(というより心リハが必要不可欠な医療であること）を理解してもらうことはたやすいことではありません．わたしたちが取り組んできたことのうち効果的だったと思えるものとして，病棟の看護師の心リハ室研修，理学療法士による病棟看護師への講義，病院レジデントの2カ月間のリハ研修，外科の朝カンファレンスへのリハスタッフの参加，病棟心リハ回診によるリハ必要患者の早期洗い出しなどが挙げられます．

**●今後の展望・方針**

この数年，循環器医療が大きく変わろうとしています．AMIは急性期血行再建療法により重症例が減り，入院期間も極端に短縮しています．また，心大血管手術も体外循環非使用冠動脈バイパス術（OPCAB）を初め，侵襲度の低い手術が多くなり，術後経過も良好となっています．このような症例に望まれているのは，今まで行われてきたような，週に2～3回通院し運動療法をするようなリハとは違うのではないかと感じるようになりました．また，一方で「患者の高齢化」「重症救急症例に対するチャレンジケースにみられがちな重篤な術後合併症や治療抵抗性の心不全例」などへの対応が必要となります．前者には，欧米でも注目されている在宅リハを含めた新しいリハの形や生活習慣そのものに働きかけることのできるようなヘルスプロモーションの整備が必要になるでしょうし，後者には，チームとしての退院支援，生活支援を意識したリハが必要となってくると考えています．

**●心臓リハビリテーションに携わるスタッフの育成**

心リハで最も重要なことは，急変が起こる可能性の高い心疾患患者のリスク管理だと思います．そのためには基本的な知識の習得とスタッフ間での患者情報の共有が重要です．週1回の多職種によるカンファレンスでは，新規導入患者の診療情報の整理や疑問点の解消，個々の患者における注意点の整理などに重点を置いています．また，ハイリスク患者においてはカルテのラベルの色を変えたり，スタッフ数の多い時間帯に検討するように工夫しています．当院では他施設からの見学や研修者も多く，スタッフへの負担となっていますが，人に分かりやすく説明ができることも重要であり，自らの勉強としても積極的にかかわる姿勢を持つようにお願いしています．

表1 ● 心臓リハビリテーション室での集団講義の内容

| |
|---|
| ①心臓リハビリテーションって何だろう？ |
| ②心疾患後の生活に向けて |
| ③心臓病に運動療法は必要ですか？ |
| ④動脈硬化を抑える食生活 |
| ⑤虚血性心疾患とストレス |
| ⑥しっかり禁煙！ |



グへの参加も可能であり，特に筋持久力不足で運動耐容能の改善が悪いと考えられる例は良い適応となると考えています．

**③心臓リハビリテーション室での集団講義（患者教育）**

疾患の理解や日常生活上の注意点，自己管理の仕方などについては，入院中から病棟看護師が中心となって行っていますが，心リハ室としての取り組みは，心リハ室での集団講義として1回30分，全6コマ（表1）の講義を週3回11時から開催し，入院および外来患者向けに行っています．以前には12コマの講義を，主に外来患者を対象として行っていましたが，回数が多い割に効果が薄いように感じられ，入院中からの働きかけの重要性を感じたため変更しました．集団講義はとかく一方通行の情報提供型教育となりがちですが，スタッフが必要事項を分かりやすく患者に伝える技術を培う意味でも重要な取り組みであると考えています．また，隔週土曜日には患者家族のための心肺蘇生法（CPR）・自動体外式除細動器（AED）講習会を行っており，既に総参加者数も1,000名を優に超えています．

**●NPO法人ジャパンハートクラブ府中支部としての活動**

日本心臓リハビリテーション学会の有志が2004年5月に設立したNPO法人ジャパンハートクラブ（濱本拡理事長）[1]は，ドイツの維持期心リハシステムであるAmbulante Herzgruppeを模した組織です．わたしたちは2005年4月より府中支部としての活動を始めました．毎週木曜日の18時と19時からの約1時間，日中のリハと同じことを行いますが，ここでの活動は心臓リハビリテーション指導士が中心となって展開されています．参加者はPhase Ⅱリハ終了後の患者や，高血圧や脂質異常症を抱え心疾患の一次予防として参加する人が主であり，年会費10,000円と1回1,500円の参加費で運動療法を継続することが可能です．現在，会員は120名ほどとなり，毎回30人程度が参加者しています．運動療法のほか，年数回の会員向け講習会や高尾山へのハイキングを行ったり，市のNPOボランティアセンターの催しなどへ参加したりして

## 2. チーム内での看護師の役割

### チームにおける看護師の役割は？

病棟看護師は，入院中の患者のベッドサイドに常にいる存在であり，患者に必要なリハを実施していくこと，患者が安心した退院後の生活を送れることや再発予防・予後改善に向けた支援を行います．

チームの中での看護師の役割は以下の通りです．

①患者が心リハの実施を受け入れられるように，十分な説明と動機づけを行う．

②患者の退院後生活や再発予防・予後改善を見据えアセスメントを行う．

③退院後の生活の送り方について患者指導を行う．

④再発予防・予後改善に必要な生活習慣について患者指導を行う．

⑤患者が再発予防・予後改善に必要な専門家からの支援を受けられるように，他職種や心リハ室と連携する．

心リハ室看護師は，入院中から病棟看護師や理学療法士と連携をとり，患者に対しリハ通院への動機づけを行います．またリハ通院中は，再発予防・予後改善に向け生活習慣を是正し，維持できるように，教育・指導を行います．また，患者が再発予防・予後改善に必要な支援を各専門家から受けることができるよう，他職種との連携を図ります．

### 他職種との連携はどのように？

当院の心リハチームは，心リハ室スタッフだけではなく，患者を中心に，入院部門スタッフと心リハ室との連携によって成り立っており，患者の入院中から入院部門スタッフと心リハ室スタッフとの協力で実施しています（図1）．

AMIの急性期段階負荷試験は，負荷前後の患者の状態評価を医師と看護師が行い，段階負荷は臨床検査技師が実施します．大血管心臓術後は，理学療法士がICUにて立位負荷試験を行い，医師の許可の下，理学療法士が術後リハプログラムの選択を行います．病棟看護師は，理学療法士からの運動計画を基に段階的リハを実施します．

また，患者の状態に合わせて，スタンダードリハプログラム以外のリハや

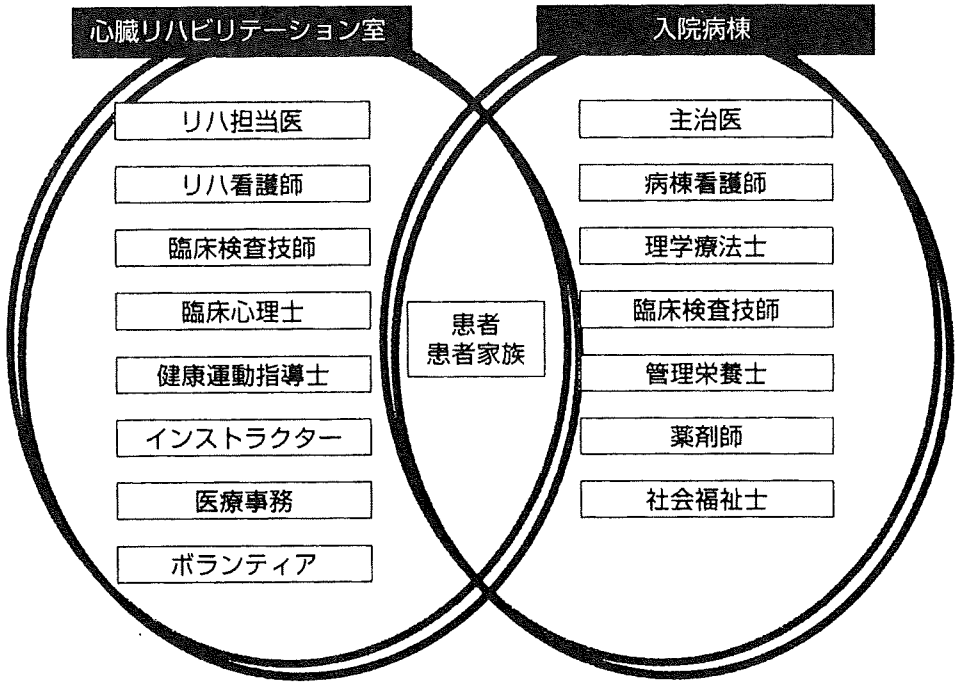


**図1 ● 心臓リハビリテーションチームにおける各職種の連携**

栄養指導，服薬指導などが必要な場合は，病棟看護師から理学療法士，管理栄養士，薬剤師に連絡をとり，個別介入を実施していきます．

なお，心リハ室では，入院中の患者に対してリハ回診を行っており，入院中から退院後のリハ導入に向けた診察を行うとともに，入院中に強化すべきリハに関して主治医や病棟看護師に情報提供しています．心リハ室看護師は定期的に病棟ラウンドを行っており，病棟看護師と患者の情報交換や退院後リハ導入に向けた協力体制をとっています．

## 困難を感じる点は？

### ●チーム活動において

患者にとって心リハは重要な治療です．患者を支援するすべての医療従事者が心疾患の再発予防や予後の改善のためには心リハが必要だと認識すべきだと考えます．しかし，入院中の患者を支える病棟では，目の前の診断・治療に重点が置かれ，長期予後の改善に向けた支援まで至っていないのが現状です．

また，外来リハに通院する患者に対しては，退院後も再発予防や生命予後改善のための継続支援を行うことができますが，外来リハに通院しない患者に対しては継続支援ができていないのが現状です．こうした患者については

外来診療でフォローできればと考えていますが，短い診察時間の中では，メディカルチェックが中心となっています．

主治医や病棟看護師が患者の一生を考えて支援できるようになるためにも，心リハの重要性を周知させ，患者にとって必要な支援は何かということについて医療従事者の認識を一致させることが重要であると考えます．

**●患者とのかかわりにおいて**

①心リハは，患者自らが生きる目標を持ち，そのために必要な再発予防策や生命予後改善に向けた行動を取り入れ，維持できるようになることが必要です．看護師は，患者が疾患罹患後や手術の後の状態を受け入れ，生きる上での目標を持てるようにかかわります．しかし，疾患罹患や手術によって変化した心身の状態を受容することが困難な症例や，生きる希望を失っている症例に対し，短い入院日数の中で考え方の転換を図ることは，当然のことながら困難であり，社会資源も巻き込んだ長期にわたる支援と支援技術のスキルアップも必要です．

