J Infect Chemother (2009) 15:424–425
DOI 10.1007/s10156-009-0720-x

NOTE

Yoshinori Hirata · Makoto Sata · Yuko Makiuchi
Keita Morikane · Akihito Wada · Nobuhiko Okabe
Hitonobu Tomoike

Comparative analysis of *Micrococcus luteus* isolates from blood cultures of patients with pulmonary hypertension receiving epoprostenol continuous infusion

Received: June 8, 2009 / Accepted: July 24, 2009

Abstract During the period 2002–2008, at the National Cardiovascular Center, Osaka, 28 *Micrococcus luteus* isolates and one *Kocuria* spp. isolate were obtained from blood cultures of pulmonary hypertension (PH) patients who were receiving continuous infusion therapy with epoprostenol. Pulsed-field gel electrophoresis patterns of the isolates were unrelated, suggesting that the infections had multiple origins. The preparation of epoprostenol solution by patients themselves was thought to be a risk factor.

Key words Pulmonary hypertension · *Micrococcus luteus*

Pulmonary hypertension (PH) is a serious illness due to the circulatory disturbance and respiratory disorders that are difficult to treat. The recent development of continuous infusion therapy with epoprostenol, a prostaglandin I_2 analogue, has improved the prognosis of this disease. Bacterial infection, however, is one of the main complications during this treatment. *Micrococcus luteus* is sometimes isolated from the bloodstream during this treatment, although this organism is rarely isolated in other situations.[1–3] The cause of the *M. luteus* infection is unclear, and there have been no molecular epidemiology reports detailing the cause. We performed pulsed-field gel electrophoresis (PFGE) analysis of *M. luteus* isolates obtained from blood cultures of patients with PH to investigate the cause(s) of this infection.

During 2002 to 2008, 29 bacterial isolates were obtained from blood cultures of patients with PH who were admitted to the National Cardiovascular Center and received continuous infusion therapy with epoprostenol. The isolates were stored at –80°C until analysis.

First, an ID 32 STAPH kit (bioMérieux, Marcy l'Etoile, France) was used for identification of the isolates. Then, 16S rDNA sequences of the isolates were determined. The method for preparing PFGE samples was described previously.[4] Lysozyme (1 mg/ml; Wako, Tokyo, Japan) and N-acetylmuramidase (200 μg/ml; Seikagaku, Tokyo, Japan) were used instead of lysostaphin. The genomic DNA of each isolate was digested with 20 units of *Xba*I (Takara, Otsu, Japan) and separated by PFGE. The direction of the electric field was changed periodically every 1 to 30 s for 18 h.

All but one of the isolates were identified as *M. luteus* by the ID 32 STAPH kit and 16S rDNA analysis. Isolate 27 belonged to the genus *Kocuria*, but it could not be identified to the species level by ID 32 STAPH or 16S rDNA analysis (data not shown). PFGE analysis revealed differences in the observed patterns of the isolates, except for isolates 16 and 28, which had indistinguishable patterns (Fig. 1). This result indicates that the bloodstream infections of *M. luteus* were not caused by a single source but had various origins.

Bacterial infections by *Micrococcus* spp. and *Staphylococcus* spp. are complications that arise during infusion therapy in patients with PH.[1–3] Continuous infusion of epoprostenol via a Hickman catheter is a major risk factor for *Micrococcus* infections.[1,3] Because *M. luteus* has been isolated from environments with a pH value of 11,[5] this organism may be able to tolerate epoprostenol, which has a pH value of 10.2 to 10.8. Although the actual source of contamination of *M. luteus* in our study was not known, the preparation of the epoprostenol solution by patients themselves may entail a potent risk. To reduce the risks of *M. luteus* infection, it is very important that patients with PH be trained and informed about the risks of catheter infection and the appropriate procedures to be followed with the

Y. Hirata (✉)
Department of Clinical Laboratory, National Cardiovascular Center, 5-7-1 Fujishirodai, Suita, Osaka 565-8565, Japan

Y. Hirata · M. Sata · Y. Makiuchi
Office of Infection Control, National Cardiovascular Center, Osaka, Japan

M. Sata · H. Tomoike
Cardiovascular Internal Medicine Department, National Cardiovascular Center, Osaka, Japan

A. Wada
Department of Bacteriology, National Institute of Infectious Diseases, Tokyo, Japan

K. Morikane · N. Okabe
Infectious Diseases Surveillance Center, National Institute of Infectious Diseases, Tokyo, Japan

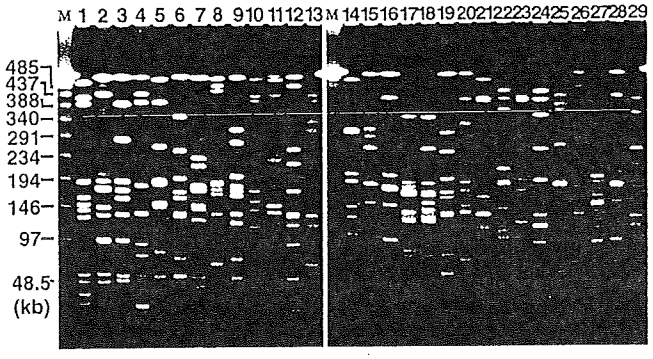


Fig. 1. Pulsed-field gel electrophoresis (PFGE) profiles of the 29 bacterial isolates. *Lane M* shows a lambda molecular size marker. The size of each fragment of the marker is shown *on the left side*

epoprostenol preparation. Further, it is mandatory for medical and laboratory staff to carefully monitor the status of patients with PH and conduct epidemiological analyses in order to prevent *M. luteus* infection.

References

1. Yap RL, Mermel LA. *Micrococcus* infection in patients receiving epoprostenol by continuous infusion. Eur J Clin Microbiol Infect Dis 2003;22:704–5.
2. Oudiz RJ, Widlitz A, Beckmann XJ, Camanga D, Alfie J, Brundage BH, Barst RJ. Micrococcus-associated central venous catheter infection in patients with pulmonary arterial hypertension. Chest 2004;126:90–4.
3. Mitsutake K, Nogami T. *Micrococcus* blood stream infection in patients receiving epoprostenol. Kansenshogaku Zasshi 2005;79:829–30.
4. Wada A, Katayama Y, Hiramatsu K, Yokota T. Southern hybridization analysis of the *mecA* deletion from methicillin-resistant *Staphylococcus aureus*. Biochem Biophys Res Commun 1991;176:1319–25.
5. Tiago I, Paula A, Veríssimo A. Bacterial diversity in a nonsaline alkaline environment: heterotrophic aerobic populations. Appl Environ Microbiol 2004;70:7378–87.

Long-Term Prognosis of Probands With Brugada-Pattern ST-Elevation in Leads V_1–V_3

Shiro Kamakura, MD, PhD; Tohru Ohe, MD, PhD; Kiyoshi Nakazawa, MD, PhD; Yoshifusa Aizawa, MD, PhD; Akihiko Shimizu, MD, PhD; Minoru Horie, MD, PhD; Satoshi Ogawa, MD, PhD; Ken Okumura, MD, PhD; Kazufumi Tsuchihashi, MD, PhD; Kaoru Sugi, MD, PhD; Naomasa Makita, MD, PhD; Nobuhisa Hagiwara, MD, PhD; Hiroshi Inoue, MD, PhD; Hirotsugu Atarashi, MD, PhD; Naohiko Aihara, MD; Wataru Shimizu, MD, PhD; Takashi Kurita, MD, PhD; Kazuhiro Suyama, MD, PhD; Takashi Noda, MD, PhD; Kazuhiro Satomi, MD, PhD; Hideo Okamura, MD; Hitonobu Tomoike, MD, PhD; for the Brugada Syndrome Investigators in Japan

Background—The prognosis of patients with saddleback or noncoved type (non–type 1) ST-elevation in Brugada syndrome is unknown. The purpose of this study was to clarify the long-term prognosis of probands with non–type 1 ECG and those with coved (type 1) Brugada-pattern ECG.

Methods and Results—A total of 330 (123 symptomatic, 207 asymptomatic) probands with a coved or saddleback ST-elevation ≥1 mm in leads V_1–V_3 were divided into 2 ECG groups—type 1 (245 probands) and non–type 1 (85 probands)—and were prospectively followed for 48.7±15.0 months. The absence of type 1 ECG was confirmed by drug provocation test and multiple recordings. The ratio of individuals with a family history of sudden cardiac death (14%) was lower than previous studies. Clinical profiles and outcomes were not notably different between the 2 groups (annual arrhythmic event rate of probands with ventricular fibrillation; type 1: 10.2%, non–type 1: 10.6%, probands with syncope; type 1: 0.6%, non–type 1: 1.2%, and asymptomatic probands; type 1: 0.5%, non–type 1: 0%). Family history of sudden cardiac death at age <45 years and coexistence of inferolateral early repolarization with Brugada-pattern ECG were independent predictors of fatal arrhythmic events (hazard ratio, 3.28; 95% confidence interval, 1.42 to 7.60; P=0.005; hazard ratio, 2.66; 95% confidence interval, 1.06 to 6.71; P=0.03, respectively, by multivariate analysis), although spontaneous type 1 ECG and ventricular fibrillation inducibility by electrophysiological study were not reliable parameters.

Conclusions—The long-term prognosis of probands in non–type 1 group was similar to that of type 1 group. Family history of sudden cardiac death and the presence of early repolarization were predictors of poor outcome in this study, which included only probands with Brugada-pattern ST-elevation. (***Circ Arrhythmia Electrophysiol.* 2009;2:495-503.**)

Key Words: death, sudden ■ prognosis ■ follow-up studies ■ electrocardiography ■ Brugada syndrome

Brugada syndrome is a hereditary arrhythmogenic disease characterized by ST-elevation in the right precordial lead of standard ECGs and an increased risk of sudden cardiac death (SCD).[1] The prognosis for this condition and the management approaches have been reported in several multicenter studies of patients with the coved type 1 ECG. However, no prospective data have been reported in patients with saddleback type or noncoved Brugada-pattern ST-elevation before, because they were excluded from previous

Clinical Perspective on p 503

studies as atypical Brugada patients showing a benign clinical course. Besides, the data from previous studies are all conflicting with regard to the prognosis of the typical Bru-

Received August 22, 2008; accepted July 14, 2009.

