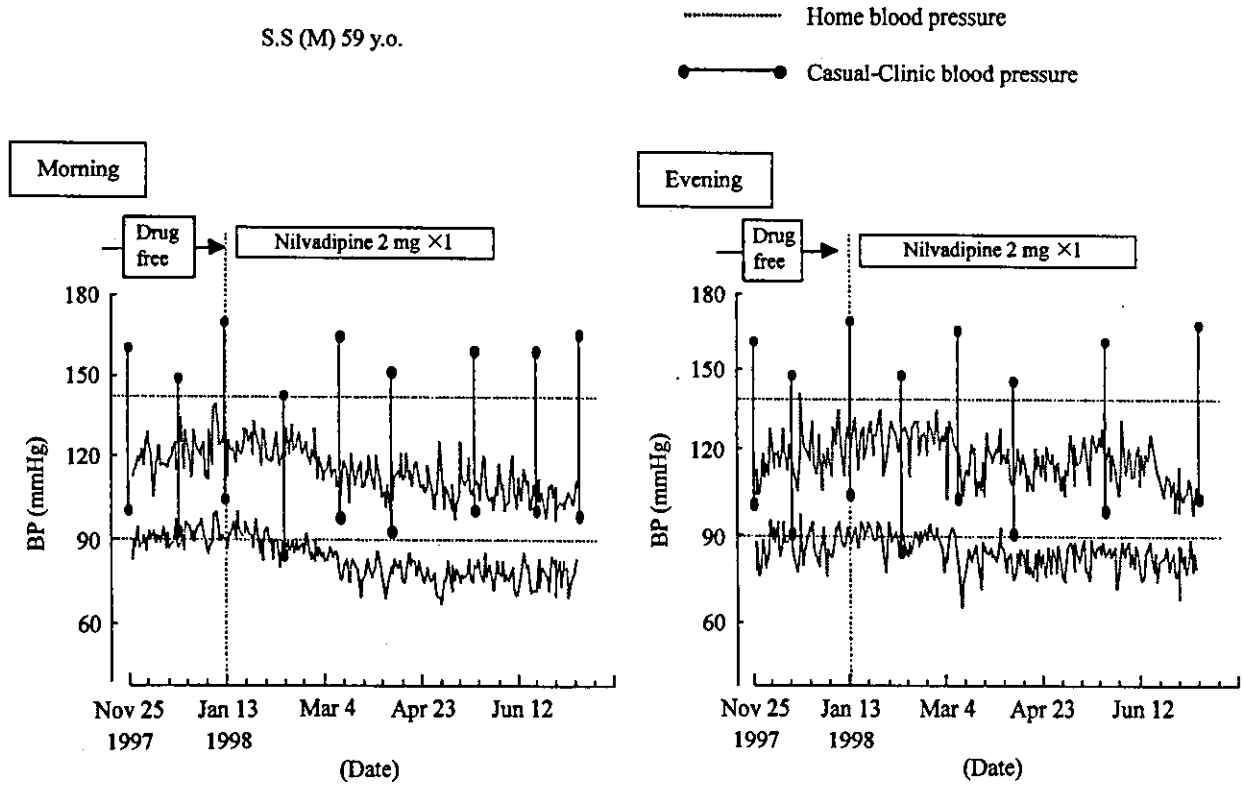

Figure 3. Effects of nilvadipine, a calcium antagonist, on white-coat effect in a hypertensive patient. Nilvadipine did not decrease casual-clinic blood pressure (CBP), but decreased home BP both in the morning and evening. SBP: systolic BP, DBP: diastolic BP, HBP: home BP, HR: heart rate.

now available from home BP measurements. However, the most common routine for home BP measurements is in the morning and in the evening.

Recently, the concept of hypertension in the morning is an issue in the management of hypertension. Morning hypertension is mediated by a morning surge and non-dipper or inverted dipper circadian BP variation. The morning surge of BP represents a mirror image of nocturnal dipping, and thus, is essentially observed in extreme dippers. In the Ohasama study, morning hypertension is primarily mediated by non-dipper or inverted dipper circadian BP variation and only 10% of patients with morning hypertension have an extreme dipper pattern, and thus, a morning surge. Recently, Kamoi et al reported that in normotensive (per CBP) patients with diabetes mellitus only those with high BP in the morning obtained during home BP measurements had severe target organ damage (18). It is well known that patients with diabetic target organ damage usually have non-dipper or inverted dipper circadian BP variation, suggesting that the morning hypertension reported by Kamoi et al reflects the overall BP load throughout 24 hours. Morning hypertension reported by Kamoi et al is also defined as reverse white-coat hypertension or white-coat normotension, which was related to poor prognosis in the Ohasama study (15). This concept was later reported as masked hypertension by Pickering et al (19). We found that masked hypertension is mediated by non-dipper circadian BP variation, inverted dipper circadian BP variation and insufficient duration of action of the antihypertensive medication (20). Home BP measurement is the only a practical method to determine the occurrence of morning hypertension.

