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A Novel *SCN5A* Gain-of-Function Mutation M1875T Associated With Familial Atrial Fibrillation

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Objectives This study describes a novel heterozygous gain-of-function mutation in the cardiac sodium (Na^+) channel gene, *SCN5A*, identified in a Japanese family with lone atrial fibrillation (AF).

Background *SCN5A* mutations have been associated with a variety of inherited arrhythmias, but the gain-of-function type modulation in *SCN5A* is associated with only 1 phenotype, long-QT syndrome type 3 (LQTS3).

Methods We studied a Japanese family with autosomal dominant hereditary AF, multiple members of which showed an onset of AF or frequent premature atrial contractions at a young age.

Results The 31-year-old proband received radiofrequency catheter ablation, during which time numerous ectopic firings and increased excitability throughout the right atrium were documented. Mutational analysis identified a novel missense mutation, M1875T, in *SCN5A*. Further investigations revealed the familial aggregation of this mutation in all of the affected individuals. Functional assays of the M1875T Na^+ channels using a whole-cell patch-clamp demonstrated a distinct gain-of-function type modulation; a pronounced depolarized shift (+16.4 mV) in $V_{1/2}$ of the voltage dependence of steady-state inactivation; and no persistent Na^+ current, which is a defining mechanism of LQTS3. These biophysical features of the mutant channels are potentially associated with increased atrial excitability and normal QT interval in all of the affected individuals.

Conclusions We identified a novel *SCN5A* mutation associated with familial AF. The mutant channels displayed a gain-of-function type modulation of cardiac Na^+ channels, which is a novel mechanism predisposing to increased atrial excitability and familial AF. This is a new phenotype resulting from the *SCN5A* gain-of-function mutations and is distinct from LQTS3. (J Am Coll Cardiol 2008;52:1326–34) © 2008 by the American College of Cardiology Foundation

The cardiac sodium (Na^+) channel plays a crucial role in cardiac excitation/contraction via initiating the action potential of the conduction system and working myocytes. Mutations in *SCN5A*—which encodes the α-subunit of voltage-gated cardiac Na^+ channels—have been associated with a variety of cardiac arrhythmias. The loss-of-function mutations result in Brugada syndrome (1), idiopathic ventricular fibrillation (2), cardiac conduction disease (3), or congenital sick sinus syndrome (4), whereas the gain-of-function type modulation in *SCN5A* is associated with only 1 phenotype, long-QT syndrome type 3 (LQTS3) (5).

We reported on the screening for *SCN5A* mutations in Japanese patients with Brugada syndrome (6) and now have extended the cohort to various inherited arrhythmias, given the wide spectrum of clinical phenotypes of cardiac Na^+ channelopathies. In the present study, in a Japanese family with lone atrial fibrillation (AF), we identified a novel missense mutation of *SCN5A* (M1875T). Until recently, only potassium channel mutations have been linked to familial AF (7–10); however, 3 recent reports have identified *SCN5A* loss-of-function mutations: D1275N in 2 families with atrial arrhythmias (AF, cardiac conduction disease, or sick sinus syndrome) plus dilated cardiomyopathy (11,12), and N1986K in a family with lone AF (13). Thus, this is the first report to identify an *SCN5A* gain-of-function type mutation in familial AF.

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Methods

Clinical evaluation. This study was approved by the Institutional Ethics Committee, and all patients provided informed consent. Affected individuals were considered as having AF or premature atrial contractions (PACs) when documented by 12-lead electrocardiograms (ECGs). Lone AF was defined as onset of AF at age <65 years without structural heart disease, hypertension, hyperthyroidism, myocardial infarction, or congestive heart failure. Paroxysmal AF was defined as sporadic AF lasting >30 s for <7 days. When sustained beyond 7 days, AF was considered persistent. Atrial fibrillation refractory to cardioversion or not attempted was classified as permanent. In both sinus rhythm and AF, the mean QT and RR intervals were measured from 3 and 6 consecutive beats, respectively.

Deoxyribonucleic acid (DNA) isolation and mutation analysis. Genomic DNA was isolated from blood lymphocytes and screened for candidate genes by denaturing high-performance liquid chromatography with a WAVE System Model 3500 (Transgenomic, Omaha, Nebraska). Abnormal conformers were amplified by polymerase chain reaction, and sequencing was performed on an ABI PRISM 3100 DNA sequencer (Applied Biosystems, Foster City, California).

Site-directed mutagenesis and electrophysiology. To construct the *SCN5A* mutant, we adopted site-directed mutagenesis performed via a kit, QuickChange II XL (Stratagene, La Jolla, California). The human cell line HEK293 cultured in a 35-mm dish was transiently transfected with 0.5 μg of either pRcCMV-WT or mutant complementary DNA in combination with 0.5 μg of the bicistronic plasmid (pEGFP-IRES-hβ1) encoding enhanced green fluorescent protein and the human β1-subunit (hβ1). The Na^+ currents were recorded 48 h after transfection with the whole-cell patch-clamp technique at 22°C to 23°C as described elsewhere (14). Results are expressed as mean ± SEM, and statistical significance was established with the Student *t* test. Statistical significance was assumed for $p < 0.05$.

Abbreviations and Acronyms

AF = atrial fibrillation
AT = atrial tachycardia
ECG = electrocardiogram
LQTS3 = long-QT syndrome type 3
PAC = premature atrial contraction
WT = wild-type

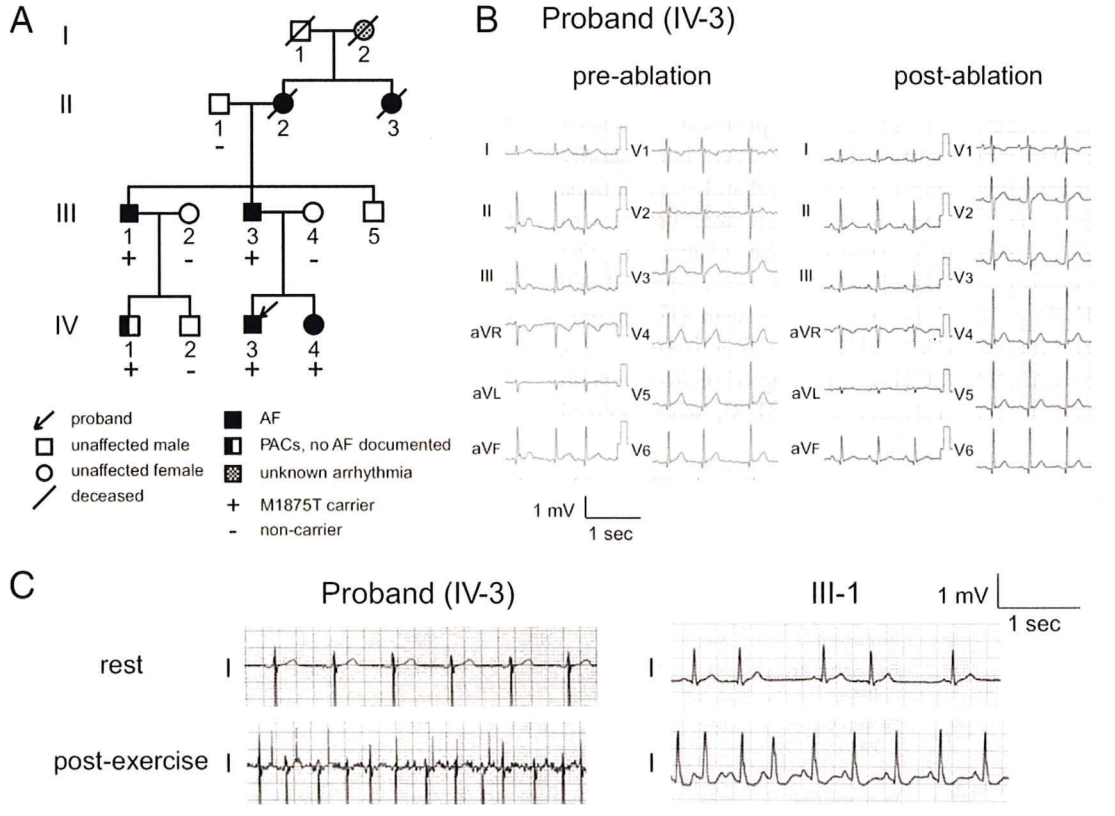

Figure 1 **Pedigree and Clinical Features**

(A) Pedigree of the family. Phenotypic traits are designated within pedigree symbols. **(B)** Electrocardiograms (ECGs) obtained from the proband, IV-3, before (**left panel**, atrial fibrillation [AF]) and after (**right panel**, sinus rhythm) he underwent radiofrequency catheter ablation. **(C)** At rest and post-exercise ECGs recorded from the proband (IV-3) and his uncle (III-1). Exercise-induced AF for the proband and atrial tachycardia for the uncle can be observed. *Continued on next page.*

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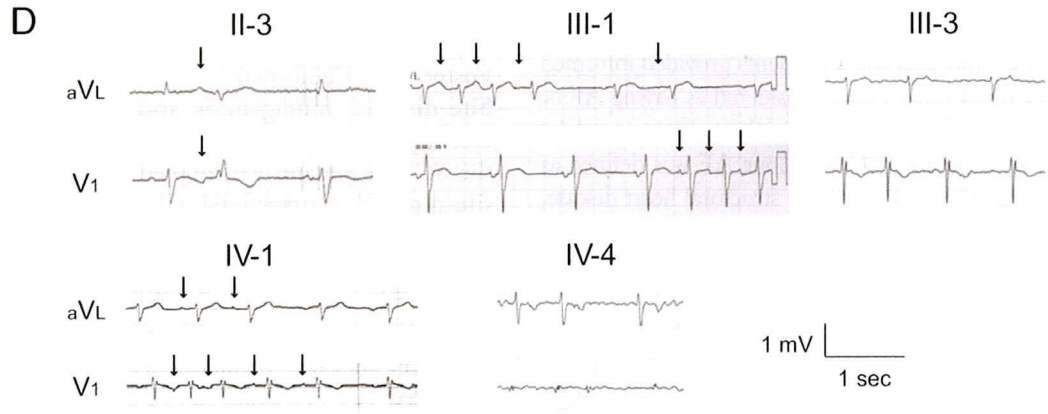

Figure 1 Continued

(D) Electrocardiograms recorded from other affected individuals. **Arrows** indicate P waves of premature atrial contractions (PACs) in II-3, III-1, and IV-1. III-3 and IV-4 demonstrated AF.

Results

Clinical features. We studied a Japanese family with autosomal dominant hereditary AF that spanned 3 generations (Fig. 1A). The proband (IV-3) (Fig. 1A), a 31-year-old man, first experienced repetitive palpitation due to frequent PACs at age 18, which later progressed to paroxysmal AF and atrial tachycardia (AT) at age 27 years (pre-ablation) (Fig. 1B).

