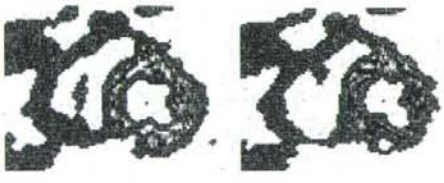
領域を設定する操作に要する時間は約 1 分，その後の処理を自動的に行い結果を出力するのに要する時間は約 2 秒（実験環境：Intel® Xeon™ CPU 3.60 GHz, GNU Linux）であった．

スライス 2 における 3 フレーム目（収縮早期）と 8 フレーム目（拡張早期）における，左室心筋中層部の圧縮ひずみ速度分布を図 9 に示す．図中で輝度値の高い部分は短縮部分を，低い部分は伸展部分を表し，グレーの部分は速度 0 または処理対象外であることを表す．

スライス 3 における 2 フレーム目（収縮早期）と 5 フレーム目（収縮後期）における，局所心筋のずりひずみ変化速度分布を図 10 に示す．図中で輝度値の高い部分はずりひずみ量 Δx_s が半時計回りの向きであること，すなわち内膜側の反時計回りの回転速度が外膜側の回転速度よりも速いことを示し，輝度値の低い部分はその逆であることを表す．

短軸断面において，図 11 に示すように **3.2.1** で求めた左室内腔中心から断面内右方に水平にひいた半直線を基準とした ±45 度の範囲を左室自由壁部分とする．左室自由壁部分における圧縮ひずみ速度すなわち局所心筋短縮・伸展速度の各心時相における平均値を，スライス 1 からスライス 4 まで四つの断面に対してそれぞれ求めた結果を図 12 に示す．収縮期において左室自由壁の局所心筋短縮速度が最大となるのは四つの断面ともに 3 フレーム目（収縮中期）であり，最大 2.3，最小 1.9，平均 2.0（単位 s^{-1}）であった．また，拡張期において左室自由壁の局所心筋伸展速度が最大となるのは四つの断面とも 8 フレーム目（拡張早



(a) (b)

図 9 左室心筋中層部における局所心筋圧縮ひずみ速度分布，スライス位置 2 (a) 3 フレーム目（収縮早期），(b) 8 フレーム目（拡張早期）

Fig. 9 Distribution of compressional strain rate in the left midcardium, slice no.2, (a) 3rd frame (early systole), (b) 8th frame (early diastole).

(a) (b)

図 10 左室心筋中層部における局所心筋ずりひずみ速度分布，スライス 3 (a) 2 フレーム目（収縮早期），(b) 5 フレーム目（収縮後期）

Fig. 10 Distribution of shearing strain rate in the left midcardium, slice no. 3, (a) 2nd frame (early systole), (b) 5th frame (late systole).

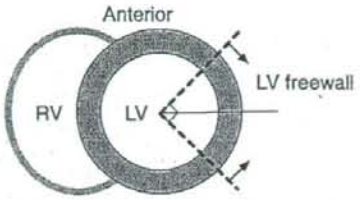


図 11 短軸断層像における左室自由壁部分

Fig. 11 Diagram showing the location of the left ventricular free wall on the short-axis slice.

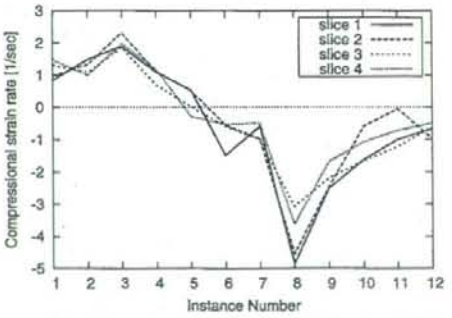


図 12 左室自由壁における心筋短縮・伸展速度の平均値

Fig. 12 Compressional strain rate in the left ventricular free wall.

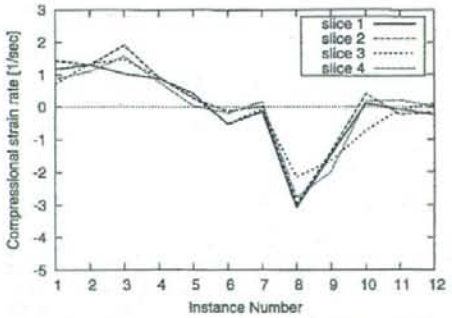


図 13 左室壁における心筋短縮・伸展速度の平均値

Fig. 13 Compressional strain rate in the whole left ventricle.

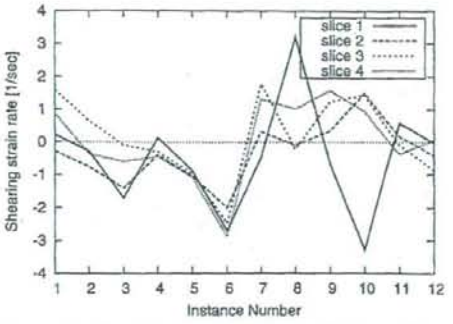


図 14 左室自由壁における心筋ずりひずみ速度の平均値
Fig. 14 Shearing strain rate in the left ventricular free wall.

期）であり，最大 4.8，最小 3.0，平均 4.0（単位 s^{-1}）であった．

図 13 に，左室心筋中層部全体における局所心筋短縮・伸展速度の各心時相における平均値を示す．3 フレーム目（収縮中期）における局所心筋短縮速度は，最大 1.9，最小 1.0，平均 1.5（単位 s^{-1}）であった．

ずりひずみ速度の各心時相における平均値を左室自由壁に対して求めた値を，図 14 に示す．

5. 考　　察

提案手法と開発したプログラムにより，MR-PC 法により得られた強度画像及び位相画像を用いて，自動的に短軸断面内における局所心筋の短縮伸展速度とずりひずみ速度を求め可視化することができた．

本手法により得られた局所心筋の収縮期最大短縮速度の左室自由壁における値は，カテーテル左室造影により算出した円周方向心筋線維短縮速度の報告 [18] や，MR-PC 画像より用手的に算出した報告 [15], [19] とよく一致した．一方，拡張期最大伸展速度の左室自由壁における値を他報告 [15], [19] と比較すると，最大短縮速度よりも大きい値をとる傾向は一致するものの，2 倍程度大きな値を示した．その理由として，本論文が健常男性 1 名のデータのみを対象としていること，撮影条件の違い，計算に用いる点数の違い，計測に用いた短軸断面位置の違いが考えられる．他報告 [15], [19] では，左室短軸断面内の 8 点のみの速度情報を用いて局所心筋・短縮速度を計測している．本手法と比較して，計算に用いる点の数や近傍 2 点間の距離の違いや，MRI 装置の空間分解能，短軸断面を定める際のスライス位置選択の精度など撮影条件の違いが結果の差違につながっていることが考えられる．また，他報告 [15], [19] では計測に用いた短軸断面は一つのみでその位置は不明であるが，本手法では四つの断面位置を用いて局所心筋・短縮速度を求めている．図 12 において，収縮期最大短縮速度にはスライス位置による違いは見られないが，拡張期最大伸展速度にはスライス位置による違いが見られる．心尖部側のスライス 1，2 における値の方が，心基部側のスライス 3，4 における値より大きい傾向を示している．スライス位置の違いにより拡張期最大伸展速度に差が生じる可能性を示している．

左室自由壁における局所心筋短縮・伸展速度に比べて，左室全体における局所心筋短縮・伸展速度が全般的に低い値を示している．また，収縮早期における速度分布（図 9 (a)）において，左室と右室とが結合する部分の近辺に輝度値の低い部分（伸展部分）が多く見られる．左室と右室とが結合する部分では心筋線維の収縮が解剖学的に制限され，左室全体における心筋短縮速度の平均値を低下させていると考えられる．MR-PC 法で心筋の収縮機能を解析する際には心筋線維方向だけでなく，両心室の結合部といった解剖学的条件も考慮する必要があることを示している．

図 9 において，収縮期・拡張期いずれにおいても短縮部分と伸展部分が入り交じって存在している．先行研究 [20] において，円周方向角速度が部位ごとに揺らぎをもつ場合にこのような収縮伸展速度分布を示すことを数値モデルにより示しているが，図 9 は，そのような揺らぎが今回の提案手法における処理スケール（圧縮ひずみを求めるための初期量 $x = 5.0\,\mathrm{mm}$）でも存在することを示していると考えられ，今後解析していく必要がある．