From the Division of Cardiology (S.K., N.A., W.S., T.K., K.S., T.N., K.S., H.O., H.T.), National Cardiovascular Center, Suita, Japan; the Department of Cardiovascular Medicine (T.O.), Okayama University Graduate School of Medicine, Okayama, Japan; the Department of Cardiology (K.N.), St Marianna University, Kawasaki, Japan; the Division of Cardiology (Y.A.), Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan; the Division of Cardiology (A.S.), Yamaguchi University Graduate School of Medicine, Ube, Japan; the Department of Cardiovascular Medicine (M.H.), Shiga University of Medical Science, Otsu, Japan; the Department of Cardiopulmonary Medicine (S.O.), Keio University, Tokyo, Japan; the Second Department of Internal Medicine (K.O.), Hirosaki University School of Medicine, Hirosaki, Japan; the Second Department of Internal Medicine (K.T.), Sapporo Medical University School of Medicine, Sapporo, Japan; the Division of Cardiovascular Medicine (K.S.), Toho University Medical Center Ohashi Hospital, Tokyo, Japan; the Department of Cardiovascular Medicine (N.M.), Hokkaido University Graduate School of Medicine, Sapporo, Japan; the Department of Cardiology (N.H.), Tokyo Women's Medical University; the Second Department of Internal Medicine (H.I.), Toyama University, Toyama, Japan; and the Department of Internal Medicine, Nippon Medical School, Tama-Nagayama Hospital, Tokyo, Japan.

Correspondence to Shiro Kamakura, MD, PhD, Division of Cardiology, Department of Internal Medicine, National Cardiovascular Center, 5-7-1 Fujishiro-dai, Suita, Osaka, 565-8565, Japan. E-mail kamakura@hsp.ncvc.go.jp

Circ Arrhythmia Electrophysiol is available at http://circep.ahajournals.org

DOI: 10.1161/CIRCEP.108.816892

gada syndrome.[2-5] This may be caused by cohort studies that included a significant number of family members other than probands, in which the prognosis of pedigree members can be affected by the disease severity of probands. Furthermore, a selection bias can be present if the data are analyzed retrospectively. Therefore, we aimed to investigate the long-term prognosis of probands with noncoved type ST-elevation in leads V_1–V_3, prospectively, and compared it with that of probands with the type 1 ST-elevation.

Methods

Patient Population

A total of 330 individuals with spontaneous ST-elevation were registered consecutively in this study, namely, "a multicenter study for risk stratification and management in patients with Brugada syndrome." The study was conducted at 26 institutions across Japan beginning in July 2001. These individuals were prospectively followed up for more than 12 months to the end of March 2007. Subjects were enrolled in this study if they met the following inclusion criteria: (1) proband, (2) J-point (QRS-ST junction) amplitude of ≥0.1 mV (1 mm) with either coved or saddle back type ST-segment elevation in at least 2 of the 3 precordial leads (V_1–V_3) on resting standard 12-lead ECG, (3) normal findings on physical examination, and (4) no abnormality in either right or left ventricular morphology and/or function demonstrated by chest radiography and echocardiography. Patients with vasospastic angina and those with vasovagal syncope were excluded from this study. Patients were not administered antiarrhythmic drugs and did not have electrolyte abnormalities at the time of baseline ECG recording and other examinations.

Classification of Groups

We divided the 330 patients with Brugada-pattern ECG into 3 groups according to their symptoms: The ventricular fibrillation (VF) group consisted of 56 probands with aborted sudden death and/or documented VF, the syncope group consisted of 67 probands with syncope without documented arrhythmias that was not typical for vasovagal syncope, and the asymptomatic group consisted of 207 asymptomatic individuals whose ECGs were mainly detected by individual annual medical checkup or health screening in their place of employment.

We also divided these patients into 2 groups according to ECG morphology: The type 1 group consisted of 245 probands with a spontaneous type 1 ECG or those who developed type 1 ECG with a drug provocation test. The non–type 1 group consisted of the remaining 85 probands who never showed type 1 ST-elevation even with the drug provocation test (Figure 1) and during the follow-up on standard 12-lead ECGs.

Clinical Data, ECG, and Electrophysiological Testing

Clinical data including age at the enrollment, sex, family history of SCD, and the presence of atrial fibrillation were collected for all patients. The standard ECGs were recorded more than 5 times during the follow-up period in all patients. ECG recording on higher intercostals spaces (third and/or second) in leads V_1–V_3[6] was encouraged in patients who had cardiac events during the follow-up period.

A type 1 ECG was defined as a prominent coved ST-segment elevation displaying J-point wave amplitude or ST-segment elevation ≥2 mm or 0.2 mV.[7,8] ECG patterns with a prominent coved ST-elevation ≥2 mm followed by a positive or flat T wave were also included in type 1 group (Figure 2A through C). A non–type 1 ECG was defined as one of the following: type 2 ECG,[7] type 3 ECG,[7] and ECG displaying coved or saddleback ST-elevation with J-wave amplitude ≥1 mm and <2 mm (Figures 1 and 2D through 2G).

The presence of early repolarization in the inferolateral leads[9] was evaluated by baseline 12-lead ECGs at the time of enrollment to elucidate ECG findings associated with Brugada syndrome. Early repolarization was defined as an elevation of the J point in at least 2 leads. The amplitude of the J wave or J-point elevation had to be at least 1 mm above the baseline level, either as QRS slurring or notching in the inferior lead (II, III, and aVF), lateral (I, aVL, and V_4–V_6) lead, or both.[9]

ECGs were evaluated by 3 independent investigators (S.K., N.A., and W.S.) who were unaware of the patients' other clinical information. The ECG type or morphology was established by the evaluation in which at least 2 of the 3 observers were in agreement.

Sodium channel blocker pilsicainide (1 mg/kg body weight at a rate of 5 to 10 mg/min), disopyramide (1.5 mg/kg, 10 mg/min), flecainide (2 mg/kg, 10 mg/min), or procainamide (10 mg/kg, 100 mg/min) was administered intravenously in 270 (82%) patients (233, 15, 14, and 8, respectively) to test the conversion to typical coved ST-elevation.[8,10,11]

Baseline electrophysiological studies (EPS) were performed in 232 (70%) patients. A maximum of 3 ventricular extrastimuli were delivered from 2 right ventricular (RV) sites (RV apex and RV outflow tract) unless VF or polymorphic ventricular tachycardia (VT) (lasting ≥10 beats) that terminated spontaneously within 30 seconds, causing syncope, or requiring intervention to be terminated was elicited at a previous step. Premature beats were started in late diastole; coupling intervals were then reduced in 10-ms decrements until refractoriness was reached. Stimulation was performed at twice the diastolic threshold. Patients with inducible ventricular arrhythmias lasting less than 10 beats were classified as noninducible. The indices including age, sex distribution, a family history of SCD at

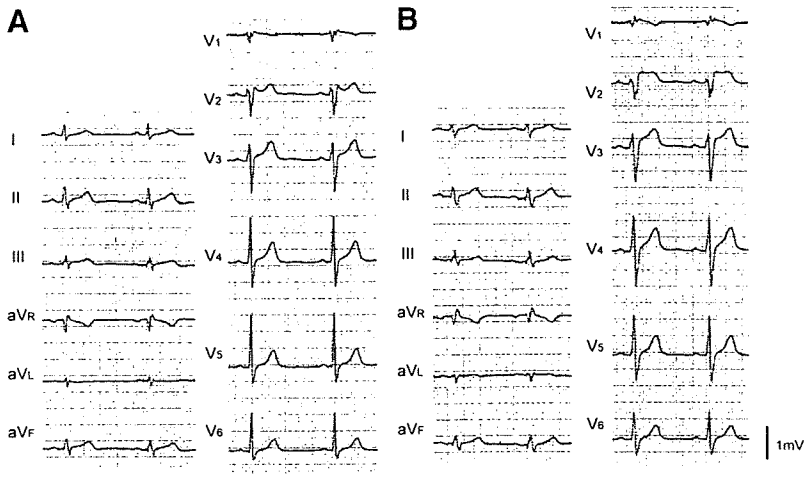


Figure 1. Presentation of 12-lead ECGs of a patient with non–type 1 ST-elevation. A, Baseline 12-lead ECG; B, 12-lead ECG after provocation by intravenous administration of 50 mg pilsicainide in the same patient. Saddleback-type ST-elevation in leads V_1 and V_2 was enhanced after pilsicainide but was not changed to type 1 ST-elevation. This 46-year-old male patient with a history of syncope but with no family history of SCD had inducible VF by electrophysiological study. He had spontaneous VF 11 months after enrollment.

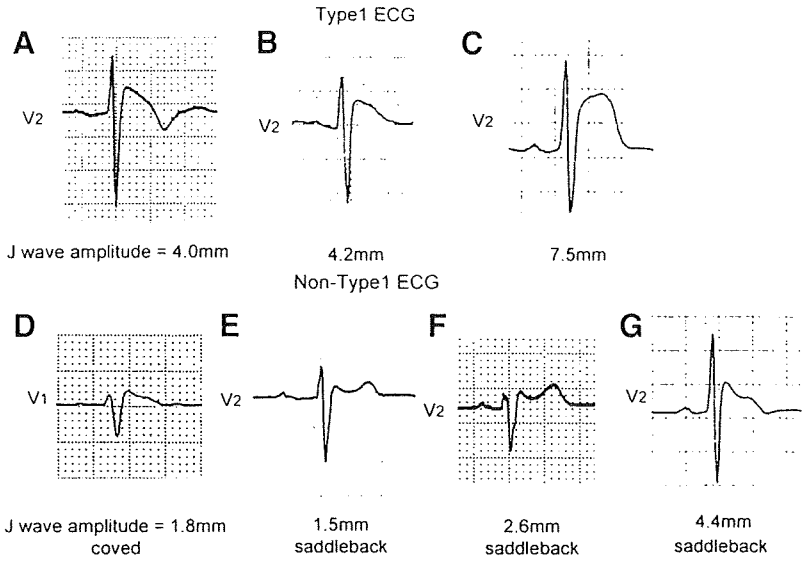


Figure 2. Presentation of type 1 and non-type 1 ECG. Coved-type ST-elevation with a J-wave amplitude ≥2 mm followed by a negative T wave (A) or a positive/flat T wave (B), and a coved ST-elevation followed by a smaller J wave than T wave (C) were defined as type 1 ECG. Coved (D) or saddleback-type ST-elevation (E) with a J-wave amplitude <2 mm, a saddleback ST-elevation with a J-wave amplitude ≥2 mm (F), and a saddleback ST-elevation displaying bigger J wave than T wave (G) were defined as non-type 1 ECG.

less than 45 years of age, and VF/polymorphic VT inducibility were compared with those reported in previously published studies[2,3,5] (Table 1). In addition to these parameters, the presence of atrial fibrillation, cardiac events at night, and inferolateral early repolarization were compared between type 1 and non-type 1 groups.