②よい生活習慣を取り入れて継続していくためには，長期的支援を行い，評価と状態に合った介入を行っていく必要があります．短い入院日数では，再発予防と予後改善に向けた一般的な情報提供までのかかわりとなり，心リハではよい習慣を取り入れるところまでの支援となっています．現在の支援体制では，心疾患を有する患者すべてに必要な心リハの実施や望ましい生活習慣の維持を支援することが困難です．より多くの患者に，長期にわたる心リハを提供できる体制を構築することが課題であると考えます．

## ■ やりがいは？

**●チーム活動において**

心リハを受ける患者は，疾病を罹患したことによるショックを引きずっていたり，動くことへの恐怖を感じていたりして，心リハが進まないことがあります．また，再発予防や生命予後改善に必要な食事療法や運動療法，禁煙といった行動は，患者の嗜好や習慣を変えることであり，「分かっているけれど，取り入れられない」と訴える患者も少なくありません．

このような，患者が抱えるさまざまな問題に対して，看護師だけで対応するのは困難です．患者の危機心理やストレスのアセスメント方法などについては，臨床心理士に相談しながら進めると，より患者に寄り添った支援につながります．また，動きたくない患者の気持ちを理解し，動けるように支援

する支援や動くための手法については，理学療法士に相談するとよりよい方法を導くことができます．

患者の抱える問題や患者が必要としている支援をチームで共有し，具体的な方法をさまざまな専門家と相談しながら支援することで，よりよい支援を展開することができます．多職種間でのこのような協力は，互いの力の弱部分を補い合い，専門性をさらに生かして患者支援を行うことにつながっていると思います．

**●患者とのかかわりにおいて**

「こんなに痛いのに動きたくない」「自分にはそんな必要はない」．心リハの重要性を伝えていく過程で，心リハに対する否定や拒絶の言葉がきかれることがあります．患者への介入スキルが未熟であったころは，このような患者を「病識がない」と評価し，それ以上の介入をあきらめることもありました．しかし，患者への介入方法についての学びを深めていく中で，否定や拒絶をする患者の奥にある“体験”や“思い”，“価値観”に触れることにより，上記のような反応への理由を知ることができ，その患者に合った介入方法を見いだすことができるようになりました．いつ，どんなことで困るのか，どんなことでつまずくのか，困難やつまずきにどのように対応するかを患者と一緒に考えていくことで，さまざまな工夫方法を学ぶことができました．このような経験の積み重ねが，自分の支援力を高めていると思います．

また，心リハを取り入れ，生き生きと活動する患者から「あのとき嫌がる自分に根気よく付き合ってくれてありがとう」「自身が持てるようになった」との言葉をいただけるようになると，この活動をやっていてよかったと思います．

現在，看護の介入の質の違いにより，患者の疾患受容や生活習慣の是正，維持における効果に違いがあります．患者の再入院率の減少や予後改善において確実に効果があると言えるようになることが今後の課題です．看護支援による効果のエビデンスを得ることで，さらにやりがいを持てるようになると考えます．


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*Original Article*

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# Plasma B-type natriuretic peptide levels reflect the presence and severity of stable coronary artery disease in chronic haemodialysis patients

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## Abstract

**Background.** Coronary artery disease (CAD) is one of the leading causes of morbidity and mortality in haemodialysis (HD) patients. Although the plasma B-type natriuretic peptide (BNP) levels may be a strong marker of long-term mortality in HD patients, what plasma BNP levels reflect is not well known in this setting. Therefore, we examined the relationship between plasma BNP levels and the presence and severity of stable CAD based on coronary angiography (CAG) in chronic HD patients.

**Methods.** Plasma BNP levels were measured in 179 consecutive HD patients who were referred for CAG due to symptoms or objective signs of stable CAD. Left ventricular end-diastolic wall stress (LV EDWS) was also calculated as a crucial haemodynamic determinant of plasma BNP.

**Results.** Plasma BNP levels were significantly higher in patients with CAD than in those with non-CAD. The area under the receiver operating characteristic curve for BNP to predict CAD was 0.837. Plasma BNP levels increased progressively with the extent of CAD [1-vessel disease (VD), 496 ± 49 pg/ml; 2-VD, 932 ± 119 pg/ml; 3-VD, 2073 ± 317 pg/ml; $P < 0.01$]. LV EDWS was well correlated with plasma BNP levels ($r = 0.61$, $P < 0.01$), and a multivariable regression analysis that took into account EDWS demonstrated a significant association between the extent of CAD and BNP ($P < 0.01$).

**Conclusions.** These results suggest that the presence and severity of stable CAD determine plasma BNP levels in chronic HD patients. Plasma BNP levels may be a useful marker in the management of HD patients.

**Keywords:** BNP; coronary artery disease; haemodialysis; left ventricular diastolic wall stress

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## Introduction

The high mortality rate in end-stage renal disease (ESRD), particularly in patients on dialysis, has been well documented by several investigators [1,2]. Cardiovascular diseases account for >50% of ESRD deaths, and the cardiovascular death rates in patients who are receiving dialysis are substantially higher than those in the general population [3]. Coronary artery disease (CAD) is one of the leading causes of death among cardiovascular diseases [4,5]. Therefore, the screening or early diagnosis and aggressive management of CAD are required in long-term dialysis patients.

B-type natriuretic peptide (BNP) is synthesized in the ventricular myocardium in response to ventricular stretching and wall stress (WS) [6,7]. BNP as well as NT-proBNP is widely used as a marker for various cardiovascular diseases. In heart failure, they are used for diagnosis, risk stratification or prognosis and treatment monitoring. In the setting of acute coronary syndrome (ACS), BNP/NT-proBNP has been reported to be an extremely powerful prognostic indicator [8]. Hypoxia, independent of stretching, might also stimulate peptide release [9,10]. Recently, BNP/NT-proBNP has also been shown to be useful in stable CAD patients. Bibbins-Domingo *et al.* reported that elevated plasma BNP levels are independently associated with inducible ischaemia in patients with stable CAD [11] and that they predict cardiovascular morbidity and mortality, independent of other prognostic markers, in the same population [12]. Weber *et al.* demonstrated that NT-proBNP is closely correlated with disease severity in patients with stable CAD [13]. However, their utility and validity in patients with ESRD is not yet established, since their levels are recognized to be strikingly elevated and variable even in most asymptomatic patients with ESRD [14,15]. Recently, the prognostic potential of plasma BNP levels has been investigated in several studies on patients with chronic kidney disease (CKD), haemodialysis (HD) and peritoneal dialysis [16–18]. However, in this setting, it is not yet

clear what plasma BNP levels reflect and whether previous observations in patients without CKD could be applied to ESRD patients.

Accordingly, we tested the hypothesis that plasma BNP levels might reflect the presence and severity of stable CAD in chronic HD patients. We also examined left ventricular end-diastolic wall stress (LV EDWS), which we previously found is a crucial haemodynamic determinant of plasma BNP levels [7], to clarify the contribution of haemodynamic factors in the regulation of plasma BNP in this setting.

## Subjects and methods

### *Study patients*

One hundred seventy-nine chronic HD patients who had been referred for CAG due to symptoms of angina pectoris, or with objective evidence of ischaemia (positive exercise electrocardiogram or nuclear test), were enrolled in the present study. Patients with ACS including unstable angina and acute myocardial infarction were excluded from this study. All patients underwent regular 4-h sessions of HD using polysulfone membrane filters three times weekly. After dialysis, they were shown to have a condition that entailed no clinical signs of hypervolaemia such as oedema, dyspnea or an excessive increase in arterial blood pressure, which was established under the supervision of experienced nephrologists.

Plasma BNP levels, haemoglobin, serum albumin, serum C-reactive protein (CRP) and serum creatinine were determined in blood samples withdrawn immediately before coronary angiography (CAG). Echocardiographic examination was performed after an HD session on the day before CAG.

### *CAG and lesion morphology*

CAG was performed following a standard technique. Two experienced cardiologists who were blinded to plasma BNP levels assessed the coronary angiograms. If they disagreed, a third expert examined the angiogram to determine the characteristics of the lesions. Diameter stenosis of $\geq$70% by quantitative angiography was accepted as significant. Extension of CAD was classified as 1-, 2- or 3-vessel disease (VD) by the standard method. We also estimated the degree of CAD using the Gensini score and the CAD prognostic index. The former is a measure of the extent and severity of CAD and is computed by assigning a severity score to each coronary segment according to the degree of luminal narrowing and its geographic importance [19]. The latter considers the number of diseased vessels, the presence of left anterior descending or left main coronary disease, which have been validated in an overlapping heart failure population [20,21]. Left ventricular pressure was recorded with a 5-F pigtail catheter connected to a fluid-filled transducer. Left ventricular volume and ejection fraction (EF) were determined by left ventriculography with a contrast medium using Kennedy's formula.

### *Echocardiography*

Echocardiographic examinations were performed in all patients with a Sonos 5500 machine equipped with a 2.5 MHz probe. M-mode images were obtained to measure left atrial and ventricular dimensions [22]. The left ventricular mass index (LVMI) was estimated using the formula of Devereux *et al.* In patients with sinus rhythm, the pulsed Doppler transmitral flow velocity was recorded to measure the ratio of peak mitral E-wave velocity to peak mitral A-wave velocity (E/A ratio) and the deceleration time of the mitral E-wave velocity. Based on haemodynamic and echocardiographic data, end-diastolic and systolic meridional WS were calculated as follows: $WS = 0.334 \times P(LVID)/WT(1 + WT/LVID)$, where $P$ = LV pressure (i.e. peak systolic pressure or end-diastolic pressure (EDP), which was obtained during cardiac catheterization), LVID = left ventricular internal dimension and WT = wall thickness [7].