局所心筋ずりひずみ速度について，図 12 において，1 フレーム目から 3 フレーム目にかけてはグラフの傾きが負であり，4 フレーム目では 0 付近の値をとる．また，スライス 2 を除いて 3 フレーム目までに符号が変わる．MR タギング画像によるずりひずみの検討 [19] によれば，左室自由壁におけるずりひずみ量は収縮早期に最大となり，収縮中期から後期にかけてゼロに復帰するとされる．また，心筋の収縮を引き起こす電気的興奮波は内膜側から外膜側へ伝搬することを考慮すると，左室自由壁の収縮過程は図 15 のようにまず先にずりひずみが優位となり，続いて圧縮ひずみが優位になると考えられる．ずりひずみ速度の時間積分がずりひずみ量であるので，図 12 の 1 フレーム目から 4

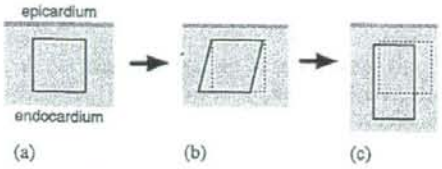


図 15　収縮期における心筋ひずみの模式図 (a) 拡張末期, (b) 収縮早期におけるずりひずみ (c) 収縮早期～収縮末期における圧縮ひずみ

Fig. 15　Schematic diagrams showing the myocardial strain at systole, (a) end diastole, (b) shearing strain at early systole, (c) compressive strain during systole.

フレーム目までの傾向は，この心筋収縮過程におけるずりひずみの優位性の変化を反映したものと考えられる．一方で，1 フレーム目におけるすりひずみ速度はスライスごとに様々な値をとる．収縮過程においてスライス間で相互に及ぼし合う影響や電気的興奮波に対応する時間のずれなどが原因として考えられるが，今後ともより詳細な検討が必要である．

本手法では，ある断面における局所心筋収縮機能情報を得るために，2 回の息止めで撮影した位相画像から合成した速度場を用いている．したがって，画像位置ずれの影響及び撮影時の生理学的なコンディションの差が計算結果に影響を与えている可能性も考えられる．今後は多数の症例に対して本手法を適用し，画像位置合せのアルゴリズムの改善や，画像解像度を落として 1 回の息止めで 2 方向の速度情報を得るなどの撮影方法の検討が必要である．

本手法では，MR 強度画像において心筋部分と心臓外部分の境界部分が不明りょうであること，ひずみ速度は心筋中層部において求めるので心筋部分の正確な抽出は必ずしも必要でないことを考慮したアルゴリズムを検討し実装している．しかしながら，乳頭筋も含まれる心筋部分の距離画像を用いて左室心筋中層部を求めているために，乳頭筋が存在する部分ではより内腔側の領域まで処理対象に含めていると考えられる．心筋中層部と比較して内腔側では心筋線維方向は短軸断面に対して大きな角度をとるので，短縮・伸展速度は低く計算されると予想されるが，図 12 に示す左室自由壁における心筋短縮・伸展速度の平均値は従来報告 [15], [19] と比較して低くはなっていない．その理由として，統計的な処理の中で乳頭筋の存在の影響が埋もれている可能性が考えられる．今後，より狭い範囲別に詳細な検討を行っていく際には，乳頭筋の存在に影響されずに左室中層部を抽出するアルゴリズムが必要と考えられる．

本手法は，求心性左室肥大における心筋の疲弊状態，あるいは肥大型心筋症における心筋の線維化といった心筋収縮機能の異常の程度を評価する際に有効であると考えられる．心筋の収縮機能特性は，収縮の程度よりも速度によりいっそう反映される．MR タギング法による標識追跡や，近年提案されている SENC [21], [22], DENSE [23], [24] 法により得られる変位情報から速度情報を得ることはできるが，変位を微分して速度を計算する，あるいは逆に速度を積分して変位を計算するよりも，目的に応じてそれぞれの指標の直接計測を行う方が誤差の拡大を招かず有利であると考えられる．例えば，1 周期にわたって心臓の変形を追跡する目的や，ひずみテンソルを計算する目的には，変位を直接計測できる撮影方法が有利であると考えられる．一方，心筋の収縮機能特性を反映するひずみ速度を計算する目的には，速度を直接計測できる MR-PC 法が有利であると考えられる．更に，短軸断面における左室心筋中層部に対してひずみ速度を求めることは，心筋線維に沿った方向の短縮伸展速度を考えることになり，医学・生理学的な意味を直観的にとらえることが可能で，臨床的有用性という観点からも重要と考えられる．

6. むすび

MR-PC 法で得られた速度情報を提案手法により解析することで，左室心筋中層部における局所短縮・伸展速度及びずりひずみ速度を自動的に抽出し可視化することができた．任意断面において三次元的に速度を直接算出できる MR-PC 法は，心筋収縮機能を評価する上でエコーや心筋タギング法といった他手法より有利な点があり，臨床的にも有用となることが期待される．

局所心筋短縮・伸展速度及びずりひずみの指標は，正常心と肥大心との差異が指摘されており，開発したプログラムを用いての自動的な局所心筋ひずみ速度の計算と可視化は，正常心と病的心との比較など今後の検討に有効であると考えられる．

今後の課題として，正常心と病的心との差違の検討，短軸断面を貫く方向の速度成分の考慮及び断面間のずりひずみの検討が挙げられる．撮影時の息止め回数の増加を抑えながら，MR-PC 法でしか得られない心筋収縮機能情報や材料特性の指標を取得できるよう今後

多くの臨床例での評価を行っていきたい．

謝辞　本研究は，文部科学省科学研究費補助金（基盤研究（B）18300157），文部科学省リーディングプロジェクト「細胞・生体機能シミュレーションプロジェクト」による成果の一部である．

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（平成 19 年 9 月 14 日受付，20 年 1 月 25 日再受付）

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Original Article

Impact of Metabolic Syndrome Components on the Incidence of Cardiovascular Disease in a General Urban Japanese Population: The Suita Study

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Abdominal obesity is a prerequisite for some definitions of metabolic syndrome (MetS). We investigated the impact of MetS defined by two different criteria, which either did or did not require abdominal obesity as a prerequisite, on cardiovascular disease (CVD) incidence in an urban Japanese cohort study. We studied 5,332 Japanese (aged 30–79 years, without CVD at baseline), who completed a baseline survey (September 1989 to March 1994) and were followed up through December 2005. MetS was defined by the NCEP-ATPIII (modified by Asian obesity criteria) and the Japanese criteria. After 61,846 person-years of follow-up, we documented 317 CVD incidences. The MetS frequencies of the Japanese and of the modified NCEP-ATPIII criteria were 17.7% and 25.1% for men and 5.0% and 14.3% for women, respectively. The multivariate hazard ratios (HRs; 95% confidence intervals [CI]) of CVD incidence for MetS by the modified NCEP-ATPIII criteria were 1.75 (1.27–2.41) in men and 1.90 (1.31–2.77) in women, and those for MetS by the Japanese criteria were 1.34 (0.96–1.87) in men and 2.20 (1.31–3.68) in women. The multivariate HRs of CVD incidence for MetS for the Japanese and for the modified NCEP-ATPIII criteria were 2.92 (1.54–5.55) and 1.94 (0.98–3.82) in men under 60 years old, respectively. The CVD incidence risks increased according to the number of MetS components. The risks were similar among participants with the same number of MetS components, regardless of abdominal obesity. In conclusion, the number of MetS components (modified NCEP-ATPIII criteria) may be more strongly associated with CVD incidence than the abdominal obesity essential criteria (the Japanese criteria) in a general urban Japanese population. (*Hypertens Res* 2008; 31: 2027–2035)

Key Words: **metabolic syndrome, cardiovascular risk factor, cohort study, general population**

Introduction

Metabolic syndrome (MetS) is a clustering of impaired glucose metabolism, abdominal fat accumulation, dyslipidemia, and elevated blood pressure (*1*). Previous papers have shown an association between MetS and cardiovascular disease (CVD) (*2*), but most studies conducted thus far have been based on Western populations. There have been several well-designed prospective studies of Asian populations, and those

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This study was supported by grants-in-aid from the Japanese Ministry of Health, Labour and Welfare (H14-Kouka-027, H19-SeiShu-017, and H18-SeiShu-012) and by a Research Grant for Cardiovascular Disease from the Ministry of Health, Labour and Welfare (18S-2, 19K-8), Japan.

