7 医療機器の治験

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背　景

わが国では，国民皆保険制度のもと，高い保健医療水準が達成されてきたが，急速な少子高齢化に直面して診療報酬改定を含めた医療構造改革の必要性が強調されているなかで，エビデンスに基づいた医療の質に関する議論の重要性が増している．

医療機器は，虚血性心疾患に対する薬剤溶出型冠動脈ステント，致死性不整脈に対する除細動器，そして重症心不全に対する補助人工心臓の臨床試験結果に代表されるように，その規格や性能を示すのみならず，生活の質(QOL)や生命予後の改善効果を示すことで，医療のなかでの重要性が高まっている[1]．

しかしながら，臨床試験の大半は海外で実施されており，治療用医療機器の輸入超過傾向[2]や，国産の医療機器ですら欧米での開発が先行している例などから，国内における医療機器治験実施体制の強化が急務とされている．

医療機器と治験

薬事法では，医療用具を「人若しくは動物の疾病の診断，治療若しくは予防に使用されること又は人若しくは動物の身体の構造若しくは機能に影響を及ぼすことが目的とされている器具機械であって，政令で定めるもの」と定義している．

平成17(2005)年4月の改正薬事法施行により，法制上の名称が「医療用具」から「医療機器」に変更されるとともに，国際基準に準拠してクラス分類が整備された(図1)．

医療機器には，メス，ピンセット，はさみから，歯科インプラントや人工関節などの医療材料，そしてMRIやPETなどの大型診断用機器など，幅広い範囲が含まれており，多くの国で法規制の対象となっている．生体への接触部位，接触時間，および不具合が生じたときの危険性の大きさなどを考慮して，承認の要/不要，あるいは臨床試験の要/不要が判断されている．

医療機器治験の問題点

米国では，1993年に米国食品医薬品局(Food and Drug Administration；FDA)の臨床検討委員会がいわゆるTemple報告をまとめ，医療機器治験の計画・実施・解析の各段階における問題点を指摘した．

- 明確な仮説の欠如
- 事前に計画がなされていない
- 試験の目的が不明確
- 適切な対照群が設定されていない
- 症例数が不十分
- 選択・除外基準の設定理由が不明確
- 試験のエンドポイント(成功，失敗，complication)が不明確
- エンドポイントが主観的な場合にバイアスが最小化されていない

これらは申請者にも審査当局にもネガティブな結果をもたらすことから，事前に計画を立てることでコストをかけることなくより多くの情報が得られるように，以下の勧告を行い，医療機器の臨床試験の統計的指針が発表[3]され，申請資料の質も飛躍的に向上した．

- 早い時期から審査官と申請者が相談する
- 審査に生物統計専門家を加える
- 企業に対するガイダンスを整備する

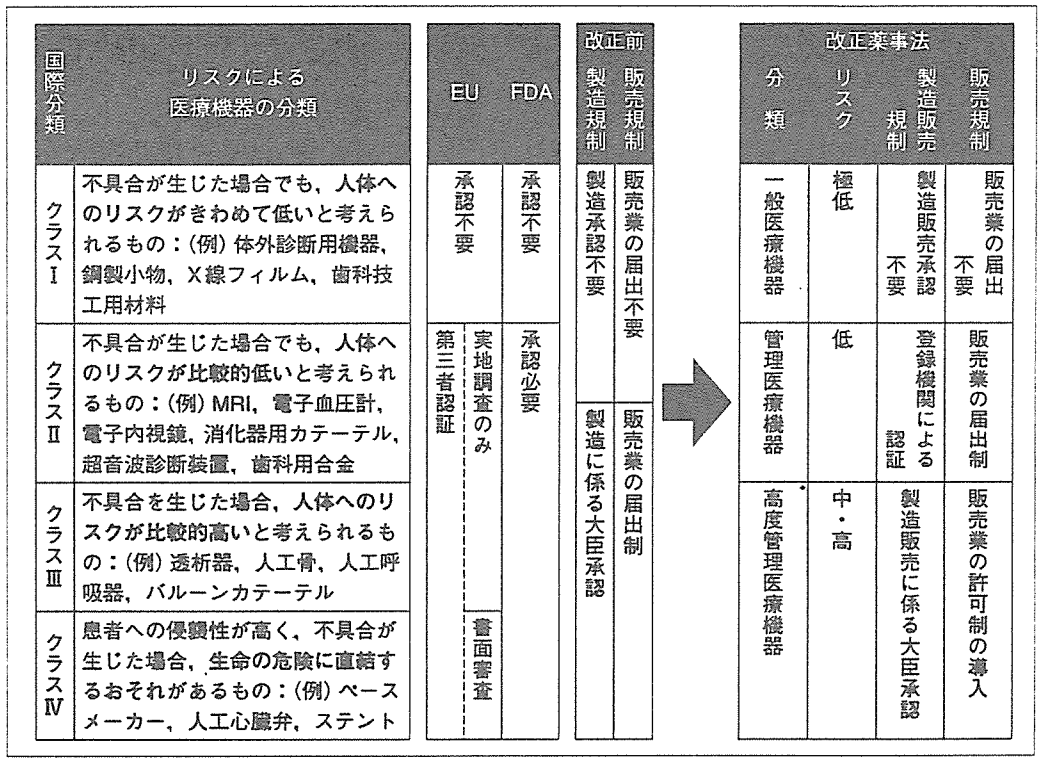

図1 医療機器のクラス分類

- 規制当局のスタッフに対しても教育やガイダンスを整備する
- 開発中から審査期間まで一貫したアドバイスを提供する

わが国でも，医薬品医療機器総合機構が設立され，従来の2施設60症例といった画一的な医療機器治験のデザインにこだわらず，早い段階から生物統計家を交えて治験実施計画書(プロトコル)を作成するために，治験相談を活用することが期待されている．

◆◆◆医療機器治験のデザイン◆◆◆

臨床的意義と統計的意義

試験の目的は簡潔で明確に記載する．開発段階に応じて，パイロット・スタディ(探索的試験)やフィジビリティー・スタディ(妥当性検討試験)，そしてピボタル・スタディ(検証的試験)を実施する[3]．

パイロット・スタディは，当該機器の使用目的を決めるとともに，適切な評価指標を設定し，さらなる研究計画作成やプロトタイプ機器改良，そして評価指標の反応性評価のために重要である．

試験のエンドポイントはあらかじめ明確に記載する．たとえば，経皮的冠動脈形成術を対象とした試験では，手技の成功，6か月目の開存率，狭心症の改善，心筋梗塞・うっ血性心不全・死亡などの主要な心血管イベントの予防など，診療ガイドラインなどを参考に，主要評価項目，副次的評価項目，解析計画を決定する．

試験母集団は選択・除外基準により規定されるが，対象患者は冠疾患をもつすべての患者なのか，そのなかで症候性の狭心症がある患者か，他の治療法が無効な患者に限定するか，特殊な狭窄形態や病変部位に限るのか，事前に明確にする．

医療機器の治験ではしばしば対照群の設定

が大きな問題になる．介入の効果を評価する場合には，患者背景や予後因子をそろえた対照が必要である．質の高いヒストリカル・コントロールが利用可能な場合もあるが，新規医療機器は無治療の場合よりもよいか，現時点における最善の手術・薬物療法・類似機器と比較してどうかなど，比較可能性という観点からは，同時対照のほうが有利である．

必要症例数の設定は，対象母集団における評価指標(結果変数)の平均値，機器治療群と対照群での差とその統計的意義，さらにはその臨床的意義について，あらかじめ医学専門家と生物統計家が十分に検討する．市販後の市場規模などから予算の枠内で実施可能な症例数を決めることには問題が多い．

医療機器の治験では，マスキングあるいは盲検化が難しい．とくに，治験実施医師や患者をマスキングする二重盲検法の実施はしばしば困難である．評価者に起因するバイアスを最小限にするために，主観的ではなく客観的指標で評価することや，画像フィルム解析や検査データの評価者に割り付けを知らせない第三者盲検法(コアラボ)が採用される．

無作為化(ランダム化)は，選択バイアスを排除して予後因子の不均衡を最小にするために有用である．

解析方法については，ヒストリカル・コントロールを用いる場合には観察研究と同様にプロペンシティ・スコア(Propensity Score；PS)を用いた因果推論を実施するとか，すでに類似医療機器が承認されている場合にBayesian法*を活用するなどの試みが報告されている．

実行可能性

統計的検出力の維持には症例数確保とプロトコル遵守が必要であるため，実施医療機関と治験実施医師の選択は試験デザイン中で最も重要な項目の一つである[3]．

施設選定にあたっては，機器のターゲットとなる適格患者が十分に存在すること，プロトコル診療が可能な設備および経験と能力をもったスタッフが必要である．

一般に試験に参加する可能性がある医師は患者数あるいは施設の実力を過剰評価する傾向があるため，被験者パネルを常に見直すことが大切である．

治験責任医師については，医療機器の治験に特有の問題[3]として，手術手技を伴うこと，施設・術者の違いによるバイアス，学習曲線(ラーニング・カーブ)の影響を受けやすいなどに注意が必要である．どのような理由があってもプロトコルや医薬品の臨床試験の実施の基準(Good Clinical Practice；GCP)を厳格に遵守する気のない医師は臨床試験に参加してはならない．

倫理性

ヒトを対象とした臨床試験は，十分な教育を受けた医師が，ヘルシンキ宣言に基づいて基礎研究や疫学研究などの結果を踏まえた実施計画書(プロトコル)を作成し，治験審査委員会(Institutional Review Board；IRB)で第三者による審査を受けたうえで，被験者への説明と同意(インフォームド・コンセント)を確認しつつこれを行う．

有害事象とは，因果関係の有無にかかわらず被験者に生じたあらゆる好ましくない，あるいは意図しない徴候，症状，または病気をいうが，とくに埋込型の医療機器の場合，周術期に手術関連有害事象が多発することか