Patient treatment was based on clinical judgment of the participating hospital. Twenty-eight (8%) probands received antiarrhythmic drugs (quinidine sulfate ≤400 mg, bepridil ≤200 mg, disopyramide ≤300 mg, aprindine ≤30 mg, and amiodarone ≤200 mg/d) for prevention of atrial fibrillation or VF. Calcium antagonists were administered in 18 (5%) probands for hypertension. Quinidine and bepridil were administered only after a documentation of VF during follow-up. Among the 330 patients, 125 (38%) received an implantable cardioverter-defibrillator (ICD). During follow-up, patients were considered to have an arrhythmic event if sudden death occurred or VF was documented.

Statistical Analysis

Data are presented as mean±standard deviation. The Fisher exact test or the χ^2 test was used for categorical variables. One-way ANOVA was used for comparisons of continuous variables among the different groups. Survival curves were plotted by the Kaplan-Meier method and analyzed by the log-rank test. Cox proportional hazards models were used to analyze factors associated with the time to the first arrhythmic event during follow-up in all probands as well as in type 1, non-type 1, VF, and non-VF (syncope and asymptomatic) groups. Variables were included in the multivariate analysis with the use of a forward stepwise procedure with a criteria of $P<0.05$ for inclusion and $P>0.15$ for removal from the model. A probability value of $P<0.05$ was considered statistically significant.

This study was performed under the ethical code approved by the Health, Labor, and Welfare Ministry of Japan. Written informed consent was obtained from all individuals.

Results

Clinical Profiles of All Probands

The mean age of the 330 probands was 51.4±14.8 years (median, 53 years; range, 4 to 86 years). The majority (315; 95%) of probands were male. A low percentage (14%) of patients had a family history of SCD occurring before the age of 45 years. The induction rate of VF/polymorphic VT by EPS was higher (77/109: 72%, $P<0.005$) in symptomatic than asymptomatic probands (61/123: 50%) (Table 1).

Comparison of Clinical Characteristics Between Type 1 and Non-Type 1 Groups

Type 1 ECG was found in 245 probands (VF group: 45, 18%; syncope group: 46, 19%; and asymptomatic group: 154, 63%). Of these 245 probands, 173 (71%) showed type 1 ECG spontaneously and the remaining 72 (29%) showed characteristic type 1 morphology after class Ic or Ia antiarrhythmic drug administration. In 85 probands of the non-type 1 group (VF group: 11, 13%; syncope group: 21, 25%; and asymptomatic group: 53, 62%), non-type 1 ECG remained during the drug provocation test (type 2: 61,

Table 1. Comparison of Patient Characteristics Among 3 Large Registries

	Brugada et al[2]		Eckardt et al[5]		Kamakura et al	
	Sympt	Asympt	Sympt	Asympt	Sympt (VF, S)	Asympt
No.	144	190	89	123	123 (56, 67)	207
Age, y	41±16*	40±16	46±14	44±14	50.4±16.6	51.9±13.6
Men, %	83	71	76	68	96	95
FH of SCD, %	34	72	21	33	19 (25, 13)	11
VF/VT inducibility, %	73	33	63	39	71 (65, 75)	50

Values in parentheses are for the patients with aborted sudden death and an episode of syncope. Sympt indicates symptomatic; Asympt, asymptomatic; S, syncope; FH of SCD, prevalence of patients with a family history of sudden cardiac death at <45 years old; and VF/VT inducibility, induction rate of VF or polymorphic ventricular tachycardia by EPS.

*Age of patients with VF.

Table 2. Comparison of Clinical Profiles Between Probands With Type 1 ECG and Those With Non–Type 1 ECG

	Type 1 (n=245)			Non-Type 1 (n=85)			
	VF	Syncope	Asympt	VF	Syncope	Asympt	*P* Value
No.	45	46	154	11	21	53	0.33
Age, y	48.2±17.8	52.5±15.6	52.3±13.1	48.0±18.1	51.9±15.8	50.7±15.2	0.99
Men, n (%)	44 (98)	44 (96)	146 (95)	11 (100)	19 (90)	51 (96)	0.90
FH of SCD, n (%)	11 (24)	8 (17)	17 (11)	3 (27)	1 (5)	5 (9)	0.06
Event at night, n (%)	37/45 (82)	15/45 (33)		5/9 (56)	7/18 (39)		0.06
Inferolateral ER, n (%)	8 (18)	3 (7)	15 (10)	2 (18)	1 (5)	4 (8)	0.85
Prevalence of AF, n (%)	19 (42)	7 (15)	21 (14)	4 (36)	3 (14)	8 (15)	0.87
VF/VT inducibility, n (%)	27/41 (66)	31/40 (78)	52/91 (57)	7/11 (64)	12/17 (71)	9/32 (28)	0.04

n (%) indicates the number and the ratio of patients with each parameter; event at night, event developed at night (8 PM to 8 AM); inferolateral ER, inferolateral early repolarization; AF, atrial fibrillation; VF/VT inducibility, induction rate of VF or polymorphic ventricular tachycardia by EPS.

72%; coved with J-point amplitude <2 mm: 24, 28%) and the follow-up period. Most of the clinical parameters except for VF/VT inducibility, namely, age, sex distribution, the prevalence of atrial fibrillation, the presence of a family history of SCD, cardiac events at night (8 PM to 8 AM), and early repolarization, were of similar occurrence between type 1 and non–type 1 groups (Table 2). Only 8% (7/85) of probands in the non–type 1 group and 11% (26/245) of those in the type 1 group were associated with early repolarization in the inferolateral leads.

Follow-Up and Predictors of Outcome

The mean follow-up period for the entire study population was 48.7±14.9 months. Follow-up time was similar among VF (51.9±15.0 months), syncope (48.5±14.0 months), and asymptomatic (47.7±15.0 months) groups and between type 1 (48.6±15.2 months) and non–type 1 (48.9±14.2 months) groups. Twenty-four patients had fatal arrhythmic events during follow-up. The frequency of events in the type 1 group—15 of 45 (33%) in patients with VF, 1 of 46 (2%) in syncope patients, and 3 of 154 (2%) in asymptomatic patients— was similar to that in the non–type 1 group (4/11: 36%, 1/21: 5%, and 0/53: 0%, respectively, $P=0.22$; Figure 3). In 5 patients who had events in the non–type 1 group, 2 had shown a type 1 ST-elevation only in the higher (second or third) intercostal spaces—1 in a follow-up ECG and 1 after drug provocation test. The observed frequency of arrhythmic events was significantly highly in patients with early repolarization in the inferolateral leads (7/33; 21% versus 17/297; 6%, $P<0.005$), although there was no difference in risk between the 2 groups (type 1: 6/26; 23%, non–type 1: 1/7; 14%, $P=0.67$). One asymptomatic patient with type 1 ECG died suddenly 3 months after enrollment. Six patients died of nonarrhythmic causes; 3 died of cancer, 1 because of rupture of abdominal aortic aneurysm, 1 because of pneumonia, and cause of death for 1 patient was unknown. Seven percent of all patients who entered the study dropped out, the most frequent reason for drop-out was inability of follow-up due to patient's change of address.

Figure 4 shows the Kaplan–Meier analysis of arrhythmic events in probands with type 1 and non–type 1 ECG. Probands in the VF group had significantly worse prognosis than those in the syncope and asymptomatic groups. The

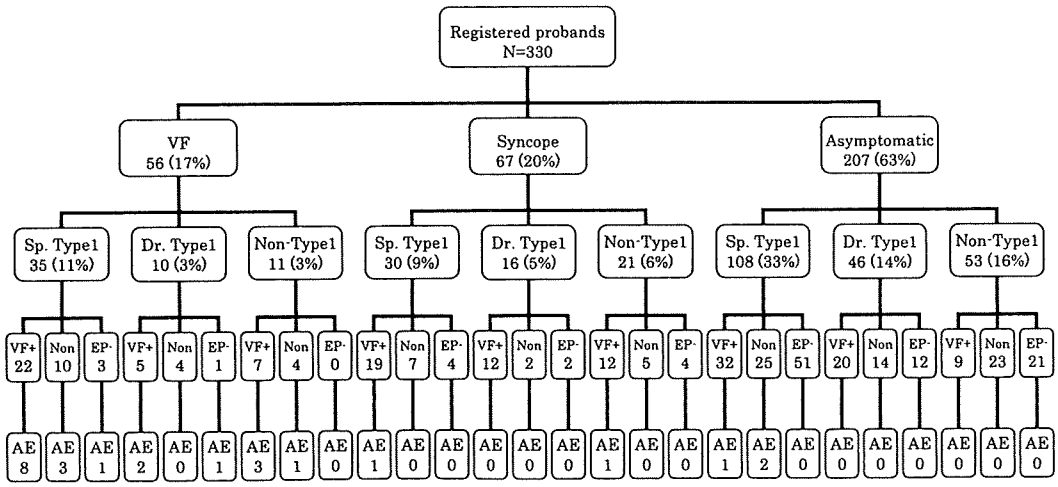


Figure 3. Flow chart of proband groups categorized according to symptom, ECG morphology, and VF/VT inducibility by electrophysiological study. Sp. Type 1 indicates spontaneous type 1 group; Dr. Type 1, drug-induced type 1 group; VF+, a group with inducible VF/VT; Non, a group with noninducible VF/VT; EP−, a group in which electrophysiological study was not performed; AE, fatal arrhythmic event during follow-up. The number indicates the number of probands in each category.