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Received May 30, 2008; Accepted in revised form September 7, 2008.

studies had various limitations, including the use of body mass index (BMI) (*3*, *4*), non-fasting triglyceride and glucose levels (*3*, *4*), mortality (*4*, *5*), or small sample size (*4*–*7*). In order to properly define MetS, it is essential to use data on waist circumference and on the levels of both fasting glucose and fasting triglycerides.

MetS has been defined in several ways by several groups, including the World Health Organization (*8*), the European Group for the Study of Insulin Resistance (*9*), the American Association of Clinical Endocrinologists, and the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATPIII) (*10*). However, these definitions are aimed mainly at Western countries. The International Diabetes Foundation (IDF) (*11*) and the American Heart Association (*12*) have recently introduced alternative definitions that can be applied worldwide (*10*). Stroke incidence is relatively higher in Japan than in Western countries (*13*). It is uncertain whether these criteria can be applied well to Japanese populations. A MetS definition needs to be tailored to the epidemiological background of the area in question.

The Japanese Committee on the Criteria for MetS has recently proposed a definition of Japanese MetS (*14*, *15*). Under both the IDF and the Japanese definitions, the presence of abdominal obesity is necessary for a diagnosis of MetS. However, no prospective study has examined the association between MetS based on the Japanese criteria and CVD, particularly in urban areas, where most Japanese live. Therefore, we undertook this study to examine the impact of MetS under the Japanese and modified NCEP-ATPIII criteria on CVD incidence in a general urban Japanese population.

Methods

Study Population

The Suita study (*16*, *17*), an epidemiological survey of cerebrovascular disease and CVD, was based on a random sampling of 12,200 residents of Suita, a city of approximately 350,000 people in northern Osaka, Japan. As a baseline, in 1989, participants between the ages of 30 and 79 were arbitrarily selected from the municipality population registry and stratified into groups by sex and age in 10-year increments. Of these, 6,406 men and women participated in regular health checkups between September 1989 and March 1994. Since then, these participants have participated in regular health checkups at the National Cardiovascular Center every 2 years and answered health questionnaires every year.

Some cohort members in the study population were excluded from these analyses because they met one or more of the following criteria: past or present CVD illness at baseline (n=208), failure to fast for at least 10 h before venipuncture or missing data (n=170), or failure to follow up after their baseline examination (n=696). After these exclusions, 5,332 individuals remained for analysis.

Baseline Survey

We performed routine blood tests that measured fasting serum total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, and glucose levels. Physicians or nurses administered questionnaires covering the subjects' personal habits and any present illnesses. The subjects were classified as current smokers if they smoked at least one cigarette per day, as non-smokers if they had never smoked, and as past smokers if they had stopped smoking. Blood pressure was measured three times in a sitting position after at least 5 min of rest. Systolic and diastolic blood pressures (SBP and DBP) were taken to be the average of the second and third measurements that were recorded at least 1 min apart by well-trained doctors. Waist circumference was measured in a standing position at the umbilical level to the nearest 1 cm by well-trained technicians. Informed consent was obtained from all participants. This study was approved by the Institutional Review Board of the National Cardiovascular Center.

Definitions of Metabolic Syndrome

MetS was defined using two criteria. First, in accordance with NCEP-ATPIII (*18*) criteria, it was defined as the presence of three or more of the following five components: 1) abdominal obesity modified by the International Obesity Task Force central obesity criteria for Asia (waist circumference ≥90 cm in men and ≥80 cm in women) (*19*), 2) elevated blood pressure (SBP/DBP ≥130/85 mmHg and/or current use of antihypertensive medication), 3) hypertriglyceridemia (serum triglyceride levels ≥1.7 mmol/L [150 mg/dL] and/or current use of cholesterol-lowering medication), 4) low HDL cholesterol (serum HDL levels of ≤1.0 mmol/L [40 mg/dL] in men and of ≤1.3 mmol/L [50 mg/dL] in women), and 5) elevated blood glucose levels (fasting blood glucose ≥6.1 mmol/L [110 mg/dL] and/or current use of insulin or oral medication for diabetes).

Second, we used the definition of MetS recommended by the Japanese Committee on the Criteria for MetS (*14*, *15*). MetS was defined by abdominal obesity (waist circumference ≥85 cm in men and ≥90 cm in women) (*20*) and least two of the following three components: 1) elevated blood pressure (SBP/DBP ≥130/85 mmHg), 2) hyperlipidemia (serum triglyceride levels ≥1.7 mmol/L [150 mg/dL] and/or HDL levels <1.0 mmol/L [40 mg/dL]), and 3) elevated blood glucose levels ≥6.1 mmol/L (110 mg/dL). Subjects taking medication for hypertension, hyperlipidemia, or diabetes were included as having that component.

Endpoint Determination

The endpoint of the follow-up period for each participant was whichever one of the following occurred first: 1) the date of the first myocardial infarction (MI) or stroke event, 2) the date of death, 3) the date the participant moved out of Suita,

Table 1. Baseline Distributions of Cardiovascular Disease Risk Factors According to Metabolic Syndrome under the NCEP-ATPIII Modified by Asian Obesity Definitions

	Men (n=2,492)			Women (n=2,840)		
	MetS(−) (n=2,043)	MetS(+) (n=449)	p*	MetS(−) (n=2,253)	MetS(+) (n=587)	p*
Age at baseline, years	55.4±13.3	58.1±11.5	<0.001	52.2±12.6	61.3±9.8	<0.001
Systolic blood pressure, mmHg	126±20	140±19	<0.001	120±20	141±20	<0.001
Diastolic blood pressure, mmHg	78±12	85±11	<0.001	73±11	83±12	<0.001
Total cholesterol, mg/dL	200±34	210±35	<0.001	210±38	227±38	<0.001
HDL cholesterol, mg/dL	51±13	40±10	<0.001	60±12	45±10	<0.001
Triglyceride, mg/dL†	121±73	241±156	<0.001	90±44	178±113	<0.001
Waist circumference, cm	81.0±7.3	89.7±7.0	<0.001	74.7±8.9	87.4±8.5	<0.001
Elevated blood pressure, %	41.8	85.8	<0.001	30.4	82.1	<0.001
Hypertriglyceridemia, %	21.6	82.9	<0.001	7.2	63.7	<0.001
Lower-HDL cholesterol, %	15.5	64.8	<0.001	18.7	80.1	<0.001
Hyperglycemia, %	8.9	43.9	<0.001	3.6	29.6	<0.001
Current smoker, %	50.5	47.6	0.278	11.9	11.8	0.958
Current drinker, %	75.5	72.6	0.207	34.6	25.4	<0.001

Elevated blood pressure: antihypertensive drug use or >130/85 mmHg; hypertriglyceridemia: antilipidemic drug use or triglyceride >150 mg/dL; lower-HDL cholesterol: HDL cholesterol <40 mg/dL. MetS, metabolic syndrome; HDL, high-density lipoprotein. *ANOVA or χ^2 tests were performed. †Log-transformed triglyceride was performed to statistical analysis.

Table 2. Age-Adjusted Hazard Ratios (Confidence Intervals) for Incidence of Cardiovascular Disease According to Abdominal Obesity at Baseline Examination

	Men				Women			
	Case, n	Person-year	HR (95% CI)	p	Case, n	Person-year	HR (95% CI)	p
Japanese criteria								
<85 cm (men)/<90 cm (women)	111	17,112	1		96	29,960	1	
≥85 cm (men)/≥90 cm (women)	77	11,247	0.97 (0.72–1.30)	0.844	33	3,890	1.64 (1.09–2.46)	0.019
Asian criteria								
<90 cm (men)/<80 cm (women)	145	23,136	1		53	21,139	1	
≥90 cm (men)/≥80 cm (women)	43	5,223	1.18 (0.84–1.67)	0.327	76	12,711	1.44 (1.00–2.07)	0.048
NCEP-ATPIII criteria								
<102 cm (men)/<88 cm (women)	182	27,976	1		91	28,730	1	
≥102 cm (men)/≥88 cm (women)	6	384	2.00 (0.88–4.54)	0.095	38	5,121	1.47 (1.00–2.17)	0.048

HR, hazard ratio; CI, confidence interval.

or 4) December 31, 2005 (censored). As a first-step survey to detect MI and stroke incidence, each participant's health status was checked during a clinical visit at the National Cardiovascular Center every 2 years. Furthermore, every year a health questionnaire was given to each participant *via* mail or telephone.