* Bayesian法(ベイズ統計学)：18世紀にイギリスのトーマス・ベイズ(1717－1761)が発表した定理に基づく統計学の体系．事前確率と新しいデータから事後確率を更新する(ベイズ改訂)ことにより，エビデンスを集積する過程で得られた情報を活用し，臨床試験の必要症例数を少なくしたり，試験期間を短くすることが期待されている．
http://www.fda.gov/cdrh/meetings/072706-bayesian.html

ら，あらかじめ重篤度，既知・未知の区別を検討しておくことが必要である．

また，医療機器に特有の用語として，「不具合」がある．これは，設計，製造販売，流通または使用の段階を問わず，破損，作動不良など広く具合のよくないこと，と定義されている．

なお，埋込型の医療機器については，承認後の特定医療用具トラッキング制度と同様に，承認前の治験についても治験期間終了後の安全性確保対策が必要と思われる．

信頼性

臨床試験の信頼性のなかで，たとえば原資料(診療録など)と症例報告書(Case Report Form；CRF)の整合性といった基本的なレベルでも，忙しい日常診療の合間においては問題が指摘されてきた．

また，医療機器の臨床試験で最大の課題となる点の一つは，在庫管理である．医薬品と異なり，医療機器は薬局などで中央管理することが難しいため，治験責任医師が治験用医療機器管理者となって手術室などで管理していることも多かった．ところが，治験用機器を廃棄したという記録が出されているにもかかわらず，治験終了後に医師が臨床で使用するためにその機器を別に保管している場合も報告されている．

平成17(2005)年に医療機器GCPが施行されたことにより，今後は医薬品と同様に治験依頼者による直接閲覧が制度化される．したがって，①モニタリングと監査，②治験医療機器の管理，③CRFの作成・提出，④記録の保存，⑤治験の契約様式の整備など，臨床試験の質が向上すると期待されている．

◆◆◆医療機器治験の実施◆◆◆

治験が必要とされる場合には医療機器GCP[2]，治験以外の臨床試験を実施する場合には臨床研究に関する倫理指針[4]に準拠する必要がある．

医療機器治験の支援体制

医療機器GCPは，日米EU医薬品規制調和国際会議-医薬品の臨床試験の実施の基準(The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use-Good Clinical Practice；ICH-GCP)に準拠した形で整備されたため，医薬品の治験とほぼ同様に，医療機関では実施医療機関の長，IRB，そして治験責任医師の3者が重要な責任を負うこととなった．

多忙を極める医療機関のなかで，それぞれがプロトコル遵守およびGCP遵守，そして被験者の保護といった責任を果たすには，「権限を委譲することは可能であるが責任まで委譲してはならない」という原則を理解したうえで，必須文書の流れに沿って，事務局やコーディネーターなどの支援体制を構築することが大切である[5]．

治験協力者は，わが国において治験コーディネーター(Clinical Research Coordinator；CRC)による治験責任医師への支援を法的に支える根拠となっている．

医療機器のGCP省令では，「治験責任医師又は治験分担医師の指導の下にこれらの者の治験に係る業務に協力する薬剤師，看護師，臨床検査技師，臨床工学技士その他の医療関係者」と定義され，臨床検査技師，臨床工学技士などにもCRCへの道が開かれた．

治験に関連した費用の負担

医療機器治験では，特定療養費制度のもとで治験に係る費用を負担する方法が医薬品治験とは異なり〔厚生労働省保険局医療課長通知，保医発第0318001号，平成14(2002)年3月18日〕，手術の前後1週間に行われた検

査・画像診断・手術などについて，治験依頼者が負担するのが原則である[2]．

さらに，医師主導の治験については，手術・処置などの前後1週間に行われたものであるか否かにかかわらず，検査・画像診断にかかる費用が特定療養費の支給対象となった．また，当該治験対象である機械器具などの費用負担を患者に求めることも可能になった[2]．

ただし，米国では治験医療機器(Investigational Device Exemptions；IDE)に指定されると有償治験を実施することが可能になる場合があるなど，制度上の相違点も散見される．わが国でも，承認条件として施設あるいは術者を限定したり，市販後の臨床試験(製造販売後臨床試験)実施を義務づけたりするなど，医療機器の臨床評価を推進する制度を整備すべきであろう．

開発の国際化とトランスレーショナル・リサーチ

国際ハーモナイゼーション

医薬品の規制については，ICHに代表される国際ハーモナイゼーションが一定の成果を上げているが，医療機器についても同様の動きが始まり，注目されている[5]．

国際標準化機構(International Organization for Standardization；ISO)では，医療機器の基本要件適合性を評価する仕組みとして，非臨床試験，臨床試験，および品質保証活動全般にわたり，ガイドラインを作成している．臨床試験の実施の基準については，医療機器の生物学的評価に関する技術委員会(ISO/TC194)が「ヒトを対象とした医療機器の臨床試験」(ISO14155)を作成した．

一方，日本，米国，EU，オーストラリア，およびカナダの規制当局が，医療機器規制の国際整合化会議(Global Harmonization Task Force；GHTF)を設立した．医療機器の特殊性に配慮しつつ，医療機器の製造販売承認の国際基準である基本要件(essential principles)，不具合報告，品質保証活動，診療的証拠や臨床評価の定義と方法論などについて，既存のガイダンス文書や各国の法体系の見直しをすすめている(www.ghtf.org)．

トランスレーショナル・リサーチとクリティカルパス研究

現在，診断系医療機器では国内企業が比較的強い競争力をもっているものの，治療系医療機器においては外国製品が大きなシェアを占めている[5]．

医療機器は，医薬品と比較して少量多品種生産であるが国際流通性は大きい(医薬品は2万品目・年6兆円，医療機器は30万品目・年2兆円)．

国内製造業者は，製造物責任法(Product Liability；PL法)とバイオマテリアル危機問題[5]に代表される危機管理体制も大きな負担になるなかで，研究開発投資額は小さく経済的基盤も零細である．

一方，欧米では研究開発への投資と同時にそのパテント戦略に対しても投資を行い，先行者利益の確保に努めており，医療機器企業の研究開発費の日米の差は拡大傾向にある．最先端医療機器の開発をすすめるためには，医療，機械，電気，化学等科学工学分野の高度な統合が必要である．

厚生労働省は，より優れた，より安全な革新的医療機器の提供を目指し，医療機器産業ビジョンを発表した．これは，橋渡し研究(トランスレーショナル・リサーチ)やFDAが提唱するクリティカルパス研究にも近い考え方で，研究，開発，生産，販売，使用のすべての段階について，問題点を洗い出しつつ重点領域を前進させる体制作りを提唱したものである．

医療従事者の役割

ISOやGHTFなどの国際ハーモナイゼーションは，基本的には規制当局と企業が向き合う形ですすめられている．しかしながら，医療の質向上という目的を実現するためには，対話のみならず実践が必要である．

とくに，医療従事者にとって多忙な毎日のなかで診療・教育・研究のバランスをとることは難しいが，しかしながら，諸外国で標準的とされる医療機器が未承認であったり，国産の医療機器がまず海外で開発されていたりする現実に直面すると，医療従事者に期待される役割は大きい．

まとめ

情報やモノは容易に国境を越えるようになり，医療機器の開発も国際化がすすんでいる．わが国では治療機器の多くを輸入に頼っており，治験開始や承認申請の段階で欧米に後れをとっていることから，今後さらに新しい治療の提供が遅れるとともに，価格の内外格差など種々の問題点が拡大する懸念がある．

医師あるいはプロフェッショナル集団としての学会は，医療機器の薬事承認や保険適用などの制度を理解しつつ，産学官連携をもとにした国際調和の実践をすすめ，臨床評価のための体制整備や人材育成を続けることが重要である．

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Uric Acid, Left Ventricular Mass Index, and Risk of Cardiovascular Disease in Essential Hypertension

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Abstract—Elevated serum uric acid (UA) is frequently encountered in individuals with hypertension, but whether the relationship between UA and cardiovascular events is circumstantial or causal remains to be answered. We examined the association between serum UA and left ventricular mass index (LVMI) and investigated prospectively whether the combination of UA and LVMI can predict the incidence of cardiovascular disease (CVD) in asymptomatic subjects with essential hypertension. A total of 619 subjects (mean age, 61 years; 52% female) free of prior CVD were included in this study. A significant association between UA and LVMI was also confirmed in multiple regression analysis (male: $F=4.29$, $P<0.04$; female: $F=4.24$, $P<0.05$). During follow-up (mean, 34 months), 28 subjects (14 female) developed CVD including myocardial infarction, angina pectoris, congestive heart failure, cerebral infarction, and transient cerebral ischemia. Sex-specific median values were used to separate the higher group from the lower group of UA and LVMI. Kaplan–Meier curves showed a significantly poorer survival rate in the group with higher UA and LVMI (LVMI, male: >126.9, female: >112.0 g/m^2; UA, male: >374.7, female: >303.3 μmol/L; log-rank $\chi^2=13.18$; $P<0.01$). Multivariate Cox regression analysis showed that the combination of higher UA and LVMI was an independent predictor for CVD events (hazard ratio, 2.38; $P<0.03$). Our findings demonstrate that UA is independently associated with LVMI and suggest that the combination of hyperuricemia combined with left ventricular hypertrophy is an independent and powerful predictor for CVD. The association between UA and CVD events may be introduced in part because of a direct association of UA with LVMI. (***Hypertension.* 2006;47:195-202.**)

Key Words: uric acid ■ cardiovascular diseases ■ hypertrophy ■ risk factors

Effective prevention of cardiovascular disease (CVD) requires the early detection and correction of predisposing conditions and risk factors in susceptible patients. Hypertension is a common risk factor for CVD, and the cardiovascular prognosis in patients with hypertension depends not only on the level of blood pressure (BP), but also on the presence of associated risk factors. Hyperuricemia is frequently encountered in hypertensive patients.[1] Several large epidemiologic studies have identified an association between increased serum uric acid (UA) and cardiovascular risk in the general population[2–6] and among patients with hypertension.[7,8] Other recent reports have also confirmed these associations by angiographic procedure.[9,10] Some studies have claimed that UA is an independent risk factor for CVD, whereas others have failed to identify UA as a significant and independent risk factor.[11–13] Thus, the status of UA as an independent risk marker remains controversial, and whether the relationship between UA level and cardiovascular events is circumstantial or causal remains to be answered.[2] On the other hand, the level of serum UA is affected by or linked to many factors, such as obesity, insulin resistance, dyslipidemia, and hypertension, all of which are also associated with left ventricular hypertrophy (LVH). In a recent report, in female subjects, UA level was independently associated with the presence of LVH detected by echocardiography.[14] These results suggest that UA level may be related to left ventricular mass index (LVMI).