### *Statistical analysis*

Groups were compared using a chi-square analysis for proportions and unpaired Student $t$ tests for continuous variables. Cut-off levels of BNP and the sensitivities and specificities of the cut-off levels were calculated using a receiver operating characteristics (ROC) curve analysis. The linearity of a relationship between two variables was assessed by linear regression analysis. Further multivariable analysis was performed to evaluate the independent relationship between severity of CAD (VD, Gensini score or CAD prognostic index) and plasma BNP levels in concert with demographic variables, haemodynamic indexes and laboratory data using JMP version 5.0. Variables included in the analysis were sex, age, BMI (body mass index), NYHA (New York Heart Association) class, HT (hypertension), DM (diabetes mellitus), HLP (hyperlipidaemia), AF (atrial fibrillation), HD etiology and duration, medications, haemodynamic and echocardiographic indexes and laboratory data (creatinine, CRP, albumin and haemoglobin); $P < 0.05$ was considered significant. Results were expressed as mean $\pm$ SEM.

## Results

### *Patient characteristics*

The baseline clinical characteristics in chronic HD patients according to the presence and extent of CAD are shown in Tables 1 and 2, respectively. In all of the studied patients, the mean age was 67.6 $\pm$ 0.7 years and 13% of the patients were female. Patients with CAD were more likely to have a history of DM and HLP. There were no significant differences in other past history, duration of HD, etiology, medications or haemoglobin and creatinine levels between CAD and non-CAD or among the three CAD-extension groups. Patients with CAD showed a higher CRP level than those with non-CAD, but there was no difference among the three CAD-extension groups. Patients with 1-VD had a higher serum albumin level than with 2-VD or 3-VD. Patients who showed NYHA functional class $\geq$2 were more prevalent in those with multivessel disease or CAD.

Geometric and functional parameters obtained by echocardiography or cardiac catheterization are shown in Table 3. In all of the studied patients, mean EF was 45.5 ± 1.1% and mean LVMI, LV end-diastolic volume index (LVEDVI) and LV EDWS were 163.5 ± 4.3 g/m$^2$, 84.4 ± 3.0 ml/m$^2$ and 35.5 ± 2.0 kdynes/cm$^2$, respectively.

**Table 1.** Patient characteristics [1]

| | Non-CAD | CAD | *P*-value |
|---|---|---|---|
| *N* | 51 | 128 | |
| Age (years) | 66.5 ± 1.3 | 68.1 ± 0.8 | 0.30 |
| Females | 9 (18%) | 15 (12%) | 0.30 |
| BMI (kg/m$^2$) | 22.6 ± 0.4 | 22.1 ± 0.5 | 0.50 |
| HT | 49 (96%) | 120 (94%) | 0.51 |
| DM | 19 (37%) | 72 (56%) | 0.02 |
| HLP | 15 (29%) | 60 (47%) | 0.02 |
| AF | 2 (4%) | 11 (9%) | 0.25 |
| OMI | 9 (18%) | 38 (30%) | 0.11 |
| CABG | 3 (6%) | 20 (16%) | 0.23 |
| NYHA class ≥ 2 | 5 (10%) | 44 (34%) | <0.01 |
| HD duration (years) | 6.5 ± 1.2 | 7.7 ± 0.7 | 0.87 |
| Etiology | | | |
| nephrosclerosis | 23 (45%) | 44 (34%) | 0.32 |
| DM | 16 (31%) | 52 (41%) | 0.56 |
| CGN | 8 (16%) | 26 (20%) | 0.61 |
| PCKD | 2 (4%) | 3 (2%) | 0.32 |
| Others | 2 (4%) | 3 (2%) | 0.32 |
| Medication | | | |
| ACEI or ARB | 23 (45%) | 60 (47%) | 0.80 |
| β-blocker | 29 (57%) | 77 (61%) | 0.54 |
| Ca blocker | 49 (96%) | 122 (95%) | 0.62 |
| Laboratory | | | |
| BNP (pg/ml) | 285 ± 30 | 1237 ± 144 | <0.001 |
| Cr (mg/dl) | 8.5 ± 0.5 | 8.7 ± 0.3 | 0.65 |
| Haemoglobin (g/dl) | 10.3 ± 0.1 | 10.1 ± 0.1 | 0.39 |
| Albumin (g/dl) | 3.70 ± 0.05 | 3.73 ± 0.04 | 0.52 |
| CRP (mg/dl) | 0.29 ± 0.05 | 0.52 ± 0.06 | 0.02 |

ACEI = angiotensin-converting enzyme inhibitor; AF = atrial fibrillation; ARB = angiotensin receptor blocker; BMI = body mass index; CABG = coronary artery bypass grafting; CGN = chronic glomerulonephritis; Cr = serum creatinine; CRP = C-reactive protein; DM = diabetes mellitus; HD = haemodialysis; HLP = hyperlipidaemia; HT = hypertension; NYHA = New York Heart Association; OMI = old myocardial infarction; PCKD = polycystic kidney disease.
Values are mean ± SEM or number (%).

*Cut-off level for detecting CAD*

Fifty-one patients who underwent CAG had no significant coronary stenotic lesions. Plasma BNP levels were significantly higher in patients with CAD than in those with non-

**Table 2.** Patient characteristics [2]

| | 1-VD | 2-VD | 3-VD | *P*-value |
|---|---|---|---|---|
| *N* | 44 | 33 | 51 | |
| Age (years) | 67.7 ± 1.2 | 68.1 ± 1.8 | 68.4 ± 1.2 | 0.92 |
| Females | 2 (5%) | 5 (15%) | 8 (15%) | 0.14 |
| BMI (kg/m$^2$) | 22.0 ± 0.4 | 21.3 ± 0.5 | 22.6 ± 1.0 | 0.28 |
| HT | 39 (89%) | 33 (100%) | 48 (96%) | 0.09 |
| DM | 20 (46%) | 18 (56%) | 34 (68%) | 0.09 |
| HLP | 17 (39%) | 16 (49%) | 27 (53%) | 0.31 |
| AF | 4 (9%) | 2 (6%) | 5 (10%) | 0.82 |
| OMI | 12 (27%) | 9 (27%) | 17 (33%) | 0.56 |
| CABG | 7 (16%) | 2 (6%) | 11 (22%) | 0.12 |
| NYHA class ≥ 2 | 4 (9%) | 13 (44%) | 27 (53%) | 0.01 |
| HD duration (yrs) | 9.9 ± 1.6 | 7.6 ±1.4 | 6.2 ± 0.8 | 0.10 |
| Etiology | | | | |
| nephrosclerosis | 16 (36%) | 9 (28%) | 19 (37%) | 0.32 |
| DM | 12 (27%) | 15 (46%) | 25 (49%) | 0.18 |
| CGN | 13 (29%) | 7 (21%) | 6 (12%) | 0.08 |
| PCKD | 1 (2%) | 1 (3%) | 1 (2%) | 0.32 |
| Others | 1 (2%) | 1 (3%) | 1 (2%) | 0.32 |
| Medication | | | | |
| ACEI or ARB | 20 (45%) | 14 (44%) | 26 (51%) | 0.78 |
| β-blocker | 28 (63%) | 23 (72%) | 26 (51%) | 0.14 |
| Ca blocker | 41 (93%) | 32 (96%) | 47 (93%) | 0.42 |
| Laboratory | | | | |
| BNP (pg/ml) | 496 ± 49 | 932 ± 119 | 2073 ± 317 | <0.001 |
| Cr (mg/dl) | 8.2 ± 0.8 | 9.1 ± 0.5 | 8.8 ± 0.4 | 0.32 |
| Haemoglobin (g/dl) | 10.3 ± 0.2 | 10.2 ± 0.3 | 10.0 ± 0.2 | 0.58 |
| Albumin (g/dl) | 3.90 ± 0.06 | 3.65 ± 0.06 | 3.66 ± 0.06 | 0.01 |
| CRP (mg/dl) | 0.41 ± 0.07 | 0.49 ± 0.15 | 0.65 ± 0.11 | 0.23 |
| Gensini score | 33.6 ± 3.4 | 49.0 ± 4.0 | 84.7 ± 5.8 | <0.001 |
| CAD prognostic index | 27.2 ± 1.3 | 42.6 ± 1.9 | 68.8 ± 1.7 | <0.001 |

ACEI = angiotensin-converting enzyme inhibitor; AF = atrial fibrillation; ARB = angiotensin receptor blocker; BMI = body mass index; CABG = coronary artery bypass grafting; CAD = coronary artery disease; CGN = chronic glomerulonephritis; CGN = chronic glomerulonephritis; Cr = serum creatinine; CRP = C-reactive protein; DM = diabetes mellitus; HD = haemodialysis; HLP = hyperlipidaemia; HT = hypertension; NYHA = New York Heart Association; OMI = old myocardial infarction; PCKD = polycystic kidney disease.
Values are mean ± SEM or number (%).

**Table 3.** Echocardiographic and haemodynamic parameters

| | Non-CAD | 1-VD | 2-VD | 3-VD | *P*-value |
|---|---|---|---|---|---|
| LVEDD (mm) | 48.9 ± 1.0 | 51.6 ± 1.2 | 52.7 ± 1.3 | 54.6 ± 1.0 | <0.01 |
| PWT (mm) | 11.3 ± 0.3 | 10.3 ± 0.3 | 11.2 ± 0.3 | 10.3 ± 0.3 | 0.01 |
| LAD (mm) | 42.3 ± 0.8 | 43.8 ± 1.0 | 43.0 ± 1.6 | 42.7 ± 1.1 | 0.79 |
| E/A | 0.80 ± 0.05 | 0.98 ± 0.12 | 0.83 ± 0.07 | 0.93 ± 0.09 | 0.44 |
| DCT (msec) | 249 ± 9 | 219 ± 11 | 226 ± 12 | 203 ± 10 | 0.03 |
| LVMI (g/m$^2$) | 152 ± 10 | 157 ± 6 | 183 ± 10 | 167 ± 7 | 0.02 |
| EF (%) | 52.7 ± 1.6 | 45.8 ± 2.2 | 44.0 ± 2.6 | 40.1 ± 1.9 | <0.01 |
| LVEDVI (ml/m$^2$) | 73.5 ± 4.1 | 88.0 ± 5.8 | 85.0 ± 7.4 | 90.8 ± 6.5 | 0.18 |
| LVSP (mmHg) | 147 ± 3 | 145 ± 4 | 146 ± 4 | 140 ± 4 | 0.52 |
| LVEDP (mmHg) | 12.4 ± 0.6 | 13.7 ± 1.0 | 14.7 ± 1.1 | 18.4 ± 0.9 | <0.01 |
| EDWS (kdynes/cm$^2$) | 23.8 ± 1.6 | 34.0 ± 5.1 | 32.5 ± 3.1 | 48.7 ± 3.8 | <0.01 |

DCT = deceleration time of early diastolic filling; EDWS = end-diastolic wall stress; EF = ejection fraction; E/A = ratio of peak mitral E-wave velocity to peak mitral A-wave velocity; LAD = left atrial dimension; LVEDD = left ventricular end-diastolic dimension; LVEDP = left ventricular end-diastolic pressure; LVEDVI = left ventricular end-diastolic volume index; LVMI = left ventricular mass index; LVSP = left ventricular peak systolic pressure; PWT = posterior wall thickness.
Values are mean ± SEM.