Day-by-day variability of blood pressure

Short-term BP variability is a risk factor for cardiovascular diseases (21). Such short-term information is available from ambulatory BP monitoring, while the information on day-by-day variability is obtained only with home BP measurements. The Ohasama study demonstrated that day-by-day variability reflects the risk of cardiovascular diseases. Thus, home BP measurements can now replace ambulatory BP monitoring.

Treatment of hypertension based on home blood pressure measurements

Evaluation of antihypertensive effect

Since home BP is measured under controlled conditions using a standardized method, the reproducibility of home BP is assured and no placebo effect is observed in the measurements (22). Therefore, the accuracy and validity of home BP measurements reflects the physiological response to the clinical pharmacology of antihypertensive drugs; e.g., a decrease in systolic BP by 6 mmHg is determined by 15 subjects when based on the home BP measurements. Home BP measurement improves the quality in clinical pharmacological studies.

Evaluation of duration of action of antihypertensive effect

Home BP measurements can be used to evaluate medication effects and the duration of action. Figure 4 demonstrates that when trichlomethiazide was administered, once in the morning, home BP was decreased when measured before taking the next dose; this suggested that the duration of action for this drug is more than 24 hours. Such characteristics of home BP measurements provide an index of duration of action of drugs, i.e., the morning effect vs. evening effect ratio (M/E ratio), which is comparable to trough/peak (T/P) ratio obtained by ambulatory BP monitoring. The M/E ratio is more reliable than the T/P ratio, since the former is obtained by the average of multiple measurements of the difference between the period before treatment and the treated period.

JSH Guidelines for self-monitoring of blood pressure at home

Home BP measurements are indispensable for the improvement of management of hypertension in medical practice as well as for the recognition of hypertension in the population. Therefore, practice of self-measurement of BP is the first priority and for this purpose it is not necessarily expected that strict measurement conditions will be set. However, the presence of a standard for home BP measurements may be convenient and useful for practitioners as well as for patients. Such standards should be intended to instruct patients and subjects in the general population on how to measure BP at home and may provide a shared basis of information for clinical decision making. The Working Group for Establishment of Guidelines for Measurement Procedure of Self-Monitoring of Blood Pressure at Home of the JSH has established standard for all techniques and procedures of home BP measurements (6).

The recommendations are as follows:

1) For home BP use, arm-cuff devices are recommended. They should be based on the cuff-oscillometric method, validated officially, and confirmed for accuracy in each individual.
2) BP should be measured in the upper arm. Finger-cuff devices and wrist-cuff devices should not be used for home BP measurements.
3) Devices for home BP measurement should be adapted from the American Association Medical Instrumentation (AAMI) standard and the British Hypertension Society (BHS) guidelines. In addition, the difference between the BP measured by the auscultatory method and that measured using the device should be 5 mmHg or less in each individual. Accuracy and function of the home measurement device should be validated before use and at regular intervals.
4) Home BP should be monitored under the following conditions.

   The morning measurements:

   · within 1 hour after waking

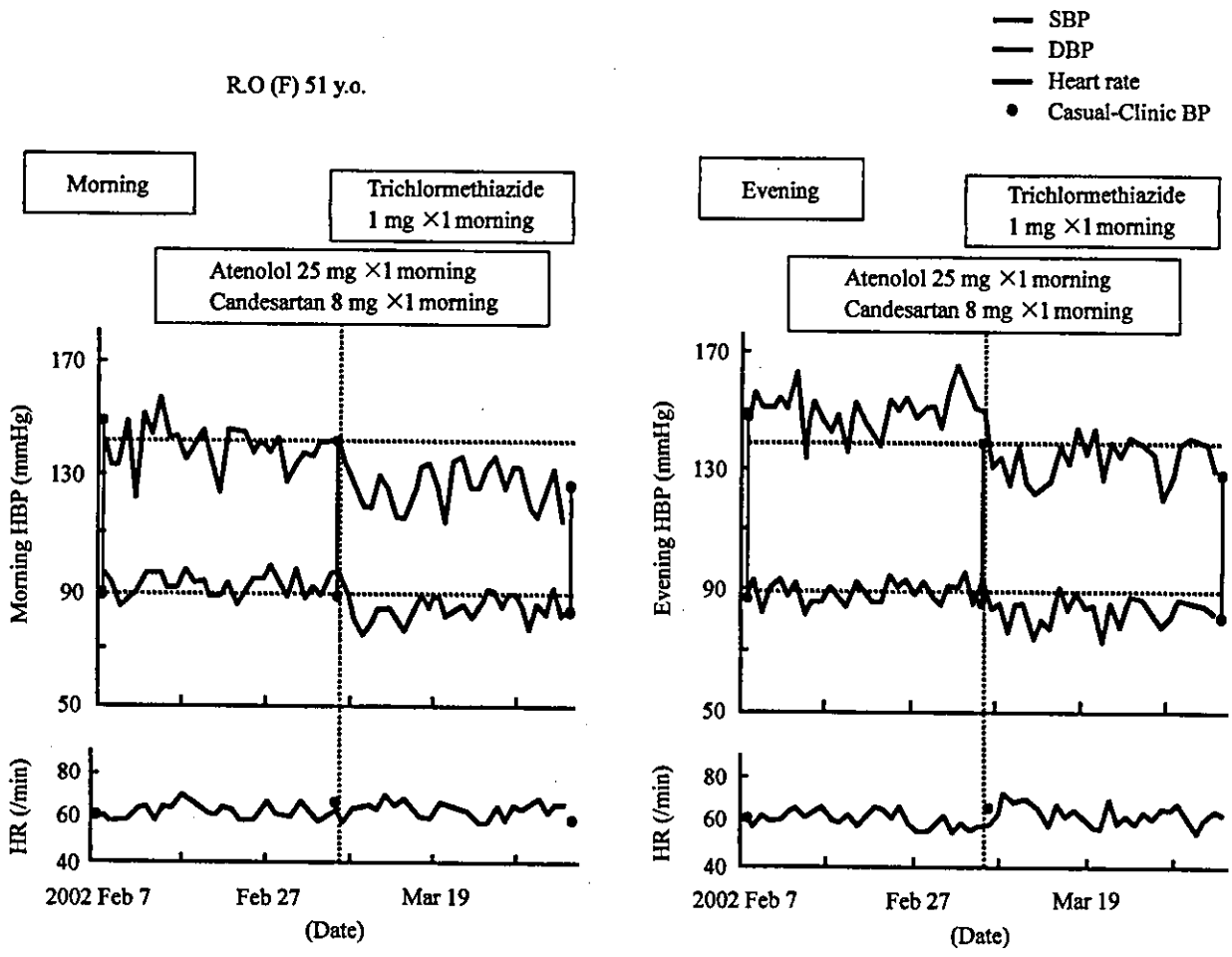

Figure 4. Effect of trichlormethiazide, administered once in the morning, on home blood pressure (HBP) in the morning and evening in a patient with essential hypertension. The HBP in the morning was measured before morning dose, thus, reflecting that the duration of action of thiazide is over 24 hours.

- after micturition
- after 1 to 2 minutes of sitting at rest
- before drug ingestion
- before breakfast

The evening measurements:

- just before going to bed
- after 1 to 2 minutes of sitting at rest

5) Home BP should be measured at least once in the morning and once in the evening.
6) All home BP measurements should be documented without selection or omission and include the date, time, and pulse rate. Use of a device with a printer or an integrated circuit memory is useful to avoid selection bias.
7) The home BP in the morning and that in the evening should be averaged separately for a certain period. The first measurement on each occasion should be used for totaling.
8) Home BP values that average 135/80 mmHg and over, for a certain period, indicate hypertension. Average values of 135/85 mmHg and over indicate definite hypertension. Normotension is defined as less than 125/80 mmHg and definite normotension as less than 125/75 mmHg.