In hypertension, LVH is initially a compensatory process against abnormal loading conditions, but it is also the first step toward the development of overt clinical disease, such as CVD.[15] In essential hypertension, the risk of future CVD complications is higher in patients with LVH on echocardiography than in those with normal left ventricular (LV) mass.[15,16] Thus, assessment of LV mass by echocardiography is a well-established procedure to estimate the risk of CVD in hypertensives.

The hypothesis that the combination of serum UA level and LVMI may be a strong predictor of CVD has never been examined. In this study, we investigated the relationship between UA level and LVMI in essential hypertensive subjects. Furthermore, we also examined prospectively the relations of UA level, LVMI, and their combination to the incidence of CVD during follow-up in asymptomatic hypertensive subjects.

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Methods

Study Subjects

A total of 619 hypertensive subjects who had good-quality echocardiographic recordings were enrolled and monitored for 33.5±0.8 months in this study. All of the subjects were selected from patients who were admitted and underwent medical investigation at the National Cardiovascular Center in Osaka, Japan. Hypertension was defined as a systolic BP of ≥140 mm Hg and/or a diastolic BP of ≥90 mm Hg on repeated measurements or receiving antihypertensive treatment. Diabetes mellitus was defined according to the American Diabetes Association criteria.[17] Smoking was defined as current smoking or having a history of habitual smoking. Ischemic heart disease was defined as a ≥75% organic stenosis of ≥1 major coronary artery as confirmed by coronary angiography or a history of myocardial infarction or percutaneous transluminal coronary angioplasty. Renal insufficiency was defined as a serum creatinine concentration >176.8 μmol/L. All of the subjects enrolled in this study had essential hypertension. Exclusion criteria included ischemic heart disease, acute coronary syndrome, congestive heart failure (CHF; New York Heart Association class II or greater), chronic renal insufficiency, valvular heart disease, old cerebral infarction, and history of transient ischemic attack. Participants with moderate or severe aortic or mitral regurgitation or a heart rate >100 bpm were also excluded. The study protocol was approved by the ethics committee of our institution. All of the subjects enrolled in this study were Japanese, and all of the subjects gave informed consent to participate in this study.

Baseline Clinical Characteristics

After fasting overnight, BP was measured with an appropriate arm cuff and a mercury column sphygmomanometer on the left arm after a resting period of ≥10 minutes in the supine position. After BP measurements, venous blood sampling from all of the subjects was performed. Height and body weight were measured, and body mass index (BMI) was calculated. Insulin sensitivity was estimated using the homeostatic model assessment index; that is, plasma glucose level×(plasma insulin level/22.5). Urine samples were collected for 24 hours and used to evaluate creatinine clearance (Ccr). The following parameters were also determined: total cholesterol (T-chol), triglycerides (TG), high-density lipoprotein cholesterol (HDL-chol), serum UA, serum creatinine, and C-reactive protein (CRP) levels. Serum UA levels were determined by the uricase-peroxidase method.[18]

Echocardiographic Methods and Calculation of Derived Variables

Imaging and Doppler echocardiography were performed in all of the participants in this study. Studies were performed with phased-array echocardiography with M-mode, 2D, pulsed, and color-flow Doppler capabilities. LV internal dimension and septal and posterior wall thickness were measured at end-diastole and end-systole according to the American Society of Echocardiography recommendations.[19,20] Color-flow Doppler recordings were used to check for aortic and mitral regurgitation, as described previously.[21] End-diastolic dimensions were used to calculate LV mass by a previously reported formula.[22] LV mass was considered an unadjusted variable and was normalized by body surface area and expressed as LVMI.

The LV diastolic filling pattern was recorded from the apical transducer position with subjects in the left lateral decubitus position, with the sample volume situated between the mitral leaflet tips. The leading edge of the transmitral Doppler flow pattern was traced to derive the peak of early diastolic and atrial phase LV filling (E-velocity and A-velocity, respectively), their ratio (E/A ratio), and the deceleration time of early diastolic LV filling (DcT). All of the measurements were performed by a trained investigator who was blinded to the clinical data of the subjects.

Clinical End Points

For survival analysis, observation began on the date of echocardiography, with verified dates updated through March 2004. All of the subjects were followed at the National Cardiovascular Center in Osaka and treated by implementation of standard lifestyle and pharmacological measures. All of the subjects were periodically referred to our institution for BP control and other diagnostic procedures. CVD events of interest in this study were myocardial infarction and angina pectoris confirmed by electrocardiographic changes, coronary angiography and/or myocardial scintigraphy findings, stroke and transient cerebral ischemia confirmed by clinical symptoms, computed tomography and magnetic resonance angiography and/or cerebrovascular angiography findings, and CHF requiring hospitalization. CHF was diagnosed from clinical symptoms and findings (paroxysmal nocturnal dyspnea or cough, pulmonary rales because of pulmonary congestion, distended jugular veins, neck vein distension, enlarging heart size, pleural effusion and/or acute pulmonary edema on chest radiography, hepatojugular reflux, bilateral ankle edema, shortness of breath on ordinary exertion, and/or heart rate of ≥120 bpm). The cause of death was classified as CVD if there was sudden death from CVD by an independent review panel of physicians who were unaware of the echocardiographic and clinical findings. Events that were more equivocal, such as unrecognized myocardial infarction, angina pectoris, and transient cerebral ischemia, were not included as CVD for this analysis. Furthermore, patients with clinical evidence of pneumonia or uremia were excluded. For patients who experienced multiple nonfatal episodes of CVD, the analysis included only the first event.

TABLE 1. Baseline Clinical Characteristics of Study Subjects

Variables	Male	Female
n	296	323
Age, y	60.2±0.7	62.5±0.7*
BMI, kg/m^2	24.6±0.2	24.2±0.2
Duration of hypertension, y	14.7±0.6	14.8±0.6
Smoking, %	72.0	18.7†
Systolic BP, mm Hg	142.5±0.9	144.6±0.8
Diastolic BP, mm Hg	83.2±0.6	80.8±0.6†
Pulse pressure, mm Hg	59.3±0.8	63.8±0.7†
Heart rate, bpm	66.4±0.5	67.4±0.5
Diabetes, %	26.7	18.9*
T-chol, mmol/L	5.16±0.04	5.37±0.04†
TG, mmol/L	1.69±0.06	1.29±0.06†
HDL-chol, mmol/L	1.24±0.02	1.43±0.02†
UA, μmol/L	378.5±4.8	313.2±4.6†
Ccr, Ml/min	101.6±2.3	94.2±2.2*
HOMA-index	1.79±0.10	1.63±0.10
CRP, mg/L	2.1±0.4	1.5±0.4
Septal wall thickness, mm	11.2±0.1	10.2±0.1†
Posterior wall thickness, mm	11.1±0.1	10.2±0.1†
LV internal diameter, mm	47.1±0.2	43.8±0.2†
LVMI, g/m^2	130.5±1.7	116.0±1.7†
Peak E-velocity, m/s	0.68±0.01	0.72±0.01†
Peak A-velocity, m/s	0.77±0.01	0.86±0.01†
DcT, ms	229.3±2.8	230.1±2.6
E/A ratio	0.92±0.02	0.87±0.02*

HOMA indicates homeostatic model assessment. Data are mean±SE.
*P<0.05 and †P<0.01 vs male subjects.

Statistical Analysis

Parametric data are presented as mean±SE. The relations between LVMI or serum UA and various parameters were assessed using univariate linear regression analysis and Pearson's correlation coefficient. Multiple linear regression analysis was applied to identify independent determinants of LVMI after adjustment for potential confounding factors affecting LVMI.

Serum UA level and LVMI were stratified into 4 groups according to median values of baseline serum UA level and LVMI by each sex. One-way ANOVA with Dunnett multiple comparison posttest was used to analyze data among 4 groups. Event-free survival analysis was performed with the Kaplan–Meier method to plot the cumulative incidence of CVD, and the groups were compared by the Mantel log rank test. Cox proportional hazard analysis was used to examine the association between variables and the cumulative incidence of CVD. With respect to serum UA and LVMI, the cumulative incidence of CVD was calculated using the group with lower UA and LVMI as a reference for each other. These effects were measured by hazard ratios (HRs) and their 95% CIs based on Cox regression models. We used multivariable Cox proportional hazards regression models to examine the relations of serum UA and LVMI to CVD events, after accounting for relevant variables using a P value of <0.05 as the selection criterion. A P value <0.05 was considered statistically significant. All of the calculations were performed using a standard statistical package (JMP 4.0, SAS Institute).

Results

Association Between UA and LVMI

The baseline clinical and biochemical characteristics of the study subjects, analyzed on the basis of sex, are shown in Table 1. UA level and LVMI were significantly higher in men than in women. At baseline, 78.5% of the study patients were taking antihypertensive drugs, and 21.5% were complying with lifestyle measures only. Diuretics, β-blockers, angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, and calcium-channel blockers were used alone or in various combinations in 11.3%, 26.7%, 33.9%, and 62.8% of the study patients, respectively. In addition, 9.0% of the study subjects were taking urate-lowering medication (allopurinol and probenecid).