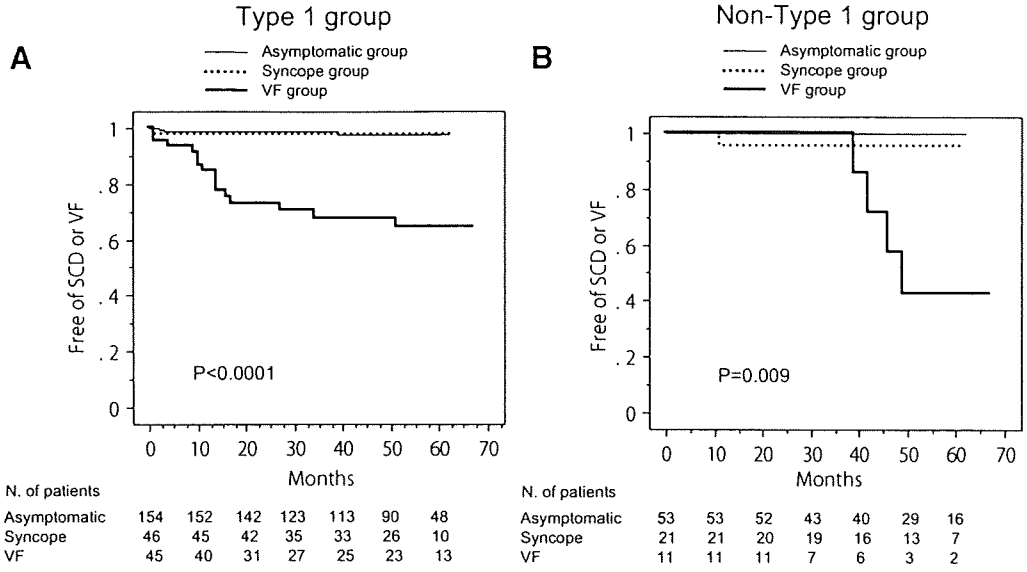


Figure 4. Kaplan–Meier analysis of arrhythmic events (SCD or documented VF) during follow-up depending on the clinical presentation (VF/aborted sudden death, syncope, or asymptomatic) in probands with type 1 ECG (A) and those with non–type 1 ECG (B). $P<0.0001$ represents overall comparison, and $P=0.009$ is for comparison between the VF group and the syncope group. There was no statistically significant difference ($P=0.95$) in the events-free survival of VF probands comparing type 1 and non–type 1 groups.

annual rate of arrhythmic events in probands with type 1 ECG was 10.2% in the VF group, 0.6% in the syncope group, and 0.5% in the asymptomatic group (Figure 4A). The cumulative rate of arrhythmic events in probands with non–type 1 ECG was similar to those with type 1 ECG. The annual arrhythmic event rate was 10.6%, 1.2%, and 0%, respectively (Figure 4B).

By univariate analysis, a family history of SCD was a predictor for arrhythmic events in the type 1 group (hazard ratio [HR], 5.1; 95% CI, 2.0 to 12.8; $P=0.0004$) and the non–type 1 group (HR, 12.3; 95% CI, 2.0 to 74.8; $P=0.006$). Coexistence of posterolateral early repolarization with precordial Brugada-pattern ECG was another predictor in the type 1 group (HR, 4.2; 95% CI, 1.6 to 11.2; $P=0.003$); however, other parameters were not reliable. Figure 5 shows the Kaplan–Meier curves of arrhythmic events in the type 1 group during follow-up, depending on the presence of a family history of SCD (Figure 5A), inferolateral early repolarization (Figure 5B), a spontaneous type 1 ST-elevation (Figure 5C), and inducibility of ventricular arrhythmias by EPS (Figure 5D). Multivariate analysis in all probands identified that the former 2 parameters were independent risk factors for arrhythmic events (a family history of SCD: HR, 3.28; 95% CI, 1.42 to 7.60; $P=0.005$; early repolarization: HR, 2.66; 95% CI, 1.06 to 6.71; $P=0.03$, Table 3) as well as a family history of SCD in analysis of probands without VF (syncope and asymptomatic groups) (HR, 12.5; 95% CI, 2.0 to 75.0; $P=0.005$).

Discussion

Main Findings

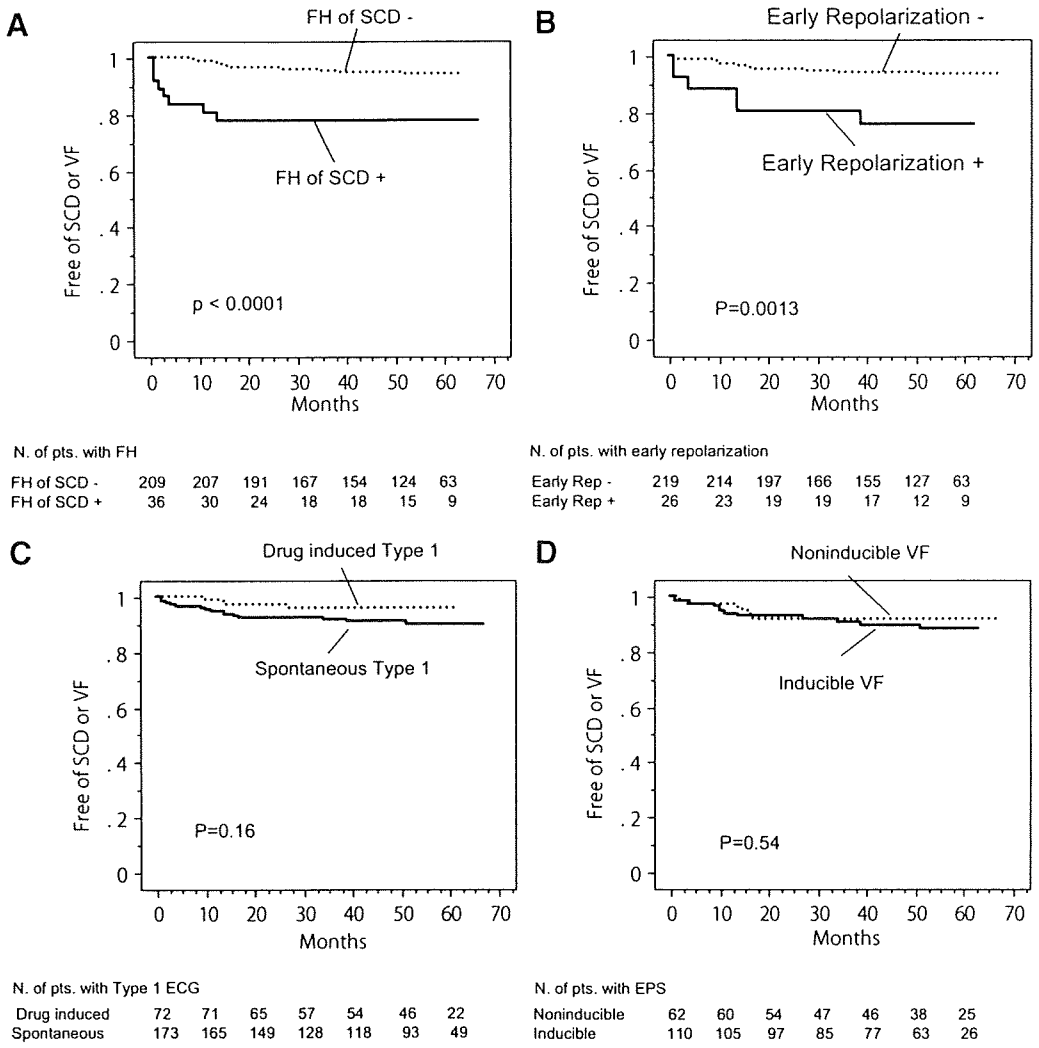
We present one of the largest series of consecutive patients with Brugada-pattern ECG. Importantly, in the present study only probands were included. Also, this study has the longest follow-up ever reported. The main finding is that probands who have a non–type 1 ECG, even after challenged with a sodium channel blocker, do not necessarily have a better prognosis than patients with spontaneous or drug-induced type 1 ECG. Patients presenting with aborted cardiac arrest had a grim prognosis and those presenting with syncope or no symptoms had an excellent prognosis irrespective of their ECG pattern (that is, type 1 versus non–type 1). Also, a family history of sudden death at age <45 years and coexistence of early repolarization in the inferolateral leads were predictors of poor outcome. In contrast, VF/VT inducibility during EPS was not a predictor of outcome.

Comparison With Previous Studies

In this study, the follow-up time was uniform among the 3 groups. The mean follow-up time for the asymptomatic individuals was the longest (47.7±15.0 months) compared with the studies by Brugada et al[2] (27±29 months), Priori et al[3] (34±44 months), and Eckardt et al[5] (33.7±52.2 months). The percentage of female patients (5%) and patients with a family history of SCD (14%) was significantly smaller than 2 of these previous reports (5% versus 24% to 28%[2,3,5]; $P<0.001$, and 14% versus 28% to 54%[2,3,5]; $P<0.001$), although the percentage (14%) of a family history of SCD was similar to that of probands (20%) that Priori et al[3] had reported. The values observed in the present study may reflect the true profile of the probands of Brugada syndrome in contrast to previous studies in which a significant number of family members were also enrolled.