The guidelines aimed to establish practical advice which would not restrict casual daily life of the subjects. For example, one to two minutes of rest before measurements would be acceptable by the majority of people who measure BP at home every day. In the guidelines, prohibition of smoking and taking coffee before measurement was not addressed. Since BP values obtained after smoking and taking coffee would reflect daily behavior and lifestyle, regulation may actually interfere with the validity of the BP readings. Room temperature was also not addressed by the guidelines, since casual temperature *per se* is an important factor for daily BP level. In the present guidelines, it has been emphasized that home BP should be routinely measured at least once per occasion. "At least once" means that more than one measure-

ment during that occasion is also permissible. Actually subjects measure their BP repeatedly, until a reasonable value is obtained when their home BP is high. We must evaluate all values recorded. However, to compare data among individuals, groups and institutes a standardized measurement is necessary. The first measurement would be considered a common denominator in all cases. Therefore, the average of the first measurement for a certain period is an important commonality for clinical decision making.

Conclusion

At present, international reference values have been established. However, the treatment goal for home BP level has not yet been established. The normotensive value of home BP is set at the level of 125/80 mmHg. This value is approximately equivalent to a CBP level of 140/90 mmHg. Therefore, it seems that a value of less than 125/80 mmHg would be the goal for home BP. However, setting the goal for home BP must be based on the results of large-scale intervention studies. Among such studies the Hypertension Objective Treatment on Measurements by Electrical Device of BP (HOMED-BP) study is ongoing in Japan (23)

Standardization of the measurement procedure may elevate the position of home BP measurements in the practice of diagnosing and treating hypertension, and as a result, home BP measurements may bring greater accuracy in the screening for hypertension and assessment of BP control during treatment and improved drug compliance. Home BP measurements under such controlled conditions are expected to have a beneficial effect on the economics of the diagnosis and treatment of hypertension.

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Original Article

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

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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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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

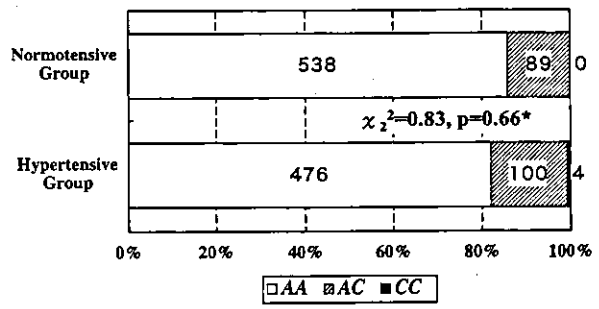

Fig. 1. *Frequencies of AT1R genotypes. The frequency of subjects with* AC+CC *genotypes was higher in hypertensives than in normotensives.* * p *value was after adjusted for age, sex, BMI, current smoking habit and current drinking. Odds ratio for hypertension* (AA vs. AC+CC) *after adjusted for sex 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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Adiponectin I164T Mutation Is Associated With the Metabolic Syndrome and Coronary Artery Disease

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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

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Abbreviations and Acronyms

BMI	= body mass index
CAD	= coronary artery disease
HbA1C	= hemoglobin A1C
HDL-chol	= high-density lipoprotein cholesterol
HOMA	= homeostasis model assessment
PCR	= polymerase chain reaction
SNP	= single nucleotide polymorphism
T-chol	= total cholesterol
TG	= triglyceride
TNF	= tumor necrosis factor

(SNP94) in the Japanese population (19,20). Among these mutations, the I164T mutation correlated with type 2 diabetes (19); SNP94 was reported to be associated with type 2 diabetes and obesity (21,22). A weak association was observed between SNP94 and plasma adiponectin levels in French Caucasians, although no significant association was found in the Japanese population (23). Recently, SNP at position 276 (SNP276) was reported to be associated with type 2 diabetes (21); SNP276 was associated with plasma adiponectin levels in French Caucasians and only in obese Japanese subjects (21,23). In addition, the haplotype identified by SNP94 and SNP276 was related with obesity and other features of the insulin resistance syndrome in Caucasians (24). A susceptibility locus for type 2 diabetes was mapped on chromosome 3q27, which harbors the adiponectin gene (25). A genome-wide scan for CAD replicated linkage with the metabolic syndrome on the region 3q27, suggesting that adiponectin might be one of the candidate genes susceptible for the metabolic syndrome-linked CAD (26). Although the metabolic syndrome includes insulin resistance, it is very important to elucidate the genetic contribution of adiponectin in the development of CAD.

In the present study, we investigated the frequency and the clinical significance of I164T, SNP94, and SNP276 of adiponectin gene in consecutive CAD patients and age- and body mass index (BMI)-matched non-CAD subjects.