We first examined the simple correlations between serum UA and clinical variables after dividing the subjects into 2 groups according to sex (Table 2). In both male and female subjects, UA level was significantly associated with BMI, TG, HDL-chol, Ccr, and LVMI. In addition, only in female subjects, there was a significant association between UA level and CRP, smoking, taking diuretics, and taking urate-lowering medication.

The simple correlations between LVMI and clinical variables were also examined (Table 2). In male subjects, LVMI was significantly correlated with duration of hypertension, systolic BP, pulse pressure, heart rate, T-chol, UA, and CRP and was significantly higher in smokers and those taking urate-lowering medication. In female subjects, LVMI was significantly correlated with age, BMI, duration of hyperten-

TABLE 2. Simple Correlation Among Serum UA, LVMI, and Clinical Characteristics

	UA, μmol/L		LVMI, g/m²	
Characteristics	Male	Female	Male	Female
Age	0.10	0.04	0.10	0.16†
BMI	0.13*	0.17†	0.10	0.18†
Duration of hypertension	0.10	0.09	0.13*	0.13*
Smoking, yes vs no	381.0±5.7 vs 369.0±9.1	332.6±10.5 vs 309.2±5.1*	133.3±2.1 vs 123.5±3.4*	119.7±3.8 vs 115.2±1.8
Systolic BP	0.01	0.05	0.14*	0.26†
Diastolic BP	0.01	0.06	0.01	0.04
Pulse pressure	0.03	0.01	0.17†	0.26†
Heart rate	0.03	0.01	−0.21†	−0.12*
Diabetes, yes vs no	381.0±5.7 vs 368.5±9.3	322.0±10.5 vs 311.1±5.1	131.0±3.5 vs 130.4±2.1	128.5±3.7 vs 113.1±1.8†
T-chol	0.03	0.02	0.12*	0.01
TG	0.25†	0.32†	0.08	0.19†
HDL-chol	−0.19†	−0.27†	−0.10	−0.15†
Ccr	−0.14*	−0.19†	−0.11	−0.01
HOMA-index	0.11	0.11	0.04	0.18†
CRP	0.03	0.15†	0.13*	0.08
LVMI	0.15†	0.16†		
Taking diuretics, yes vs no	397.1±15.3 vs 375.5±5.1	353.3±12.7 vs 307.5±4.8†	132.0±5.7 vs 130.4±1.9	123.7±4.6 vs 114.8±1.7
Taking urate-lowering medication, yes vs no	385.9±12.5 vs 376.2±5.3	431.7±22.6 vs 308.6±4.5†	140.2±4.6 vs 128.9±1.9*	121.2±8.5 vs 115.8±1.7
Peak E-velocity	−0.04	−0.01		
Peak A-velocity	0.01	0.06		
DcT	0.08*	0.12*		
E/A ratio	−0.02	−0.05		

HOMA indicates homeostatic model assessment. Data indicate correlation coefficients and mean±SE.
*$P<0.05$.
†$P<0.01$.

sion, systolic BP, pulse pressure, heart rate, TG, HDL-chol, homeostatic model assessment index, and UA and was significantly higher in diabetics.

Multiple linear regression analysis was performed including age, duration of hypertension, BMI, systolic and diastolic BP, heart rate, T-chol, TG, HDL-chol, Ccr, CRP, smoking, and diabetes and revealed that UA was independently associated with LVMI in male and female subjects (Table 3). In addition, even after adjustment for taking diuretics and taking urate-lowering medication, UA was still independently associated with LVMI (male, F=4.831, P=0.0290; female, F=4.591, P=0.0330).

To exclude the effect of drugs on UA level, we next examined the association between UA and LVMI after excluding subjects receiving diuretics and urate-lowering medication (male; n=232, female; n=273). Even after excluding these subjects, a significant association between UA and LVMI was observed (male: r=0.16, female: r=0.17, P<0.01 respectively).

LVH was considered to be present when LVMI was >125 for men and >110 g/m^2 for women.[23] UA level was significantly higher in subjects with LVH (male, 383.4±6.2 versus 363.5±6.6; female, 323.5±6.2 versus 303.0±6.5 μmol/L, P<0.03 respectively). A significant association between UA and LVH was also confirmed in multiple regression analysis including age, duration of hypertension, BMI, systolic and diastolic BP, heart rate, T-chol, TG, HDL-chol, Ccr, CRP, smoking, and diabetes (male, 384.5±7.6 versus 363.7±8.0, F=4.3, P<0.04; female, 329.7±9.1 versus 302.0±10.5 μmol/L, F=5.8, P<0.02).

The association between LV diastolic function and UA level was examined, and a significant association between UA and DcT was observed (Table 2). On the other hand, UA was not significantly associated with E-velocity, A-velocity, and E/A ratio. It is well described that early diastolic relaxation decreases with increasing age.[24] In the present study, we also found that DcT had a significant positive relationship with age (male: r=0.36, female: r=0.30, P<0.01 respectively), but not heart rate (male: r=−0.05, female: r=−0.01) and body surface area (male: r=0.07, female: r=0.05). Even after adjustment for age, DcT was significantly related to UA level (male: F=4.34, P<0.04; female: F=3.99, P<0.05).

TABLE 3. Independent Determinants of LVMI by Each Sex in Multiple Linear Regression Analysis

	Male		Female	
Variables	F	*P* Value	F	*P* Value
Age	0.068	0.7946	9.410	0.0024
BMI	4.718	0.0309	1.903	0.1689
Duration of hypertension	1.489	0.2236	0.026	0.8711
Smoking	3.935	0.0485	0.204	0.6516
Systolic BP	6.362	0.0124	20.479	0.0001
Diastolic BP	0.086	0.7702	0.011	0.9150
Heart rate	8.872	0.0032	5.859	0.0162
Diabetes	0.007	0.9357	9.837	0.0019
T-chol	2.826	0.0942	3.071	0.0808
TG	1.182	0.2781	6.239	0.0131
HDL-chol	0.294	0.5881	0.498	0.4812
UA	4.285	0.0396	4.244	0.0403
Ccr	1.886	0.1710	5.516	0.0196
CRP	0.246	0.6206	0.468	0.4944
	R^2=0.162; F=3.095; P=0.0002		R^2=0.249; F=6.344; P<0.0001	

Predictive Value of Serum UA and LVMI for CVD

Because of the sex difference in serum UA levels and LVMI values, different median values for men and women were used to separate the higher group from the lower group in each variable. Demographic and hemodynamic data of the subjects grouped according to the median value of serum UA (male: 374.7; female: 303.3 μmol/L) and LVMI (male: 126.9; female: 112.0 g/m^2) in each sex. As a result, the total subjects were divided into 4 groups as follows; lower LVMI and UA, lower LVMI and higher UA, higher LVMI and lower UA, and higher LVMI and UA. The baseline clinical and biochemical characteristics of the study subjects are shown in Table 4. There was a trend toward higher age, longer duration of hypertension, higher systolic BP, higher pulse pressure, and lower heart rate with increasing LVMI. On the other hand, the groups with higher UA showed higher BMI and lower Ccr. In addition, the group with higher LVMI and UA showed significantly lower HDL-chol and Ccr than that with higher LVMI and lower UA. At the follow-up contact, the proportions of subjects treated with diuretics, alone or combined with other agents, during follow-up were 6.8%, 11.9%, 9.6%, and 16.6% (P<0.05 versus lower LVMI and UA), respectively, in the 4 groups. The proportions of subjects treated with urate-lowering medication were 3.7%, 9.8%, 13.2% (P<0.05 versus lower LVMI and UA), and 10.7%, respectively.

During the follow-up period, 28 patients (4.5%; 14 female) developed CVD. There were 11 subjects with CHF, 1 with myocardial infarction, 8 with angina pectoris, 7 with cerebral infarction, and 1 with transient cerebral ischemia. Serum UA level and LVMI were significantly higher in patients who developed CVD during the follow-up period than in event-free subjects (UA: 385.3±16.8 versus 341.8±3.6 μmol/L, LVMI: 139.5±5.8 versus 122.1±1.3 g/m^2, P<0.01, respectively). Life table analyses of CVD throughout the follow-up period according to the 4 groups of baseline serum UA and LVMI are plotted in Figure 1. These curves illustrate significantly poorer survival in the group with higher UA and LVMI.

We next performed Cox regression analysis to examine whether the influence of higher UA and LVMI on CVD events was independent of other risk factors. As shown in Table 5, the risk for CVD was significantly higher in the group with higher UA and LVMI compared with that with lower UA and LVMI (HR, 2.70). In addition, age, duration of hypertension, pulse pressure, and Ccr were also significantly associated with the incidence of CVD. In multivariate Cox regression analysis, the combination of serum UA level and LVMI was an independent predictor for CVD (HR, 2.38).