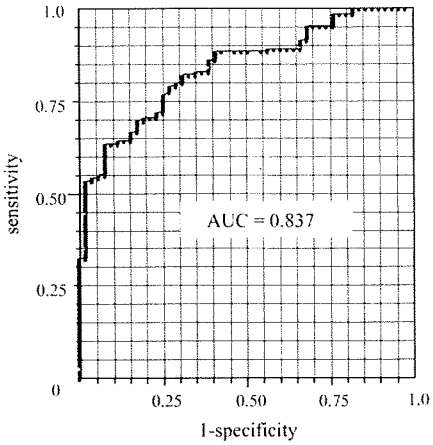


**Fig. 1.** Receiver operating characteristic (ROC) curve for plasma BNP as a predictor of relevant coronary artery disease. AUC = area under the ROC curve.

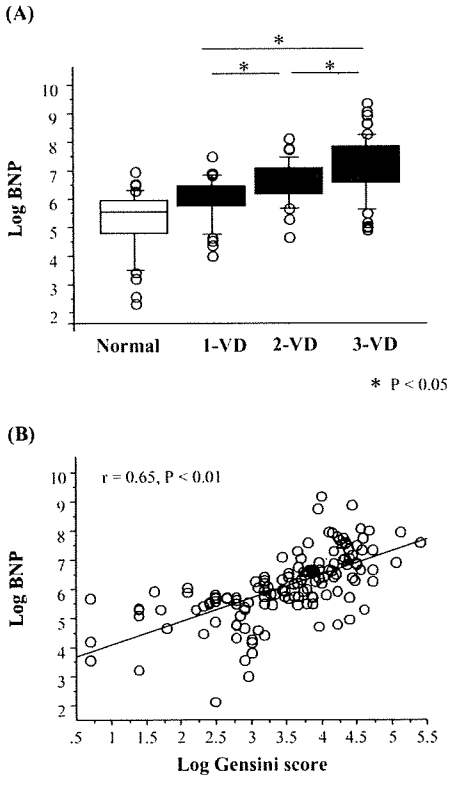


**Fig. 2.** **(A)** Plasma BNP levels in relation to the number of coronary arteries with >70% diameter stenosis. The box defines the interquartile range with the median indicated by the crossbar. The error bars indicate the 10th and 90th percentiles. **(B)** Correlation between log plasma BNP level and log Gensini score.

CAD (1237 ± 144 and 285 ± 30 pg/ml, respectively; $P < 0.01$). The ROC curve for BNP as an indicator of the presence of CAD is shown in Figure 1. The area under the ROC curve was 0.837 (95% confidence interval 0.778–0.895). The optimal value of BNP as an indicator of CAD was 366 pg/ml, with a sensitivity of 79%, a specificity of 73%, an accuracy of 77%, a positive predictive value of 88% and a negative predictive value of 58%.

*CAD extension and plasma BNP levels*

Of the 128 patients in the CAD groups, 44, 33 and 51 patients had 1-VD, 2-VD and 3-VD, respectively. As shown in Figure 2, plasma BNP levels increased progressively with the extent of CAD (1-VD, 496 ± 49 pg/ml; 2-VD, 932 ± 119 pg/ml; 3-VD, 2073 ± 317 pg/ml; $P < 0.01$). Furthermore, they correlated well with the Gensini score ($r = 0.65$, $P < 0.01$) or the CAD prognostic index ($r = 0.60$, $P < 0.01$). Thus, plasma BNP levels were directly correlated to the extent of CAD, and the difference between each category was highly significant.

*Haemodynamic parameters and plasma BNP levels*

In comparisons among the CAD groups, there were no significant differences in LVMI ($P = 0.08$), LV volume ($P = 0.83$) or EF ($P = 0.15$). However, higher LV EDWS and EDP were observed in the 3-VD group ($P < 0.01$), as shown in Table 3. Also, the non-CAD group showed lower LV volume and EDWS and higher EF than CAD group.

As demonstrated in Figure 3, LV EDWS and EF were well correlated with plasma BNP levels ($r = 0.61$ and 0.53, $P < 0.01$, respectively) and LVMI was significantly, but poorly, correlated ($r = 0.27$, $P = 0.009$). Furthermore, a multivariable regression analysis that took into account EDWS demonstrated a significant positive association between the Gensini score, the extent of CAD (the number of diseased vessels), or the CAD prognostic index and plasma BNP levels. In addition, EF was independently associated with plasma BNP level, whereas LVMI and NYHA $\geq 2$ were unrelated to plasma BNP once the effects of the EDWS, Gensini score and EF were accounted for (Table 4). The fit ($R$-square) of the model including these variables was 0.53.

## Discussion

CAD is one of the leading causes of morbidity and mortality in chronic HD patients. However, since most patients remain asymptomatic because of deconditioning, limited activity levels and the effects of long-standing DM, the early detection of CAD is difficult. Recently, Charytan *et al.* studied 67 asymptomatic HD patients who volunteered for CAG with a median follow-up of 2.7 years [23]. They showed that 41.7% of the patients had CAD and that the proximal CAD, multivessel disease or the CAD prognostic index >48 was associated with higher mortality. CAG is a definitive diagnostic tool, but it is invasive. It is essential to diagnose the presence and severity of CAD by non-invasive tests as early as possible in HD patients. In the present study, although the area under the ROC curve was <0.85, which makes a BNP test of limited clinical value for detecting CAD, the present result seems to be superior to other reports on patients with no CKD [13] or on those with CKD not requiring dialysis [24]. Also, the diagnostic utility

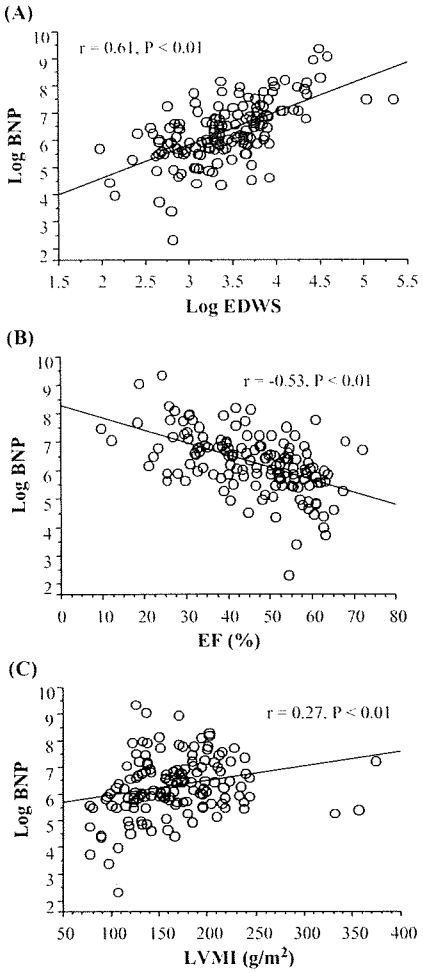


**Fig. 3.** Correlations between log BNP level and (**A**) log LV end-diastolic wall stress (EDWS), (**B**) LV ejection fraction (EF) and (**C**) LV mass index (LVMI) in all studied patients.

**Table 4.** Predictors for BNP levels in regression analysis

| | Log BNP | |
|---|---|---|
| Parameter | β-coefficient | *P*-value |
| NYHA ≥ 2 | | NS |
| LVMI | | NS |
| EF | −0.018 | 0.009 |
| Log EDWS | 0.694 | < 0.001 |
| Log Gensini score | 0.012 | < 0.001 |

NYHA = New York Heart Association; LVMI = left ventricular mass index; EF = ejection fraction; EDWS = end-diastolic wall stress.
Significant univariable predictors were included into the multivariable regression model as continuous and NYHA ≥ 2 as a binary variable.

of other non-invasive tests, including exercise ECG, dobutamine stress echocardiography and scintigraphy for patients with CKD, is reported to be less than that observed in those with no CKD [25]. Thus, the measurement of plasma BNP levels in combination with other non-invasive investigations might help in assessing CAD involvement and aggressive management in this high-risk population.

Several recent studies have suggested that plasma BNP levels may have prognostic potential in chronic HD patients. Zoccali *et al.* demonstrated that BNP was an independent predictor of overall and cardiovascular mortality in HD patients [17]. Cataliotti *et al.* reported that BNP was significantly higher in dialysis patients who died of cardiovascular causes than in survivors [26]. Although LV mass and function have been considered to be important associated factors, ischaemia was not sufficiently considered in these reports. There have been few studies on the association between plasma BNP level and ischaemia itself in chronic HD patients. Although both Osajima *et al.* and Nishikimi *et al.* reported elevated plasma BNP levels in HD patients with CAD [27,28], the diagnostic evaluation of CAD does not seem to be sufficient and the sample number was relatively small. In the present study, we used CAG in all patients for a thorough evaluation of disease severity. The number of diseased coronary arteries, the Gensini score or the CAD prognostic index well correlated with the plasma BNP levels in our study population. Plasma BNP levels may achieve prognostic potential, at least in part, by reflecting the presence and severity of CAD in chronic HD patients. Recently, BNP/NT-proBNP has also been shown to be useful in stable CAD patients with normal renal function. Plasma BNP levels could predict the extent of angiographic coronary artery stenosis and prognosis in patients with stable angina pectoris [13,29] as well as in those with ACS [30]. McClure *et al.* reported that, in patients with coronary ischaemia, removal of coronary stenosis by percutaneous coronary revascularization resulted in decrease of plasma NT-proBNP [31]. Although the pathophysiological mechanism behind the relation between CAD and elevated BNP levels is not well defined, Goetze *et al.* reported that tissue hypoxia alone could trigger release of BNP in the absence of LV dysfunction [10]. We recently demonstrated that LV EDWS is a crucial haemodynamic determinant of plasma BNP levels in patients with chronic heart failure [7]. Therefore, we measured LV EDWS and tried to clarify the independent role of chronic ischaemia on plasma BNP from haemodynamic load in the setting of CAD in HD patients. As a result, LV EDWS was associated with plasma BNP levels, but to a lesser extent than in patients with heart failure [7,32]. The extent of CAD (including the Gensini score or the CAD prognostic index) was correlated with plasma BNP levels independent of the haemodynamic load according to a multivariable analysis. Chronic ischaemia itself might contribute to the elevated BNP levels in the present setting.