Confirmation of Strokes and Myocardial Infarctions

In total, five hospitals in this area were capable of performing computed tomographic scans and/or magnetic resonance imaging, and all were major hospitals that admitted acute stroke and MI patients. Medical records were reviewed by registered hospital physicians or research physicians who were blinded to the baseline information. Strokes and MI events were registered if they occurred after the date on which the baseline health examination was held and before January 1, 2006. Strokes were defined according to the National Survey of Stroke criteria (*21*). These criteria require the rapid onset of a constellation of neurological deficits lasting at least 24 h or until death. For each stroke subtype (cerebral infarction [thrombotic or embolic infarction], intracerebral hemorrhage, and subarachnoid hemorrhage), a definite diagnosis was established based on examination of computed tomographic scans, magnetic resonance images, or autopsy. Defi-

Table 3. Age-Adjusted Hazard Ratios (95% Confidence Intervals) for Incidence of Cardiovascular Disease, Myocardial Infarction, and All Strokes According to Metabolic Syndrome under the Japanese and NCEP-ATPIII Definitions

	Men			Women		
	MetS(−)	MetS(+)	*p* value	MetS(−)	MetS(+)	*p* value
Cardiovascular disease						
MetS Japanese definition						
Cases, *n*	140	48		110	19	
Person-year	23,542	4,817		32,325	1,526	
Age-adjusted	1	1.31 (0.94–1.82)	0.109	1	2.16 (1.31–3.54)	0.002
Multivariate-adjusted	1	1.34 (0.96–1.87)	0.080	1	2.20 (1.31–3.68)	0.003
<60 years old						
Cases, *n*	27	15		25	4	
Person-year	14,752	2,366		22,085	529	
Age-adjusted	1	2.76 (1.46–5.23)	0.002	1	5.39 (1.82–15.98)	0.002
Multivariate-adjusted	1	2.92 (1.54–5.55)	0.001	1	6.25 (2.08–18.79)	0.001
≥60 years old						
Cases, *n*	113	33		85	15	
Person-year	8,790	2,451		10,240	997	
Age-adjusted	1	1.04 (0.70–1.53)	0.841	1	1.83 (1.05–3.18)	0.033
Multivariate-adjusted	1	1.06 (0.71–1.57)	0.764	1	1.80 (1.01–3.20)	0.046
MetS NCEP-ATPIII (Asian) definition						
Cases, *n*	133	55		73	56	
Person-year	23,373	4,986		27,405	6,446	
Age-adjusted	1	1.70 (1.23–2.34)	0.001	1	1.93 (1.35–2.77)	<0.001
Multivariate-adjusted	1	1.75 (1.27–2.41)	<0.001	1	1.90 (1.31–2.77)	<0.001
<60 years old						
Cases, *n*	30	12		19	10	
Person-year	14,509	2,606		19,872	2,742	
Age-adjusted	1	1.79 (0.91–3.52)	0.089	1	2.72 (1.23–5.99)	0.013
Multivariate-adjusted	1	1.94 (0.98–3.82)	0.055	1	2.96 (1.34–6.57)	0.007
≥60 years old						
Cases, *n*	103	43		54	46	
Person-year	8,864	2,381		7,533	3,704	
Age-adjusted	1	1.67 (1.16–2.40)	0.005	1	1.78 (1.19–2.66)	0.005
Multivariate-adjusted	1	1.73 (1.20–2.48)	0.003	1	1.70 (1.12–2.59)	0.012
Myocardial infarction						
MetS Japanese definition						
Cases, *n*	56	22		32	7	
Person-year	22,962	4,663		31,697	1,457	
Age-adjusted	1	1.48 (0.90–2.44)	0.117	1	2.36 (1.02–5.46)	0.043
Multivariate-adjusted	1	1.51 (0.91–2.48)	0.105	1	2.70 (1.15–6.35)	0.023
MetS NCEP-ATPIII (Asian) definition						
Cases, *n*	52	26		18	21	
Person-year	22,833	4,795		26,944	6,211	
Age-adjusted	1	2.09 (1.30–3.37)	0.002	1	2.68 (1.41–5.10)	0.003
Multivariate-adjusted	1	2.12 (1.31–3.43)	0.002	1	2.77 (1.44–5.32)	0.002
All strokes						
MetS Japanese definition						
Cases, *n*	84	26		78	12	
Person-year	23,177	4,659		32,078	1,487	
Age-adjusted	1	1.21 (0.78–1.89)	0.381	1	2.09 (1.12–3.88)	0.019
Multivariate-adjusted	1	1.27 (0.81–1.97)	0.292	1	2.05 (1.07–3.92)	0.031
MetS NCEP-ATPIII (Asian) definition						
Cases, *n*	81	29		55	35	
Person-year	23,010	4,826		27,266	6,299	
Age-adjusted	1	1.52 (0.99–2.34)	0.053	1	1.70 (1.09–2.64)	0.018
Multivariate-adjusted	1	1.58 (1.02–2.43)	0.037	1	1.62 (1.02–2.58)	0.041

Multivariate adjusted for age, smoking and drinking status. MetS, metabolic syndrome.

nite and probable MI was defined according to the criteria set out by the MONICA (Monitoring Trends and Determinants of Cardiovascular Disease) project (*22*), which requires evidence from ECGs, cardiac enzymes, and/or autopsy. Sudden deaths of unknown origin were deaths that occurred within 24 h from onset and were included in MI. However, there was little difference in hazard ratios between the groups with and without sudden death from CVD, because sudden death constituted a small sample size ($n=6$).

To complete surveillance for fatal stroke and MI, we also systematically searched for death certificates, the purpose of which were permitted to use by the Ministry of Health, Labour and Welfare. We checked for possible stroke and MI using data from 1) the health examination and questionnaire for the stroke and MI registry, without informed consent for the medical records survey and 2) death certificates without registration of CVD incidence, which were defined as probable stroke or MI. CVD was defined as stroke and MI in this study. Informed consent to review in-hospital medical records was obtained from 86.2% of participants who were suspected of having any signs or information suggesting the incidence of stroke or MI. For 13.8% of subjects from whom informed consent was not obtained, final diagnoses of CVD were confirmed by physicians or epidemiologists who had been involved in the diagnostic process throughout the study, in order to avoid the misclassification of diagnoses.

Statistical Analysis

Analyses of variance and χ^2 tests were used to compare mean values and frequencies by sex, respectively, according to MetS based on the modified NCEP-ATPIII criteria. For each subject, the person-years of follow-up were calculated from September 1, 1989, to whichever came first: the first endpoint, MI or stroke event, death, emigration, or December 31, 2005. A Cox proportional hazards regression model was used to detect associations between abdominal obesity for Japanese (≥85 cm in men or ≥90 cm in women), Asian (≥90 cm in men or ≥80 cm in women), and American criteria (≥102 cm in men or ≥88 cm in women) and CVD during the follow-up period. The Cox proportional hazard regressions were fitted to the grouping (positive or negative MetS) after adjusting for age and the other potential confounding factors: baseline age, smoking status (never, ex-smoker, or current smoker), and drinking status (never, ex-drinker, or current drinker). Trend tests were conducted by assigning the number of MetS components to test the significance of these variables. All statistical analyses were conducted using the SAS statistical package (release version 8.2; SAS Institute Inc., Cary, USA).

Results

During the follow-up period (averaging 12.5 years), 200 strokes were documented (160 definite strokes and 40 probable strokes). These strokes comprised 130 cerebral infarctions, 31 intracerebral hemorrhages, 22 subarachnoid hemorrhages, and 17 unclassified strokes. In addition, 117 MIs were documented (61 definite MIs and 56 probable MIs or sudden cardiac deaths).

Table 1 shows the distribution of CVD risk factors at the baseline according to MetS as defined by the modified NCEP-ATPIII criteria. Compared with the non-MetS groups, men and women with MetS were more likely to be older and to have higher frequencies of each MetS component.

Table 2 presents the age-adjusted HRs (95% confidence intervals [CI]) for the incidence of CVD according to waist circumference by the NCEP-ATPIII, Japanese, and Asian obesity criteria. Regardless of the criteria set, abdominal obesity was associated with CVD only in women.

Table 3 shows the association of MetS by the Japanese and the modified NCEP-ATPIII criteria with CVD incidence according to age category and sex. Using the Japanese criteria, MetS was associated only in women with the incidence of CVD, MI, and all strokes (HR [95% CI]: 2.20 [1.31–3.68], 2.70 [1.15–6.35], and 2.05 [1.07–3.92], respectively), whereas in men overall MetS was not associated with the incidence of CVD or its subtypes. However, among men under 60 years old, MetS based on the Japanese criteria was associated with CVD incidence (HR=2.92, 95% CI: 1.54–5.55). Using the modified NCEP-ATPIII definition, MetS was associated with each CVD subtype in both men and women. Multivariate adjusted HRs of CVD incidence for MetS based on the NCEP-ATPIII criteria were 1.94 (0.98–3.82) and 1.73 (1.20–2.48) in men less than or equal to and over 60 years old, respectively.