Prognosis of Probands Presenting With Syncope and Without Symptoms

The prognosis of probands in the syncope and asymptomatic groups was very good, and the annual rate of arrhythmic events was ≤1.2%. In the syncope group, this rate is far less than reported in previous studies,[2–5] although the



N. of pts. with FH

FH of SCD -	209	207	191	167	154	124	63
FH of SCD +	36	30	24	18	18	15	9

N. of pts. with early repolarization

Early Rep -	219	214	197	166	155	127	63
Early Rep +	26	23	19	19	17	12	9

N. of pts. with Type 1 ECG

Drug induced	72	71	65	57	54	46	22
Spontaneous	173	165	149	128	118	93	49

N. of pts. with EPS

Noninducible	62	60	54	47	46	38	25
Inducible	110	105	97	85	77	63	26

Figure 5. Kaplan–Meier analysis of fatal arrhythmic events during follow-up depending on a family history (FH) of SCD (FH of SCD− versus FH of SCD +) (A), inferolateral early repolarization (early repolarization− versus early repolarization+) (B), a spontaneous type 1 ST-elevation (drug-induced type 1 versus spontaneous type 1) (C), and inducibility of ventricular arrhythmias by EPS (noninducible VF versus inducible VF) (D).

rate in the asymptomatic group is similar to that in the Eckardt registry[5] and the rate of around 10% for the VF group is comparable to the rate reported in the Brugada registries.[2,8] The reason that the patients in the syncope group showed excellent prognosis is not entirely clear but may be related to the method of registry. Poor prognosis in prior studies is possibly related to the retrospective design of the studies consisting of probands and family members,[2,3,5] in which only severe syncope directly linked to VF tends to be categorized later as a syncope, despite difficulty to determine the cause of syncope at the onset. Even so, we cannot exclude the possibility that some patients with vasovagal syncope were inevitably included in the syncope group because not a few patients have undefined syncope and >30% of Brugada patients are reported to have both vasovagal syncope and the syncope due to ventricular arrhythmia.[12] Another reason for the good prognosis is the difference of genetic background. Brugada syndrome is known to be common in Asian people, which possibly relates to the higher prevalence of polymorphism of haplotype B, associated with the cardiac sodium channel.[13,14] The average prognosis of Asian patients with Brugada syndrome may be better than that of the white population, because individuals without a critical genetic defect are easily detected as a Brugada patient in a routine medical checkup. Further genetic studies are required to clarify the racial difference of outcome. Nevertheless, the patients in this study with an aborted sudden death showed worse prognosis than European people in the study by Eckardt et al[5] and had a similar outcome to those who underwent ICD implantation.[15]

Prognosis of Probands With Non–Type 1 ECG

The outcome of probands with non–type 1 ECG was similar to those with type 1 ECG and the rate of arrhythmic events in the VF group was considerably higher. Some of these patients had shown a coved (type 1) ST-elevation only in the higher (second or third) intercostal spaces during the drug provocation test or follow-up. Miyamoto et al[16] reported that men with a spontaneous type 1 ECG

Table 3. Probability of Sudden Death or VF During Follow-Up Depending on Clinical and Electrophysiological Variables in All Probands (Type 1 and Non–Type 1 Groups)

	Univariate Analysis			Multivariate Analysis		
	HR	95% CI	*P* Value	HR	95% CI	*P* Value
Prior VF	21.46	8.00–57.53	<0.0001	17.48	6.22–49.11	<0.0001
FH of SCD	6.35	2.84–14.19	<0.0001	3.28	1.42–7.60	0.005
Inferolateral ER	4.14	1.71–10.00	0.001	2.66	1.06–6.71	0.03
AF	2.15	0.92–5.03	0.07	0.87	0.36–2.09	0.75
Syncope	0.35	0.08–1.09	0.15			
Sp. type1	2.31	0.67–7.94	0.18			
VF induc. (apex/OT)	1.81	0.72–4.70	0.20			
VF induc. (apex)	1.58	0.60–4.11	0.34			
Male		NA				

FH indicates family history; inferolateral ER, inferolateral early repolarization; AF, atrial fibrillation; Sp. type 1, spontaneous type 1 ST-elevation on 12-lead ECG at baseline; VF induc. (apex/OT), VF induction by programmed pacing at the RV apex or RV outflow tract; and VF induc. (apex), VF induction by programmed pacing at the RV apex.

recorded only at the higher leads V_1 and V_2 showed a prognosis similar to that of men with a type 1 ECG when using standard leads. In the past, patients with non–type 1 ST-elevation in standard ECG had been excluded from studies as a benign entity of Brugada syndrome. However, if patients had a history of aborted sudden death or agonizing nocturnal dyspnea, non–type 1 Brugada-pattern ECG should not be disregarded. Careful follow-up including ECG recording at the higher intercostals spaces and the implantation of ICD is probably required in such a patient to prevent SCD.

Clinical Features of Probands With Non–Type 1 ECG

The clinical profiles of probands were very similar between the non–type 1 group and the type 1 group (Table 2). Inferolateral early repolarization occurred equally in small percentage of patients in both groups (8% and 11%, respectively), which is comparable to the prevalence (12%) of early repolarization that Letsas et al[17] reported in patients with Brugada syndrome. This means that the patient characteristics of the non–type 1 group are much closer to Brugada syndrome than early repolarization syndrome reported by Haïssaguerre et al,[9] in which the VF occurrence rate during sleeping was low (19%) and VF inducibility by EPS was only 34%. Moreover, they reported that several aspects including the relapsing VF and the efficacy of isoproterenol and quinidine,[9,18] which were observed in some patients with early repolarization, were exactly like those of typical Brugada syndrome. Haïssaguerre et al[9] excluded patients with Brugada syndrome, defined as right bundle-branch block and ST-segment elevation >0.2mV in leads V_1–V_3, at the enrollment. However, considering that they possibly included patients with non–type 1 ECG as non-Brugada pattern in their study, some patients with prior VF and early repolarization might have represented non–type 1 Brugada patients of high risk.

Predictors of Outcome

It was reported that male sex, a previous episode of syncope, a spontaneous type 1 ECG, and inducibility of ventricular arrhythmias by EPS are predictors for poor outcome.[2–4] Brugada et al demonstrated that inducibility of ventricular arrhythmias was a reliable marker in patients with and without VF/SCD,[2,4] although Priori et al[3] did not find any significant difference in the analysis of all patients. A spontaneous type 1 ECG was also indicated as a reliable marker of poor prognosis by Brugada et al[4] in the analysis of patients without VF/SCD and by Eckardt et al[5] in all patients.[5] However, we could not find any reliability in these markers (Figures 3 and 5). Inducibility of ventricular arrhythmias was not a significant predictor even if it was evaluated by programmed pacing only from the RV apex (type 1 group: HR, 1.9 [95% CI, 0.7 to 5.2], $P=0.18$; all probands: HR, 1.5 [95% CI, 0.6 to 4.1], $P=0.34$, by univariate analysis).

In contrast, a family history of SCD occurring at age of <45 years is an independent risk factor of a poor prognosis in probands of any groups irrespective of their ECG type (type 1 or non–type 1) or symptoms (with VF or without VF). This was probably caused by a smaller proportion of probands with a family history of SCD as compared with previous studies[2–5] A family history was not found to be a marker in studies that enrolled many patients with SCD or a family history of Brugada syndrome. These results indicate that we should evaluate risks for arrhythmic events cautiously in studies with a significant number of family members.

Early repolarization pattern in the inferolateral leads was another indicator of poor prognosis, although Letsas et al[17] did not find any association with arrhythmic events in the data collected from 3 European centers, which also included ≈30% of patients with a family history of SCD. The reason for the poor outcome in probands with early repolarization in this study is not clear. However, it is conceivable that the combination of precordial Brugada-pattern ST-elevation with inferolateral early repolarization may represent electric heterogeneity in extensive regions of ventricles, which can result in lethal ventricular arrhythmias.

Study Limitations

In this study, premature ventricular electric stimulation was given until refractoriness was reached. The minimal

coupling interval of extrastimuli was not constant between participating hospitals and was sometimes shortened to <200 ms to induce ventricular arrhythmias.

We did not show the results of genetic analysis in this report, although more than half of the patients underwent genetic screening. Detailed results will be presented in a future report. So far, no positive relationship between genetic findings and patient outcomes has been found.[3,19]

We did not record ECGs at the higher intercostals spaces systematically except for probands with cardiac events, because the importance of "high-recording" became apparent in the course of this study.[6] Therefore, some patients of the non–type 1 group may have shown type 1 ST-elevation at the higher precordial positions.

Conclusions

This study described the long-term prognosis of probands with noncoved (non–type 1) Brugada-pattern ECG compared with type 1 ECG. The annual incidence of fatal arrhythmic events was similar between the 2 groups, which reached 10.6% in probands with non–type 1 ECG and a prior episode of VF. A family history of SCD occurring at age of <45 years and the presence of early repolarization were indicators of poor outcome although VF inducibility and a spontaneous type 1 ST-elevation were not reliable indicators in this prospective study including only probands.

Appendix

The following investigators and institutions participated in this study: A. Hukui, Yamagata University, Yamagata; M. Hiraoka, Tokyo Dental and Medical University, Tokyo; S. Takata, Kanazawa University, Kanazawa; H. Sakurada, Hiroo Metropolitan Hospital, Tokyo; Y. Eki, Ibaragi-higashi National Hospital, Tokai; Y. Sasaki, Nagano National Hospital, Ueda; Y. Tomita, Nagoya Medical Center, Nagoya; U. Shintani, Mie-chuo Medical Center, Tsu; T. Hashizume, Minami-Wakayama Medical Center, Tanabe; Y. Fujimoto, Okayama Medical Center, Okayama; W. Matsuura, Higashihiroshima Medical Center, Higashihiroshima; K. Sakabe, Zentuuji National Hospital, Zentuuji; and I. Matsuoka, Kagoshima Medical Center, Kagoshima, Japan.

Sources of Funding

This work was supported by a research grant for cardiovascular diseases (13A-1, 16C-3) from the Ministry of Health, Labor, and Welfare of Japan.

Disclosures

None.