Six family members, along with the proband, presented with either AF or frequent PACs (Fig. 1A). The majority shared a similar clinical course with the proband—palpitations due to PACs start in their teens, which later progress to paroxysmal and ultimately persistent AF (Table 1). The proband and his uncle (III-1) showed exercise-induced AT and/or AF (Fig. 1C). The proband's cousin (IV-1) presented with frequent PACs (Fig. 1D) that have not yet progressed to AF. The ECGs of the affected relatives—with the exception to the proband's aunt (II-3), who received disopyramide—did not show any QT prolongation (Fig. 1D, Table 1). Interestingly, the analysis of P wave morphology in the affected family members revealed that the majority of PAC foci were localized in the right atrium (Fig. 1D). The affected individuals received various antiarrhythmic agents (Table 1) but, in most cases, to no avail. There was neither structural heart disease nor a history of major ventricular arrhythmias or sudden cardiac death in this family.

At age 27 years, the proband underwent radiofrequency catheter ablation. Intravenous administration of isoproterenol induced repetitive ATs from multiple origins in the right atrium (Fig. 2A). We successfully ablated the major origin, located in the lower-right atrial septum (Figs. 2B and 2C).

Three years later, the second ablation session was performed due to a relapse of persistent AF. This time, we identified 2 other PAC foci in the right atrium (Fig.

Table 1 Clinical Characteristics of Affected Individuals

Individual	Gender	Age (yrs)	Arrhythmias	Onset of PAC (yrs)	Onset of AF (yrs)	Mutation Carrier	HR (beats/min)	QRS (ms)	QTc	LAD (mm)	LVEF (%)	Antiarrhythmic Agents Used to Treat AF
I-2	F	90*	Unknown	NA	NA	ND	NA	NA	NA	NA	NA	NA
II-2	F	75*	Permanent AF	NA	NA	ND	88	74	427	36	79	—
II-3	F	75*	Paroxysmal AF, PACs	NA	NA	ND	77†	104†	478†	30	75	Disopyramide
III-1	M	60	Paroxysmal AF, PACs	NA	48	Yes	70	92	394	32	75	Disopyramide, cibenzoline, aprindine
III-3	M	57	Permanent AF	15	51	Yes	71	82	385	NA	NA	—
IV-1	M	34	PACs	23	—	Yes	68	82	399	ND	ND	—
IV-3	M	31	Persistent AF	18	27	Yes	65	81	388	30	63	Pilsicainide, flecainide
IV-4	F	29	Permanent AF	12	26	Yes	88	87	429	31	61	Pilsicainide

*Age of death; †disopyramide administration.
AF = atrial fibrillation; HR = heart rate; LAD = left atrial dimension; LVEF = left ventricular ejection fraction; NA = records not available; ND = not determined; PAC = premature atrial contraction.

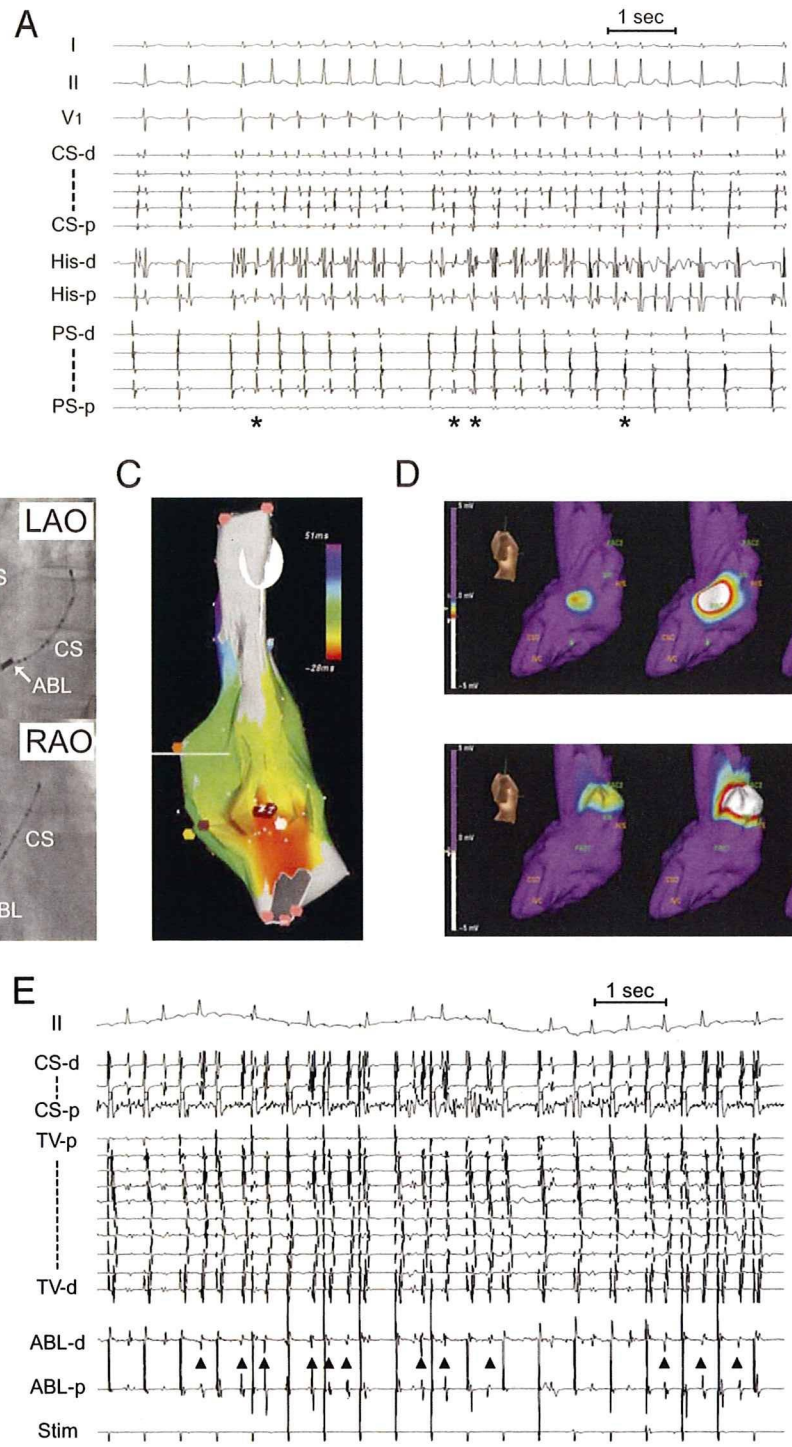

Figure 2 **Radiofrequency Catheter Ablation Recordings**

The first **(A to C)** and the second **(D and E)** ablation sessions on the proband. **(A)** Repetitive atrial tachycardias (ATs) from multiple origins. Shown are intracardiac recordings from the coronary sinus (CS), the His bundle (His), and a catheter placed along the posterior septum (PS) in the right atrium. Note that atrial activation sequences in CS during ATs were consistently proximal to distal, suggesting right atrial origins. **Asterisks** indicate the successfully ablated AT originating from the lower-right atrial septum. **(B)** Fluoroscopic images of the electrodes in the left anterior oblique (LAO) and right anterior oblique (RAO) views. Position of the ablation catheter (ABL; indicated by **arrows**); successful ablation site of the AT. **(C)** Three-dimensional electroanatomic map of the AT in the right posterior oblique view. The AT focus in the lower-right atrial septum was successfully ablated in the first session. **(D)** Noncontact mapping of PACs in the right lateral view. Two PAC foci in the middle of the crista terminalis **(upper panel)** and the high posterolateral wall **(lower panel)** ablated successfully in the second session are shown. **(E)** Numerous electrical firings (▲) from the contact site during radiofrequency energy delivery in generating tricuspid valve isthmus block. Continuous pacing was performed from proximal CS. Stim = stimulator; TV = tricuspid valve annulus; other abbreviations as in Figure 1.

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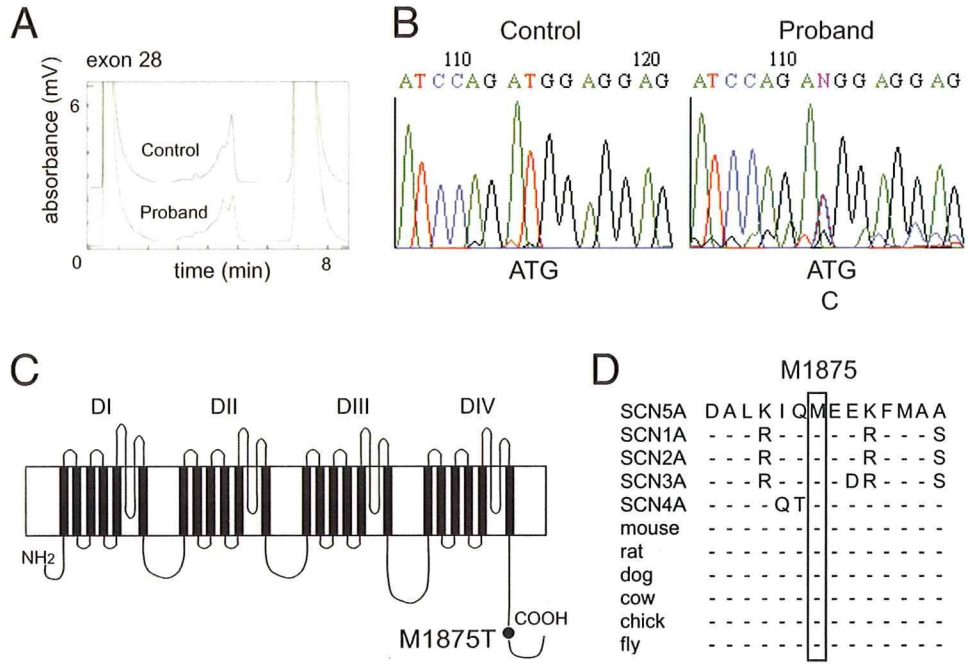

Figure 3 Genetic Analysis

(A) The denaturing high-performance liquid chromatography elution profiles for an *SCN5A* mutation, M1875T, detected in the affected family members. **(B)** Deoxyribonucleic acid sequencing electropherograms demonstrate heterozygosity for a *SCN5A* mutation. **(C)** Topology of the α-subunit of voltage-gated cardiac sodium (Na^+) channel. The location of the detected mutation is shown. **(D)** Amino acid sequence alignments of *SCN5A* with related Na^+ channels. The *SCN5A* indicates human heart; *SCN1A*, human brain type I; *SCN2A*, human brain type II; *SCN3A*, human brain type III; *SCN4A*, human skeletal muscle. The sequences of other species represent the cardiac isoforms of Na^+ channels.

2D)—both were successfully ablated. During the subsequent procedure to generate a cavotricuspid isthmus block, we noticed that energy delivery from the catheter induced numerous electrical firings from the contact sites (Fig. 2E). After the second ablation session, he maintained a sinus rhythm under medication, yet even after which he experienced occasional episodes of paroxysmal AF. Interestingly, after each attempt of cardioversion, ATs that lasted for several seconds were observed immediately after the shock was delivered and before sinus rhythm conversion (data not shown). All of these features strongly suggest the proband's increased vulnerability to atrial arrhythmias.