Figure 1 shows that the frequency of MetS increased with age for men and women based on the NCEP-ATPIII (A) and Japanese (B) criteria, respectively. The frequency based on the NCEP-ATPIII modified by the Asian obesity criteria (25.1% for men and 14.3% for women) was higher than that based on the Japanese criteria (17.7% for men and 5.0% for women), especially in women.

The risk of CVD incidence increased according to the number of components combined in men and women with and without abdominal obesity (Fig. 2). In addition, compared with the non-abdominal obesity and non-component groups, the risks of CVD incidence were similar among participants who had the same numbers of components, regardless of the presence or absence of abdominal obesity in men and women combined.

Figure 3 shows the multivariate HRs for MetS based on the Japanese and NCEP-ATPIII definitions modified by the obesity criteria for waist circumference. When the Japanese definition was adopted and the risk of MetS was monitored through sequential waist circumference changes, the cut-off points for waist circumference, which conferred a risk of CVD in men and women, were 84 cm and 92 cm, respectively. When the definition of MetS-indicative waist circumference was higher than those values, the risk was not statistically significant. When the NCEP-ATPIII definition

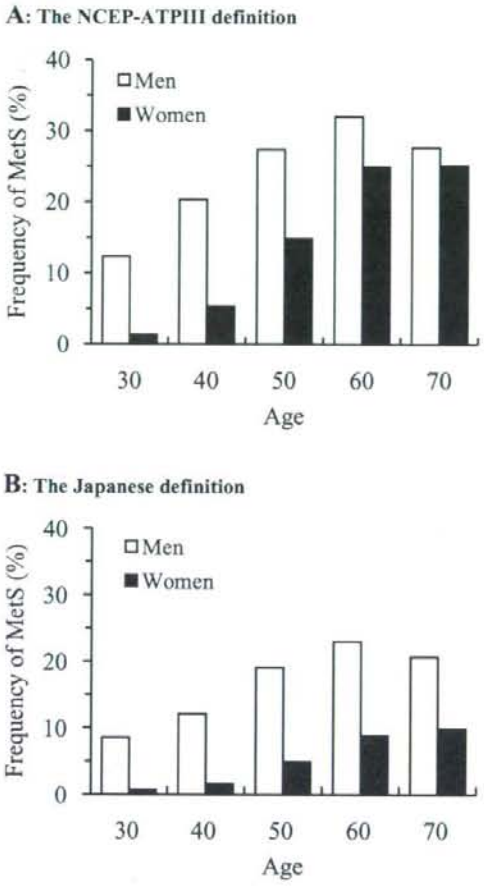

Fig. 1. *Frequencies of MetS components (A: the NCEP-ATPIII definition; and B: the Japanese definition, modified by the Asian waist circumference criteria) by sex. White and solid bars indicate men and women, respectively.*

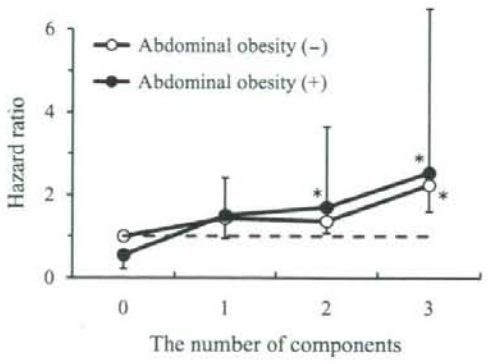

Fig. 2. *Multivariate HRs for the risks of CVD incidence according to the number of components based on the NCEP-ATPIII definition with and without abdominal obesity. White and solid circles indicate non-abdominal and abdominal obesity according to the Asian obesity criteria. *$p<0.05$ compared to the reference of non-abdominal obesity and no-components. Bars show 95% CI for the HRs.*

was used, the value of waist circumference did not modify the risk of CVD, implying that the clustering of risk factors may be more important than waist circumference itself for determining CVD risk.

Discussion

In the current cohort study of a general urban Japanese population, the association between MetS and CVD was significant when the NCEP-ATPIII (modified by the Asian criteria) definition was applied. MetS based on the Japanese criteria was associated with CVD incidence in women, whereas in men the association was found only in those under 60 years old. In addition, the risk of CVD incidence was similar among participants who had the same numbers of components regardless of whether they were abdominally obese. To the best of our knowledge, this is the first study of an urban Japanese cohort.

Compared to the previous studies, this study has several methodological strengths. First, previous Japanese cohort studies associating MetS with CVD were based predominantly on BMI (*3*, *4*), non-fasting blood collection (*3*, *4*), and mortality as the endpoint (*4*, *5*). Our baseline subjects were observed in the fasting state, and we used waist circumference and a wide age range. Second, we evaluated a large prospective cohort of people randomly selected from a general Japanese population. A prospective study has little recall bias as well as results from a general population cohort that is more representative than occupational, hospital-based, or volunteer cohorts. Third, our sample size was relatively large for a cohort study and we could therefore perform sub-analysis by age and CVD subtypes. Fourth, our cohort population was selected at random from an urban population, in contrast to most of the other MetS cohort populations, which were selected from rural populations. Our study is the first of its kind in an urban area. Finally, our study examined the risk of CVD incidence, which is a more direct measure of CVD risk than the rate of CVD mortality, because the time to death from CVD is influenced by treatment.

Abdominal obesity induces inflammation in adiposities (*23*), endothelial dysfunction (*24*, *25*), and oxidative stress (*26*), thereby contributing to CVD development (*27*, *28*).

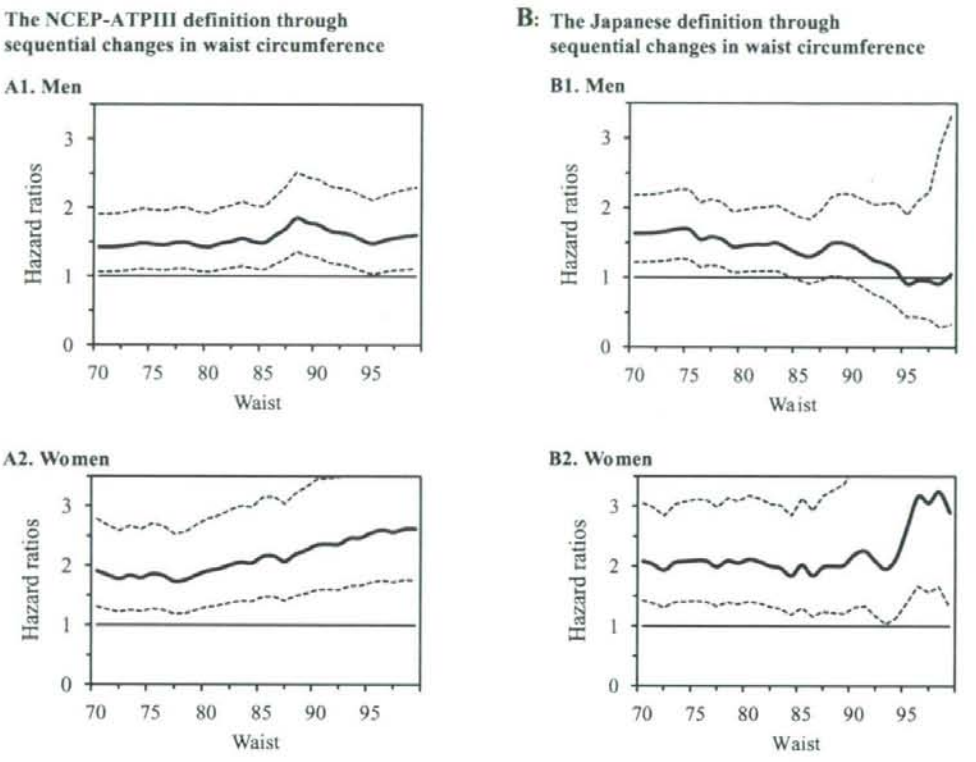

Fig. 3. *Multivariate HRs for MetS based on the NCEP-ATPIII (A) and Japanese (B) definitions through sequential changes in waist circumference by sex. Solid and dotted lines indicate HRs and 95% CI, respectively.*

Accumulating evidence suggests that MetS increases the risk of CVD (*29*). However, there has been a lack of convincing evidence (*29*) that MetS is associated with CVD in Japan. Iso *et al.* reported that MetS was associated with a risk for ischemic CVD in Japan (*3*), although they used BMI as well as non-fasting blood glucose and triglyceride levels to define MetS. Ninomiya *et al.* reported that MetS was a significant risk factor for CVD in a rural Japanese population (*6*). However, that study examined a rural population half the size of that in our study. Takeuchi *et al.* reported that MetS was a risk factor for cardiac disease in a rural cohort (*7*), but their data were based on a small sample that comprised only men. Kadota *et al.* reported that MetS, defined by BMI and non-fasting blood samples, was associated with CVD mortality (*4*).