Several limitations should be considered in interpreting our results. First, the study population was relatively small, especially in the non-CAD group. Any negative findings could thus be caused by a low statistical power. Second, only plasma BNP levels were considered in our study. Recently, the measurement of NT-proBNP was increasingly used clinically because of its longer half-life and larger size. NT-proBNP might be more dependent on renal clearance than BNP [33]. However, most studies have demonstrated that both are equally useful, even in CKD and HD patients [34,35]. Third, echocardiography was typically performed

the day before cardiac catheterization. This time lag could have influenced the results. Last, in the present study, a cohort of HD patients consisted of those who had been referred for CAG due to symptoms or objective evidence of ischaemia, and asymptomatic patients with lack of objective evidence of ischaemia were not included. Therefore, the applicability of our results to the screening for CAD in all HD patients might be limited.

The present study clearly showed that plasma BNP levels were closely correlated with disease severity as assessed by the number of stenotic coronary arteries, the Gensini score and CAD prognostic index in chronic HD patients with CAD. In addition, they had significantly higher plasma BNP levels than those with non-CAD. We also analyzed EDWS and showed that chronic ischaemia itself might contribute to the increased BNP levels in addition to the EDWS in this setting. Therefore, our data suggest that plasma BNP levels may be a useful marker in the diagnosis and follow-up of stable CAD in patients with chronic HD by reflecting both the haemodynamic load and the presence and severity of ischaemia.

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*Conflict of interest Statement.* None declared.

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# Identification of Genetic Markers Associated With High-Density Lipoprotein-Cholesterol by Genome-Wide Screening in a Japanese Population

## —— The Suita Study ——

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***Background:*** Recent genome-wide association studies (GWAS) have identified genes or loci affecting lipid levels. Given the difference in allele frequencies and linkage disequilibrium patterns across the populations, a GWAS was conducted using the Illumina 550K in a Japanese population (n=900) in search of population-specific genetic variations associated with high-density lipoprotein (HDL)-cholesterol.

***Methods and Results:*** Among the 368,274 single nucleotide polymorphisms (SNPs) with a minor allele frequency of at least 0.1, 43 SNPs exceeded the arbitrary threshold of $-\log_{10}P > 4.0$. The most significant SNP was rs3764261, located 5'upstream of *CETP*, exhibiting a $-\log_{10}P$ value of 6.17. Increasing the sample size by genotyping in the additional Suita sample (n=1,810) further improved the level of significance, with each additional copy of the minor allele being associated with an increase in HDL-cholesterol by 6.2 mg/dl ($P=3.4\times10^{-12}$). Interestingly, the minor allele was more prevalent in cases with myocardial infarction than in controls (0.221 vs 0.196, nominal P=0.02).

***Conclusions:*** The association between genetic variants at *CETP* and HDL-cholesterol was replicated in our sample. None of the genetic variants exerted a greater influence on HDL levels than those at *CETP*. Associations for the top-ranked SNPs need to be tested for further replication in an independent sample. (*Circ J* 2009; **73:** 1119–1126)

**Key Words:** Genetics; HDL-cholesterol; Single nucleotide polymorphism

High-density lipoprotein (HDL)-cholesterol is one of the well-established independent risk factors for cardiovascular disease, and an inverse association between circulating HDL-cholesterol levels and the risk of coronary heart disease has been consistently demonstrated in epidemiological studies[1–5] Because HDL-cholesterol is a routinely measured quantitative trait with substantial heritability, genetic determinants of HDL-cholesterol have been previously studied by a candidate gene approach[6] Common polymorphisms in *CETP*, *LPL*, *LIPC*, *LIPG* and *APOA1* have been reported to be significantly associated with HDL-cholesterol levels in Japanese[7–11]

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## Editorial p 1016

There have been an increasing number of genome-wide association studies (GWAS) being conducted, followed by replication studies in independent populations. This GWAS approach has led to the identification of previously unrecognized associations between genetic variants and common diseases[12–16] or phenotypic traits[17,18]

In the previously performed genome-wide association analyses of HDL-cholesterol, the most strongly and consistently associated single nucleotide polymorphisms (SNPs) with genome-wide significance, have been reported to be near or in the *CETP* gene at 16q13[18–21] The genome-wide screening of 341,518 SNPs in 6,382 white women has found rs3764261 located upstream of *CETP* to be the most strongly associated SNP ($P=1.05\times10^{-41}$), accounting for 3% of the residual variance in HDL-cholesterol[19] A recent meta-analysis based on 3 GWAS results of 8,816 white individuals, and the subsequent replication involving 11,569 individuals, have not only confirmed the previously reported associations between HDL-cholesterol and genetic variations in *CETP*, *LPL*, *LIPC*, *LIPG* and *ABCA1* genes but have also identified novel genetic loci for HDL-cholesterol; near *MVK-MMAB* and *GALNT2*[18]

While GWAS have been increasingly utilized to identify

**Table 1. Clinical Characteristics of the Study Populations**

| | GWAS sample | | Suita sample | |
|---|---|---|---|---|
| | Men | Women | Men | Women |
| No. of subjects | 406 | 494 | 1,468 | 1,760 |
| Age (year) | 59.8±7.3 | 58.2±6.8 | 66.0±10.7 | 63.8±10.5 |
| BMI (kg/m$^2$) | 23.3±2.8 | 22.2±3.0 | 23.4±2.9 | 22.4±3.2 |
| HDL-cholesterol (mg/dl) | 54.9±13.5 | 65.3±15.5 | 54.8±14.3* | 64.6±15.0* |
| LDL-cholesterol (mg/dl) | 123.5±29.6 | 136.6±32.1 | 121.1±28.7* | 134.3±30.4* |
| Triglyceride (mg/dl) | 123.2±90.6 | 93.3±54.6 | 119.0±84.8* | 93.0±55.6* |
| % Medication for dyslipidemia | 0 | 0 | 11.0 | 18.5 |

Continuous variables are mean±standard deviation (SD).
*Subjects with lipid-lowering medication were excluded.
GWAS, genome-wide association studies; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

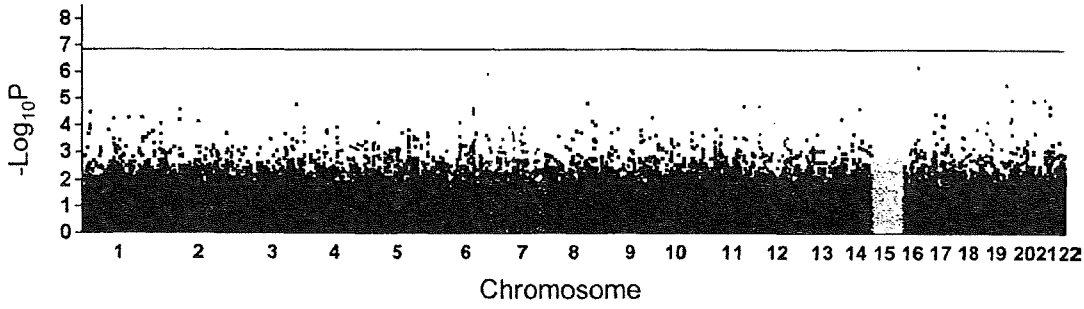


**Figure 1.** Genome-wide association analysis for high-density lipoprotein (HDL) levels. The levels of significance expressed as $-\log_{10}P$ on the basis of the association analysis of 368,274 single nucleotide polymorphisms with HDL-cholesterol adjusted for sex, body mass index and daily ethanol consumption (g) are plotted against chromosomal position. The genome-wide significance level is indicated by the line.

previously unrecognized genotype–phenotype associations, GWAS have been predominantly conducted in populations of European ancestry and the data on non-European populations are scarce. Given the difference in allele and genotype frequencies and linkage disequilibrium (LD) patterns across the populations, the results obtained in white populations might not be directly applicable to the Japanese population. Population-specific GWAS might identify additional loci or genes influencing HDL-cholesterol levels, revealing pathways or underlying mechanisms for anti-atherogenic property of HDL. Thus, we conducted a genome-wide association analysis in a Japanese population in search of population-specific genetic variations associated with HDL-cholesterol levels.

## Methods

### Study Population

Subjects who were between 40 and 75 years of age and not on medication for dyslipidemia were randomly selected from the Suita Study for genotyping by Illumina 550K (n=900; age range, 42–73 years). The study design of the Suita Study has been described previously[22–29] In brief, the sample consisted of 14,200 men and women (30 to 79 years of age at enrolment), stratified by gender and 10-year age groups (10 groups and 1,420 subjects in each group) who had been randomly selected from the municipal population registry. They were all invited by the use of a letter, to attend regular cycles of follow-up examinations (every 2 years).

To examine the association between genetic variations influencing HDL-cholesterol levels and the risk of myocardial infarction (MI), allele and genotype frequencies of HDL-associated SNPs (rs3764261 and rs467571) were compared between controls and MI cases. Subjects who were recruited into the Suita Study between April 2002 and February 2004 and were free from coronary artery disease served as controls (n=3,097). Patients with MI were randomly selected in- and outpatients with documented MI and were enrolled in the Division of Cardiology at the National Cardiovascular Center between May 2001 and April 2003 (n=589). Both controls and MI cases were of the same ethnicity (Japanese).

Only those who gave written informed consent were included in the study. The study protocol was approved by the Institutional Ethics Committee, and the Committee on Genetic Analysis and Gene Therapy of the National Cardiovascular Center.

Subjects were asked to estimate the amount and frequency of alcohol intake per week. Alcohol consumption was expressed as ethanol (g) per day.