Genetic analysis. We identified a novel missense mutation, c.5624T>C, p.M1875T, in the *SCN5A* gene in the proband. Figure 3 shows the denaturing high-performance liquid chromatography and sequence results (Figs. 3A and 3B) and an illustration showing the position of the identified mutation (Fig. 3C). The amino acid at codon 1875 (methionine) is highly conserved among different Na^+ channel isoforms and species (Fig. 3D). Furthermore, this mutation was absent in 210 Japanese control individuals (420 chromosomes). We failed to identify mutations in any other potential candidate genes of familial AF (*KCNQ1*, *KCNH2*, *KCNE1*, *KCNE2*, *KCNE3*, *KCNJ2*, and *KCNA5*). Further analysis of the family members revealed that the M1875T mutation in SCN5A perfectly matched their clinical phenotypes (Figs. 1A and 1D, Table 1).

Functional analysis of M1875T-*SCN5A*. We performed biophysical assays for the novel *SCN5A* mutation with a heterologous expression system in HEK293 cells. Figure 4A illustrates representative whole-cell current traces from cells expressing wild-type (WT) and M1875T Na^+ channels in the presence of the coexpressed Na^+ channel β subunit.

Notably, M1875T channels showed an apparently slower inactivation compared with WT. The time constants for both fast and slow inactivation across a wide range of test potentials were significantly larger with M1875T in comparison with WT (Fig. 4B), indicating impaired inactivation. Figure 4C shows the peak current-voltage relation for WT and M1875T channels. The maximum current density of WT was observed at −20 mV but shifted to −30 mV for M1875T. In addition, the peak current density of M1875T was significantly larger than WT (WT, 326.2 ± 28.2 pA/pF, n = 23; M1875T, 484.6 ± 49.6 pA/pF, n = 31, $p < 0.01$) (Fig. 4D). As in WT, M1875T channels showed no persistent inward Na^+ currents at the end of a 200-ms depolarization (Fig. 4E), which is one of the defining mechanisms of QT interval prolongation in patients with LQTS3. The subtracted amplitude at the end of the 200-ms depolarization was 0.046 ± 0.009% (n = 5) of the peak current for WT and 0.048 ± 0.038% (n = 7) for M1875T.

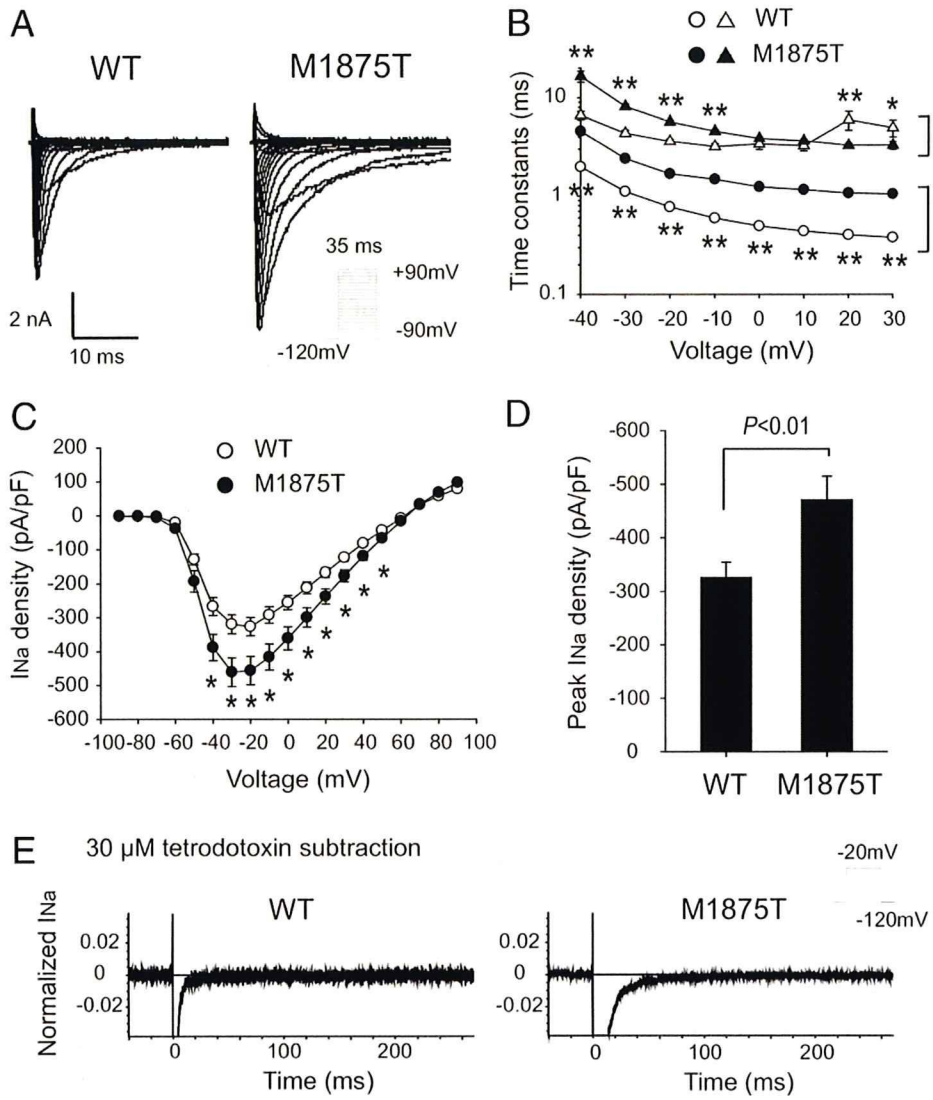

Figure 4 **Macroscopic Na^+ Currents of M1875T Channels**

(A) Representative whole-cell current traces of wild-type (WT) and M1875T sodium (Na^+) channels. Cells were transfected with human β1-subunit (protocol shown as an inset). **(B)** Voltage dependence of inactivation time constants. The time course of inactivation was fit with a 2 exponential equation: $I/I_{max} = A_f \times \exp(-t/\tau_f) + A_s \times \exp(-t/\tau_s)$. Lower and upper bundles of symbols indicate fast (τ_f) and slow (τ_s) time constant values, respectively. Statistically significant differences are indicated (*$p < 0.05$, **$p < 0.01$). **(C)** Average current-voltage relationship for WT and M1875T channels. The current is normalized to cell capacitance to give a measure of Na^+ current density. **Asterisks** indicate the voltages at which the current density was statistically different (*$p < 0.05$). **(D)** Average peak Na^+ current density of WT and M1875T channels. The peak current density was significantly larger in M1875T (WT at −20 mV, 326.2 ± 28.2 pA/pF, n = 23; M1875T at −30 mV, 484.6 ± 49.6 pA/pF, n = 31, $p < 0.05$). **(E)** Representative Na^+ current traces recorded in the absence or presence of 30 μmol/l tetrodotoxin. Tetrodotoxin-sensitive persistent currents were calculated by digital subtraction. M1875T channels showed no persistent inward Na^+ currents.

Figure 5A shows the conductance-voltage and steady-state inactivation curves for WT and M1875T channels. Numerical data pertaining to the biophysical properties therein are summarized in Table 2. The parameters for the activation gate were similar between WT and M1875T. In contrast, the half-maximal potential ($V_{1/2}$) for the steady-state inactivation of M1875T showed a marked positive shift (+16.4 mV) compared with that of WT (WT, $V_{1/2}$ = −78.08 ± 0.94 mV, n = 22; M1875T, $V_{1/2}$ = −61.68 ± 0.76 mV, n = 33, $p < 0.01$). The slope factor (k) for M1875T was significantly larger than that of WT (WT, k = −7.13 ± 0.12, n = 22; M1875T, k = −6.07 ± 0.16, n = 33, $p < 0.01$). The pronounced depolarizing shift of the inactivation gate is likely to increase Na^+ channel availability during excitation.

We also investigated the other kinetic properties of Na^+ channels: recovery from inactivation, onset of slow inactivation, and closed-state inactivation. Parameters of recovery from inactivation and onset of slow inactivation were identical between WT and M1875T (Figs. 5B and 5C,

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Table 2 Biophysical Properties of WT and M1875T Channels

	WT	M1875T
Activation (mV)	(n = 23)	(n = 37)
$V_{1/2}$	−43.61 ± 0.79	−44.09 ± 0.72
k	6.53 ± 0.16	5.98 ± 0.18
Steady-state inactivation (mV)	(n = 22)	(n = 33)
$V_{1/2}$	−78.08 ± 0.94	−61.68 ± 0.76†
k	−7.13 ± 0.12	−6.07 ± 0.16†
Recovery from inactivation	(n = 16)	(n = 25)
A_f	0.84 ± 0.01	0.84 ± 0.01
A_s	0.15 ± 0.01	0.15 ± 0.01
τ_f (ms)	8.95 ± 0.95	8.32 ± 0.83
τ_s (ms)	338.6 ± 30.4	271.6 ± 31.6
Onset of slow inactivation	(n = 15)	(n = 16)
A	0.12 ± 0.01	0.12 ± 0.01
τ (ms)	773.9 ± 90.0	647.2 ± 70.6
Closed-state inactivation	(n = 9)	(n = 9)
A	0.13 ± 0.03	0.05 ± 0.02*
τ (ms)	97.1 ± 7.8	212.7 ± 22.3†

Data are mean ± SEM. Parameters were obtained from fitting individual experiments illustrated in Figure 4. *p < 0.05; †p < 0.01 versus wild-type (WT).

A and τ = fractional amplitude and time constant, respectively; n = number of tested cells; $V_{1/2}$ and k = midpoint potential and slope factor, respectively.

Table 2). With regard to closed-state inactivation, the extent was significantly less (WT, A = 0.13 ± 0.03 ms, n = 9; M1875T, A = 0.05 ± 0.02 ms, n = 9, $p < 0.05$), and the time constant was larger in the M1875T channels when compared with WT (WT, τ = 97.1 ± 7.8 ms, n = 9; M1875T, τ = 212.7 ± 22.3 ms, n = 9, $p < 0.01$) (Fig. 5D, Table 2). These data suggest that the number of inactivated M1875T channels is reduced near the resting potential.

Collectively, the M1875T mutation exhibited a gain-of-function type modulation in the cardiac Na^+ channels without persistent inward Na^+ currents: increased peak Na^+ channel density; prolonged time constants of both fast and slow inactivation; a large depolarizing shift in $V_{1/2}$ of the steady-state inactivation; and a lesser extent and a larger time constant with regard to closed-state inactivation. In short, the M1875T mutation clearly demonstrates characteristics that make it distinct from the LQTS3-type gain-of-function modulation.

Discussion

In the present study, we identified a novel gain-of-function *SCN5A* mutation that causes a familial form of AF. The clinical course of AF development materialized in a similar fashion among all affected family members (i.e., palpitations due to frequent PACs and ATs in their teens, followed by paroxysmal and then persistent AF). During the clinical electrophysiological study in the proband, we recognized multifocal activity sites and increased excitability in the right atrium. These distinguishing features are presumably associated with the unique biophysical properties of the mutant Na^+ channels. It should be noted, however, that the size of the pedigree analyzed in this study is limited.