TABLE 4. Baseline Clinical Characteristics of Study Subjects

	Lower LVMI		Higher LVMI	
Variables	Lower UA	Higher UA	Lower UA	Higher UA
N	166	145	138	170
Male, %	50.6	45.1	50.0	45.3
Age, y	59.3±0.9§	59.5±1.0§	63.9±1.0†	62.8±0.9*
BMI, kg/m^2	23.5±0.3‡	24.7±0.3†	24.4±0.3*	25.2±0.3†
Duration of hypertension, y	12.3±0.8§	14.9±0.9	16.0±0.9†	16.3±0.8†
Smokers, %	41.4	42.4	45.6	47.7
Systolic BP, mm Hg	140.8±1.2§	141.4±1.3‡	146.1±1.3†	145.9±1.1†
Diastolic BP, mm Hg	82.0±0.8	82.1±0.9	82.8±0.9	81.1±0.8
Pulse pressure, mm Hg	58.9±1.0‡	59.3±1.1‡	63.3±1.1*	64.9±1.0†
Heart rate, bpm	68.6±0.7§	68.0±0.7‡	65.4±0.8†	65.8±0.7*
Diabetes, %	18.9	18.8	27.2	26.5
T-chol, mmol/L	5.29±0.06	5.37±0.06	5.26±0.06	5.17±0.06
TG, mmol/L	1.28±0.08	1.76±0.09†‡	1.35±0.09	1.56±0.08†
HDL-chol, mmol/L	1.43±0.03	1.33±0.03	1.38±0.03	1.23±0.03†§
UA, μmol/L	280±5	400±5†	287±6	406±5†§
Ccr, mL/min	102.8±3.1	96.8±3.3	102.9±3.4	89.8±3.1†‡
HOMA-index	1.80±0.26	1.93±0.27	1.59±0.27	2.05±0.24
CRP, mg/L	1.42±0.36	1.25±0.37	1.62±0.37	1.83±0.33
Septal wall thickness, mm	9.6±0.1§	9.9±0.1§	11.5±0.1†	11.8±0.1†
Posterior wall thickness, mm	9.8±0.1§	9.9±0.1§	11.3±0.1†	11.5±0.1†
LV internal diameter, mm	43.7±0.3§	43.4±0.3§	46.9±0.3†	47.4±0.3†
LVMI, g/m^2	99.9±1.6§	99.9±1.7§	143.1±1.7†	147.8±1.6†
Peak E-velocity, m/s	0.71±0.01	0.71±0.01	0.69±0.01	0.70±0.01
Peak A-velocity, m/s	0.78±0.01§	0.81±0.02	0.84±0.02†	0.84±0.01†
DcT, ms	223.7±3.7	226.1±3.9	230.0±4.0	237.4±3.6*
E/A ratio	0.95±0.02§	0.92±0.02	0.85±0.02†	0.85±0.02†
No. of CVD events	2	5	5	16

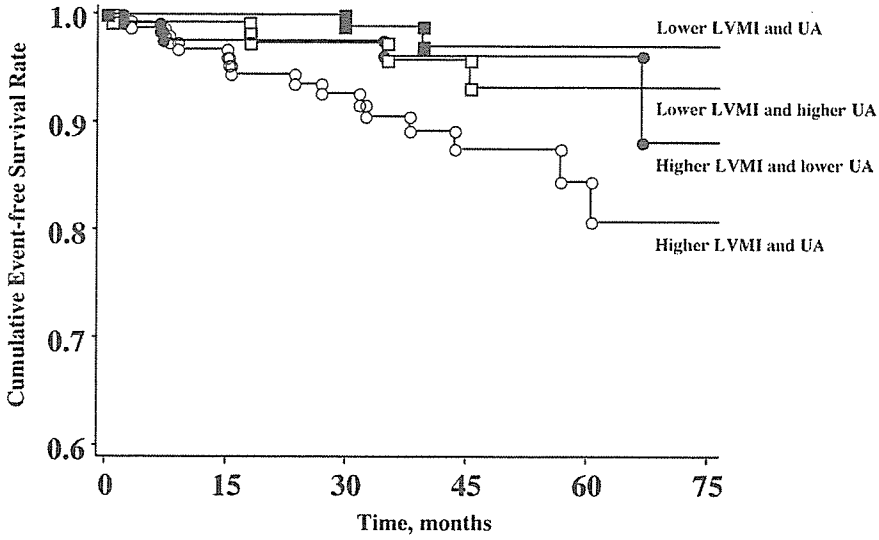
HOMA indicates homeostatic model assessment. Data are mean±E.
*$P<0.05$ and †$P<0.01$ vs lower LVMI and lower UA.
‡$P<0.05$ and §$P<0.01$ vs higher LVMI and lower UA.

In addition, the influence of the combination of UA and LVMI on CVD events was also examined by dividing the 4 groups according to the normal levels of UA (UA level ≤420 in men and ≤390 μmol/L in women) and with/without LVH; that is, normal UA and without LVH (n=244), hyperuricemia and without LVH (n=53), normal UA and LVH (n=245), and hyperuricemia and LVH (n=77). The independent predictive value of the hyperuricemia and LVH for CVD events was also confirmed by the Kaplan–Meier method (log-rank $\chi^2=5.58$; $P=0.0355$) and by Cox regression analysis (HR, 1.7; 95% CI, 0.78 to 3.41; $P<0.03$). In multivariate Cox regression analysis, the combination of hyperuricemia and LVH was an independent predictor for CVD events (HR, 1.8; 95% CI, 0.85 to 3.48; $P<0.05$).

Discussion

This study documented and validated that serum UA level is associated with LVMI, and the results of multiple linear regression analysis indicated that serum UA is independently associated with LVMI. Compared with the group with lower UA and LVMI, the group with higher UA and LVMI showed a condition of increased risk for cardiovascular and renal morbidity, such as significantly longer duration of hypertension, higher pulse pressure, worse dyslipidemia, and lower Ccr. Even after adjustment for other clinical factors, higher UA level and LVMI and age were independent predictors for CVD.

Our results suggest that serum UA is independently associated with LVMI, whereas an elevation of UA is associated with an actual metabolic disorder, and whether an elevation of serum UA level is the cause or result of LVH is unclear. The association between UA and LVMI might relate to an association of UA with other risk factors, especially including renal dysfunction, oxidative stress, severity of hypertension, and obesity. Renal dysfunction increases serum UA and activates the renin–angiotensin system, and angiotensin II is essential for the development of LVH.[25] UA is the final breakdown product of dietary or endogenous purines and is generated by xanthine oxidase (XO). A net release of urate in coronary heart disease[26] and the presence of XO in the human



Kaplan–Meier plots showing cumulative CVD-free survival in subjects according to 4 groups divided by median values of UA and LVMI (log-rank χ^2=13.18; P=0.0042). Marker groups for LVMI (g/m^2): lower-LVMI, ≤126.9 for men and ≤112.0 for women; higher-LVMI, >126.9 for men and >112.0 for women. Marker groups for UA (μmol/L): lower-UA, ≤374.7 for men and ≤303.3 for women; higher-UA, >374.7 for men and >303.3 for women.

heart has been demonstrated.[27] UA may reflect the generation of superoxide and resultant oxidative stress via the XO system.[28] Furthermore, the independent association between UA and the severity of hypertension is well accepted.[1] On the other hand, there is a possibility that UA itself may induce LVH. Previous reports have shown that UA impaired NO generation and induced endothelial dysfunction and smooth muscle cell proliferation.[29,30] In experimental and in vitro systems, UA appears to have the ability to induce inflammatory mediators, such as tumor necrosis factor α,[31] and potentially stimulates mitogen-activated protein kinases,[32] which are known to induce cardiac hypertrophy.[33,34] These results suggest that cardiac hypertrophy may be, at least in part, attributable to an increase in UA itself, via stimulation of endothelial dysfunction, smooth muscle cell proliferation, and inflammation.

Our results showed that the incidence of CVD in subjects with higher UA and LVMI was ≈2.4-fold higher than that in subjects with lower UA and LVMI, even after adjustment for confounding factors. Thus, our results indicate that hyperten-

TABLE 5. Predictors for CVD Events by Cox Regression Analysis

	Univariate		Multivariate	
Variables, Unit of Increase	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
LVMI and UA	χ^2=12.79	0.0051	χ^2=9.08	0.0282
Lower LVMI and UA	1 (reference)		1 (reference)	
Lower LVMI and higher UA	1.01 (0.43 to 2.17)		1.14 (0.48 to 2.47)	
Higher LVMI and lower UA	1.02 (0.43 to 2.19)		1.01 (0.49 to 2.06)	
Higher LVMI and higher UA	2.70 (1.51 to 5.08)		2.38 (1.31 to 4.55)	
Age, 1 y	1.07 (1.03 to 1.12)	0.0004	1.05 (1.01 to 1.11)	0.0260
Sex, male	1.07 (0.74 to 1.56)	0.7212		
BMI, 1 kg/m^2	1.04 (0.93 to 1.16)	0.4566		
Duration of hypertension, 1 y	1.06 (1.03 to 1.10)	0.0003	1.03 (0.99 to 1.07)	0.0931
Smoking, yes	1.14 (0.78 to 1.66)	0.4844		
Systolic BP, 1 mm Hg	1.01 (0.98 to 1.03)	0.5092		
Diastolic BP, 1 mm Hg	1.03 (0.99 to 1.07)	0.0502		
Pulse pressure, 1 mm Hg	1.03 (1.00 to 1.05)	0.0343	1.01 (0.98 to 1.03)	0.5577
Heart rate, 1 bpm	0.98 (0.94 to 1.02)	0.4500		
Diabetes, yes	1.40 (0.93 to 2.05)	0.1005		
T-chol, 1 mmol/L	0.87 (0.52 to 1.43)	0.5757		
TG, 1 mmol/L	1.07 (0.69 to 1.34)	0.7261		
HDL-chol, 1 mmol/L	0.81 (0.28 to 2.07)	0.6685		
Ccr, 1 mL/min	0.99 (0.98 to 1.00)	0.0439	1.00 (0.99 to 1.01)	0.9661
HOMA-index, 1	1.01 (0.82 to 1.10)	0.8801		
CRP, 1 mg/L	1.00 (0.85 to 1.03)	0.7561		

HOMA indicates homeostatic model assessment.

sive subjects with LVH and hyperuricemia have an increased risk of developing CVD and suggest that the assessments of serum UA level and LVMI by echocardiography are useful and sensitive for predicting the risk for CVD. Many epidemiologic studies have attempt to identify whether hyperuricemia is an independent risk factor for CVD, but the results obtained were controversial after adjusting for other CVD risk factors, especially including LVH determined by electrocardiography.[7,8,13] Although hyperuricemia itself may have the ability to increase the risk of CVD, our results suggest that the association between UA and CVD events may be introduced in part because of a direct association of UA with LVMI. On the other hand, all of the antihypertensive drugs failed to show a cardioprotective effect in this study. Previous epidemiologic studies have also shown that UA level was independently predictive for the development of CVD even after antihypertensive treatment.[7,8,35,36] Furthermore, in the Systolic Hypertension in the Elderly Program trial, a subanalysis showed that the cardioprotection by diuretics was lost in those treated patients in whom UA levels increased.[36]