We have shown that the components of MetS synergistically increase CVD risk. Abdominal obesity did not affect the association between the number of MetS components and the risk of CVD incidence. The risk of CVD was also not related to waist circumference when the NCEP-ATPIII definition was applied (data not shown), suggesting that the combination of risk factors *per se* is more important than abdominal obesity for conferring risk.

The definition of MetS may be reconsidered on the basis of age and sex. According to our results, lifestyle modifications may not be needed for older men who are free of cardiovascular risk factors even if they have abdominal obesity. Therefore, to prevent CVD, it is not adequate for only subjects with MetS to change their lifestyles; subjects with one or two MetS components, even without abdominal obesity, should modify their lifestyles.

When the waist-circumference threshholds were sequentially changed in the Japanese criteria for MetS, our data showed that the clustering of metabolic risk factors was statistically significant for CVD at waist circumferences less than 85 cm for men and 93 cm for women. When the definition of MetS-indicative waist circumferences was higher than those values, the risk clustering was not statistically significant for

CVD in men, and the 95% CI was much wider but still significant in women. Subjects with high risks and non-abdominal obesity with risk clustering aside from abdominal obesity will drop out when the waist-circumference definitions are raised.

Our study has several limitations. First, the annual emigration rate (1.5%) is relatively higher than that in rural areas. Second, about 10% of the subjects who underwent a baseline examination did not respond to our questionnaires afterward. We found no clinical background difference between participants and non-participants, because the main denial reason for participation in this study was not health problems. The frequencies of MetS according to NCEP-ATPIII modified by Asian criteria were 19% and 21% for participants and non-participants, respectively (χ^2 test $p=0.09$). In this study, the main reasons for emigration included job transfer, but not health problems.

In conclusion, the current prospective study for a general urban population showed that MetS, as defined by the Japanese criteria, was associated with CVD in women and middle-aged men; a stronger association was found when the NCEP-ATPIII definition modified by the Asian obesity criteria was applied. The number of MetS components may be more strongly associated with CVD incidence than the essential waist-circumference criteria.

Acknowledgements

We would like to express our deepest gratitude to all members of the Suita City Health Center and the Suita Medical Association. We also thank all researchers and the staff members of the Division of Preventive Cardiology for performing medical and follow-up examinations. We also thank Satsuki-Junyukai, the volunteer administrator for the Suita study.

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Original Article

Associations of Hypertension and Its Complications with Variations in the Xanthine Dehydrogenase Gene

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Hyperuricemia and oxidative stress participate in the pathophysiology of hypertension and its complications. Xanthine dehydrogenase (XDH) produces urate and, in its oxidase isoform, reactive oxygen species. Here we have studied whether or not the genetic variations in *XDH* could be implicated in hypertension and its complications. By sequencing the promoter region and all exons of *XDH* in 48 subjects, we identified three missense mutations (G172R, A932T, N1109T) in a heterozygous state in addition to 34 variations, including 15 common single nucleotide polymorphisms (SNPs). The three missense mutations and eight common SNPs (11488C>G, 37387A>G, 44408A>G, 46774G>A, 47686C>T, 49245A>T, 66292C>G, and 69901A>C) were genotyped in 953 hypertensive Japanese subjects and in 1,818 subjects from a general Japanese population. Four hypertensive patients with rare missense mutations (G172R or N1109T) in homozygous form had severe hypertension. Multivariate logistic regression analysis showed a significant association of three SNPs with hypertension in men: 47686C>T (exon 22, odds ratio [OR]: 1.52, $p=0.047$) and 69901A>C (intron 31, OR: 3.14, $p=0.039$) in the recessive model, and 67873A>C (N1109T) (exon 31, OR: 1.84, $p=0.018$) in the dominant model. After full adjustment for all confounding factors, only one polymorphism (69901A>C) was found to be associated with carotid atherosclerosis in the dominant model ($p=0.028$). Multiple logistic regression analysis showed that one SNP (66292C>G) was significantly associated with chronic kidney disease (CKD: estimated creatinine clearance <60 mL/min) in the recessive model ($p=0.0006$). Our results suggest that genetic variations in *XDH* contribute partly to hypertension and its complications, including atherosclerosis and CKD. (*Hypertens Res* 2008; 31: 931–940)

***Key Words*: xanthine dehydrogenase gene, missense mutation, single nucleotide polymorphism, hypertension, atherosclerosis, chronic kidney disease**

Introduction

Hypertension is one of the most common and important risk factors for stroke, coronary heart diseases (CHD), and chronic kidney disease (CKD). The major contribution to the etiology of this disorder is proposed to come from the combined effects of genes that modify the response of blood pressure to environmental stresses, including diet and environmental susceptibility genes (*1*). This multifactoral trait increases the

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This study was supported by the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO) of Japan, and by a research grant from the Japanese Ministry of Health, Labour and Welfare.

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Received August 7, 2007; Accepted in revised form December 28, 2007.

affected individuals' risks of stroke, CHD, and CKD, and is one of the leading causes of morbidity and mortality in adults (*2*). The population-wide application of preventative measures and analyses of candidate genes to predict modifiable risks, in addition to developing new treatments for hypertension and its complications, are thus very worthwhile (*3*, *4*).

Xanthine oxidoreductase (XOR), best known as the rate-limiting enzyme of the purine degradation pathway, converts hypoxanthine to xanthine and xanthine to uric acid (UA) *via* its two interconvertible isoforms, xanthine dehydrogenase (XDH) and xanthine oxidase (XO); in its oxidase isoform, XOR produces reactive oxygen species (ROS) (*5*). Hyperuricemia is commonly seen in hypertensive patients (*6*). Several large epidemiologic studies have identified an association between increased serum UA levels and cardiovascular risk in the general population (*7–10*), among patients with hypertension (*11*, *12*), between increased serum UA levels and renal failure in the general population (*13*, *14*), and among patients with hypertension (*15*). ROS plays critical roles in the pathogenesis of a number of cardiovascular diseases, including atherosclerosis, hypertension, diabetes mellitus, and heart failure (*16*). They have also been implicated as important mediators of the progression of renal injury in different animal models of hypertension (*17–20*). The conversion of XDH to XO and increased XO activity have been reported in some pathological conditions, including hypertension (*21–23*) and atherosclerosis (*24*). Importantly, treatments with XO inhibitors were recently reported to normalize ROS levels in microvessels from rats fed a high-salt diet (*25*) and to promote endothelial-dependent relaxation in arteries from SHR (*26*). These findings suggest that XO is an important source of ROS in patients with hypertension. Therefore, the XDH gene is suspected to be associated with constitutional susceptibility to hypertension and its complications.

So far, there are no reports about the relation between variations in human XDH gene (*XDH*) and hypertension and its complications. Human *XDH*, located on chromosome 2 at p23.1 (*27*), consists of 36 exons that encode a 1,333-amino acid protein. The aim of the present study was to screen for possible genetic variations in the promoter and all exon regions of *XDH* in 48 patients with hypertension. By genotyping the missense mutations and common single nucleotide polymorphisms (SNPs) in a large hypertensive population and the general population, we further assessed the role of these genetic variations in hypertension and clarified the contributions of common SNPs to hypertension and its complications, including atherosclerosis and CKD.