METHODS

Study subjects. Consecutive 383 CAD patients were recruited from the inpatients who were admitted to Osaka University Hospital. The criteria for CAD were a 75% ≤ organic stenosis of at least one segment of a major coronary artery confirmed by coronary angiogram. The control subjects were selected from people who received medical check in Osaka University Hospital or our affiliated hospitals. In these latter subjects, it was unethical to perform coronary angiography to rule out the presence of asymptomatic CAD. Therefore, the following inclusion criteria were used: no history of angina or other atherosclerotic vascular diseases, and normal exercise electrocardiogram stress testing. They were matched with CAD patients for age and BMI. All patients and subjects enrolled in this study were Japanese and gave written informed consent. This study was approved by the Ethics Committee of Osaka University.

Laboratory methods. Venous blood was drawn from all patients and control subjects after an overnight fast. Plasma samples were kept at −80° centigrade for subsequent assay. Plasma concentration of adiponectin was evaluated by a sandwich ELISA system (Adiponectin ELISA Kit, Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan) as previously reported (27). Serum total cholesterol (T-chol) and triglyceride (TG) concentrations were determined by an enzymatic method. High-density lipoprotein cholesterol (HDL-chol) was also measured by an enzymatic method after heparin and calcium precipitation. Plasma glucose was measured by a glucose oxidase method. The value of hemoglobin A1c (HbA1c) was determined by high-performance liquid chromatography. Insulin resistance was assessed by homeostasis model assessment (HOMA) (insulin resistance index = [fasting glucose (mmol/l) × fasting insulin (U/ml)]/22.5 (28). Body mass index was calculated as weight/height^2.

Definitions of risk factors. Diabetes mellitus was defined according to World Health Organization criteria, and/or having received treatment for diabetes mellitus (29). Dyslipidemia was defined as a T-chol concentration >5.69 mmol/l, a TG concentration >1.69 mmol/l, an HDL-chol concentration <1.03 mmol/l, and/or having received treatment for dyslipidemia. Hypertension was defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or having received treatment for hypertension. We did not exclude the subjects under medical treatment for diabetes mellitus, dyslipidemia, and hypertension.

DNA extraction and genotyping. Genomic DNA was prepared from frozen whole blood with the use of a QIAamp DNA Blood Mini Kit (QIAGEN, Valencia, California). We determined the missense mutation I164T and the SNP276 of adiponectin gene by the TaqMan (Roche Molecular Systems Inc., Pleasanton, California) polymerase chain reaction (PCR) chemistry method as previously described (30). The TaqMan probe is a fluorogenic probe that consists of an oligonucleotide labeled with both a fluorescent reporter dye and a quenched dye. The fluorescent reporter dye, such as VIC and FAM (Applied Biosystems Inc., Foster City, California), is covalently linked to the 5′ end of the nucleotide. Each of the reporters is quenched by minor groove binder, typically located at the 3′ end. The following primers were used for the missense mutation I164T: a forward primer, 5′-AACATTCCTGGGCTGTACTACTTTG-3′; a reverse primer, 5′- GGCTGACCTTCACATCCTTCATA-3′; a T-allele-specific probe, 5′-VIC-ACCACATCA-CAGTCTA-MGB-3′; a C-allele-specific probe, 5′-FAM-CCACACCACAGTCT-MGB-3′. The following primers were used for the G/T SNP at position 276: a forward primer, 5′-AGAATGTTTCTGGCCTCTTTCATC-3′; a reverse primer, 5′- TTCTCCCTGTGTCTAGGCCTTAGT-3′; a G-allele-specific probe, 5′-FAM-CTATATGAAGGCAT-TCATTA-MGB-3′; T-allele-specific probe, 5′-VIC-

