Table 3 IVUS Results

	Baseline				Follow-Up			
	Both Groups (n = 252)	Pitavastatin (n = 125)	Atorvastatin (n = 127)	p Value Between Groups	Both Groups (n = 252)	Pitavastatin (n = 125)	Atorvastatin (n = 127)	p Value Between Groups
Plaque volume (mm^3)	56.9 ± 32.2	49.8 ± 28.8	63.9 ± 33.9	<0.001	47.5 ± 29.1	41.6 ± 25.0	53.3 ± 31.7	0.0013
Percent plaque volume (%)	50.0 ± 10.3	49.4 ± 10.8	50.5 ± 9.7	0.4	44.0 ± 10.8	43.7 ± 11.0	44.3 ± 10.7	0.7
Normalized plaque volume (mm^3)	54.6 ± 18.9	52.7 ± 19.4	56.4 ± 18.3	0.1	45.3 ± 18.0	43.9 ± 17.7	46.6 ± 18.3	0.2
Vessel volume (mm^3)	113.0 ± 59.3	100.2 ± 54.2	125.6 ± 61.5	<0.001	105.4 ± 55.0	93.3 ± 48.7	117.2 ± 58.3	<0.001
Lumen volume (mm^3)	56.1 ± 31.5	50.5 ± 29.7	61.6 ± 32.3	0.0046	57.8 ± 30.5	51.7 ± 27.9	63.9 ± 31.7	0.0013
IVUS lesion length (mm)	6.7 ± 3.0	6.1 ± 2.8	7.3 ± 3.1	0.0021	S/B	S/B	S/B	—

	Nominal Change						
	Both Groups (n = 252)	p Value Compared With Baseline	Pitavastatin (n = 125)	p Value Compared With Baseline	Atorvastatin (n = 127)	p Value Compared With Baseline	p Value Between Groups
Plaque volume (mm^3)	−9.4 ± 9.8	<0.001	−8.2 ± 8.9	<0.001	−10.6 ± 10.6	<0.001	0.05
Percent plaque volume (%)	−6.0 ± 6.2	<0.001	−5.7 ± 6.3	<0.001	−6.3 ± 6.1	<0.001	0.5
Normalized plaque volume (mm^3)	−9.3 ± 8.4	<0.001	−8.7 ± 8.2	<0.001	−9.8 ± 8.6	<0.001	0.3
Vessel volume (mm^3)	−7.7 ± 14.9	<0.001	−7.0 ± 15.2	<0.001	−8.3 ± 14.7	<0.001	0.5
Lumen volume (mm^3)	1.8 ± 10.6	0.0093	1.2 ± 10.5	0.2	2.3 ± 10.5	0.019	0.4
IVUS lesion length (mm)	S/B	—	—	—	—	—	—

	Percent Change (%)						
	Both Groups (n = 252)	p Value Compared With Baseline	Pitavastatin (n = 125)	p Value Compared With Baseline	Atorvastatin (n = 127)	p Value Compared With Baseline	p Value Between Groups
Plaque volume (mm^3)	−17.5 ± 14.0	<0.001	−16.9 ± 13.9	<0.001	−18.1 ± 14.2	<0.001	0.5
Percent plaque volume (%)	NA	<0.001	NA	<0.001	NA	<0.001	0.5
Normalized plaque volume (mm^3)	NA	<0.001	NA	<0.001	NA	<0.001	0.3
Vessel volume (mm^3)	−6.0 ± 11.4	<0.001	−5.9 ± 11.8	<0.001	−6.2 ± 11.1	<0.001	0.8
Lumen volume (mm^3)	6.5 ± 20.4	<0.001	6.4 ± 21.5	0.0012	6.6 ± 19.4	<0.001	0.9
IVUS lesion length (mm)	S/B	—	—	—	—	—	—

Continuous variables were represented by mean ± SD.
IVUS = intravascular ultrasound; NA = not applicable; S/B = same as the baseline.

patients with ACS have many greater-risk nonculprit plaques (16–19). The plaques in the most-diseased 10-mm segments showed more regression than whole coronary artery in the REVERSAL trials (4), and the %PV at the baseline in this study was relatively large compared with those in both trials (JAPAN-ACS, ~50%; REVERSAL, ~40%). Furthermore, there was relatively greater proportion of the patients who were administered statins de novo after the entry of this trial. It is also possible there are genetic, racial, or ethnic differences in terms of response to statins.

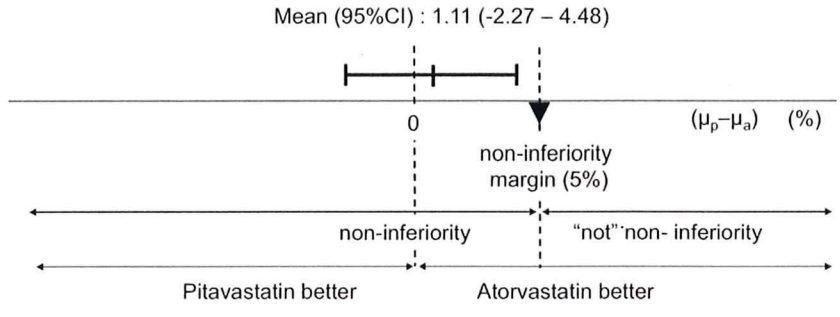

Figure 2 Primary End Point (Noninferiority Test)

The difference of drug effects on percent change in plaque volume (μp − μa) adjusted for sex, the presence of diabetes mellitus, and total cholesterol level, where μp represents percent change in plaque volume of the pitavastatin group and μa represents that of the atorvastatin group. Noninferiority of pitavastatin to atorvastatin was evident for this end point.