***SCN5A* mutations and familial AF.** Mutations in *SCN5A* have been reported to cause a wide variety of cardiac arrhythmias. The gain-of-function mutations result in LQTS3 (5), whereas the loss-of-function mutations result in various phenotypes: 1) Brugada syndrome; 2) idiopathic ventricular fibrillation; 3) cardiac conduction disease; and 4) congenital sick sinus syndrome. We previously reported that *SCN5A*-linked Brugada syndrome is a high-risk group of bradyarrhythmias, linked predominantly to sick sinus syndrome (6). Although AF is a common complication of Brugada syndrome (10% to 30%) (6,15), there is a scarcity of reports on *SCN5A*-positive Brugada syndrome and AF.

Atrial fibrillation is the most common form of cardiac arrhythmia, characterized by rapid irregular activation of the atrium, and a common cause of morbidity and mortality. Atrial fibrillation occurs predominantly in elderly persons and is frequently associated with underlying cardiac diseases. In 15% to 30% of patients, however, an etiology is absent (i.e., lone AF) (16,17). Although AF has been regarded a sporadic and acquired disease, the familial aggregation of AF has been shown to be more frequent than previously recognized (18,19). Chen et al. (7) found the first gene mutation responsible for familial AF in *KCNQ1*, which encodes the α-subunit of slow delayed rectifier potassium (K^+) channels. Since then, 3 additional genes—all of which encode cardiac K^+ channels—responsible for familial AF have been identified: *KCNE2* (8), *KCNJ2* (9), and *KCNA5* (10). Recently, loss-of-function *SCN5A* mutations (D1275N) were reported to be associated with 2 families who have atrial arrhythmias (AF, cardiac conduction disease, and sick sinus syndrome) with dilated cardiomyopathy (11,12). More recently, an *SCN5A* mutation (N1986K) was identified in a family with lone AF (13). Functional assays on the N1986K channels revealed a hyperpolarized shift of steady-state inactivation, indicating a loss-of-function type modulation. One of the affected members underwent pacemaker implantation due to sick sinus syndrome, suggesting the underlying conduction disturbance resulting from the Na^+ channel loss-of-function. In addition, a common polymorphism (H558R) in *SCN5A*, present in 20% of the population (20), reduces Na^+ current density (21). The screening for the polymorphism in 157 patients with lone AF revealed that the R558 allele was more common in patients with lone AF than in the control subjects and as such was considered to be a risk factor for lone AF (22). However, none of the M1875T-positive individuals carried the R558 allele.

These reports implicate a potential relationship between decreased Na^+ currents and AF; however, to date, an *SCN5A* gain-of-function mutation has never been linked to AF.

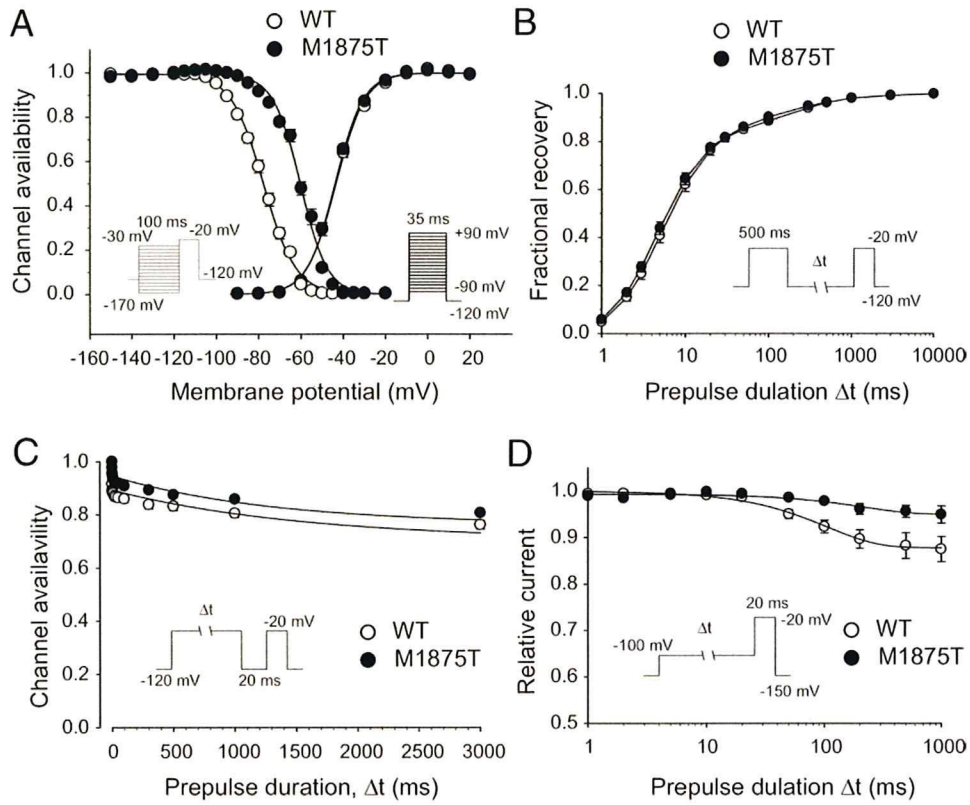

Figure 5 **Gating Properties for M1875T Channels**

Detailed parameters are given in Table 2. **(A)** Voltage dependence of relative sodium (Na^+) conductance activation and steady-state inactivation were determined by means of the voltage protocols, as shown in the inset. Curves were fit with the Boltzmann equation, $I/I_{max} = (1 + \exp[V-V_{1/2}]/k)^{-1}$ to determine the membrane potential for half-maximal inactivation or activation ($V_{1/2}$) and the slope factor *k*. Note that M1875T channels showed a pronounced depolarized shift (+16.4 mV) in the $V_{1/2}$ of steady-state inactivation compared with wild-type (WT). **(B)** Time course of recovery from inactivation was elicited with a double pulse protocol. Data were fit with a 2 exponential equation: $I/I_{max} = A_f \times (1 - \exp[-t/\tau_f]) + A_s \times (1 - \exp[-t/\tau_s])$, where A_f and A_s are fractions of fast and slow inactivation components, and τ_f and τ_s are the time constants of fast and slow inactivation components, respectively. **(C)** Onset of slow inactivation. Time course of entry into the slow inactivation state was obtained by a double pulse protocol. Curves were fit with a single exponential equation: $I/I_{max} = y0 + A \times \exp(-t/\tau)$. **(D)** Closed-state inactivation. The transfer rate of Na^+ channels from closed-state to inactivated closed-state without an intervening opening state was measured by a double pulse protocol. Time course for development of closed-state inactivation was fit with a single exponential equation: $I/I_{max} = y0 + A \times \exp(-t/\tau)$. The extent of closed-state inactivation was significantly less and the time constant larger in M1875T channels in comparison with WT.

Unique gain-of-function properties of M1875T Na^+ channels. To date, *SCN5A* gain-of-function mutations have been reportedly linked to only 1 phenotype, LQTS3. Persistent inward Na^+ currents observed in these mutant channels are considered to cause QT prolongation. However, M1875T channels did not display persistent inward Na^+ currents (Fig. 4E). This might explain why all of the affected and mutation-positive individuals in our study exhibited normal QT interval, with the exception of 1 individual who received disopyramide therapy. The functional properties of M1875T Na^+ channels were quite distinct from those of LQTS3. The most prominent change was a +16.4 mV shift in the steady-state inactivation. This is, to the best of our knowledge, the greatest depolarization shift in all of the previously reported SCN5A mutants. Some of the LQTS3 mutants (E1295K, A1330P, A1330T, and I1768V) showed a similar depolarizing shift of the steady-state inactivation without persistent inward Na^+ currents; however, the extent of the depolarizing shift was much less than M1875T (all were <+10 mV). The M1875T channels displayed the increased peak Na^+ current density (Fig. 4D), perhaps due to the large depolarized shift in steady-state inactivation. Interestingly, the location of the mutation is within a complex region that includes Ca^{2+} binding EF-hand like motifs and a putative binding site for calmodulin (23), and thus the mutation might disrupt inactivation by altered calcium sensitivity.

The potential mechanisms by which the identified gain-of-function mutation might lead to PAC or AF could be explained as the following: first, increased inward Na^+ currents might cause repolarization failure or early afterdepolarizations, thereby inducing triggered activities; and second, the increased Na^+ currents might increase the conduction velocity and facilitate the maintenance of the fibrillation wave. However, further studies are needed to elucidate the underlying mechanisms.

Genotype–phenotype relationship and clinical implications. Quite impressively, the affected family members shared a similar clinical course with high penetrance—frequent PACs and ATs first appeared during their teens and subsequently progressed to AF (Fig. 1A). Increased automaticity and irritability in the atrium was demonstrated by recurrent atrial arrhythmias that were resistant to ablation or drug therapy, induced PACs during exercise (Fig. 1C), and numerous ectopic firings and increased excitability throughout the right atrium during catheter ablation (Fig. 2). Because Na^+ channels encoded by *SCN5A* are expressed in both the atrium and ventricle, it remains unknown why our patients showed only atrial arrhythmias but not ventricular arrhythmias. The different electrophysiological properties between atrial and ventricular cells might be the underlying cause. Resting membrane potential is more depolarized, and the peak Na^+ current density is larger in atrial cells than in ventricular cells in dogs (24). The critical depolarization and current threshold for action potential initiation are smaller in atrial cells than in ventricular cells, indicating that atrial cells are more readily excitable than ventricular cells (25).

Conclusions

We identified a novel *SCN5A* gain-of-function mutation that causes a familial form of AF without any underlying structural heart diseases, which provides us with new insight into the pathogenesis of the commonly occurring form of AF.

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Key Words: arrhythmia ■ atrial fibrillation ■ genetics ■ ion channels ■ sodium.

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Better Survival With Statin Administration After Revascularization Therapy in Japanese Patients With Coronary Artery Disease

—— Perspectives From the CREDO-Kyoto Registry ——

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Background The importance of statins in cardiovascular prevention has been demonstrated in various patient subsets. This study aimed to evaluate the effects of statins on long-term outcomes of Japanese patients undergoing their first coronary revascularization.

Methods and Results A total of 9,225 patients undergoing their first coronary revascularizations during 2000–2002 were divided into 2 groups according to the use of statins at discharge; patients with acute myocardial infarction were not included. Statins was administered to only 28.5% (n=2,630) of the patients. The median follow-up period was 3.5 years. Patients on statin therapy showed lower all-cause (5.2% vs 10.0%; p<0.0001) and cardiovascular (3.2% vs 6.2%; p<0.0001) mortality than those without statins (n=6,595) by Kaplan-Meier analysis and log-rank test. After adjustment by multivariate analysis according to 29 variables, statin therapy remained as an independent predictor of reduced all-cause (relative risk ratio (RR) 0.71, 95% confidence interval (CI) 0.59–0.86, p=0.0005) and cardiovascular (RR 0.72, 95% CI 0.56–0.91, p=0.0067) mortality. The validity of RR of statin therapy in multivariate analysis was further confirmed by risk adjustment using propensity scores (all-cause mortality: propensity-adjusted RR 0.70, 95% CI 0.58–0.85, p=0.0003; cardiovascular mortality: propensity-adjusted RR 0.70, 95% CI 0.54–0.89, p=0.0038).