One notable result of this study is that, in the group with higher LVMI, the risk of CVD became higher with increasing UA level. This result may have been introduced because of decreased renal function and HDL-chol level, which are established risk factors for CVD, in subjects with hyperuricemia and LVH. Apart from renal function and lipid metabolism, there are other possible mechanisms by which the risk for CVD became higher with increasing UA levels. Several mechanisms have been proposed to account for the association between hyperuricemia and CVD, including the following: (1) the direct relationship of UA with severity of hypertension,[1] in which the predictive relationship of UA with BP is dose dependent;[37] (2) increased oxidative stress;[38] (3) a subtle reduction in glomerular filtration rate leading to impaired renal UA clearance;[39] (4) impaired NO production,[38] which activates the renin–angiotensin system[40] and induces endothelial dysfunction and smooth muscle cell proliferation;[29,30] (5) impaired platelet adhesiveness, disturbed hemorheology, and aggregation;[38] and (6) synthesis of monocyte chemoattractant protein-1 in vascular smooth muscle cells,[41] which is a chemokine that is importantly involved in CVD.[42] On the other hand, the close association between LVH and CVD events may be explained by decreased myocardial contractility, severe diastolic filling abnormalities, and increased oxygen requirement of the myocardium.[43] Our results showed that more severe relaxation impairment was observed in hyperuricemic subjects with LVH, and this "impaired relaxation" is known to be associated with increased risk of CVD.[44] In addition, a weak but significant association between UA and DcT, a marker of relaxation impairment, was observed in this study, and higher UA levels may contribute to the progression of LV dysfunction. Consequently, we propose the idea that, in subjects with LVH, severe hypertension, activation of oxidative stress and the renin–angiotensin system, stimulation of production of cytokines from leukocytes and chemokines from vascular smooth muscle cells, and more impaired relaxation may occur with increasing UA levels and enhance the risk for CVD.

The limitations of this study include missing baseline data and potentially important characteristics, such as menopause, alcohol intake, and a high-purine diet, which are also associated with a higher serum UA level. Because our data were obtained in subjects with treated essential hypertension at the start of the study, these results could underestimate the involvement of BP itself in the development of LVH and CVD events.

Perspectives

Our results demonstrate that UA is independently associated with LVMI and suggest that the combination of hyperuricemia with LVH is a powerful independent predictor for CVD. The association between UA and CVD events may be introduced in part because of a direct association of UA with LVMI. In hypertensive as well as LVH subjects, assessment of UA levels may help to refine CVD risk stratification. A crucial next step is to investigate whether UA is causally linked to LVH in a longitudinal setting. If so, hypouricemic agents might be used in clinical practice for LVH risk reduction in hypertensive patients. A large prospective population–based study will be important to confirm our preliminary observations.

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Original Article

Genetic Variations of *HSD11B2* in Hypertensive Patients and in the General Population, Six Rare Missense/Frameshift Mutations

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Mutations in the gene encoding 11β-hydroxysteroid dehydrogenase type 2, *HSD11B2*, cause a rare monogenic juvenile hypertensive syndrome called apparent mineralocorticoid excess (AME). In AME, defective HSD11B2 enzyme activity results in overstimulation of the mineralocorticoid receptor (MR) by cortisol, causing sodium retention, hypokalemia, and salt-dependent hypertension. Here, we have studied whether genetic variations in *HDS11B2* are implicated in essential hypertension in Japanese hypertensives and the general population. By sequencing the entire coding region and the promoter region of *HDS11B2* in 953 Japanese hypertensives, we identified five missense mutations in 11 patients (L14F, *n*=5; R74H, *n*=1; R147H, *n*=3; T156I, *n*=1; R335H, *n*=1) and one novel frameshift mutation (4884Gdel, *n*=1) in a heterozygous state, in addition to 19 genetic variations. All genetic variations identified were rare, with minor allele frequencies less than 0.005. Four of 12 patients with the missense/frameshift mutations showed renal failure. Four missense mutations, L14F, R74H, R147H, and R335H, were successfully genotyped in the general population, with a sample size of 3,655 individuals (2,175 normotensives and 1,480 hypertensives). Mutations L14F, R74H, R147H, and R335H were identified in hypertensives (*n*=6, 8, 3, and 0, respectively) and normotensives (*n*=8, 12, 5, and 0, respectively) with a similar frequency, suggesting that these missense mutations may not strongly affect the etiology of essential hypertension. Since the allele frequency of all of the genetic variations identified in this study was rare, an association study was not conducted. Taken together, our results indicate that missense mutations in *HSD11B2* do not substantially contribute to essential hypertension in Japanese. (*Hypertens Res* 2006; 29: 243–252)

Key Words: **HSD11B2, missense mutation, genetic variation, essential hypertension, salt-sensitivity**

Introduction

In mineralocorticoid target organs, the 11β-hydroxysteroid dehydrogenase (HSD11B) catalyzes the interconversion of the endogenous cortisol and cortisone in humans. Two distinct forms, HSD11B1 and HSD11B2, of HSD11B have been characterized and cloned (*1–3*). HSD11B1 is expressed in most tissues. In contrast, HSD11B2 has been identified in a limited range of tissues, such as the distal tubules of the kid-

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ney (*2*, *4*, *5*). In mineralocorticoid-responsive cells, HSD11B2 converts cortisol to cortisone, which is not a ligand for the mineralocorticoid receptor, permitting aldosterone to occupy the receptor.

Apparent mineralocorticoid excess syndrome (AME) is an autosomal recessive disorder that results in severe low-renin hypertension and other characteristic clinical features (*6–8*). Typical patients present with severe hypertension, hypokalemia, and undetectable aldosterone. Most patients also have low birth weight, polyuria and polydipsia, failure to thrive, and nephrocalcinosis. The syndrome has been associated with sudden fatality. The HSD11B2 deficiency has been demonstrated in patients with AME and explains the pathogenesis of the disease, which results from excess cortisol binding to the mineralocorticoid receptor due to a failure to convert cortisol to cortisone (*9–11*). Over the last two decades, various genetic mutations in the *HSD11B2* gene have been reported (*12–17*). In Japanese patients with AME, two missense mutations (S180F, R208H) and a deletion of 3 nucleotides resulting in R337H and delta Y338 have been identified (*14*, *18*).

In 1998, a mild form of this disease characterized by P227L mutation in the *HSD11B2* gene was reported (*19*). In contrast to the patients with AME, this patient had low-renin hypertension and hypoaldosteronism but no other phenotypic features that would lead to the diagnosis of AME. Afterwards, it was reported that the defective allele frequency in a cohort of Mennonites was 1.7% (*20*). The genetic mutation in the *HSD11B2* gene, which results in a mild HSD11B2 deficiency, may represent an important cause of low-renin hypertension, the diagnostic basis of which is mostly unknown. Together, these findings suggest that, because 40% of patients with essential hypertension have low renin, these patients may have a mild form of AME.

In the *HSD11B2* gene, the 535G>A polymorphism (synonymous mutation at E178) in exon 3, which can be distinguished by *Alu* I cleavage and the polymorphic microsatellite marker (*21*), have been reported. The minor allele frequency of the 553G>A polymorphism was 0.086 in a healthy Caucasian population and 0.180 in a group of renal transplant patients (n=61), indicating association of this polymorphism with end-stage renal disease. This polymorphism was not associated with essential hypertension (*22*). As for the microsatellite marker, a total of 12 alleles were detected. The urinary ratio of cortisol to cortisone metabolites was higher in subjects homozygous for the A7 microsatellite allele than in the corresponding control subjects. Thus, the association of a polymorphic microsatellite marker of the *HSD11B2* gene with reduced HSD11B2 activity suggests that variants of the *HSD11B2* gene contribute to enhanced blood pressure response to salt in humans (*23*). The study demonstrated that a salt-induced blood pressure increase is associated with impaired HSD11B2 activity, as measured by the urinary excretion ratio of cortisol to cortisone metabolites in young Caucasian salt-sensitive men.

The present study was undertaken 1) to identify the genetic variants in the *HSD11B2* gene in Japanese hypertensives, 2) to address whether individuals with heterozygous missense/frameshift mutations show hypertension or renal impairment, and 3) to explain the genetic contribution to a mild form of hypertension including low-renin hypertension and hypoaldosteronism. We sequenced the promoter and exon regions of *HSD11B2* in Japanese hypertensives and genotyped the rare missense/frameshift mutations in the general population. We assessed the role of these genetic variations in hypertension and clarified their contribution to hypertension in Japanese.

Table 1. General Characteristics of Patients with Hypertension

Number	953
Age (years)	65.1±10.5
Gender (M/F)	522/431
Body mass index (kg/m^2)	24.2±3.3
SBP (mmHg)	145.5±19.2
DBP (mmHg)	84.8±13.4
Essential hypertension	880
Secondary hypertension	73
Renal hypertension	36
Renovascular hypertension	23
Primary aldosteronism	11
Hypothyroid-induced hypertension	2
Renal impairment/failure*	110
Ischemic heart disease	102
Stroke	145

Values are expressed as mean±SD. *Serum creatinine ≥1.4 mg/dl. M, male; F, female; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Methods

Hypertensive Patients

A total of 953 hypertensive patients (522 men and 431 woman; average age: 65.0±10.5 years) were recruited from the Division of Hypertension and Nephrology at the National Cardiovascular Center as reported previously (*24–27*). Briefly, 92% of study subjects (880 subjects) were diagnosed with essential hypertension, and the rest had secondary hypertension (Table 1). Hypertension was defined as systolic blood pressure (SBP) of ≥140 mmHg, and/or diastolic blood pressure (DBP) of ≥90 mmHg, or current use of antihypertensive medication. Hyperlipidemia was defined by total cholesterol ≥220 mg/dl or current use of antihyperlipidemia medication. Diabetes mellitus was defined by fasting plasma glucose ≥126 mg/dl or HbA1c ≥6.5% or current use of anti-diabetic medication. Study subjects had routine laboratory tests including electrolytes, renal function, blood glucose, HbA1c, plasma renin activity and plasma aldosterone concentration.