Table 1. Clinical Characteristics of Control Subjects and CAD Patients

	Control Subjects (n = 368)	CAD Patients (n = 383)	p Value
Age, yrs	62.3 ± 0.6	63.0 ± 0.4	NS
Gender, M/F	240/128	270/113	NS
Adiponectin, μg/ml	7.7 ± 0.2	6.1 ± 0.2	< 0.001
BMI, kg/m²	23.8 ± 0.2	24.1 ± 0.2	NS
Family history of diabetes mellitus, n (%)	(15.8)	(18.5)	NS
Diabetes mellitus, n (%)	58 (10.3)	71 (48.0)	< 0.001
FPG, mmol/l	38 ± 0.04 5.40	184 ± 0.14 6.67	< 0.001
HbA1c, %	5.11 ± 0.04	6.09 ± 0.08	< 0.001
Dyslipidemia, n (%)	179 (48.6)	259 (67.6)	< 0.001
T-chol, mmol/l	5.23 ± 0.05	5.29 ± 0.05	NS
TG, mmol/l	1.57 ± 0.05	1.77 ± 0.06	<0.05
HDL-chol, mmol/l	1.52 ± 0.03	1.19 ± 0.02	< 0.001
Hypertension, n (%)	272 (73.9)	264 (68.9)	NS
SBP, mm Hg	134.6 ± 1.0	132.9 ± 0.9	NS
DBP, mm Hg	80.1 ± 0.7	75.4 ± 0.8	< 0.001

Data represent means ± SE.
BMI = body mass index; CAD = coronary artery disease; DBP = diastolic blood pressure; FPG = fasting plasma glucose; HbA1c = hemoglobin A1C; HDL-chol = high-density lipoprotein cholesterol; SBP = systolic blood pressure; T-chol = total cholesterol.

AAACTATATGAAGTCATTCATTA-MGB-3′. The fluorescence level of PCR products was measured with the ABI PRISM 7200 Sequence Detector (Applied Biosystems, Inc.). We determined the SNP94 in exon 2 of adiponectin gene by a PCR-based assay for the analysis of restriction fragment length polymorphism as previously described (20).

Statistical methods. For continuous variables, results are presented as mean ± SE. Differences in continuous parameter, such as BMI, between two groups were calculated by the Student *t* test, and differences in continuous parameter, such as plasma adiponectin level, among more than three groups were evaluated by analysis of variance. Because plasma adiponectin level, HOMA, and TG were skewed, these three parameters were log-transformed before analysis, and the parameters presented were back-transformed. Categorical variables were presented using frequency counts, and intergroup comparisons were analyzed by chi-square test. A level of $p < 0.05$ was accepted as statistically significant. All calculations were performed using a standard statistical package (JMP for Macintosh, version 4.0, SAS Institute Inc., Cary, North Carolina).

RESULTS

The clinical characteristics of CAD patients and non-CAD control subjects are shown in Table 1. The mean plasma adiponectin level in CAD patients was significantly lower than the control ($p < 0.001$), as we described previously (10). Patients with CAD had significantly higher levels of fasting plasma glucose, HbA1c, TG, numbers of diabetes mellitus, dyslipidemia, and lower levels of HDL-chol and diastolic blood pressure than the control group. There were no significant differences in age, gender, BMI, number of family history for diabetes, T-chol, systolic blood pressure, and number of hypertension between the two groups.

The frequency of I164T mutation in CAD patients (11 [2.9%] of 383) was significantly higher than that in non-CAD subjects (3 [0.8%] of 368, $p < 0.05$) (Table 2). All subjects with the mutation were heterozygotes. In contrast to this mutation, no significant differences in the distribution of SNP94 and SNP276 genotypes were observed between the two groups. The plasma adiponectin levels in subjects carrying the I164T mutation (3.2 ± 0.5 μg/ml) were significantly lower than in subjects without the mutation (6.9 ± 0.2 μg/ml, $p < 0.0001$) (Fig. 1A), although no

Table 2. Frequency of Mutation and Polymorphism in Adiponectin Gene

		Control Subjects	CAD Patients	
n		368	383	p Value
I164T, n (%)		3 (0.8)	11 (2.9)	<0.05
SNP94, n (%)	G/G	29 (7.9)	33 (8.6)	NS
	G/T	148 (40.2)	140 (36.6)	
	T/T	191 (51.9)	210 (54.8)	
SNP276, n (%)	G/G	190 (51.6)	185 (48.3)	NS
	G/T	149 (40.5)	164 (42.8)	
	T/T	29 (7.9)	34 (8.9)	

CAD = coronary artery disease; SNP = single nucleotide polymorphism.