### Genotyping Assays

The genome-wide scan was carried out in 900 Japanese patients of both sexes using the Illumina Sentrix Human Hap550 BeadChip (Illumina Inc, San Diego, CA, USA). Genotyping was performed by Illumina Inc (San Diego, CA, USA). SNPs with a call rate of less than 90% and/or with a minor allele frequency (MAF) of less than 0.1 were excluded from the study, leaving 368,274 autosomal SNPs for the analysis. Deviation from the Hardy–Weinberg Equilibrium and the degree of LD were analyzed using HaploView 4.0 (http://www.broad.mit.edu/mpg/haploview/)[30]

Twenty-two SNPs were genotyped in the remaining Suita sample (n=1,000–1,500) for validation of the associations

**Table 2. Summary of the Top-Ranked SNPs Associated With HDL-Cholesterol in the Initial GWAS Results**

| SNP | P value† | HWE | Call rate | MAF | [A/B] | Frequency | | | Mean±SD | | | β | P value‡ | Ch | Gene symbol | Position (Mb) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | AA | AB | BB | AA | AB | BB | | | | | |
| rs3764261* | 6.17 | 0.852 | 100 | 0.196 | [A/C] | 37 | 280 | 583 | 8.54±15.71 | 1.94±13.07 | −1.47±13.06 | 5.6 | 1.9E−04 | 16 | *CETP* | 55.6 |
| rs10945991* | 5.90 | 0.176 | 100 | 0.135 | [A/C] | 666 | 223 | 11 | −0.11±13.03 | −0.68±12.82 | 20.64±25.81 | 14.0 | 1.9E−07 | 6 | | 164.8 |
| rs6509732* | 5.50 | 0.139 | 100 | 0.164 | [A/G] | 618 | 264 | 18 | −0.21±13.88 | −0.57±10.9 | 15.58±19 | 10.7 | 4.7E−07 | 19 | *ZNF665* | 58.4 |
| rs6133175* | 4.98 | 0.489 | 100 | 0.327 | [A/G] | 103 | 382 | 415 | 4.60±13.83 | 0.88±13.21 | −1.95±13.07 | 3.4 | 2.2E−04 | 20 | *SLC23A2* | 4.8 |
| rs467571* | 4.97 | 0.731 | 100 | 0.101 | [T/C] | 733 | 156 | 11 | −0.35±13.02 | 0.30±13.56 | 18.94±20.38 | 12.7 | 2.9E−06 | 21 | *FLJ45139* | 39.2 |
| rs10485472* | 4.90 | 1.000 | 100 | 0.201 | [A/G] | 36 | 294 | 570 | 10.13±13.43 | 0.14±13.07 | −0.71±13.29 | 6.9 | 4.5E−06 | 20 | | 58.9 |
| rs1469918 | 4.82 | 0.700 | 100 | 0.191 | [A/G] | 33 | 273 | 594 | −10.34±9.89 | 1.22±13.84 | 0.02±13.09 | −7.3 | 3.6E−06 | 8 | | 108.8 |
| rs6790597* | 4.78 | 0.980 | 100 | 0.207 | [T/C] | 38 | 301 | 561 | −4.50±13.20 | −2.38±12.69 | 1.59±13.5 | −2.8 | 5.6E−02 | 3 | | 177.0 |
| rs12225506 | 4.72 | 0.553 | 100 | 0.363 | [A/G] | 123 | 403 | 374 | 3.39±15.93 | −2.16±12.51 | 1.22±13 | 2.6 | 2.8E−03 | 11 | | 114.5 |
| rs1544669* | 4.70 | 0.009 | 100 | 0.171 | [A/C] | 630 | 230 | 40 | 1.07±13.72 | −1.53±12.24 | −8.02±10.31 | −5.2 | 3.2E−04 | 12 | *BCL2L14* | 12.1 |
| rs12206635 | 4.64 | 0.412 | 99.4 | 0.410 | [T/G] | 142 | 444 | 309 | −4.63±11.86 | 1.30±13.89 | 0.11±12.87 | −3.6 | 1.2E−05 | 6 | | 130.8 |
| rs2246454 | 4.62 | 0.681 | 100 | 0.243 | [A/C] | 54 | 329 | 517 | 6.89±12.43 | −1.83±12.68 | 0.45±13.64 | 5.1 | 5.1E−05 | 14 | *C14orf118* | 75.7 |
| rs980861* | 4.58 | 0.841 | 100 | 0.394 | [T/C] | 329 | 435 | 136 | −1.56±12.74 | −0.26±13.38 | 4.62±13.91 | 3.7 | 8.2E−06 | 2 | | 57.5 |
| rs12134357* | 4.49 | 0.405 | 100 | 0.366 | [T/C] | 351 | 436 | 113 | 2.52±13.24 | −1.51±13.71 | −1.98±11.17 | −1.7 | 6.3E−02 | 1 | *RAP1GAP* | 21.8 |
| rs17059002 | 4.45 | 0.506 | 100 | 0.411 | [A/G] | 310 | 446 | 144 | 0.10±12.84 | 1.36±13.79 | −4.41±12.29 | −3.4 | 2.2E−05 | 6 | *TMEM200A* | 130.8 |
| rs11654690* | 4.44 | 0.770 | 100 | 0.243 | [T/C] | 52 | 343 | 505 | 7.40±18.62 | 0.57±13.05 | −1.15±12.69 | 5.1 | 5.4E−05 | 17 | *PSMB6, PLD2* | 4.7 |
| rs2236639 | 4.42 | 0.436 | 100 | 0.358 | [T/C] | 108 | 428 | 364 | 1.23±13.99 | −2.08±12.19 | 2.09±14.15 | 0.8 | 3.7E−01 | 22 | *CCT8L2* | 15.5 |
| rs280049* | 4.34 | 0.760 | 100 | 0.259 | [T/C] | 62 | 344 | 494 | 4.00±13.04 | 1.77±13.59 | −1.73±13.02 | 2.7 | 2.3E−02 | 17 | *ACCN1* | 29.0 |
| rs7547186* | 4.31 | 0.276 | 100 | 0.301 | [T/C] | 444 | 365 | 91 | −0.52±13.27 | −0.84±13.33 | 5.88±12.72 | 4.4 | 8.8E−06 | 1 | *ESRRG* | 215.0 |
| rs7550051* | 4.31 | 0.989 | 100 | 0.161 | [T/C] | 639 | 238 | 23 | 1.26±13.99 | −2.98±11.51 | −4.14±6.97 | −2.2 | 2.4E−01 | 1 | | 210.4 |
| rs4656747 | 4.30 | 0.650 | 100 | 0.122 | [A/G] | 14 | 189 | 697 | −11.38±11.17 | 2.82±13.55 | −0.53±13.19 | −8.4 | 4.9E−04 | 1 | | 168.4 |
| rs2813397 | 4.29 | 0.802 | 100 | 0.296 | [T/C] | 442 | 381 | 77 | 1.07±13.78 | 0.02±12.51 | −6.24±13.59 | −4.6 | 1.9E−05 | 10 | *ADARB2* | 1.6 |
| rs12586473* | 4.27 | 0.435 | 99.2 | 0.374 | [A/G] | 358 | 405 | 130 | 2.47±13.71 | −1.72±12.84 | −0.97±13.26 | −0.9 | 2.9E−01 | 14 | | 27.5 |
| rs3914810 | 4.27 | 0.949 | 100 | 0.351 | [A/G] | 381 | 406 | 113 | −2.00±13.14 | 0.80±13.24 | 3.88±13.56 | 3.0 | 8.3E−04 | 20 | *SLC23A2* | 4.9 |
| rs10493889* | 4.24 | 0.560 | 100 | 0.204 | [T/C] | 566 | 299 | 35 | −0.99±12.8 | 0.86±13.84 | 8.75±15.01 | 5.9 | 1.3E−04 | 1 | | 97.2 |
| rs9956878* | 4.17 | 0.774 | 100 | 0.245 | [A/C] | 53 | 340 | 507 | 7.74±15.48 | −0.24±13.22 | −0.65±13.01 | 5.5 | 1.5E−05 | 18 | | 71.7 |
| rs10496565* | 4.14 | 0.342 | 100 | 0.441 | [A/G] | 167 | 459 | 274 | −4.06±11.46 | 0.85±13.45 | 1.05±13.89 | −3.4 | 1.3E−05 | 2 | *CLASP1* | 121.9 |
| rs4815298 | 4.13 | 0.699 | 100 | 0.452 | [A/G] | 272 | 446 | 182 | 2.32±12.97 | −1.92±13.17 | 1.23±13.84 | 0.7 | 3.5E−01 | 20 | *TMC2* | 12.5 |
| rs6990139 | 4.13 | 0.820 | 100 | 0.279 | [T/C] | 66 | 357 | 477 | 1.66±13.60 | 2.13±13.81 | −1.82±12.76 | 1.0 | 3.8E−01 | 8 | | 123.6 |
| rs9359845 | 4.12 | 0.546 | 100 | 0.232 | [T/C] | 52 | 309 | 539 | −5.16±10.38 | −1.66±13.22 | 1.45±13.50 | −3.4 | 8.0E−03 | 6 | *GABRR1* | 89.9 |
| rs2242225 | 4.11 | 0.806 | 100 | 0.259 | [A/G] | 495 | 345 | 60 | −0.63±13.58 | 1.89±13.08 | −5.68±11.26 | −4.2 | 3.9E−04 | 5 | *UGT3A1* | 36.0 |
| rs450286 | 4.04 | 0.155 | 100 | 0.161 | [A/G] | 28 | 229 | 643 | 0.88±13.35 | 3.22±14.66 | −1.18±12.71 | −0.1 | 9.6E−01 | 2 | *TAF1B* | 9.9 |
| rs12453139 | 4.04 | 0.849 | 99.9 | 0.293 | [T/G] | 446 | 379 | 74 | 1.55±13.46 | −2.24±12.68 | 2.07±14.81 | 1.6 | 1.3E−01 | 17 | | 17.5 |
| rs4404877 | 4.01 | 0.900 | 100 | 0.213 | [A/C] | 550 | 309 | 41 | −1.17±12.72 | 1.14±13.36 | 7.19±18.51 | 4.8 | 6.9E−04 | 8 | | 129.2 |

Position (Mb) is obtained from NCBI Build 36.

SNPs with asterisk (*) were further genotyped in the Suita sample as summarized in Table 3.

†P values were derived from the association analysis of the initial GWAS with adjustment for sex, BMI and ethanol consumption and expressed as $-\mathrm{Log}_{10}P$.

‡P values were obtained from the linear regression analysis under the additive genetic model with adjustment for sex, BMI and ethanol consumption.