References

1. Brugada P, Brugada J. Right bundle branch block, persistent ST segment elevation and sudden cardiac death: a distinct clinical and electrocardiographic syndrome: a multicenter report. *J Am Coll Cardiol.* 1992;20:1391–1396.
2. Brugada J, Brugada R, Antzelevitch C, Towbin J, Nademanee K, Brugada P. Long-term follow-up of individuals with the electrocardiographic pattern of right bundle-branch block and ST-segment elevation in precordial leads V1 to V3. *Circulation.* 2002;105:73–78.
3. Priori SG, Napolitano C, Gasparini M, Pappone C, Della Bella P, Giordano U, Bloise R, Giustetto C, De Nardis R, Grillo M, Ronchetti E, Faggiano G, Nastoli J. Natural history of Brugada syndrome: insights for risk stratification and management. *Circulation.* 2002;105:1342–1347.
4. Brugada J, Brugada R, Brugada P. Determinants of sudden cardiac death in individuals with the electrocardiographic pattern of Brugada syndrome and no previous cardiac arrest. *Circulation.* 2003;108:3092–3096.
5. Eckardt L, Probst V, Smits JP, Bahr ES, Wolpert C, Schimpf R, Wichter T, Boisseau P, Heinecke A, Breithardt G, Borggrefe M, LeMarec H, Bocker D, Wilde AA. Long-term prognosis of individuals with right precordial ST-segment-elevation Brugada syndrome. *Circulation.* 2005;111:257–263.
6. Shimizu W, Matsuo K, Takagi M, Tanabe Y, Aiba T, Taguchi A, Suyama K, Kurita T, Aihara N, Kamakura S. Body surface distribution and response to drugs of ST segment elevation in Brugada syndrome: clinical implication of eighty-seven-lead body surface potential mapping and its application to twelve-lead electrocardiograms. *J Cardiovasc Electrophysiol.* 2000;11:396–404.
7. Wilde AA, Antzelevitch C, Borggrefe M, Brugada J, Brugada R, Brugada P, Corrado D, Hauer RN, Kass RS, Nademanee K, Priori SG, Towbin JA; Study Group on the Molecular Basis of Arrhythmias of the European Society of Cardiology. Proposed diagnostic criteria for the Brugada syndrome: consensus report. *Circulation.* 2002;106:2514–2519.
8. Antzelevitch C, Brugada P, Borggrefe M, Brugada J, Brugada R, Corrado D, Gussak I, LeMarec H, Nademanee K, Perez Riera AR, Shimizu W, Schulze-Bahr E, Tan H, Wilde AA. Brugada syndrome: report of the second consensus conference: endorsed by the Heart Rhythm Society and the European Heart Rhythm Association. *Circulation.* 2005;111:659–670.
9. Haïssaguerre M, Derval N, Sacher F, Jesel L, Deisenhofer I, de Roy L, Pasquié JL, Nogami A, Babuty D, Yli-Mayry S, De Chillou C, Scanu P, Mabo P, Matsuo S, Probst V, Le Scouarnec S, Defaye P, Schlaepfer J, Rostock T, Lacroix D, Lamaison D, Lavergne T, Aizawa Y, Englund A, Anselme F, O'Neill M, Hocini M, Lim KT, Knecht S, Veenhuyzen GD, Bordachar P, Chauvin M, Jais P, Coureau G, Chene G, Klein GJ, Clémenty J. Sudden cardiac arrest associated with early repolarization. *N Engl J Med.* 2008;358:2016–2023.
10. Hattori Y, Inomata N. Modes of the Na channel blocking action of pilsicainide, a new antiarrhythmic agent, in cardiac cell. *Japan J Pharmacol.* 1992;58:365–373.
11. Morita H, Morita ST, Nagase S, Banba K, Nishii N, Tani Y, Watanabe A, Nakamura K, Kusano KF, Emori T, Matsubara H, Hina K, Kita T, Ohe T. Ventricular arrhythmia induced by sodium channel blocker in patients with Brugada syndrome. *J Am Coll Cardiol.* 2003;42:1624–1631.
12. Letsas KP, Efremidis M, Gavielatos G, Filippatos GS, Sideris A, Kardaras F. Neurally mediated susceptibility in individuals with Brugada-type ECG pattern. *Pacing Clin Electrophysiol.* 2008;31:418–421.
13. Nademanee K, Veerakul G, Nimmannit S, Nimmannit S, Chaowakul V, Bhuripanyo K, Likittanasombat K, Tunsanga K, Kuasirikul S, Malasit P, Tansupasawadikul S, Tatsanavivat P. Arrhythmogenic marker for the sudden unexplained death syndrome in Thai men. *Circulation.* 1997;96:2595–2600.
14. Bezzina CR, Shimizu W, Yang P, Koopmann TT, Tanck MWT, Miyamoto Y, Kamakura S, Roden DM, Wilde AA. Common sodium channel promoter haplotype in Asian subjects underlies variability in cardiac conduction. *Circulation.* 2006;113:338–344.
15. Sacher F, Probst V, Iesaka Y, Jacon P, Laborderie J, Mizon-Gérard F, Mabo P, Reuter S, Lamaison D, Takahashi Y, O'Neill MD, Garrigue S, Pierre B, Jaïs P, Pasquié JL, Hocini M, Salvador-Mazenq M, Nogami A, Amiel A, Defaye P, Bordachar P, Boveda S, Maury P, Klug D, Babuty D, Haïssaguerre M, Mansourati J, Clémenty J, Le Marec H. Outcome after implantation of a cardioverter-defibrillator in patients with Brugada syndrome: a multicenter study. *Circulation.* 2006;114:2317–2324.
16. Miyamoto K, Yokokawa M, Tanaka K, Nagai T, Okamura H, Noda T, Satomi K, Suyama K, Kurita T, Aihara N, Kamakura S, Shimizu W. Diagnostic and prognostic value of a type 1 Brugada electrocardiogram at higher (third or second) V1 to V2 recording in men with Brugada syndrome. *Am J Cardiol.* 2007;99:53–57.
17. Letsas KP, Sacher F, Probst V, Weber R, Knecht S, Kalusche D, Haïssaguerre M, Arentz T. Prevalence of early repolarization pattern in inferolateral leads in patients with Brugada syndrome. *Heart Rhythm.* 2008;5:1685–1689.
18. Haïssaguerre M, Sacher F, Nogami A, Komiya N, Bernard A, Probst V, Yli-Mayry S, Defaye P, Aizawa Y, Frank R, Mantovan R, Cappato R, Wolpert C, Leenhardt A, de Roy L, Heidbuchel H, Deisenhofer I, Arentz T, Pasquié JL, Weerasooriya R, Hocini M, Jais P, Derval N, Bordachar P,

Clémenty J. Characteristics of recurrent ventricular fibrillation associated with inferolateral early repolarization: role of drug therapy. *J Am Coll Cardiol*. 2009;53:612–619.
19. Kusano KF, Taniyama M, Nakamura K, Miura D, Banba K, Nagase S, Morita H, Nishii N, Watanabe A, Tada T, Murakami M, Miyaji K, Hiramatsu S, Nakagawa K, Tanaka M, Miura A, Kimura H, Fuke S, Sumita W, Sakuragi S, Urakawa S, Iwasaki J, Ohe T. Atrial fibrillation in patients with Brugada syndrome relationships of gene mutation, electrophysiology, and clinical backgrounds. *J Am Coll Cardiol*. 2008;51:1176–1180.

CLINICAL PERSPECTIVE

The prognosis of patients with saddleback or noncoved type (non–type 1) ST-elevation in Brugada syndrome is unknown. We compared the long-term prognosis of 85 probands with non–type 1 ECG with 245 probands with coved (type 1) Brugada-pattern ECG prospectively. The absence of type 1 ECG was confirmed by drug provocation test and multiple recordings. Clinical profiles and outcomes did not differ between the non–type 1 and type 1 groups. The annual rate of fatal arrhythmic events was very low in asymptomatic probands and those with syncope but was higher in probands with ventricular fibrillation. A family history of sudden cardiac death at age <45 years and the presence of inferolateral early repolarization were indicators of poor prognosis, although ventricular fibrillation inducibility and a spontaneous type 1 ST-elevation were not reliable parameters in this prospective study including only probands.

Circ J 2009; **73:** 1119–1126

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

— The Suita Study —

Yumiko Hiura, PhD*; Chun-Shen Shen, MD*; Yoshihiro Kokubo, MD**; Tomonori Okamura, MD**; Takayuki Morisaki, MD†; Hitonobu Tomoike, MD**; Teruhiko Yoshida, MD††; Hiromi Sakamoto, PhD††; Yoichi Goto, MD‡; Hiroshi Nonogi, MD‡; Naoharu Iwai, MD*,‡

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]

(Received November 26, 2008; revised manuscript received January 5, 2009; accepted January 12, 2009; released online April 10, 2009)

**Department of Epidemiology, Research Institute, **Division of Preventive Cardiology, †Department of Biosciences, Research Institute, National Cardiovascular Center, Suita, ††Genetics Division, National Cancer Center Research Institute, Tokyo and ‡Division of Cardiology, National Cardiovascular Center, Suita, Japan*

Mailing address: Naoharu Iwai, MD, Department of Epidemiology, Research Institute, National Cardiovascular Center, 5-7-1 Fujishirodai, Suita 565-8565, Japan. E-mail: iwai@ri.ncvc.go.jp

Editorial p1016

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.

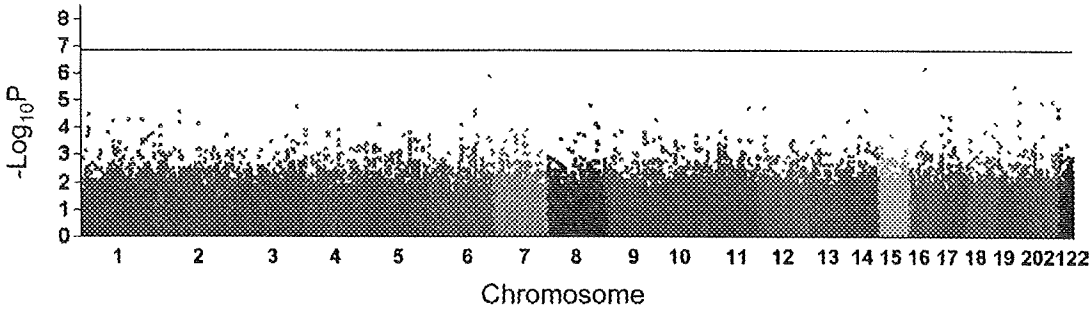


Figure 1. Genome-wide association analysis for high-density lipoprotein (HDL) levels. The levels of significance expressed as $-\log_{10}P$ on the basis of the association analysis of 368,274 single nucleotide polymorphisms with HDL-cholesterol adjusted for sex, body mass index and daily ethanol consumption (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 $-\text{Log}_{10}P$.

‡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.