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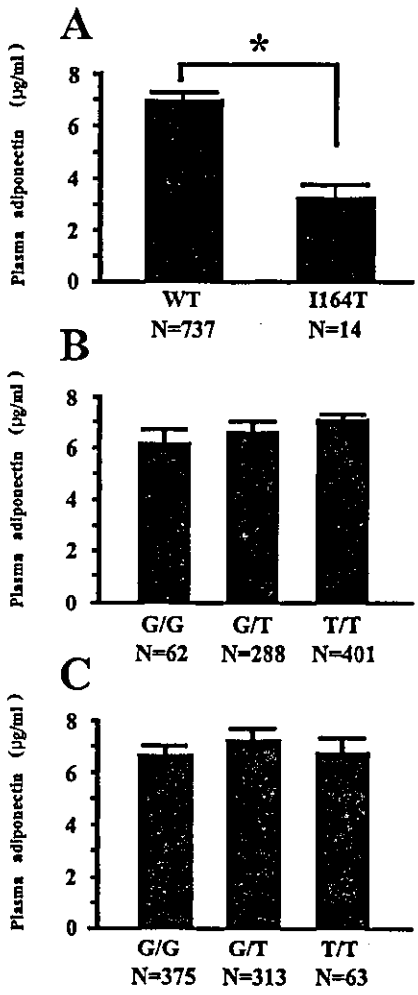

Figure 1. Association of I164T mutation, SNP94, and SNP276 with plasma adiponectin concentrations. (A) Plasma adiponectin levels in the subjects with wild type (WT) or I164T mutation in adiponectin gene. (B) Relationship between SNP94 genotypes and plasma adiponectin levels. (C) Relationship between SNP276 genotypes and plasma adiponectin levels. Columns and vertical bars denote mean and SE of the indicated sample numbers. *$p < 0.05$ vs. WT.

significant difference was observed in BMI between the subjects with and without I164T mutation (24.4 ± 1.2 vs. 24.0 ± 0.1 kg/m^2). The plasma adiponectin levels in subjects with the mutation were markedly low in both CAD and control groups (2.9 ± 0.6 vs. 4.3 ± 1.2 μg/ml, respectively), and did not correlate with BMI. The negative correlation between plasma adiponectin levels and BMI was observed in subjects without the mutation (data not shown). These data indicated that hypoadiponectinemia in subjects with the mutation was independent of BMI. The plasma adiponectin levels of the subjects with G/G, G/T, and T/T allele at SNP94 were 6.2 ± 0.6, 6.6 ± 0.2, and 7.1 ± 0.2 μg/ml, respectively (Fig. 1B). The plasma adiponectin level in the subjects having G allele at SNP94 tended to be lower, but it was not statistically significant. On the other hand, no differences were observed in plasma adiponectin levels of the subjects with G/G, G/T, and T/T allele at SNP276 (6.6 ± 0.2, 7.2 ± 0.2, and 6.7 ± 0.5 μg/ml, respectively) (Fig. 1C).

As shown in Table 3, all subjects carrying I164T had at least one risk factor including diabetes mellitus, hypertension, and dyslipidemia. Six (case 4 to 8, and 11) of the 11 CAD patients with the I164T mutation and 75 of 372 wild type CAD patients had all three metabolic abnormalities, which is a key feature of the metabolic syndrome. The percentage of the subjects with all three metabolic abnormalities was significantly higher in I164T mutation (54.5%) than that in wild type (20.2%) ($p < 0.01$). Nine (case 4 to 8 and 11 to 14) of 14 subjects with I164T mutation had diabetes mellitus, and cases 13 and 14 had received insulin treatment. However, except three cases (3, 4, and 8), six diabetic I164T patients had no apparent insulin resistance assessed by HOMA-insulin resistance (IR) compared with CAD patients (n = 383, HOMA-IR; 2.4 ± 0.2). In addition, there were no differences in HOMA-IR levels between nondiabetic I164T subjects (case 1 to 3, 9, and 10) and control subjects (n = 368, HOMA-IR; 1.8 ± 0.1).

DISCUSSION

In the present study, we found that the I164T mutation of adiponectin gene was associated with CAD prevalence and hypoadiponectinemia in the Japanese population. In contrast, the genotypes of SNP94 and SNP276, which were reported to be present in type 2 diabetes, influenced neither the prevalence of CAD nor the plasma adiponectin level.