SNP, single nucleotide polymorphism; HWE, deviation from Hardy-Weinberg Equilibrium was analyzed by an exact test and P values are presented; MAF, minor allele frequency; β, β-coefficients; Ch, chromosome. For other abbreviations see Table 1.

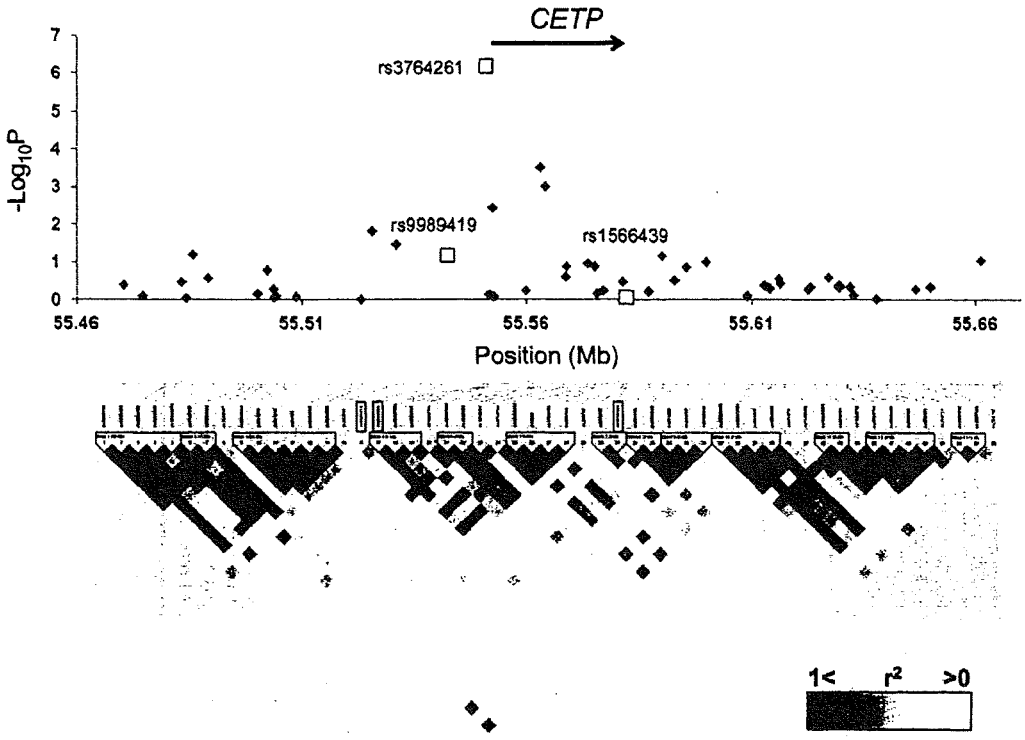


**Figure 2.** Linkage disequilibrium plot for single nucleotide polymorphisms (SNPs) encompassing the *CETP* region. The strength of the association of each SNP with high-density lipoprotein-cholesterol and its genomic position are presented in the upper panel. The lower panel shows pair-wise linkage disequilibrium ($r^2$) between 53 SNPs present on the Illumina 550K BeadChip. Open squares represent previously reported SNPs (rs3764261, rs9989419 and rs1566439).

observed in the initial subpopulation: 18 were selected from the top-ranked SNPs with a $-\log_{10}P$ of >4.0 and 4 SNPs had a $-\log_{10}P$ less than 4.0, ranging from 3.2 to 3.7. Genotyping was performed by using TaqMan allelic discrimination assays (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions.

Concordance rates between genotypes determined by Illumina and the TaqMan method were calculated by the re-genotyping of 213 SNPs present on the Illumina chip in 900 subjects by the TaqMan system, and were 99.3% on average.

### Statistical Analysis

Data are expressed as mean±standard deviation (SD). Continuous variables were tested for normality of distribution, and logarithmic transformation was applied for those with skewed distributions. Residuals, defined as the observed minus predicted values on the basis of confounding factors, were used for the genotype–phenotype association analysis by using one-way analysis of variance (ANOVA) tests. Covariates included in the model were derived from multiple logistic regression analysis and used to calculate a residual value for each variable. The level of genome-wide significance, expressed as a $-\log_{10}P$ value, was adjusted for multiple testing by Bonferroni correction and set at a $-\log_{10}P$ value of >6.87. Effect sizes were estimated for associations, meeting an arbitrary threshold of a $-\log_{10}P$ value of >4.0 by linear regression analysis under an additive genetic model, with adjustment for sex, body mass index (BMI) and ethanol consumption. The least significant number (n) was estimated to obtain the sample size required to attain approximately 50% power for the given significance level (alpha), SD of the error (sigma), and the effect size (delta). Statistical analysis was performed using a JMP statistical package 7.0 (SAS Institute, Cary, NC. USA).

## Results

Clinical characteristics of the Suita sample and a subgroup of subjects included for the initial GWAS are shown in **Table 1**. As specified by the selection criteria, subjects included for the initial GWAS were younger and free from medication for dyslipidemia.

**Figure 1** summarizes the genome-wide association analyses for HDL-cholesterol adjusted for sex, BMI and ethanol consumption. Among the 368,274 SNPs examined, none of the markers achieved genome-wide significance after Bonferroni correction. There were 43 SNPs exceeding the arbitrary threshold of $-\log_{10}P$ >4.0. The SNPs in LD as defined by the $r^2$>0.6 were categorized into a single group, and only 1 representative SNP for each region is shown in **Table 2**. The most significant SNP was rs3764261, which was located 5'upstream of *CETP* at chromosome 16q13, exhibiting a $-\log_{10}P$ value of 6.17. Each additional copy of the minor A allele at rs3764261 was associated with an increase in HDL-cholesterol by 5.6 mg/dl (P=0.0002). This observation is in good agreement with the previous GWAS where genetic variants in (rs711752, rs1864163) or near the *CETP* gene (rs3764261, rs9989419, rs1800775, rs1566439, rs12596776)[14,17–21] showed highly significant associations with HDL-cholesterol. The degree of LD ($r^2$) between SNPs across the *CETP* region and the strength of the association

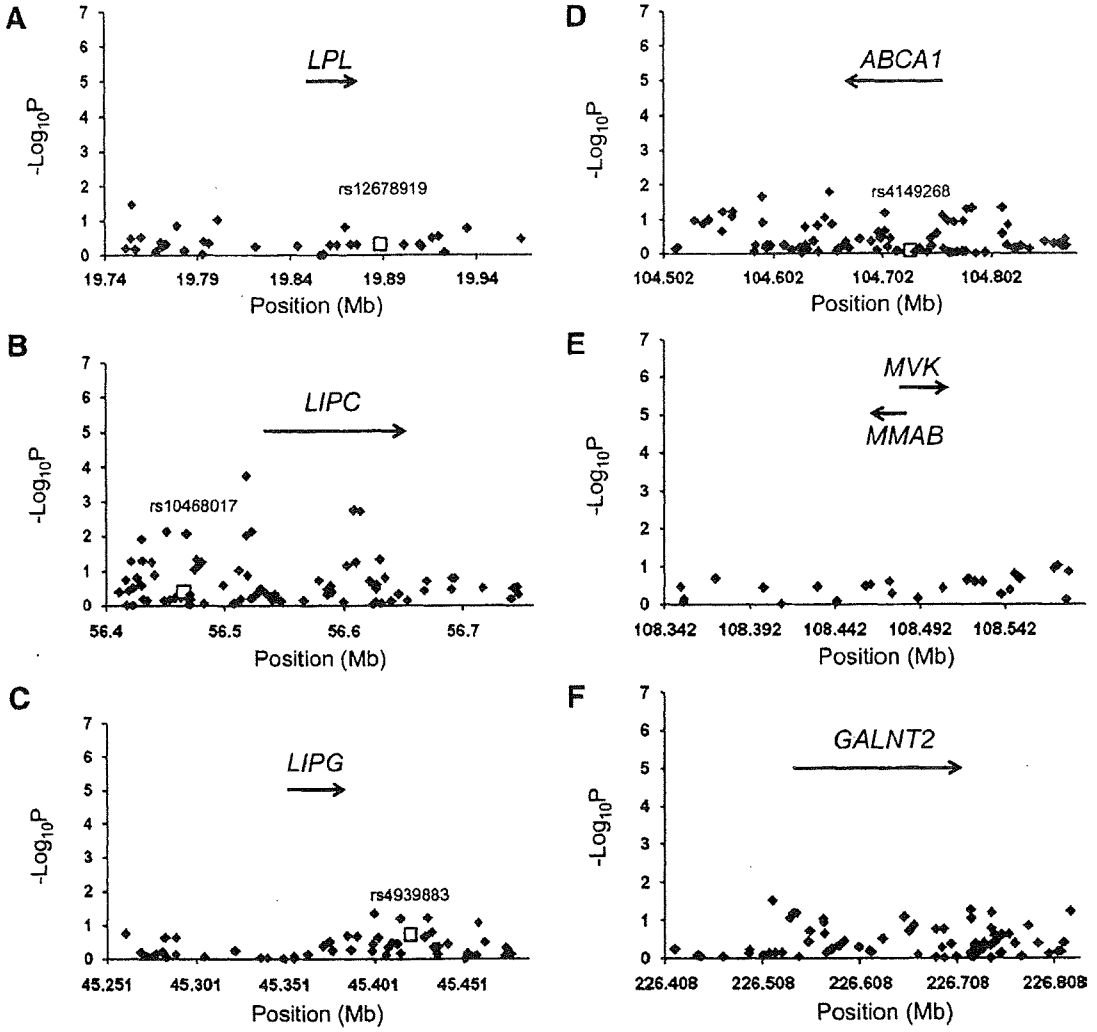


**Figure 3.** Genome-wide association study results for the previously reported genes; (**A**) *LPL*, (**B**) *LIPC*, (**C**) *LIPG*, (**D**) *ABCA1*, (**E**) *MVK-MMAB* and (**F**) *GALNT2*. Association results for genetic variants within or near (±100kb) the candidate or novel genes identified through genome-wide association studies (GWAS) approach are plotted (**A–F**). Identical single nucleotide polymorphisms genotyped in the previous GWAS by Willer et al[18] are represented by open squares.

of each SNP with HDL-cholesterol adjusted for sex, BMI and ethanol consumption are plotted in **Figure 2**. Although rs9989419 and rs1566439 have been reported to be strongly associated with HDL-cholesterol levels in previous studies,[17,18,20] we could not confirm the association in our initial screening of 900 subjects; $-\log_{10}P$ for rs9989419 and rs1566439 was 1.18 and 0.07, respectively. The most significant SNP, rs3764261, was not correlated with rs9989419 or rs1566439 ($r^2<0.1$). Despite the non-significant association, the effect size for rs9989419 ($\beta$, −1.13; P=0.37) suggests that the direction of the association is consistent with that found in previous studies, with the minor allele being associated with decreased HDL levels. It is possible that the significant association for rs9989419 was not detected because of the lower frequency of minor A allele in our Japanese sample (0.25) compared with those in white populations (0.35–0.40).[17,18,20]

While the previously reported association of *CETP* with HDL-cholesterol was confirmed in our GWAS, we failed to replicate the association for recently identified HDL-associated genes such as *MVK-MMAB* and *GALNT2*.[18] Furthermore, well-established genes contributing to HDL levels including *LPL*, *LIPC*, *LIPG*, and *ABCA1* were not listed among the top-ranked SNPs with a $-\log_{10}P$ of >4.0 from our genome-wide scan (**Table 2**). An examination of genetic variants within or near these genes (±100kb) present on the Illumina 550K BeadChip did not reveal any significant association with HDL-cholesterol (**Figuers 3A–F**).