Conclusions Statin therapy started at hospital discharge was associated with increased chance of survival in Japanese patients undergoing their first coronary revascularization. (*Circ J* 2008; **72:** 1937–1945)

Key Words: Coronary artery disease; Mortality; Revascularization; Statins

Optimized medical therapy, as well as appropriate lifestyle modification, is important to reduce cardiovascular risks in the secondary prevention of coronary artery disease (CAD). Common medications for cardiovascular prevention in contemporary clinical practice include antiplatelet drugs, HMG-CoA reductase inhibitors (statins), inhibitors of the rennin–angiotensin system (ie, angiotensin-converting enzyme inhibitors (ACEI) and angiotensin II type I receptor blockers (ARB)), and β-adrenergic blockers[1,2] In particular, consistent prognostic benefits of statins have been shown in a number of primary as well as secondary prevention trials[3–8] The study subjects have been widely distributed from hypercholesterolemic patients without CAD to specific high-risk groups such as diabetic patients, patients with acute coronary syndrome and patients undergoing percutaneous coronary intervention (PCI) or coronary artery bypass graft surgery (CABG)[9–13] Cardiovascular events can be further prevented when the low-density lipoprotein-cholesterol level is intensively decreased below the normal range with statin therapy[11,14] Much of this evidence has been obtained from randomized trials in the United States or Europe where the prevalence and mortality of CAD are higher and higher doses of statins are approved by public medical insurance systems, relative to Japan. Therefore, the data may not be directly applicable to practice in different clinical and genetic back grounds such as Japanese patients. Recently, coronary risk reduction by pravastatin has been shown in Japanese patients in the setting of primary prevention[15] In addition, recent trials have demonstrated effective cardiovascular prevention by

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Investigators in the Coronary REvascularization Demonstrating Outcome study in Kyoto (CREDO-Kyoto) registry are listed in Appendix 1.

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Table 1 Baseline Characteristics of the Patients According to Statin Treatment at Hospital Discharge

Characteristic	Statin-treated (n=2,630)	Statin-non-treated (n=6,595)	p value
Demographic characteristics			
Age (mean±SD)	65.2±10.0	67.8±9.9	<0.0001
≥75 years (%)	18.1	26.4	<0.0001
Male gender (%)	62.6	73.7	<0.0001
Mode of revascularization PCI (%)	79.8	66.3	<0.0001
BMI (kg/m^2)	24.4±3.2	23.4±3.2	<0.0001
≥25 kg/m^2 (%)	39.4	27.8	<0.0001
Family history of CAD (%)	19.1	14.4	<0.0001
Current smoking (%)	27.9	28.5	NS
Coexisting conditions			
Unstable angina (%)	7.7	7.5	NS
Prior MI (%)	22.6	26.5	0.0001
History of CHF (%)	11.8	18.4	<0.0001
History of CVA (%)	13.6	17.4	<0.0001
Peripheral vascular disease (%)	6.5	7.6	NS
Atrial fibrillation (%)	4.8	7.5	<0.0001
Anemia: Hb <10 g/dl (%)	3.9	7.8	<0.0001
COPD (%)	1.2	2.9	<0.0001
Liver cirrhosis (%)	1.6	3.7	<0.0001
Serum TC	211.9±44.4	193.1±35.3	<0.0001
≥220 mg/dl (%)	39.5	21.0	<0.0001
Serum LDL-C	130.1±41.1	119.0±31.2	<0.0001
≥130 mg/dl (%)	46.6	34.2	<0.0001
Serum HDL-C	50.2±14.8	47.3±13.5	<0.0001
<40 mg/dl (male); <50 mg/dl (female) (%)	32.8	37.8	<0.0001
Serum TG	163.6±103.3	137.2±84.2	<0.0001
≥150 mg/dl (%)	46.2	30.9	<0.0001
Hypertension (%)	71.3	68.4	0.0059
Diabetes mellitus (%)	40.6	38.0	0.0235
Insulin Tx (%)*	19.5	22.5	0.0495
CKD: GFR <60 ml/min (%)	31.8	42.9	<0.0001
Left main CAD (%)	7.3	10.3	<0.0001
Proximal LAD lesion (%)	41.1	42.6	NS
Multivessel disease (%)	63.6	63.6	NS

Values are presented as frequencies (%) within diabetic patients.
PCI, percutaneous coronary intervention; BMI, body mass index; CAD, coronary artery disease; NS, not statistically significant; MI, myocardial infarction; CHF, congestive heart failure; CVA, cerebrovascular accident; Hb, hemoglobin; COPD, chronic obstructive pulmonary disease; TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglycerides; Tx, therapy; CKD, chronic kidney disease; GFR, glomerular filtration rate; LAD, left anterior descending.

statin therapy in Japanese patients with acute myocardial infarction (AMI).[16,17] The association of statin therapy with better outcomes has also been shown in Japanese patients with AMI or angiographically proven CAD.[18,19]

The aim of this study was to assess the preventive effects of medical therapies prescribed at hospital discharge on the long-term prognosis in Japanese CAD patients undergoing their first coronary revascularization by PCI or CABG, with an emphasis on statins. The patients have been registered from 30 institutions cooperating in the Coronary REvascularization Demonstrating Outcome study in Kyoto (CREDO-Kyoto) registry.

Methods

This study was approved by the institutional review boards or ethics committees of all participating institutions. Because the study subjects were retrospectively enrolled, written informed consent was not obtained, in concordance with the guidelines for epidemiologic studies issued by the Ministry of Health, Labor and Welfare of Japan. However, 73 patients were excluded because of their refusal to participate in the study when contacted for follow-up.

CREDO-Kyoto Registry and the Study Subjects

Consecutive patients who underwent their first coronary revascularization during 2000–2002 have been enrolled in the CREDO-Kyoto registry. Patients with AMI within 1 week after onset have not been included. Thirty institutions (Appendix 1) participated in the multicenter registry, and the baseline and follow up data for 9,877 patients have been obtained.[20] After excluding patients with malignant diseases (n=496), those who died in hospital (n=62) and those without precise information about their medical treatment at discharge or follow-up data (n=94), 9,225 patients were subjected to the analyses.

Data Collection, Definition and Follow-up

Clinical and analytical information of the study patients were collected from hospital charts or databases in each center by independent clinical research coordinators (Appendix 2) according to predetermined definitions. The baseline information of the patients included age, sex, smoking habit, body mass index (BMI), mode of revascularization, biochemistry before revascularization procedure, and comorbidities and background conditions such as hypertension, diabetes mellitus (DM), chronic kidney disease (CKD), anemia, peripheral vascular disease, history of heart failure, prior myocardial infarction (MI) or cerebrovascular acci-

Table 2 Medications at Hospital Discharge

Medication	*All patients (n=9,225)*	*Statin-treated (n=2,630)*	*Statin-non-treated (n=6,595)*	*p value*
Statins (%)	*28.5*	*100*	*0*	*<0.0001*
ACEI or ARB (%)	*32.9*	*38.9*	*30.6*	*<0.0001*
ACEI (%)	*20.4*	*24.5*	*18.7*	*<0.0001*
ARB (%)	*13.4*	*15.6*	*12.5*	*<0.0001*
β-adrenergic blocker (%)	*16.6*	*22.6*	*14.2*	*<0.0001*
Calcium-channel blockers (%)	*59.4*	*60.2*	*59.0*	*NS*
Nitrates (%)	*62.3*	*63.2*	*61.9*	*NS*
Antiplatelet medications (%)	*96.3*	*97.3*	*95.9*	*0.0014*
Aspirin (%)	*88.3*	*89.4*	*87.9*	*0.0382*
Ticlopidine (%)	*56.5*	*62.6*	*54.0*	*<0.0001*

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker. Other abbreviation see in Table 1.

dent. CKD was regarded as present when the glomerular filtration rate estimated by Cockcloft-Gould formula was <60 ml/min. Anemia was defined as blood hemoglobin level <10 g/dl. Peripheral vascular disease was as present when carotid, aortic and/or other peripheral vascular disease was being treated or was scheduled for surgical or endovascular interventions. Angiographic data of CAD, as well as precise information of the medical treatment at hospital discharge, were also obtained. The patients were followed up with respect to mortality for a median of 3.5 years. An independent clinical events committee adjudicated the events. All deaths were confirmed by medical records or telephone interview of the patients' families, and death was regarded as cardiovascular in origin unless obvious noncardiovascular causes could be identified. MI was adjudicated according to the definition in the Arterial Revascularization Therapy Study.[21] Within 1 week of the index procedure, only Q-wave MI was adjudicated as MI. Stroke during follow-up was defined as symptomatic stroke.

Statistical Analysis

All continuous variables are expressed as means±SD. Statistical significance of differences in subject baseline demographics between the patients with statin therapy and the patients without statin therapy at hospital discharge were assessed by a Student t-test for parametrically distributed continuous variables, the Wilcoxon signed-ranks test for nonparametrically distributed continuous variables or a Pearson's χ^2 test for categorized data analyses.

Following the descriptive statistics, we used Kaplan–Meier estimates to plot the percentage of patients in each group free from any death, cardiovascular death, MI, stroke or any revascularization procedure. The log-rank test was used to identify significant differences in unadjusted survival rates. To determine the significant and independent prognostic factors for mortality, we listed 22 potential baseline variables: mode of revascularization, old-old age (≥75 years), male gender, BMI (≥25 kg/m^2), current smoking, hypertension, DM, peripheral vascular disease, cerebrovascular disease, atrial fibrillation, chronic obstructive pulmonary disease, CKD, liver cirrhosis, anemia, unstable angina, prior MI, history of congestive heart failure, high serum total cholesterol (TC ≥220 mg/dl) or triglycerides (≥150 mg/dl) level, left main coronary artery (LMCA) disease, proximal left anterior descending artery lesion and multivessel disease, and 7 potential risk-reducing pharmacotherapies at hospital discharge: statins, ACEI, ARB, β-adrenergic blockers, antiplatelet drugs, nitrates and calcium-channel blockers. Thus, all continuous variables were dichotomized for fitting proportional assumption according to the predetermined clinical contexts. The relationships between the 29 variables and each of the endpoints were assessed using a multivariate Cox proportional hazards model with stepwise procedure. Variable selection was carried out, stepping up with $p<0.05$ as the requirement for inclusion and stepping down by deleting the term with the highest p-value until all remaining terms were significant with p-values<0.05. In the multivariate analyses assessing the relationships between the 29 variables and MI, stroke and revascularization as the end-

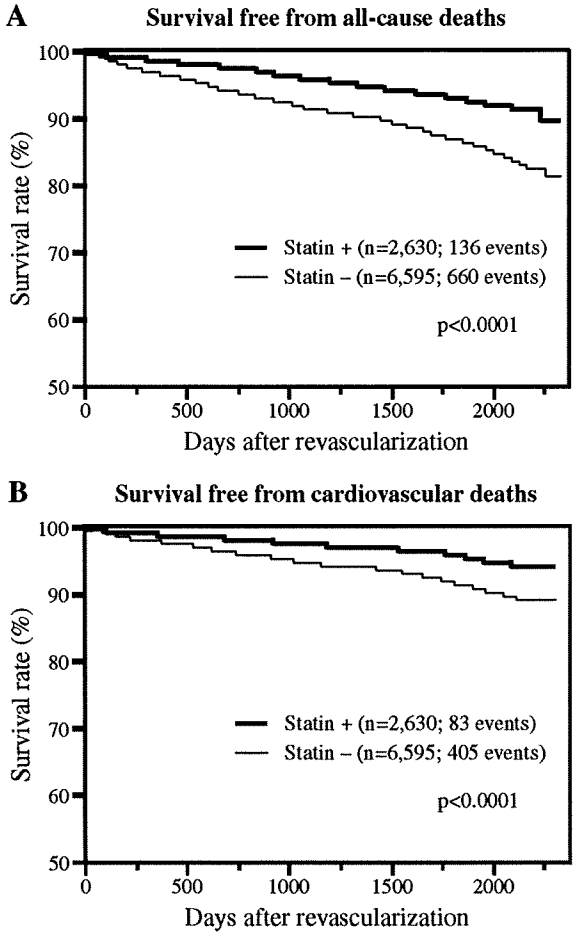

Fig 1. Kaplan–Meier analysis of cumulative rates of survival in coronary artery disease patients undergoing their first revascularization with vs without statin therapy at hospital discharge for all-cause (A) and cardiovascular (B) mortality.