Table 3. Clinical Profile of the Subjects With I164T Mutation

Case Subject	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Age, yrs	53	65	78	52	59	59	61	69	71	72	65	67	70	73
Gender	M	M	F	M	M	M	M	M	M	M	F	F	F	F
Plasma adiponectin, μg/ml	2.7	6.7	3.7	0.4	2.7	2.8	2.6	3.7	3.5	0.9	4.4	2.0	1.6	7.2
BMI, kg/m^2	23.6	ND	24.0	27.0	25.4	29.2	25.6	21.7	19.2	23.8	34.1	25.0	19.0	19.2
FPG, mmol/l	3.5	4.7	5.3	7.6	16.6	5.8	8.2	8.6	5.2	5.4	6.3	6.1	4.8	7.5
FIRI, μU/ml	8.0	4.0	5.0	13.0	5.7	6.0	3.0	10.9	6.2	4.8	6.4	10.2	3.5	2.3
HOMA-IR	1.8	0.8	1.2	3.3	4.2	1.5	2.3	4.2	1.4	1.2	1.4	2.8	0.7	0.8
Number of risk factors*	1	2	2	3	3	3	3	3	1	1	3	2	2	2
Coronary artery disease	-	-	-	AP	AP	AP	AP	MI	AP	MI	AP	AP	AP	AP

*Risk factors: diabetes mellitus, hypertension, and dyslipidemia.
AP = angina pectoris; BMI = body mass index; FIRI = fasting immunoreactive insulin; FPG = fasting plasma glucose; HOMA-IR = homeostatis model assessment of insulin resistance; MI = myocardial infarction.

Importantly, all subjects carrying I164T in the present study including CAD and non-CAD subjects had at least one or more metabolic disorders including diabetes mellitus, hypertension, and dyslipidemia. Among CAD patients, the prevalence of the metabolic syndrome was significantly higher in I164T mutation than that in wild type. These findings suggest that the I164T mutation of adiponectin gene is associated with the development of the metabolic syndrome-linked CAD. Importantly, the severe hypoadiponectinemia in subjects with the I164T mutation was independent of BMI. Recently, we have demonstrated that intimal thickening was accelerated in mechanically injured arteries of adiponectin knockout mice, and that adenovirus-mediated supplement of adiponectin completely abolished the enhanced neointimal formation (15). These results suggest that hypoadiponectinemia directly contributes to abnormal vascular remodeling. Therefore, the I164T mutation plays a pivotal role in the development of atherosclerosis.

We have reported that the plasma adiponectin levels were significantly low in subjects with obesity (27), diabetes mellitus (31), and hypertension (32). In addition, we reported that plasma adiponectin level was predictive of the development of type 2 diabetes in the Pima Indian population (33). These observations suggest that the plasma adiponectin levels might be closely associated with the development of the metabolic syndrome. In adiponectin knockout mice, glucose metabolism was normal under standard diet, and severe insulin resistance, hyperglycemia, and hypertension were developed after two weeks' feeding of atherogenic diet (18,34). In the present study, all subjects carrying I164T had at least one or more coronary risk factors. However, HOMA-IR levels of nondiabetic I164T mutation were no different than those of control subjects. These results suggest that the hypoadiponectinemia caused by I164T mutation might lead to diabetes mellitus, hypertension, and atherosclerosis only under overnutrition in the modern industrialized countries.

A recent study demonstrated that the I164T mutation was not found in the type 2 diabetic and obese French Caucasian subjects and that the genotypes of SNP94 and SNP276 affected plasma adiponectin levels (23). Higher plasma adiponectin levels were associated with the T allele of SNP94 and the G allele of SNP276 in Caucasians (23). We and others demonstrated that the I164T mutation was observed in the Japanese population (19,21). In the present study, the G allele of SNP94 tended to be associated with lower plasma adiponectin levels, and SNP276 did not correlate with plasma adiponectin levels in CAD and non-CAD Japanese subjects whose mean BMI were approximately 24 kg/m^2. Recently, the genotypes of SNP276 were reported to be associated with plasma adiponectin levels only in the obese subgroup of Japanese subjects (21). These differences between the French and Japanese populations may be due to ethnic background, although a larger population study is required to elucidate the discrepancy.

In the current study, three of the 14 subjects with the I164T mutation did not suffer from CAD, although they had at least one coronary risk factor and markedly low plasma adiponectin level. The follow-up study will be necessary to clarify whether the non-CAD subjects with I164T mutation develop CAD in the future.

In summary, we demonstrated that the I164T mutation of adiponectin gene affects CAD prevalence and the clustering of multiple risk factors for atherosclerosis. Our results indicate that screening the common genetic background of hypoadiponectinemia is helpful in evaluating the risk of the metabolic syndrome and CAD.

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