Methods

Hypertensive Population

The characteristics of the hypertensive population analyzed in the present study are summarized in Table 1. A total of 953 hypertensive subjects (522 men and 431 woman, average age: 65.1±10.5 years old) were recruited from the Division of Hypertension and Nephrology at the National Cardiovascular Center, as reported previously (*28*, *29*). Briefly, 92% of study subjects (880 subjects) were diagnosed with essential hypertension, and the rest had secondary hypertension. The hypertension criteria were a systolic blood pressure (SBP) above 140 mmHg and/or a diastolic blood pressure (DBP) above 90 mmHg, or the use of antihypertensive agents. Hyperlipidemia was defined by a total cholesterol level ≥220 mg/dL or the taking of antihyperlipidemia medication. Diabetes mellitus was defined by a fasting plasma glucose level ≥126 mg/dL, nonfasting plasma glucose ≥200 mg/dL, HbA1c ≥6.5%, or the taking of antidiabetic medication. Smoking was defined as current smoking. Total cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, and low-density lipoprotein (LDL) cholesterol levels were measured as previously described (*30*). Study subjects underwent routine laboratory tests, including examinations of electrolytes, renal function, blood glucose, HbA1c, plasma renin activity (PRA), and plasma aldosterone concentration (PAC) by radioimmunoassay.

Table 1. Characteristics of Patients with Hypertension

Number	953
Age, years	65.1±10.5
Gender (male/female)	522/431
Body mass index, kg/m^2	24.2±3.3
Systolic blood pressure, mmHg	145.5±19.2
Diastolic blood pressure, mmHg	84.8±13.4
Essential hypertension	880
Secondary hypertension	72
Renal hypertension	36
Renovascular hypertension	23
Primary aldosteronism	11
Hypothyroid-induced hypertension	2
Ischemic heart disease	102
Stroke	145

Values are expressed as mean±SD.

Evaluation of Atherosclerosis and CKD in the Hypertensive Population

Carotid ultrasonography was used to measure mean intima-medial thickness (IMT) using ultrasonography (SSA-390A; Toshiba, Tokyo, Japan) as previously described (*31*). IMT above 1.0 mm in either the left or right common carotid artery defined with the presence of an atherosclerotic lesion. We also assessed arterial stiffness using brachial-ankle-pulse wave velocity (ba-PWV) measured by form ABI (Omron Health Care, Kyoto, Japan) as described in a previous report (*32*). Estimated creatinine clearance (Ccr) determined with the Cockcroft-Gault formula (*33*) was used for the evaluation of CKD. We defined CKD as Ccr <60 mL/min according to

the guidelines of the National Kidney Foundation (*34*).

Screening of Genetic Variations in *XDH*

We sequenced the promoter region and all exons of *XDH* in 48 randomly chosen patients with hypertension. Blood samples were obtained from all hypertensive patients, and genomic DNA was isolated from peripheral blood leukocytes using an NA-3000 nucleic acid isolation system (Kurabo, Osaka, Japan) (*35*). All exons with their flanking sequences and 1 kb of the promoter region were directly sequenced with an ABI PRISM 3700 DNA analyzer (Applied Biosystems, Foster City, USA) as described previously (*36*) using 38 sets of primers. Information on the primers and PCR conditions is available on request. The sequences obtained were examined for the presence of variations using Sequencher software (Gene Codes, Ann Arbor, USA), followed by visual inspection.

Genotyping of Missense Mutations and Common SNPs in Hypertensive Subjects and the General Population

Three missense mutations and eight common SNPs with a minor allelic frequency of greater than 10% were genotyped in 953 hypertensive patients and in 1,818 subjects (835 men and 983 women) participating in the Suita Study. We chose just one common SNP for genotyping among SNPs that show strong linkage disequilibrium (LD) with an r^2 above 0.5. The sample selection and study design of the Suita Study were described previously (*37*). Briefly, the subjects visited the National Cardiovascular Center every 2 years for general health checkups. In addition to a routine blood examination that included lipid profiles, glucose levels, blood pressure, and anthropometric measurements, a physician or nurse administered questionnaires covering the subject's personal history of cardiovascular diseases, including angina pectoris, myocardial infarction, and/or stroke. Nondrinkers were those who had had no drink in the past month. Current drinkers were those who were drinking at least 30 mL of ethanol per day, and past drinkers were those who used to drink that much in the past but not in the present. Subjects were regarded as having a disease if they were currently taking antihypertensive, antihyperlipidemic, or antidiabetic medication. Seven-hundred and ninety-five subjects were diagnosed as having hypertension. All of the participants were Japanese. The characteristics of the subjects in the Suita Study are summarized in Table 2.

The TaqMan-PCR (Roche Molecular Systems, Pleasanton, USA) method was used for genotyping (*35*). The sequences of PCR primers and probes for the TaqMan-PCR method are available on request. All of the participants in the genetic analysis in the present study gave their written informed consent. All clinical data, as well as the results of sequencing and genotyping, were anonymous. The study protocol was approved by the Ethics Review Committee of the National Cardiovascular Center, Japan.

Table 2. Baseline Characteristics of Subjects in Suita Study

	Women (n=983)	Men (n=835)
Age, years	63.3±11.0	66.3±11.1*
Systolic blood pressure, mmHg	128.0±19.7	131.8±19.4*
Diastolic blood pressure, mmHg	76.5±9.8	79.7±10.7*
Body mass index, kg/m^2	22.3±3.2	23.3±2.9*
Total cholesterol, mg/dL	215.6±30.6*	197.9±30.3
HDL-cholesterol, mg/dL	64.5±15.3*	55.0±14.1
Current smokers, %	6.3	30.2*
Current drinkers, %	29.6	67.2*
Present illness, %		
Hypertension	38.0	47.3*
Hyperlipidemia	54.4*	27.8
Diabetes mellitus	5.2	12.8*

*p<0.05 *vs.* women or men. HDL, high-density lipoprotein.

Statistical Analysis

Values are expressed as means±SD. The distribution of patient characteristics between men and women in the general population and in the hypertensive population was analyzed with Student's t-test or χ^2 analysis.

The associations of genetic models with blood pressures were tested with a logistic regression analysis considering potential confounding risk variables, including age, body mass index (BMI), present illness (hyperlipidemia and diabetes mellitus), lifestyle (current smoking and drinking), and antihypertensive medication by sex. For multivariate risk predictors, the adjusted odds ratios (ORs) were given with 95% confidence intervals. The relationship between genotype and risk of hypertension was expressed in terms of ORs adjusted for possible confounding factors, including age, BMI, present illness (hyperlipidemia and diabetes mellitus), and lifestyle (current smoking and drinking) by sex. The relationship between genotype and risk of atherosclerosis or CKD in hypertensive patients was expressed in terms of ORs adjusted for possible confounding factors, including age, sex, BMI, LDL cholesterol, HbA1c, SBP, and DBP for atherosclerosis; and age, BMI, SBP, DBP, and diabetes mellitus for CKD. For each pair of SNPs, the pairwise LD parameters, D' and r^2, were calculated on the basis of the genotyping data using SNPAlyze version 3.1 Pro (Dynacom, Mobara, Japan). All analyses were performed with SAS statistical software release 8.2 (SAS Institute, Cary, USA) or JMP statistical software version 4.0 (SAS Institute). Statistical significance was established at p<0.05.

Table 3. Sequence Variations in the Promoter Region and All Exons in *XDH* Identified in 48 Japanese Patients with Hypertension and/or Renal Failure