Table 3 Multivariate Analysis for Prognostic Factors of All-Cause Mortality

Parameter	*RR*	*95%CI*	*p value*
Antiplatelet drugs at discharge	*0.61*	*0.46–0.80*	*0.0003*
BMI 25 kg/m²	*0.69*	*0.57–0.84*	*0.0002*
Statins at discharge	*0.71*	*0.59–0.86*	*0.0005*
Nitrates at discharge	*0.86*	*0.74–1.00*	*0.0446*
CVA	*1.25*	*1.06–1.47*	*0.0075*
Diabetes mellitus	*1.28*	*1.11–1.48*	*0.0009*
Male gender	*1.32*	*1.12–1.55*	*0.0007*
Revascularization by PCI	*1.35*	*1.14–1.59*	*0.0004*
Multivessel disease	*1.40*	*1.19–1.66*	*<0.0001*
Liver cirrhosis	*1.65*	*1.21–2.23*	*0.0014*
COPD	*1.68*	*1.23–2.29*	*0.0010*
Peripheral vascular disease	*1.70*	*1.38–2.08*	*<0.0001*
Age ≥75 years	*1.93*	*1.65–2.25*	*<0.0001*
History of heart failure	*2.10*	*1.80–2.45*	*<0.0001*
CKD	*2.12*	*1.78–2.52*	*<0.0001*
Hb <10 g/dl	*2.28*	*1.89–2.74*	*<0.0001*

RR, relative risk ratio; CI, confidence interval. Other abbreviations see in Table 1.

Table 4 Multivariate Analysis for Prognostic Factors of Cardiovascular Mortality

Parameter	*RR*	*95%CI*	*p value*
Antiplatelet drugs at discharge	*0.63*	*0.45–0.90*	*0.0102*
Statins at discharge	*0.72*	*0.56–0.91*	*0.0067*
BMI ≥25 kg/m²	*0.76*	*0.60–0.98*	*0.0335*
Male gender	*1.26*	*1.03–1.54*	*0.0220*
Revascularization by PCI	*1.29*	*1.06–1.58*	*0.0118*
Diabetes mellitus	*1.30*	*1.08–1.56*	*0.0064*
CVA	*1.37*	*1.12–1.68*	*0.0026*
Multivessel disease	*1.51*	*1.21–1.88*	*0.0003*
Age ≥75 years	*1.62*	*1.33–2.00*	*<0.0001*
Peripheral vascular disease	*1.67*	*1.28–2.16*	*0.0001*
History of heart failure	*2.35*	*1.94–2.86*	*<0.0001*
Hb <10 g/dl	*2.42*	*1.93–3.03*	*<0.0001*
CKD	*2.94*	*2.32–3.71*	*<0.0001*

Abbreviations see in Tables 1,3.

points, statin therapy was included in the variables, irrespective of its significance and independence as a prognostic predictor.

To evaluate the consistency of the association of statin therapy with reduced all-cause and cardiovascular mortality, multivariate analyses using the Cox proportional hazards models were also performed in predetermined subgroups. These included patients ≥75 years/<75 years, male/female, PCI/CABG, diabetic/non-diabetic, hypertensive/non-hypertensive patients, and patients with/without high serum TC, high serum triglyceride, CKD, history of heart failure or prior MI. The same factors used for analysis of the total cohort were incorporated in the multivariate models for subgroup analyses. Interaction analyses were also carried out between each categorized subgroup.

Because standard adjustment measures were not always valid, because of suboptimal assessment of degree of overlapping baseline characteristics, propensity score analysis was also performed as described and implemented by Rubin and others, using the baseline characteristics as potential prognostic factors?[22–24] Essentially, this involves calculation of a propensity score, which is the probability that any given individual patient would be part of the statin-treated group as opposed to the statin-non-treated group. The prognostic factors to calculate the propensity score were the same 29 factors included as variables of the multivariate analyses.

All analyses were performed using SAS Ver 9.1.3 (SAS Institute Inc, Cary, NC, USA) and all reported p-values are 2-sided.

Results

Subject Demographics and Characteristics of Medical Therapy at Discharge

A total of 9,225 study patients were divided into 2 groups based on the use of statin therapy at hospital discharge: 28.5% (n=2,630) of the subjects were on statin therapy and 71.5% (n=6,595) were not. Because of the large number of study patients and the observational study design, statistically significant differences were observed in many variables at baseline between the 2 groups. Significantly different variables include demographic characteristics such as the proportion of patients ≥75 years, male gender and mode of revascularization, comorbidities such as history of heart failure, other atherosclerotic diseases, dyslipidemia, diabetes and CKD, and the existence of LMCA disease (Table 1).

Prescription rates for ACEI, ARB, ACEI/ARB and β-adrenergic blockers at discharge were 20.4%, 13.4%, 32.9% and 16.6%, respectively. Most patients (96.3%) were treated with antiplatelet medications. Nitrates (62.3%) and calcium-channel blockers (59.4%) were prescribed in a considerably high proportion of the subjects (Table 2).

Table 5 RR of Statin Therapy for All-Cause and Cardiovascular Mortality by Propensity Score Analysis Compared With Multivariate Analysis

Endpoints and analysis	*RR*	*95% CI*	*p value*
All-cause mortality			
Multivariate analysis	*0.71*	*0.59–0.86*	*0.0005*
Adjustment by propensity score analysis			
Quintiles category	*0.70*	*0.58–0.85*	*0.0003*
Propensity score	*0.73*	*0.61–0.89*	*<0.0001*
Stratification of quintiles category	*0.70*	*0.58–0.85*	*0.0003*
Cardiovascular mortality			
Multivariate analysis	*0.72*	*0.56–0.91*	*0.0067*
Adjustment by propensity score analysis			
Quintiles category	*0.70*	*0.54–0.89*	*0.0038*
Propensity score	*0.73*	*0.57–0.93*	*0.0102*
Stratification of quintiles category	*0.69*	*0.54–0.89*	*0.0036*

Abbreviations see in Table 3.

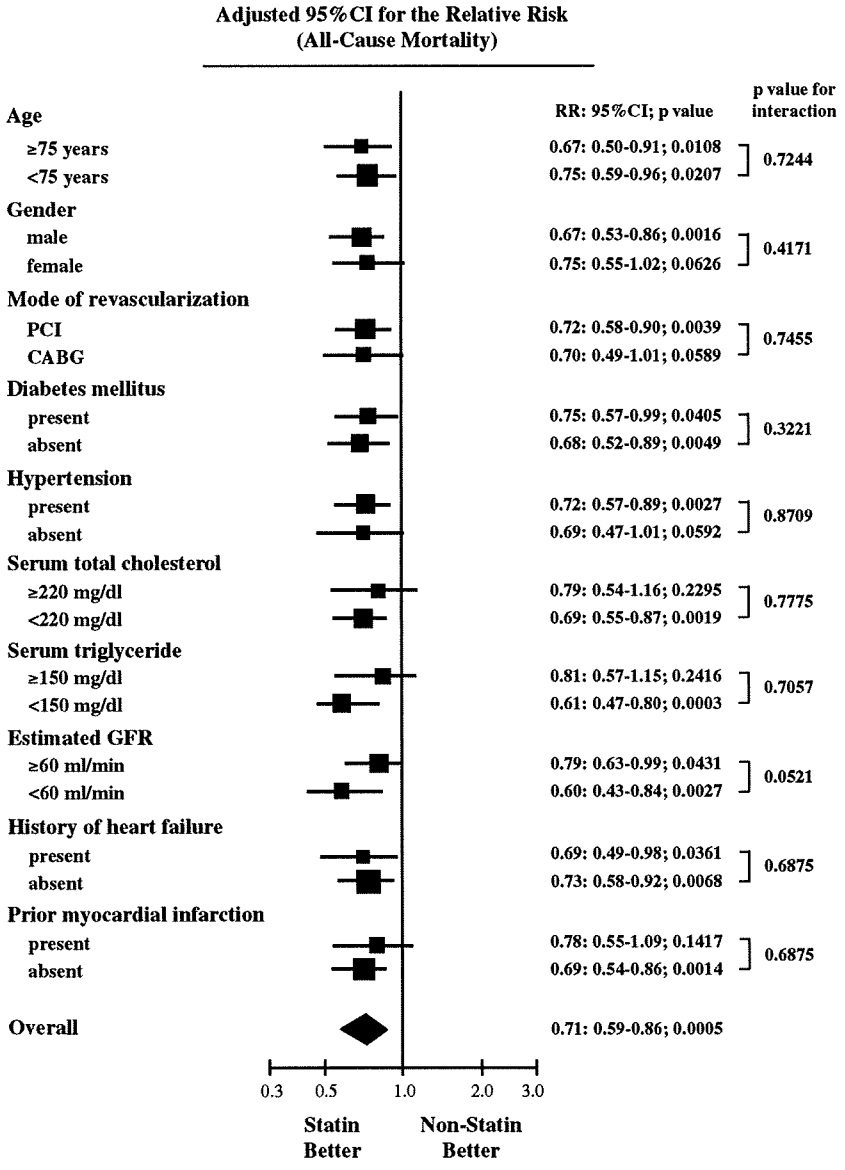

Fig 2. Relative risk ratio (RR) and 95% confidence interval (CI) for all-cause mortality for patients with vs without statin therapy in various patient subgroups. CABG, coronary artery bypass graft surgery; PCI, percutaneous coronary intervention.