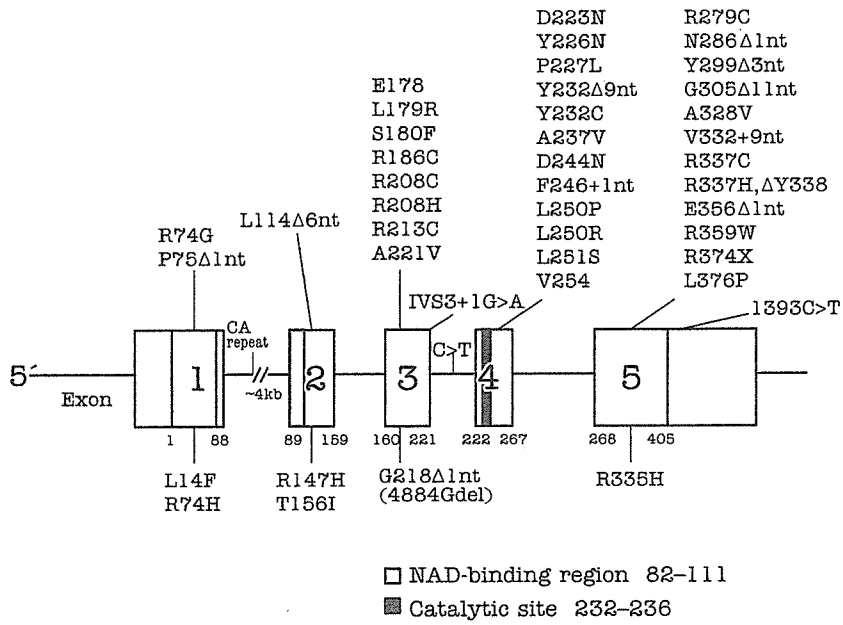

Fig. 1. *Summary of the reported genetic polymorphisms in* HSD11B2. *All polymorphisms in the upper section were reported previously, and the six polymorphisms in the lower section were identified in present study.*

Sequencing of the *HSD11B2* Gene

We sequenced all exons and the promoter region of *HSD11B2* in 953 Japanese hypertensive patients. Blood samples were obtained from hypertensive patients and genomic DNA was isolated from peripheral blood leukocytes. All exons with their flanking sequences and about 1.6 kb of the upstream region were directly sequenced with an ABI PRISM 3700 DNA analyzer (Applied Biosystems, Foster City, USA) using seven sets of primers, as described previously (*28*). Information on the primers and polymerase chain reaction (PCR) conditions is available on request. The obtained sequences were examined for the presence of variations using Sequencher software (Gene Codes Corporation, Ann Arbor, USA), followed by visual inspection. The A of the ATG of the initiator Met codon is denoted as nucleotide +1. The nucleotide sequence (GenBank Accession ID: NT_010498) was used as a reference sequence.

General Population (the Suita Study)

The sample selection and study design of the Suita Study have been described previously (*29*, *30*). Briefly, the subjects visited the National Cardiovascular Center every 2 years for general health checkups. In addition to performing a routine blood examination that included lipid profiles, glucose levels, blood pressure, anthropometric measurements, a physician or nurse administered questionnaires covering personal history of cardiovascular diseases, including angina pectoris, myocardial infarction, and/or stroke. Blood pressure was measured after at least 10 min of rest in a sitting position. SBP and DBP were means of two measurements performed by well-trained doctors using a mercury sphygmomanometer (with a 3-min interval). The subjects were classified as current drinkers if they drank at least 30 ml ethanol per day, nondrinkers if they had never drunk, and past drinkers if they previously had drunk above 30 ml ethanol per day.

Genotyping of Genetic Variations in the General Population

Genotyping was attempted for six rare missense/frameshift mutations using the TaqMan-PCR method (*31*). The sequences of PCR primers and probes for the TaqMan-PCR method are available on request. Genotyping for two of the six rare mutations—4582C>T (encoded T156I) and 4884Gdel (a frameshift mutation)—failed. Thus, four genetic variations were successfully genotyped in 3,655 participants (1,709 men and 1,946 women) of the large cohort known as the Suita Study. All of the participants for genetic analysis in the present study gave their written informed consent. All clinical data and sequencing and genotyping results were anonymous. The study protocol was approved by the Ethical Review Committee of the National Cardiovascular Center.

Table 2. Sequence Variations in the Promoter Region and All Exons in *HSD11β2* Identified in Approximately 953 Japanese Patients with Hypertension and/or Renal Failure

SNP name	Region	Amino acid substitution	Allele 1 freq.	Allele 2 freq.	Flanking sequence	Genotyping
−879C>T	promoter		0.999	0.001	TCCTCTGACA[C/T]CCCACCCTCC	
−687C>A	promoter		0.999	0.001	CAGGGGTGAG[C/A]GCGCCTTAGG	
−596 to −595 CGGCAGins	promoter		0.999	0.001	GCAGCGGCAG[CGGCAG]CGGAGACCGG	
−562G>T	promoter		0.999	0.001	TGGTTCCTCG[G/T]GGTGTTCCTG	
−74C>G	promoter		0.999	0.001	ACTCCGCGCC[C/G]CGGCCTAGAA	
40C>T	exon 1	L14F	0.997	0.003	CGCCTGGCTG[C/T]TCGTGGCTGC	done
42C>A	exon 1	L14L	0.999	0.001	CCTGGCTGCT[C/A]GTGGCTGCCC	
82C>T	exon 1	L28L	0.999	0.001	GCGCTCAGAC[C/T]TGCGTCTGGG	
221G>A	exon 1	R74H	0.999	0.001	CGCCTGGCGC[G/A]CCCGCAGCGC	done
4554G>A	exon 2	R147H	0.999	0.001	GACATTAGCC[G/A]CGTGCTAGAG	done
4582C>T	exon 2	T156I	1.000	0.000	AAGGCCCACA[C/T]CACCAGCACC	failed
4681G>A	intron 2		1.000	0.000	GCTGACCTAA[G/A]GCTTCCCTCC	
4884Gdel	exon 3	frame shift	1.000	0.000	TGACTGTGGG[G]AGCCCAGCGG	failed
4910C>G	intron 3		0.995	0.005	TGCCCCCCCC[C/G]ACTGGAGCAA	
4902insC(8–10)	intron 3		0.998	0.002	GCCCCCCCCC[C]ACTGGAGCAA	
4964C>G	intron 3		0.999	0.001	GAGCCCCTTG[C/G]CAAAGCTGAG	
5017G>A	exon 4	P227P	0.997	0.003	TGCCATATCC[G/A]TGCTTGGGGG	
5205G>A	intron 4		0.999	0.001	TATGGGGGCA[G/A]GTCAGGTTTG	
5267G>A	intron 4		0.999	0.001	CAGACCTGGC[G/A]CGGGTTAAAC	
5334C>T	intron 4		0.999	0.001	GCCACTCCTT[C/T]CCCAGAGTCA	
5422C>T	exon 5	Y295Y	1.000	0.000	TGCAGGCCTA[C/T]GGCAAGGACT	
5541G>A	exon 5	R335H	1.000	0.000	GCTCGGCCCC[G/A]CCGCCGCTAT	done
5698G>A	exon 5	Q387Q	1.000	0.000	CCCCACCACA[G/A]GACGCAGCCC	
5759A>G	3′-UTR		1.000	0.000	TCGGTGAGCC[A/G]TGTGCACCTA	
5784C>T	3′-UTR		0.996	0.004	CCAGCCACTG[C/T]AGCACAGGAG	

The A of the ATG of the initiator Met codon is denoted nucleotide +1, as recommended by the Nomenclature Working Group (*37*). The nucleotide sequence (GenBank Accession ID: NT_010498) was used as a reference sequence. UTR, untranslated region; freq., frequency. Missense mutations were genotyped for general population except two mutations of which genotypes were not determined.

Results

Identification of Genetic Variations in *HSD11B2* in a Japanese Hypertensive Population

We sequenced the promoter and exon regions of *HSD11B2* in 953 hypertensives. As a result, we did not identify the reported common genetic variations in Caucasians and causative genetic variations of AME in the *HSD11B2* gene. Instead, we identified five novel missense mutations and one frameshift mutation in *HSD11B2* (Fig. 1, Table 2). Five patients had a C-to-T substitution at nucleotide 40 in exon 1, which led to an amino acid substitution from L to F at position 14 (L14F). One patient had a G-to-A substitution at nucleotide 221 in exon 1, resulting in an amino acid substitution from R to H at position 74 (R74H). Three patients had a G-to-A substitution at nucleotide 4554 in exon 2, leading to an amino acid substitution from R to H at position 147 (R147H). One patient had a C-to-T substitution at nucleotide 4582 in exon 2, leading to an amino acid substitution from T to I at position 156 (T156I). One patient had a G-to-A substitution at nucleotide 5541 in exon 5, resulting in an amino acid substitution from R to H at position 335 (R335H). We also found one patient with a frameshift mutation that resulted from a guanine deletion at position 4884 in exon 3 (4884Gdel). These missense/frameshift mutations were all found in the heterozygous form.