SNP (allele 1>allele 2)	LD	Amino acid substitution	Region	Allele frequency		Flanking sequence	Genotyping
				Allele 1	Allele 2		
8787C>T	a		intron2	0.979	0.021	gagtgggagtga[c/t]ggagaaggggg	
11451G>T	a		intron2	0.968	0.032	gcccacagctct[g/t]cccaggcatttc	
11488C>G	b, c		intron2	0.862	0.138	cagactctctc[c/g]ctgagttcattc	done
26245G>A			intron6	0.958	0.042	ggcaggcaggat[g/a]ccctgctgttg	
26390G>A	b, c, d	Gly172Arg	exon7	0.906	0.094	ggatgctgtgga[g/a]gagatgggaata	done
26479T>A			intron7	0.958	0.042	gcctgggggtaa[t/a]ctgagacttaga	
26504C>T	c		intron7	0.625	0.375	ggagtcagtgca[c/t]gagctccatgtc	
26832G>A	b, c, d	Glu209Glu	exon8	0.915	0.085	tccaaccagga[g/a]cccatttttccc	
28272G>A			intron9	0.989	0.011	gccagggaggct[g/a]ccctggggctgc	
30863C>T	c, d	Val279Val	exon10	0.936	0.064	tcctatgattgt[c/t]tgcccagcctgg	
31503G>T			intron10	0.989	0.011	gtgattccgaac[g/t]tgcgttcccagg	
34636G>A			intron13	0.917	0.083	tttctcccccatg[g/a]ggggttcccagc	
37387A>G	f, g		intron14	0.181	0.819	tttgcagcccct[a/g]cagagcaaggtg	done
39048A>G	h		intron15	0.604	0.396	ccctgggcacac[a/g]gctctacacaaa	
44408A>G	i		intron19	0.875	0.125	tggaaaggttat[a/g]catttgcatgga	done
44426G>A			intron19	0.990	0.010	gcatggattatg[g/a]ccatcatccagt	
46476T>C			intron20	0.979	0.021	acttcaagtctg[t/c]atgtgaagcata	
46748G>C	h		intron21	0.660	0.340	ggggtggccctg[g/c]tttgcaaattaa	
46774G>A	c		intron21	0.638	0.362	ttcaagagatat[g/a]cattgaaccctg	done
47686C>T	h	Ile737Ile	exon22	0.670	0.330	ggagatatacat[c/t]ggtggccaagag	done
47804G>A			intron22	0.989	0.011	acccaggtagat[g/a]ccttttgggtca	
47879A>G	c		intron22	0.638	0.362	catgtgggaaat[a/g]ggaagagggaga	
49096G>A	i		intron23	0.875	0.125	gaaggctcacag[g/a]cttctaacactg	
49245A>T	f, g, j		intron24	0.125	0.875	tgggggcgggatg[a/t]gccatttttgtga	done
50298C>T	g, j		intron24	0.146	0.854	accttttttca[c/t]gggatgatgtgg	
50391T>C			intron24	0.917	0.083	aaacgggactta[t/c]gataaatcctc	
64606G>A		Ala932Thr	exon26	0.990	0.010	atgagtgaagtt[g/a]cagtgacctgtg	done
65050–65051insC	k		intron27	0.135	0.865	tctgctgaccccc[-/c]atataggaagct	
65747T>C	k	Phe1010Phe	exon28	0.135	0.865	tggaataagctt[t/c]acagttcctttt	
66292C>G	k		intron28	0.135	0.865	tctggcatcctt[c/g]tcttccctagg	done
67157A>G	k		intron30	0.128	0.872	tgtaaggagccc[a/g]tgggatcccgca	
67873A>C		Asn1109Thr	exon31	0.969	0.031	acaagaagaaga[a/c]tcccagtggctc	done
69901A>C			intron31	0.795	0.205	aaacctcacttc[a/c]cctgcctgatgg	done
73380C>T			intron34	0.938	0.063	agacttggccac[c/t]gatgcaccccat	
74894G>A	l		intron34	0.968	0.032	acattccaggcc[g/a]cgctgcagttgg	
75121G>A		Glu1239Glu	exon35	0.989	0.011	catccccattga[g/a]ttcagggtgtcc	
78750G>C	l	3′UTR	exon37	0.969	0.031	tgctgccttttgg[g/c]cttccatggagc	

The A of the ATG of the initiator Met codon is denoted nucleotide +1, as recommended by the Nomenclature Working Group (*Hum Mut* 1998; **11**: 1–3). The nucleotide sequence (GenBank Accession ID: NT_022184.14) was used as a reference sequence. The apparent linkage disequilibrium (LD), defined by r^2 more than 0.5, was indicated by a in the LD column. *XDH*, xanthine dehydrogenase gene; SNP, single nucleotide polymorphism; UTR, untranslated region.

Results

Identification of Genetic Variations in *XDH*

As shown in Table 3, we identified 3 missense mutations in *XDH*. Nine of the 48 individuals had a G-to-A substitution at nucleotide 26390 in exon 7, leading to an amino acid substitution from Gly to Arg at position 172 (G172R). One individual had a G-to-A substitution at nucleotide 64606 in exon 26, leading to a change from Ala to Thr at position 932 (A932T). Three of the 48 individuals had an A-to-C substitution at nucleotide 67873 in exon 31, leading to the substitution of Asn with Thr at position 1109 (N1109T). These missense

Table 4. Clinical Profiles of Four Hypertensive Patients with Two Rare Missense Mutations in Homozygous Form in *XDH*

	Case			
	1	2	3	4
SNP	26390G>A	67873A>C	67873A>C	67873A>C
(Amino acid change)	(G172R)	(N1109T)	(N1109T)	(N1109T)
Age, years old	79	70	74	67
Sex	male	male	female	female
Body mass index, kg/m^2	21.01	23.43	23.68	21.91
Diagnosis	EHT, HL	EHT, HL, HU	EHT	EHT, HL
Hypertension duration, years	5	23	22	2
Hypertension family history	mother	unknown	mother, brother	unknown
CV complications	no	no	stroke	no
Systolic blood pressure, mmHg	144	138	168	170
Diastolic blood pressure, mmHg	70	90	100	96
Medication	ARB, BB, DU	CCB, ARB, HUD	CCB, ACEI	CCB
Na^+, mEq/L	141	141	139	139
K^+, mEq/L	4.8	3.8	3.9	4.1
Cl^-, mEq/L	108	105	104	108
Creatinine, mg/dL	1	0.8	0.5	0.6
Ccr, mL/min	50.8	73.2	84.2	68.9
UA, mg/dL	5.1	4.2	4.8	4.8
Overt proteinuria	yes	no	no	no
PRA, ng/mL/h	0.1	1.3	1.3	0.4
PAC, ng/dL	9.7	35.4	19.8	4.6
FBS, mg/dL	96	115	82	92
HbA1c, %	5	5.9	4.9	5.7
ba-PWV, cm/s	2,189	no data	1,710	1,734
Average IMT, mm	1.0	no data	0.7	1.0

XDH, xanthine dehydrogenase gene; EHT, essential hypertension; HL, hyperlipidemia; HU, hyperuricemia; CV, cardiovascular; ARB, angiotensin II receptor blocker; BB, β-adrenergic blocker; DU, diuretics; CCB, calcium channel blocker; HUD, antihyperuricemic drug; ACEI, angiotensin II converting enzyme inhibitor; SNP, single nucleotide polymorphism; Ccr, creatinine clearance; UA, uric acid; PRA, plasma renin activity; PAC, plasma aldosterone conc.; FBS, fasting blood sugar; ba-PWV, brachial-ankle pulse wave velocity; IMT, intima-media thickness. Normal values: body mass index, between >18.5 and <25.0 kg/m^2; SBP, <140 mmHg; DBP, <90 mmHg; Na^+, 136 to 146 mEq/L; K^+, 3.6 to 4.9 mEq/L; Cl^-, 99 to 109 mEq/L; creatinine, 0.6 to 1.1 mg/dL; Ccr, <60 mL/min; UA, 3.6–7.0 mg/dL; PRA, 0.2 to 2.7 ng/mL/h; PAC, 2 to 13 ng/dL; FBS, <126 mg/dL; HbA1c, <6.5%; ba-PWV, <1,400 cm/s; average IMT, <1.0 mm.

mutations were all found in heterozygous form. In addition, we identified five synonymous variations (26382G>A in exon 8, 80868C>T in exon 10, 47686C>T in exon 22, 65747T>C in exon 28, and 75121G>A in exon 35) encoded for E209 (minor allelic frequency, 0.085), for V279 (0.064), for I787 (0.33), for F1010 (0.135), and for E1239 (0.011), respectively. Twenty-nine additional variations in the introns and a 3′-untranslated region were also detected. Among all the variations, there were 15 common polymorphisms with a minor allelic frequency of over 0.1 (11488C>G, 26504C>T, 37387A>G, 39048A>G, 44408A>G, 46748G>C, 46774G>A, 47879A>G, 49096G>A, 49245A>T, 50298C>T, 65050–65051 ins C, 66292C>G, 67157A>G, and 69901A>C).

Characteristics of Hypertensive Subjects with Missense Mutations in Homozygous Form

After genotyping the three missense mutations in 953 patients with hypertension, including secondary hypertension, we found one subject with G172R and three with N1109T in homozygous form. The characteristics of these four patients with rare missense mutations in the homozygous form are shown in Table 4. All four had resistant hypertension despite antihypertensive drug therapy. One of the patients with N1109T (patient 2) had hyperuricemia and was taking allopurinol. The patient with G172R (patient 1) and the two others with N1109T (patients 2 and 4) had hyperlipidemia. Patients 1 and 4 had low PRA levels (0.1 and 0.4 ng/mL/h, respectively) and high average IMT values (1.0 mm for both). Patient 1 had low Ccr (50.8 mL/min) and overt proteinurea. Three of the four patients had high ba-PWV values: no data