Association of Statin Therapy With Unadjusted Survival

During the follow-up (median=3.5 years) with 96% follow-up rate at ≥2 years, 136 deaths (5.2%) and 660 deaths (10.0%) occurred in the statin-treated and the statin-non-treated groups, respectively. Among them, 83 deaths (3.2%) in the statin-treated and 405 deaths (6.2%) in the statin-non-treated groups were from cardiovascular events. Kaplan–Meier survival method and log-rank analysis in the 2 groups indicated that the unadjusted chance of survival free from all-cause, as well as cardiovascular, deaths was significant-

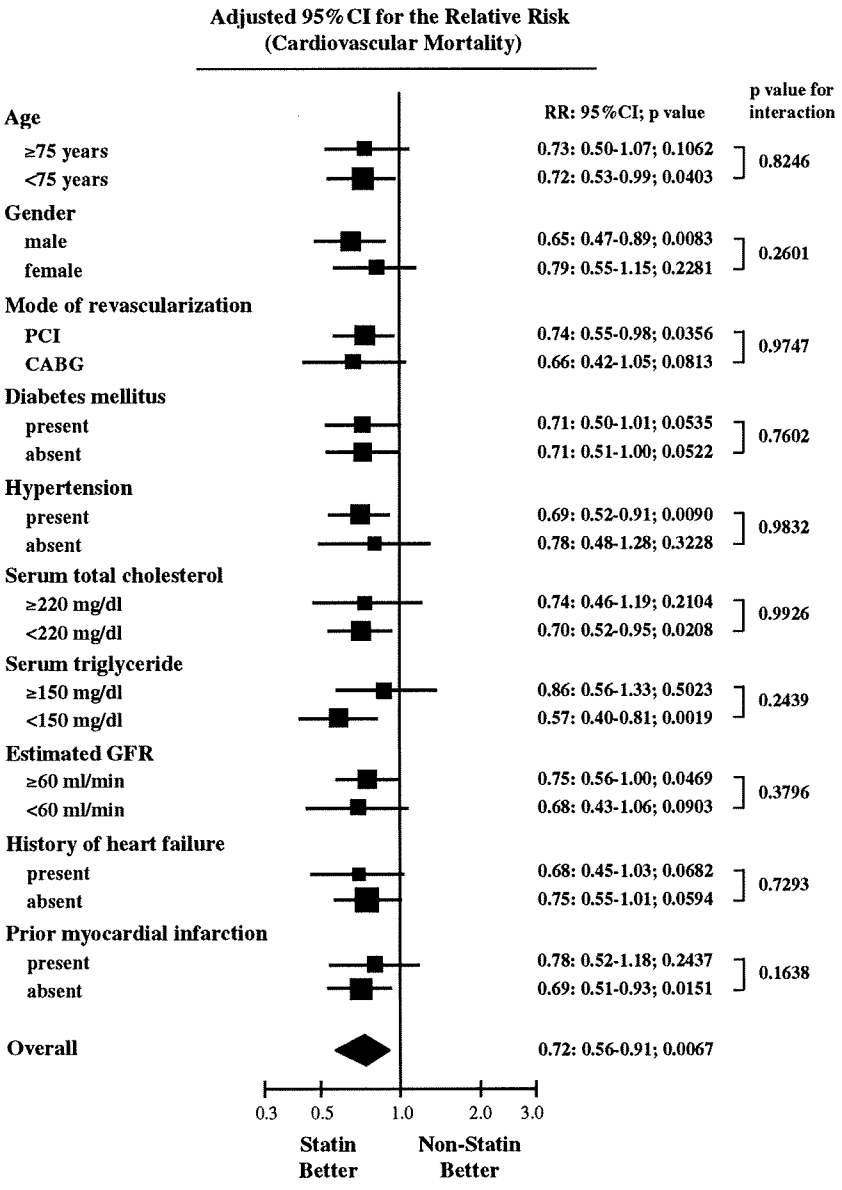

Fig 3. Relative risk ratio (RR) and 95% confidence interval (CI) for cardiovascular mortality for patients with vs without statin therapy in various patient subgroups. CABG, coronary artery bypass graft surgery; PCI, percutaneous coronary intervention.

ly greater in the statin-treated group (p<0.0001, Fig 1).

Association of Statin Therapy With Adjusted Survival

The results of the multivariate analysis of the predictors of all-cause mortality using stepwise procedure are shown in Table 3, where the relative risk ratio (RR), 95% confidence interval (CI) and p-value of each factor are given. Statin therapy at discharge remained an independent predictor of an increased chance of survival (RR 0.71, 95%CI 0.59–0.86, p=0.0005). Antiplatelets (RR 0.61, 95%CI 0.46–0.80, p=0.0003) and higher BMI (RR 0.69, 95%CI 0.57–0.84, p=0.0002) were also included as independent factors predicting increased chance of survival. Nitrates might marginally contribute to the survival of patients.

Table 4 shows the results of the multivariate analysis of predictors of cardiovascular mortality. Similar to the results of the analysis of all-cause mortality, statin therapy at discharge was an independent predictor of survival free from cardiovascular death.

The RR of statin therapy for all-cause (RR 0.70, 95%CI 0.58–0.85, p=0.0003) or cardiovascular (RR 0.70, 95%CI 0.54–0.89, p=0.0038) mortality adjusted by quintiles category of propensity score was quite similar to the result of multivariate analysis (Table 5). In the sensitivity analysis, the RR of statin therapy adjusted by propensity score as a numeric variable and the RR of statin therapy adjusted by stratification of quintiles category of propensity score also showed similar results (Table 5). Thus, the validity of the RR of statin therapy for all-cause or cardiovascular mortality in the multivariate analysis was confirmed by risk adjustment using propensity scores.

Consistency of Better Survival in Patients With Statin Therapy

Subgroup analyses indicated consistently lower all-cause, as well as cardiovascular, mortality in patients with statin therapy in all subgroups (Figs 2,3). Point-estimate of the relative risk was less than 1.0 in all subgroups and no significant interaction was found between the subgroups in each category. The relative risk for all-cause mortality was statistically significantly lower in the statin-treated group than in the statin-non-treated group in patients at ages <75 years and

≥75 years, patients treated by PCI, male patients, both diabetic/non-diabetic patients, hypertensive patients, patients with serum TC <220 mg/dl or serum triglyceride <150 mg/dl, patients with/without CKD, patients with/without heart failure, and patients without prior MI (Fig 2). The significant association of statin therapy and the lower risk for cardiovascular mortality was also seen in various subgroups (Fig 3). Despite the consistency of the association of statin therapy and better outcomes, statins were used significantly less frequently in subgroups at higher cardiovascular risk such as male patients (25.3% on statins), older patients (21.5%), patients with CKD (22.9%), patients with a history of heart failure (20.3%) or a prior MI (25.4%). Post-CABG patients were significantly less frequently prescribed statins at hospital discharge (19.3%) than post-PCI patients (32.4%).

Association of Statin Therapy With Future MI, Stroke and Coronary Revascularization

Although crude survival free from stroke was significantly better in patients with statins, by Kaplan–Meier analysis and by log-rank test (p=0.0013), statin therapy did not remain as an independent prognostic factor of stroke (444 total events: 99 in the statin-treated group, 345 in the statin-non-treated group; RR 0.83, 95%CI 0.66–1.04, p=0.0999) after adjustment by multivariate analysis. With regard to MI (257 total events: 69 in the statin-treated group, 188 in the statin-non-treated group; RR 0.89, 95%CI 0.67–1.17, p=0.4019) and any revascularization (2,835 total events: 876 in the statin-treated group, 1959 in the statin-non-treated group; RR 0.96, 95%CI 0.88–1.04, p=0.2768), both crude survival and the results of the multivariate analyses failed to prove the significant association of statin therapy with better outcomes.

Discussion

In the present study, we have shown that statin therapy at hospital discharge is associated with better outcomes in Japanese patients after their first coronary revascularization. All-cause, as well as cardiovascular, mortality was significantly lower in patients with statin therapy than in those without statins, after adjustments for coexisting coronary risk factors, mode of revascularization and concomitant medical treatments. The association of statin therapy with better survival was consistently shown among various patient subgroups.

Characteristics of the Clinical Background of the Study Patients

Our study patients consisted of a secondary prevention cohort with established CAD undergoing their first PCI or CABG. Approximately 70% of the patients underwent PCI and comprised a unique group at high risk of cardiovascular events associated with stent deployment, such as stent thrombosis and restenosis, and hemorrhagic complications related to dual antiplatelet drug therapy. Moreover, the subjects' baseline characteristics indicated a high prevalence of multiple coronary risk factors, suggesting the need for intensive risk factor management. Although racial differences in susceptibility to CAD might exist and previous observational studies have shown a lower prevalence of CAD in Japan relative to the United States[25,26] the all-cause mortality of the present patients without statins (10% at 3.5 years of median follow-up) appeared comparable with that of the control group in the 4S study (12% at 5.4 years of median follow-up), a secondary prevention study in patients with stable CAD in Europe[3] Thus, the present study has been performed in a unique Asian patient group at a mortality risk as high as a Caucasian secondary prevention cohort.

Better Survival in Patients With Statin Therapy and its Consistency

All-cause, as well as cardiovascular, mortality was significantly lower in Japanese patients with statin therapy who underwent their first coronary revascularization by PCI with bare-metal stent or by CABG. The findings are consistent with a previous small randomized trial[12] and an observational study that was carried out in Europe in patients after PCI[27] Subgroup analysis revealed a survival advantage in patients with statin therapy in various high-risk subgroups such as older patients and patients with CKD. Analysis in patients with TC ≥220 mg/dl did not indicate a significant difference in all-cause or cardiovascular mortality between the statin-treated and the non-treated groups. This unexpected result might be related to the smaller number of patients in the group with TC ≥220 mg/dl (26.4% of all subjects). Indeed, there was a trend to favor statin therapy in regard to relative risk for all-cause or cardiovascular mortality, and the RR of the statin-treated group for cardiovascular mortality was comparable between patients with TC ≥220 mg/dl and those with TC <220 mg/dl. Thus, the results added further evidence to the consistent benefits of statins for secondary cardiovascular prevention. Because an admission for a coronary revascularization procedure is a good opportunity to optimize medical therapy for CAD, most patients undergoing PCI or CABG should be considered for the indication of statins[28]

The multivariate analyses failed to show significant differences in the prevalence of MI, stroke and any coronary revascularization between the statin-treated and non-treated groups, although the relative risk appeared to favor statin therapy in the analyses for MI and stroke. Because the majority of the repeated revascularization procedures were performed within 1 year of the first revascularization, because of restenosis after stenting, the preventive role statins for restenosis appears deniable. In regard to MI and stroke, the prevalence might not be sufficiently high to show differences (2.8% for MI and 4.8% for stroke). In particular, longer follow-up or analysis in a larger patient population could increase the number of events and show a significant differences between the statin-treated and the non-treated groups in the prevalence of stroke, because a clear tendency of fewer strokes in the statin-treated group was observed. Another possible explanation may be the effect of statins on the severity of MI and stroke. It has been reported that pretreatment with statins decreases myocardial injury or periprocedural mortality during PCI or CABG[29–31] Fonarow et al have shown in a large scale observational study that new or continued statin therapy in the first 24 h of the index AMI was associated with lower mortality compared with no statin use, but not with the incidence of recurrent MI[32] Moreover, an association of prior statin use with smaller infarct size or better outcomes has been reported in patients with ischemic stroke[33,34] Thus, it is possible that statin therapy is associated with reduced mortality by its limiting of tissue damage during ischemic events and subsequent invasive therapeutic procedures for the events.