We also identified five synonymous polymorphisms, which encoded for L14 (42C>A in exon 1) with a minor allele frequency of 0.001%, L28 (82C>T in exon 1) with a minor allele frequency of 0.001%, P227 (5017G>A in exon 4) with a minor allele frequency of 0.003%, Y295 (5422C>T in exon 5) with a minor allele frequency of 0.0003% and Q387 (5698G>A in exon 5) with a minor allele frequency of 0.0003%. Fourteen additional genetic variations in the promoter, intronic, and 3′-untranslated regions were also identified. All of the genetic variations were rare, with minor allele frequencies less than 0.005 (Table 2).

Table 3. Clinical Profiles of Twelve Hypertensive Patients with Missense/Frameshift Mutations in *HSD11β2* Gene

	Case											
	1	2	3	4	5	6	7	8	9	10	11	12
Polymorphism	L14F	L14F	L14F	L14F	L14F	R74H	R147H	R147H	R147H	T156I	4884Gdel	R335H
Age (years old)	73	71	64	51	59	70	76	69	85	78	75	67
Sex	male	female	male	female	male	male	male	male	male	male	female	female
BMI (kg/m^2)	21.39	20.45	20.20	24.09	30.30	27.92	24.03	22.12	26.17	21.69	29.97	21.50
Diagnosis	EHT, HL, HU, CRF, NIDDM, hypothyroidism	Renal HT, HL, CGN	EHT	ETH, HL	EHT, HL, obesity	EHT, HL, obesity	EHT, HU, OCI	EHT, HU, OCH, CRF	EHT, AF, AAA, obesity	EHT	RVHT, NIDDM, HL, obesity	EHT, HL
HT duration (years)	24	21	24	<1	9	15	19	20	21	8	30	41
HT initial onset age (years old)	49	50	40	—	50	55	57	49	64	70	45	26
HT family history	none	none	none	father	none	father, brother	mother, brother	none	none	mother	none	farther, mother, brother
SBP (mmHg)	138	136	152	140	130	140	134	138	154	134	170	148
DBP (mmHg)	70	80	88	68	80	86	72	70	84	68	90	80
Antihypertensive drugs	CCB, ARB	CCB, ACEI	CCB, BB, diuretics	CCB, BB, AB	CCB, ARB, BB	CCB, BB	CCB, AB	CCB, ACEI, AB	CCB	CCB, ACEI, AB	CCB, ACEI	CCB, BB
Na^+ (mEq/l)	141	141	140	142	140	141	141	140	143	143	140	139
K^+ (mEq/l)	4.4	5.2	4.1	4.2	4.2	3.6	4.2	5.2	4.5	4.2	4.6	5.0
Cl^- (mEq/l)	110	109	104	107	102	107	106	108	104	111	104	103
Creatinine (mg/dl)	2.7	0.8	0.6	0.5	0.6	1.1	1.3	2.9	1.2	0.8	0.6	0.8
Overt proteinuria	+	+	–	–	+	+	+	–	–	–	–	–
PRA (ng/ml/h)	3.8	0.9	6.3	0.1	0.5	2.9	1.9	no data	3.4	13.2	19.8	3.2
PAC (ng/dl)	8.8	8.5	no data	27.6	12.4	18.9	43.5	no data	7.7	14.6	7.0	14.1
FBS (mg/dl)	128	92	105	89	113	105	95	91	95	96	137	101
HbA1c (%)	6.0	5.6	5.4	5.2	5.6	6.0	5.1	5.2	5.1	5.0	8.7	5.7

BMI, body mass index; EHT, essential hypertension; HL, hyperlipidemia; HU, hyperuricemia; CRF, chronic renal failure; NIDDM, non-insulin dependent diabetes mellitus; HT, hypertension; CGN, chronic glomerulonephritis; OCI, old cerebral infarction; OCH, old cerebral hemorrhage; AF, atrial fibrillation; AAA, abdominal aortic aneurysma; RVHT, renovascular hypertension; SBP, systolic blood pressure; DBP, diastolic blood pressure; CCB, calcium channel blocker; ARB, angiotension II receptor blocker; ACEI, angiotensin converting enzyme inhibitor; BB, β-adrenergic blocker; AB, α-adrenergic blocker; PRA, plasma renin activity; PAC, plasma aldosterone concentration; FBS, fasting blood sugar. Normal values in our institute: Na^+, 136–146 mEq/l; K^+, 3.6–4.9 mEq/l; Cl^-, 99–109 mEq/l; creatinine, 0.6–1.1 mg/dl; PRA, 0.2–2.7 ng/ml/h; PAC, 2–13 ng/dl.

Characteristics of Patients with Rare Missense/Frameshift Mutations in the Hypertensive Population

The characteristics of the 12 hypertensive patients who had missense/frameshift mutations (L14F, n=5; R74H, n=1; R147H, n=3; T156I, n=1; 4884Gdel, n=1; R335H; n=1) are shown in Table 3. Five patients out of the twelve had renal impairment including protein urea. Two (cases 1 and 2) of five patients with the L14F mutation had chronic renal failure (CRF) and chronic glomerulonephritis (CGN), and one (case 8) of three patients with the R147H mutation also had CRF. A patient with 4884Gdel (case 11) was diagnosed with renovascular hypertension caused by atherosclerosis with type 2 diabetes, hyperlipidemia and obesity (body mass index [BMI]: 29.97 kg/m^2). This patient was 75 years old, female, and had never smoked or drunk alcohol. This patient had microalbuminurea (urinary albumin excretion: 30.8 mg/g creatinine) without renal dysfunction (creatinine clearance: 112.5 ml/min) or cardiac hypertrophy (left ventricular mass index: 126.4 g/m^2). The average onset age of hypertension of the 12 patients with these missense mutations was 50.5 years. A patient with the R335H mutation (case 12) showed hypertension at her age of 26. Serum sodium levels of all patients were within normal range. There were no patients with hypokalemia as seen in AME.

Table 4. Basic Characteristics of Subjects in the General Population

	Women (n=1,946)	Men (n=1,709)
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†

Values are expressed as mean±SD. Hypertension: systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg or antihypertensive medication; hyperlipidemia: total cholesterol ≥220 mg/dl or antihyperlipidemia medication; diabetes: fasting plasma glucose ≥126 mg/dl or non-fasting plasma glucose ≥200 mg/dl or HbA1c ≥6.5% or antidiabetic medication. *p<0.05 between women and men by Student t-test. †p<0.05 between women and men by χ^2 test. HDL, high-density lipoprotein.

Characteristics of Individuals with Rare Missense/Frameshift Mutations in the General Population

The characteristics of the 3,655 subjects comprising the Japanese general population group (1,709 men, 1,946 women) are summarized in Table 4. Age, SBP, DBP, BMI, percentage of current smokers, percentage of current drinkers, and prevalence of hypertension and diabetes mellitus were significantly higher in men than in women. Total cholesterol, high-density lipoprotein (HDL)–cholesterol, and percentage of hyperlipidemia were significantly higher in women than in men. In this population, 1,480 subjects were diagnosed with hypertension.

We successfully genotyped four genetic variations in the general population, which had a sample size of 3,655 individuals (2,175 normotensives and 1,480 hypertensives), but the genotyping failed for two of the genetic variations, T156I and 4884Gdel. In the general population, a missense mutation, R335H, was not present. The remaining three mutations, L14F, R74H, and R147H, were found in both hypertensive and normotensive subjects (Table 5). We identified 14 individuals with the L14F mutation. Six individuals with the L14F mutation had hypertension and eight were normotensive. We identified 20 individuals with the R74H mutation. Among them, eight showed hypertension and 12 were normotensive. We identified 8 individuals with the R147H mutation. Among them, three showed hypertension and five were normotensive. There were no statistically significant differences in any clinical characteristics between the subjects with the three missense mutations of *HSD11B2* and the subjects in the general population (Table 5).

Comparison of Missense/Frameshift Mutations in *HSD11B2* between Normotensives and Combined Hypertensives

As seen in Table 6, there was no difference in the prevalence of missense/frameshift mutations of *HSD11B2* between the combined subjects with hypertension and the normotensives.

Discussion

A missense mutation, P227L, in *HSD11B2* was previously identified in a patient with mild low-renin hypertension (*32*). This patient did not demonstrate the typical features of AME. The authors suggested that patients with mild low-renin hypertension may carry the mutations in the *HSD11B2* gene. In our study, we did not identify the P227L mutation in 953 Japanese hypertensives.

Genetic analyses of *HSD11B2* have been reported in two Japanese AME probands (*14*, *18*). In one family, the proband had a compound heterozygous mutation with a missense mutation, R208H, and a deletion of 3 nucleotides in codons 337–338 resulting in a substitution of Arg337 to His and a deletion of Tyr338 (CGCTAT to CAT: R337H and delta Y338) (*18*). Their family members, a father, mother, and elderly sister, who carried the heterozygous mutation were all normotensive and normokalemic, and had normal ratios of urinary [THF plus aTHF]/THE (THF, tetrahydrocortisol; aTHF, allotetrahydrocortisol; THE, tetrahydrocortisone). Another Japanese patient with AME had the homozygous missense mutation, S180F. The enzymatic activity of this mutant was 1.8% compared with the wild-type enzyme when cortisol was used as the substrate and 5.7% when corticosterone was used as the substrate (*14*). Figure 1 summarizes the reported polymorphisms in *HSD11B2*. In our study, none of the three causative genetic defects was identified, indicating that those mutations were not accumulated in the Japanese population.

We identified five novel missense mutations and one frameshift mutation in *HSD11B2* (Fig. 1, Table 2). As shown in Fig. 2A, five of the missense mutations occurred in residues that were highly conserved among the three different species, indicating that these mutations may result in functional changes in *HSD11B2*. However, neither hypertensive patients nor general subjects with these novel missense mutations showed any distinctive clinical characteristics during their health-check-ups.

We identified one hypertensive patient having renal artery stenosis with a frameshift mutation (4884Gdel) in *HSD11B2*. This deletion caused the frameshift at S219 with a premature stop codon at position 270 (Fig. 2B). A recent report indicated