To assess some of the observed associations in the initial genome-wide scan, we genotyped 22 SNPs in the remaining Suita sample (n=1,000–1,500). As seen in **Table 3**, the initially observed associations became less significant by increasing the sample size, except for the genetic variants at the *CETP* locus (rs3764261 and rs1532624). Changes in HDL-cholesterol per additional copy of the minor allele at rs3764261 was estimated to be 6.2mg/dl ($P=3.4\times10^{-12}$). It is interesting to note that rs467571 located 5'upstream of *FLJ45139* at 21q22 yielded an effect size of 7.9 ($P=3.8\times10^{-5}$).

To determine whether the 2 SNPs (rs3764261 and

**Table 3. Validation of the 22 SNPs Genotyped in the Suita Sample**

| SNP | GWAS sample | | Suita sample | | Ch | Gene symbol | Position (Mb) |
|---|---|---|---|---|---|---|---|
| | $\beta$ | P value† | $\beta$ | P value† | | | |
| rs3764261 | 5.6 | 1.9E-04 | 6.2 | 3.4E-12 | 16 | *CETP* | 55.6 |
| rs10945991 | 14.0 | 2.0E-07 | 4.5 | 6.0E-03 | 6 | | 164.8 |
| rs6509732 | 10.7 | 4.7E-07 | 3.8 | 3.1E-03 | 19 | *ZNF665* | 58.4 |
| rs6133175 | 3.4 | 2.2E-04 | 2.3 | 4.3E-04 | 20 | *SLC23A2* | 4.8 |
| rs467571 | 12.7 | 2.9E-06 | 7.9 | 3.8E-05 | 21 | *FLJ45139* | 39.2 |
| rs10485472 | 6.9 | 4.5E-06 | 2.5 | 1.3E-02 | 20 | | 58.9 |
| rs6790597 | -2.8 | 5.6E-02 | -1.8 | 7.3E-02 | 3 | | 177.0 |
| rs1544669 | -5.2 | 3.2E-04 | -3.0 | 5.8E-03 | 12 | *BCL2L14* | 12.1 |
| rs980861 | 3.7 | 8.2E-06 | 1.6 | 1.6E-02 | 2 | | 57.5 |
| rs12134357 | -1.7 | 6.3E-02 | -1.1 | 9.7E-02 | 1 | *RAP1GAP* | 21.8 |
| rs11654690 | 5.1 | 5.4E-05 | 1.6 | 6.4E-02 | 17 | *PSMB6* | 4.7 |
| rs280049 | 2.7 | 2.3E-02 | 1.9 | 1.8E-02 | 17 | *ACCN1* | 29.0 |
| rs7547186 | 4.4 | 8.8E-06 | 2.0 | 4.6E-03 | 1 | *ESRRG* | 215.0 |
| rs7550051 | -2.2 | 2.4E-01 | -1.0 | 4.2E-01 | 1 | | 210.4 |
| rs12586473 | -0.9 | 2.9E-01 | 0.1 | 8.5E-01 | 14 | | 27.5 |
| rs10493889 | 5.9 | 1.3E-04 | 3.5 | 2.4E-03 | 1 | | 97.2 |
| rs9956878 | 5.5 | 1.5E-05 | 2.1 | 8.0E-03 | 18 | | 71.7 |
| rs10496565 | -3.4 | 1.3E-05 | -1.5 | 5.3E-03 | 2 | *CLASP1* | 121.9 |
| rs926130 | -1.6 | 4.8E-02 | -0.3 | 5.6E-01 | 21 | | 15.1 |
| rs3102210 | 1.5 | 2.7E-01 | 1.2 | 2.1E-01 | 13 | | 60.6 |
| rs1532624 | 2.8 | 6.2E-03 | 3.4 | 2.3E-07 | 16 | *CETP* | 55.6 |
| rs12881778 | 6.4 | 1.3E-04 | 2.2 | 3.7E-02 | 14 | | 73.9 |

Position (Mb) is obtained from NCBI Build 36.
†P values were derived from the linear regression analysis under the additive genetic model with adjustment for sex, BMI and ethanol consumption.
For abbreviations see Table 1 and 2.

rs467571), showing the minor allele being associated with higher levels of HDL-cholesterol, are protective against MI, genotype frequencies of these SNPs were compared between MI cases (n=589) and controls (n=3,097). The minor allele at rs3764261 was more prevalent in MI cases than in controls; 0.221 and 0.196 for MI cases and controls, respectively (nominal P=0.02). The association of rs3764261 with HDL-cholesterol levels was direction-consistent in the MI cases; sex-adjusted residuals for the minor homozygotes, heterozygotes and major homozyogotes were 4.36±8.11, 1.96±14.46 and -1.56±10.90, respectively (mean±SD, P=0.003). The effect size with adjustment for sex was estimated to be 2.79 (P=0.077). The non-significant difference in MAF between MI cases and controls was observed for rs467571 (0.106 vs 0.100, nominal P=0.56).

## Discussion

From our GWA scan, the strongest association for HDL-cholesterol was observed for rs3764261 located upstream of the *CETP* gene, which was further confirmed in a lager sample of the Suita study. This is one of the most replicated associations and our findings are in good agreement with the previous GWAS[17,18,20] In the present analysis, none of the genetic variants exerted a greater influence on HDL levels than those at *CETP*. Similarly, the previous GWAS conducted in the general population sample of KORA (Cooperative Health Research in the Region of Augsburg) found *CETP* to be the only gene reaching the genome-wide significance at the initial screening level (n=1,643)[20]

In contrast to the well-known anti-atherogenic property of HDL, the minor allele of rs3764261 associated with an increase in HDL-cholesterol was more frequent in MI cases than in controls. This apparently contradictory observation might be linked to the premature withdrawal of the clinical trial of the CETP inhibitor, torcetrapib[31] suggesting that the increase in HDL-cholesterol as a result of CETP deficiency might not be necessarily associated with a lower risk of cardiovascular disease. In line with this view, a 4.94-year follow-up study involving more than 8,000 subjects reported an increased risk for coronary heart disease among subjects with the A allele of the promoter SNP of the *CETP* gene at -629, which was associated with higher HDL-cholesterol levels[32] Although it has become clear that the anti-atherogenic property of HDL is not fully explained by the circulating level of HDL-cholesterol[33] the importance of the heterogeneity of HDL in terms of its function and structure has not been emphasized in GWAS.

A previously unrecognized association with HDL-cholesterol was detected for rs467571 at chromosome 21q22. The increase in HDL-cholesterol per additional minor allele was estimated to be 7.9 ($P=3.8\times10^{-5}$). Nearby genes of this intergenic variant include *FLJ45139* and *ETS2*, neither of which has been previously implicated in lipid metabolism. It remains to be confirmed in a much larger study population whether the protective allele at rs467571 is associated with the reduced risk of MI.

Lack of association between the previously reported loci and HDL levels in our GWAS can be due to the insufficient statistical power of the initial screening. Using the effect size (2.4) and SD of the error (13.2) obtained from the association analysis of rs3764261, the least significant number to attain a significant result, corresponding to approximately 50% power, was estimated to be 972, 851, 709, and 567 at the significance level ($-\log_{10}P$) of 6.8, 6.0, 5.0 and 4.0, respectively. Although our sample size (n=900) is large enough to detect an association for the effect size similar to that for the strongly associated SNP at *CETP*, it is possible that our GWAS is inadequately powered such that the modest effect was not detected. Inability to replicate the associations of well-known candidate genes and HDL levels might be accounted for by incomplete or poor coverage in

our GWAS. As discussed in the study examining the coverage of commercially available genotyping arrays,[34] it can be considered that the important causative or functional variants specific to a Japanese population are not well captured by the tag SNPs selected on the basis of HapMap data.

In our replication attempts, increasing the sample size weakened the strength of the associations for most of the SNPs genotyped in the additional Suita sample. These SNPs are likely to have a lesser impact on HDL levels compared with those in *CETP*, and the proportion explained by these genetic variations is expected to be small. The use of a cut-off of MAF>0.1 can be another limitation of the current analysis. It is possible that multiple rare alleles with a much greater influence might be involved in determining HDL levels in the Japanese population. Sequencing of the coding regions of *ABCA1*, *APOA1*, and *LCAT* in individuals with HDL-cholesterol levels less than the 5th percentile or greater than the 95th percentile demonstrated the involvement of rare sequence variations in determining low HDL,[35] suggesting that the multiple rare allele hypothesis is a valid approach. GWAS can be useful for identifying genes existing in a disease pathway, but do not allow these multiple rare allele hypotheses to be tested. Thus, deep resequencing of the responsible genes identified through GWAS in both affected and unaffected subjects might be a promising strategy for future personalized prevention.

We have successfully replicated the association of *CETP* with HDL levels. A GWAS approach is so called "hypothesis-generating"[36] Assessment of the observed associations for the top-ranked SNPs in an independent population, coupled with functional studies examining the underlying mechanisms of the identified variants will be necessary before drawing a definite conclusion.

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