Characteristics of the Medical Therapy of the Study Patients

Despite the better survival of statin-treated patients, the present study also indicated suboptimal use of statins in Japanese high-risk patients during the study period. The use of ACEI, ARB and β-adrenergic blockers at hospital discharge was also less frequent than we expected from the data for the US and Europe[27,35] The reported lower prevalence of CAD in Japan relative to the United States might cause underuse of preventive medical therapies[25,26] However, the prevalence of CAD in the general population cannot explain the future risk of patients with established CAD. Because the patients analyzed in this study were a secondary prevention cohort with CAD, the use of statins in Japan was apparently suboptimal in 2000–2002.

The use of nitrates or calcium-channel blockers was much more frequent than in the reports from Europe analyzing patients after coronary revascularization[27] This result agrees with a recent study of Japanese CAD patients[19] Higher prevalence of coronary vasospastic angina in Japanese than in Caucasians may partly explain this difference[36] Thus, our study patients had unique concomitant medical treatments in comparison with previous reports from Western countries[27] and the results of the present study enable assessment of the consistency of the beneficial effects of statins in patient groups with distinctive backgrounds and concomitant medical therapy.

Strengths and Limitations

The present study was based on data from the CREDO-Kyoto registry, a multicenter registry of 30 hospitals in Japan. The number of study patients was sufficient to assess the impact of each prognostic factor on mortality and the size of each participating hospital is variable. The data have been collected by trained clinical research coordinators based on a detailed manual and the follow-up rate was 96% at ≥2 years. Thus, the sample size, distribution of the size of the participating hospitals and the method of data acquisition strengthen the reliability of the data, and the results represent the "real world" clinical practice for CAD patients undergoing revascularization during 2000–2002 in Japan.

Several limitations that are common to all observational studies should be noted in the interpretation of the results. First, there were significant differences between the statin-treated and the statin-non-treated groups in many of the patients' baseline characteristics. Second, the statins and their doses were inconsistent and it is difficult to assess the efficacy of each statin at a particular dose, although the efficacies of statins may not be identical. Finally, the information about medical therapy was obtained at only 1 time point, hospital discharge, for each patient. Therefore, the adherence of the patients to the medications and the cross-over of patients between the statin-treated and the statin-non-treated groups have not been considered in the analyses. The lipid profiles of the patients before statin therapy are also uncertain, and the cholesterol levels without statin therapy might have been higher in the statin-treated group. Thus, the possibility that the results have been influenced by these factors cannot be eliminated.

Conclusion

In the present study of Japanese patients undergoing their first coronary revascularization therapy, starting medical treatment with statins by hospital discharge was associated with lower all-cause as well as cardiovascular mortality. The results can be seen as a rationale for the comprehensive use of statins for secondary prevention in patients with CAD.

Acknowledgements

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Appendix 1

List of Participating Centers and Investigators

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Appendix 2

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Oxidized LDL Receptor LOX-1 Binds to C-reactive Protein and Mediates its Vascular Effects

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BACKGROUND: C-reactive protein (CRP) exerts biological activity on vascular endothelial cells. This activity may promote atherothrombosis, but the effects of this activity are still controversial. Lectin-like oxidized LDL receptor-1 (LOX-1), the oxidized LDL receptor on endothelial cells, is involved in endothelial dysfunction induced by oxidized LDL.

METHODS: We used laser confocal microscopy to examine and fluorescence cell image analysis to quantify the binding of fluorescently labeled CRP to cells expressing LOX-1. We then examined the binding of unlabeled CRP to recombinant human LOX-1 in a cell-free system. Small interfering RNAs (siRNAs) against LOX-1 were applied to cultured bovine endothelial cells to analyze the role of LOX-1 in native cells. To observe its in vivo effects, we injected CRP intradermally in stroke-prone spontaneously hypertensive (SHR-SP) rats and analyzed vascular permeability.

RESULTS: CRP bound to LOX-1–expressing cells in parallel with the induction of LOX-1 expression. CRP dose-dependently bound to the cell line and recombinant LOX-1, with significant binding detected at 0.3 mg/L CRP concentration. The K_d value of the binding was calculated to be 1.6×10^{-7} mol/L. siRNA against LOX-1 significantly inhibited the binding of fluorescently labeled CRP to the endothelial cells, whereas control RNA did not. In vivo, intradermal injection of CRP-induced vascular exudation of Evans blue dye in SHR-SP rats, in which expression of LOX-1 is greatly enhanced. Anti–LOX-1 antibody significantly suppressed vascular permeability.

CONCLUSIONS: CRP and oxidized LDL-receptor LOX-1 directly interact with each other. Two risk factors for ischemic heart diseases, CRP and oxidized LDL, share a common molecule, LOX-1, as their receptor.

C-reactive protein (CRP)[7] is an acute-phase plasma protein that is synthesized by hepatocytes in response to inflammation and tissue damage; the latter can cause a 1000-fold or more increase in the human plasma concentrations of high-sensitivity CRP (hsCRP). For this reason hsCRP has long been used as an inflammatory biomarker *(1)*. CRP recognizes phosphocholine *(2)* and other various ligands, including phosphoethanolamine, chromatin, histones, fibronectin, and oxidized LDL *(3, 4)*. Recent epidemiological studies have shown that even the slightest increase in serum concentration of hsCRP can be a major risk indicator for ischemic heart disease *(5–7)*. Activation of the classical complement pathway through direct interaction with C1q is an established function of CRP. It is reported that administration of human CRP in a rat model of myocardial infarction activates complement systems, leading to increases in the size of myocardial infarction *(8)*, and that the chemical blockade of CRP prevents these deleterious effects and suppresses the myocardial infarction *(9)*. In addition, a number of recent reports have shown that CRP induces endothelial activation/dysfunction leading to atherothrombosis *(10)*. Because the earliest CRP reports may have reflected the effects of contaminants such as bacterial lipopolysaccharide and azide rather than CRP, there have been subsequent heated debates concerning CRP functions and actions

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[7] Nonstandard abbreviations: CRP, C-reactive protein; hsCRP, high-sensitivity CRP; LOX-1, lectin-like oxidized LDL receptor-1; siRNA, small interfering RNA; BAEC, bovine aortic endothelial cells; WKY, Wistar Kyoto; SHR-SP, stroke-prone spontaneously hypertensive.

(11, 12). CRP preparations free from lipopolysaccharide and azide, however, reportedly have shown significant effects on endothelial activation. Fcγ receptors are postulated to be the receptors that mediate the effects of CRP outside of complement activation *(12–14)*.

Lectin-like oxidized LDL receptor 1 (LOX-1) was originally found and identified as an endothelial receptor for oxidized LDL *(15)*. Activation of LOX-1 in endothelial cells induces the generation of superoxide, a reduction in the release of nitric oxide, and the expression of proatherogenic molecules such as endothelin-1, monocyte chemoattractant protein 1, vascular cell adhesion molecule 1, and intercellular adhesion molecule 1*(16–18)*. The overexpression of LOX-1 in mice enhances oxidative stress and the expression of adhesion molecules in blood vessels, accelerating atheroma-like lipid deposition in intramyocardial vessels *(19)*. Deletion of LOX-1 in mice preserves endothelial function, leading to reduction in atherogenesis *(20)*. In addition to oxidized LDL, LOX-1 binds various ligands, e.g., apoptotic cells, activated platelets, leukocytes, and bacteria *(21–25)*. Related functions of LOX-1 include involvement in inflammation, myocardial infarction, and intimal thickening after balloon catheter injury *(23, 26–29)*.

Because the changes induced by CRP and by LOX-1 activation overlap, we investigated the physical interactions between CRP and LOX-1 that may be related to cardiovascular pathophysiology.

Materials and Methods

CRP

Human CRP purified from pleural fluid was purchased from Chemicon (AG723). Sodium azide in the solution was removed by dialysis performed 3 times against a 3000-fold volume of Dalbecco's PBS (Wako). Gram-negative bacterial endotoxins were undetectable by limulus amebocyte lysate (Associates of Cape Cod), which can detect as low as 30 endotoxin units/L of endotoxins. CRP preparations from 2 other distributers, one purified from human plasma (C4063, Sigma) and the other from human serum (#236603, Calbiochem), were also subjected to cell-free analyses.

FLUORESCENTLY LABELED CRP

CRP was fluorescently labeled with an Alexa Fluor 546 protein-labeling kit (Invitrogen) and dialyzed 3 times against a 3000-fold volume of PBS.

CELL LINE EXPRESSING HUMAN LOX-1 (HLOX-1-CHO) DRIVEN BY TETRACYCLINE-INDUCIBLE PROMOTER

cDNA encoding the human LOX-1 (Genbank NM002543) was subcloned into pTRE2hyg (Clonetech). CHO-K1 Tet-On cells (Clonetech) were transfected with pTRE2hyg-human LOX-1 by Lipofectamin-2000 transfection reagent (Invitrogen) according to the manufacture's instructions. The stable transformants were selected with 400 mg/L of hygromycin B (Wako). The resistant clones that express LOX-1 in response to doxycycline (Calbiochem) were selected for use in these experiments. The LOX-1 expression was induced with doxycyline at the indicated concentration in Ham's F-12 medium (Gibco)/10% fetal bovine serum 24 h before the experiments. Cells were washed twice with Ham's F-12/10 mmol/L HEPES and chilled on ice for 30 min. Then, the medium was replaced with the indicated concentration of Alexa 546-CRP–containing ice-cold Ham's F-12/10 mmol/L HEPES, and cells were incubated on ice for 1 h. After being washed with ice-cold PBS, the cells were fixed with phosphate-buffered formalin (Wako). The expression of LOX-1 was visualized by immunostaining with antihuman LOX-1 antibody (TS92) *(30)* combined with Alexa 488-anti-human IgG (1:2000) (Invitrogen). Then, the specimens were subjected to microscopic analysis with confocal laser microscope (TCS SP5, Leica), and quantitative fluorescence cell image analysis with the IN Cell analyzer 1000 system (GE Healthcare).

TRANSIENT GENE EXPRESSION ASSAY

COS7 cells maintained with DMEM/10% fetal bovine serum were seeded 1 day before transfection. The cells at 80%–90% confluency were transfected with indicated plasmid by use of Lipofectamin 2000 transfection reagent (Invitrogen). After 24 h, the cells were chilled on ice for 30 min and washed with ice-cold PBS. Then, the medium was replaced with the indicated concentration of Alexa 546-CRP–containing ice-cold DMEM/10 mmol/L HEPES, and cells were incubated on ice for 1 h. After being washed with ice-cold PBS, the cells were fixed with phosphate-buffered formalin (Wako). The expression of each receptor was assessed by immunostaining with anti-V5 antibody (1:1000) (Nacalai Tesque) combined with Alexa 488-antimouse IgG (1:2000) (Invitrogen). The nuclei of the cells were counterstained with 0.5 mg/L DAPI (Sigma). Quantitative analysis was performed with an IN Cell Analyzer.

RECOMBINANT LOX-1

cDNA encoding extracellular domain of human LOX-1 (61–273) was subcloned into pcDNA4 with a chicken IgG light chain leader peptide in the N-terminal and V5-6xHis tag in the C-terminus. The plasmid was transfected into FreeStyle 293-F cells (Invitrogen). After 4 days, the recombinant protein was purified from culture supernatant with Ni-NTA superflow (Qiagen) according to the manufacturer's instructions.