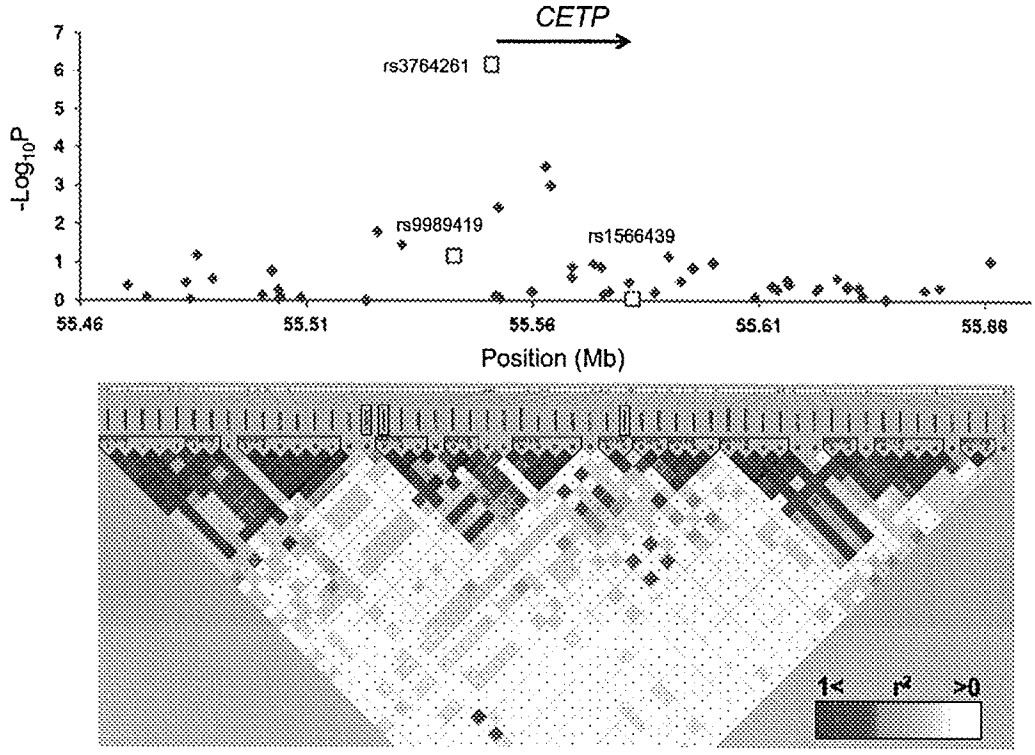


Figure 2. Linkage disequilibrium plot for single nucleotide polymorphisms (SNPs) encompassing the *CETP* region. The strength of the association of each SNP with high-density lipoprotein-cholesterol and its genomic position are presented in the upper panel. The lower panel shows pair-wise linkage disequilibrium (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

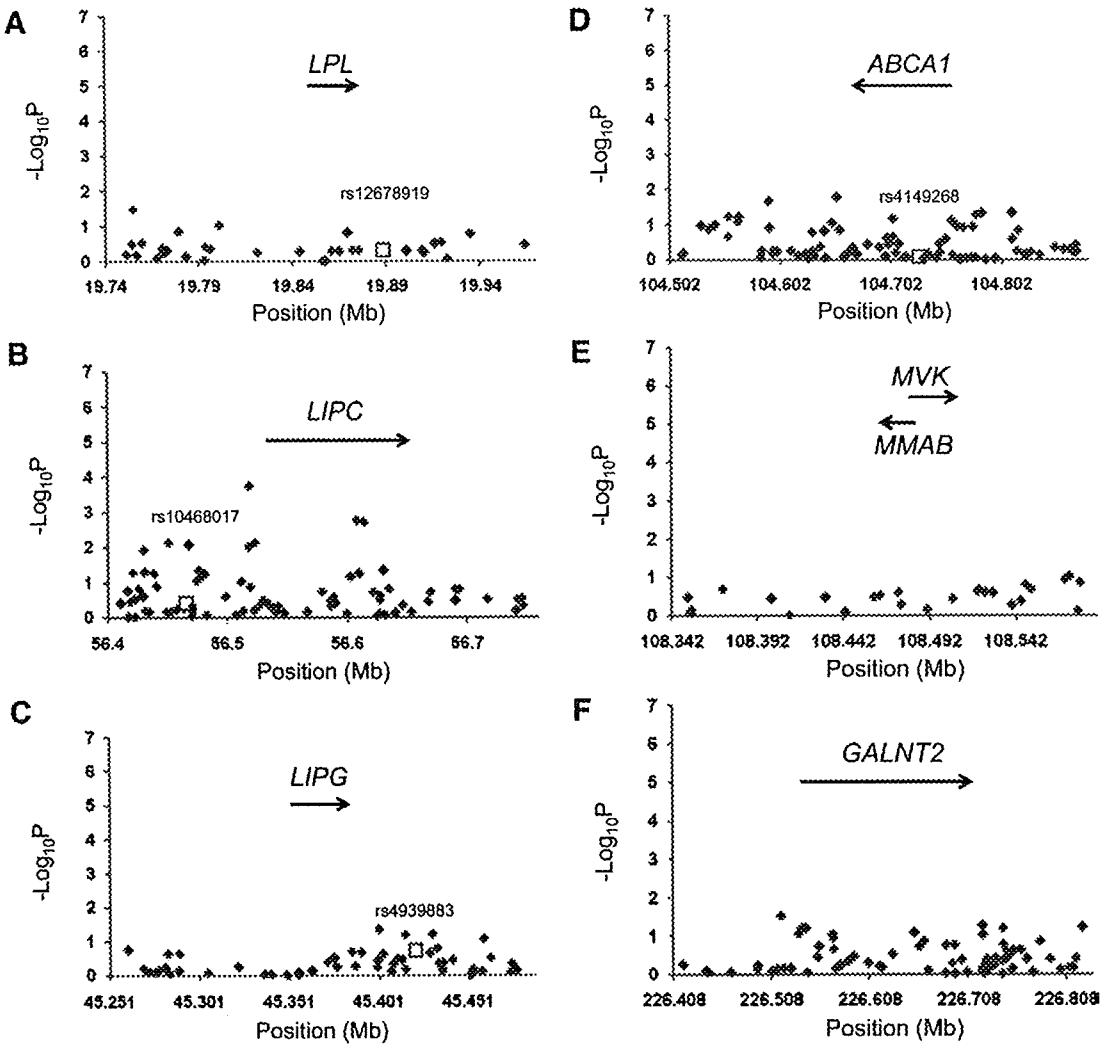


Figure 3. Genome-wide association study results for the previously reported genes; (**A**) *LPL*, (**B**) *LIPC*, (**C**) *LIPG*, (**D**) *ABCA1*, (**E**) *MVK-MMAB* and (**F**) *GALNT2*. Association results for genetic variants within or near (±100 kb) the candidate or novel genes identified through 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 (β, −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 (±100 kb) 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 6.2 mg/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)
	β	P value†	β	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 homozygotes 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;[3,4] 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;[5] 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";[6] 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.

Acknowledgment

We would like to thank all those who participated in the study. In addition, we gratefully acknowledge all the members of Suita City Health Center and the Suita Medical Association.

The present study was supported by a research grant from the Program for the Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation, Japan.

References

1. Gordon DJ, Probstfield JL, Garrison RJ, Neaton JD, Castelli WP, Knoke JD, et al. High-density lipoprotein cholesterol and cardiovascular disease: Four prospective American studies. *Circulation* 1989; **79:** 8–15.
2. Pekkanen J, Linn S, Heiss G, Suchindran CM, Leon A, Rifkind BM, et al. Ten-year mortality from cardiovascular disease in relation to cholesterol level among men with and without preexisting cardiovascular disease. *N Engl J Med* 1990; **322:** 1700–1707.
3. Wilson PW, Abbott RD, Castelli WP. High density lipoprotein cholesterol and mortality: The Framingham Heart Study. *Arteriosclerosis* 1988; **8:** 737–741.
4. Wilson PW. High-density lipoprotein, low-density lipoprotein and coronary artery disease. *Am J Cardiol* 1990; **66:** 7A–10A.
5. Teramoto T, Ohashi Y, Nakaya N, Yokoyama S, Mizuno K, Nakamura H. Practical risk prediction tools for coronary heart disease in mild to moderate hypercholesterolemia in Japan: Originated from the MEGA study data. *Circ J* 2008; **72:** 1569–1575.
6. Shimizu M, Kanazawa K, Hirata K, Ishida T, Hiraoka E, Matsuda Y, et al. Endothelial lipase gene polymorphism is associated with acute myocardial infarction, independently of high-density lipoprotein-cholesterol levels. *Circ J* 2007; **71:** 842–846.
7. Arai H, Yamamoto A, Matsuzawa Y, Saito Y, Yamada N, Oikawa S, et al. Polymorphisms in four genes related to triglyceride and HDL-cholesterol levels in the general Japanese population in 2000. *J Atheroscler Thromb* 2005; **12:** 240–250.
8. Iijima H, Emi M, Wada M, Daimon M, Toriyama S, Koyano S, et al. Association of an intronic haplotype of the LIPC gene with hyperalphalipoproteinemia in two independent populations. *J Hum Genet* 2008; **53:** 193–200.
9. Inazu A, Brown ML, Hesler CB, Agellon LB. Koizumi J, Takata K, et al. Increased high-density lipoprotein levels caused by a common cholesteryl-ester transfer protein gene mutation. *N Engl J Med* 1990; **323:** 1234–1238.
10. Inazu A, Jiang XC. Haraki T, Yagi K, Kamon N, Koizumi J, et al. Genetic cholesteryl ester transfer protein deficiency caused by two prevalent mutations as a major determinant of increased levels of high density lipoprotein cholesterol. *J Clin Invest* 1994; **94:** 1872–1882.
11. Shioji K, Mannami T, Kokubo Y, Goto Y, Nonogi H, Iwai N. An association analysis between ApoA1 polymorphisms and the high-density lipoprotein (HDL) cholesterol level and myocardial infarction (MI) in Japanese. *J Hum Genet* 2004; **49:** 433–439.
12. Helgadottir A, Thorleifsson G, Manolescu A, Gretarsdottir S, Blondal T, Jonasdottir A, et al. A common variant on chromosome 9p21 affects the risk of myocardial infarction. *Science* 2007; **316:** 1491–1493.
13. McPherson R, Pertsemlidis A, Kavaslar N, Stewart A, Roberts R, Cox DR, et al. A common allele on chromosome 9 associated with coronary heart disease. *Science* 2007; **316:** 1488–1491.
14. Saxena R, Voight BF, Lyssenko V, Burtt NP, de Bakker PI, Chen H, et al. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science* 2007; **316:** 1331–1336.
15. Sladek R, Rocheleau G, Rung J, Dina C, Shen L, Serre D, et al. A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature* 2007; **445:** 881–885.
16. The Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007; **447:** 661–678.
17. Wallace C, Newhouse SJ, Braund P, Zhang F, Tobin M, Falchi M, et al. Genome-wide association study identifies genes for biomarkers of cardiovascular disease: Serum urate and dyslipidemia. *Am J Hum Genet* 2008; **82:** 139–149.
18. Willer CJ, Sanna S, Jackson AU, Scuteri A, Bonnycastle LL, Clarke R, et al. Newly identified loci that influence lipid concentrations and risk of coronary artery disease. *Nat Genet* 2008; **40:** 161–169.
19. Chasman DI, Pare G, Zee RYL, Parker AN, Cook NR, Buring JE, et al. Genetic loci associated with plasma concentration of low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, apolipoprotein A1, and apolipoprotein B among 6382 white women in genome-wide analysis with replication. *Circ Cardiovasc Genet* 2008; **1:** 21–30.
20. Heid IM, Boes E, Muller M, Kollerits B, Lamina C, Coassin S, et al. Genome-wide association analysis of high-density lipoprotein cholesterol in the population-based KORA study sheds new light on intergenic regions. *Circ Cardiovasc Genet* 2008; **1:** 10–20.
21. Kooner JS, Chambers JC, Aguilar-Salinas CA, Hinds DA, Hyde CL, Warnes GR, et al. Genome-wide scan identifies variation in MLXIPL associated with plasma triglycerides. *Nat Genet* 2008; **40:** 149–151.
22. Mannami T, Konishi M, Baba S, Nishi N, Terao A. Prevalence of asymptomatic carotid atherosclerotic lesions detected by high-resolution ultrasonography and its relation to cardiovascular risk factors in the general population of a Japanese city: The Suita study. *Stroke* 1997; **28:** 518–525.
23. Iwai N, Katsuya T, Mannami T, Higaki J, Ogihara T, Kokame K, et al. Association between *SAH*, an acyl-CoA synthetase gene, and hypertriglyceridemia, obesity, and hypertension. *Circulation* 2002; **105:** 41–47.
24. Shioji K, Kokubo Y, Mannami T, Inamoto N, Morisaki H, Mino Y, et al. Association between hypertension and the alpha-adducin, beta1-adrenoreceptor, and G-protein beta3 subunit genes in the Japanese population; The Suita study. *Hypertens Res* 2004; **27:** 31–37.
25. Kokubo Y, Iwai N, Tago N, Inamoto N, Okayama A, Yamawaki H, et al. Association analysis between hypertension and *CYBA*, *CLCNKB*, and *KCNMB1* functional polymorphisms in the Japanese population: The Suita Study. *Circ J* 2005; **69:** 138–142.
26. Iwai N, Kajimoto K, Kokubo Y, Okayama A, Miyazaki S, Nonogi H, et al. Assessment of genetic effects of polymorphisms in the MCP-1 gene on serum MCP-1 levels and myocardial infarction in Japanese. *Circ J* 2006; **70:** 805–809.
27. Iwai N, Kajimoto K, Tomoike H, Takashima N. Polymorphism of *CYP11B2* determines salt sensitivity in Japanese. *Hypertension* 2007; **49:** 825–831.
28. Takashima N, Shioji K, Kokubo Y, Okayama A, Goto Y, Nonogi H, et al. Validation of the association between the gene encoding proteasome subunit α type 6 and myocardial infarction in a Japanese population. *Circ J* 2007; **71:** 495–498.
29. Hiura Y, Fukushima Y, Yuno M, Sawamura H, Kokubo Y, Okamura T, et al. Validation of the association of genetic variants on chromo-

some 9p21 and 1q41 with myocardial infarction in a Japanese population. *Circ J* 2008; **72:** 1213–1217.
30. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: Analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005; **21:** 263–265.
31. Barter PJ, Caulfield M, Eriksson M, Grundy SM, Kastelein JJ, Komajda M, et al. Effects of torcetrapib in patients at high risk for coronary events. *N Engl J Med* 2007; **357:** 2109–2122.
32. Borggreve SE, Hillege HL, Wolffenbuttel BH, de Jong PE, Zuurman MW, van der Steege G, et al. An increased coronary risk is paradoxically associated with common cholesteryl ester transfer protein gene variations that relate to higher high-density lipoprotein cholesterol: A population-based study. *J Clin Endocrinol Metab* 2006; **91:** 3382–3388.
33. Sviridov D, Mukhamedova N, Remaley AT, Chin-Dusting J, Nestel P. Antiatherogenic functionality of high density lipoprotein: How much versus how good. *J Atheroscler Thromb* 2008; **15:** 52–62.
34. Bhangale TR, Rieder MJ, Nickerson DA. Estimating coverage and power for genetic association studies using near-complete variation data. *Nat Genet* 2008; **40:** 841–843.
35. Cohen JC, Kiss RS, Pertsemlidis A, Marcel YL, McPherson R, Hobbs HH. Multiple rare alleles contribute to low plasma levels of HDL cholesterol. *Science* 2004; **305:** 869–872.
36. Cupples LA, Arruda HT, Benjamin EJ, D'Agostino RB Sr, Demissie S, DeStefano AL, et al. The Framingham Heart Study 100K SNP genome-wide association study resource: Overview of 17 phenotype working group reports. *BMC Med Genet* 2007; **8**(Suppl 1): S1.

Journal of the American College of Cardiology
© 2009 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 53, No. 22, 2009
ISSN 0735-1097/09/$36.00
doi:10.1016/j.jacc.2009.02.038

Natriuretic Peptides Enhance the Production of Adiponectin in Human Adipocytes and in Patients With Chronic Heart Failure

Osamu Tsukamoto, MD, PHD,*‡ Masashi Fujita, MD, PHD,‡ Mahoto Kato, MD,*
Satoru Yamazaki, PHD,* Yoshihiro Asano, MD, PHD,‡ Akiko Ogai, PHD,*
Hidetoshi Okazaki, MD, PHD,* Mitsutoshi Asai, MD,‡ Yoko Nagamachi, BS,‡
Norikazu Maeda, MD, PHD,§ Yasunori Shintani, MD, PHD,‡ Tetsuo Minamino, MD, PHD,‡
Masanori Asakura, MD, PHD,* Ichiro Kishimoto, MD, PHD,† Tohru Funahashi, MD, PHD,§
Hitonobu Tomoike, MD, PHD,* Masafumi Kitakaze, MD, PHD*

Suita, Osaka, Japan

Objectives We investigated the functional relationship between natriuretic peptides and adiponectin by performing both experimental and clinical studies.

Background Natriuretic peptides are promising candidates for the treatment of congestive heart failure (CHF) because of their wide range of beneficial effects on the cardiovascular system. Adiponectin is a cytokine derived from adipose tissue with various cardiovascular-protective effects that has been reported to show a positive association with plasma brain natriuretic peptide (BNP) levels in patients with heart failure.

Methods The expression of adiponectin messenger ribonucleic acid (mRNA) and its secretion were examined after atrial natriuretic peptide (ANP) or BNP was added to primary cultures of human adipocytes in the presence or absence of HS142-1 (a functional type A guanylyl cyclase receptor antagonist). Changes of the plasma adiponectin level were determined in 30 patients with CHF who were randomized to receive intravenous ANP (0.025 μg/kg/min human ANP for 3 days, n = 15) or saline (n = 15).

Results Both ANP and BNP dose-dependently enhanced the expression of adiponectin mRNA and its secretion, whereas such enhancement was inhibited by pre-treatment with HS142-1. The plasma adiponectin level was increased at 4 days after administration of human ANP compared with the baseline value (from 6.56 ± 0.40 μg/ml to 7.34 ± 0.47 μg/ml, $p < 0.05$), whereas there was no change of adiponectin in the saline group (from 6.53 ± 0.57 μg/ml to 6.55 ± 0.56 μg/ml).

Conclusions Natriuretic peptides enhance adiponectin production by human adipocytes in vitro and even in patients with CHF, which might have a beneficial effect on cardiomyocytes in patients receiving recombinant natriuretic peptide therapy for heart failure. (J Am Coll Cardiol 2009;53:2070–7) © 2009 by the American College of Cardiology Foundation

Plasma natriuretic peptide levels are increased in patients with congestive heart failure (CHF), and the measurement of these peptides is used widely to assess the presence, severity, and prognosis of CHF (1,2). Both atrial natriuretic peptide and brain natriuretic peptide (ANP and BNP, respectively) have a beneficial effect in patients with heart failure because of their various biological actions (3–5).

See page 2078

Adiponectin is a circulating cytokine derived from adipose tissue that has attracted considerable interest because of its identification as a risk factor for cardiovascular disease (6,7) and CHF (8). Adiponectin production is downregulated in patients with coronary risk factors that are associated with the development of heart failure (9,10).

From the Cardiovascular Division of *Medicine and †Biochemistry, National Cardiovascular Center, Suita, Osaka, Japan; and the Departments of ‡Cardiovascular Medicine and §Metabolic Medicine, Osaka University Graduate School of Medicine, Suita, Osaka, Japan. This work is supported by grants-in-aid from the Ministry of Health, Labor, and Welfare-Japan, grants-in-aid from the Ministry of Education, Culture, Sports, Science and Technology-Japan, grants from the Japan Heart Foundation, and grants from the Japan Cardiovascular Research Foundation (all to Dr. Kitakaze) and Takeda Medical Research Foundation (to Dr. Funahashi). Drs. Tsukamoto, Fujita, and Kato contributed equally to this work.

Manuscript received August 27, 2008; revised manuscript received January 22, 2009, accepted February 19, 2009.