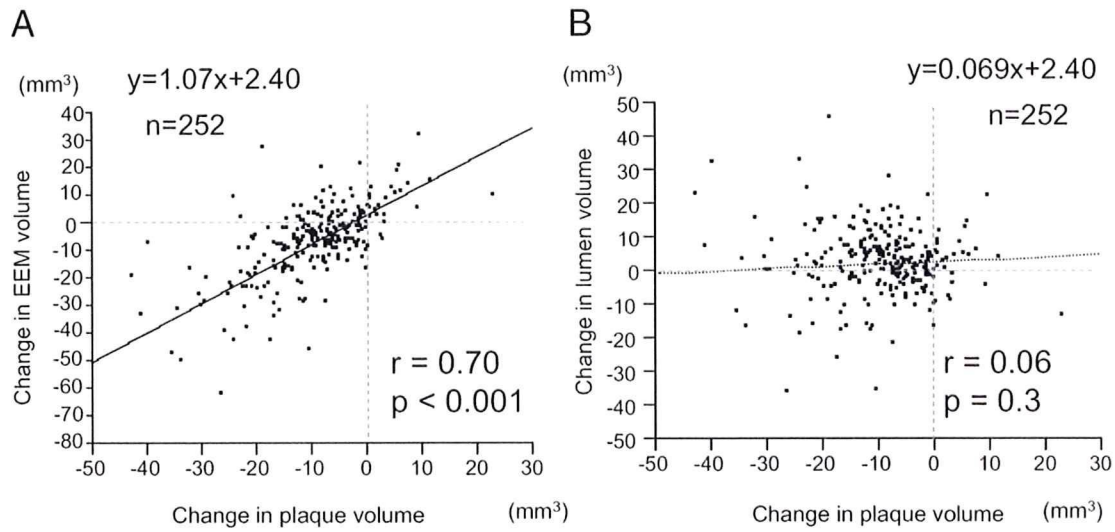

Figure 3 **Correlation Between the Change in Plaque Volume and the Change in Lumen Volume and EEM Volume**

There were significant correlations between the change in plaque volume and the change in external elastic membrane (EEM) volume **(A)**, whereas no significant correlation was observed between the change in plaque volume and the change in lumen volume **(B)**. The regression of plaque volume was associated with negative vessel remodeling.

The correlation between the reduction of LDL-C and the regression of PV was not significant in the present study as compared with previous placebo-controlled studies (Fig. 5) (6,20). One of the reasons might be that this study did not have a placebo arm of patients not receiving lipid-lowering therapy, which was not included for ethical reasons. Regression in PV was observed in a broad spectrum of patients regardless of the baseline LDL-C level. Pleiotropic

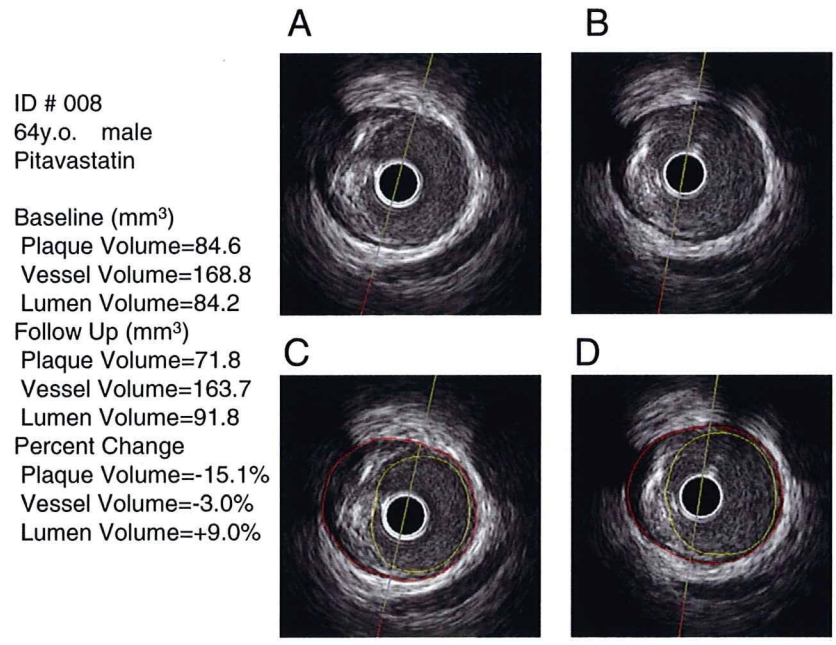

Figure 4 **Representative Cases of IVUS Analysis**

Shown are intravascular ultrasound (IVUS) images of the same cross section at the baseline **(A)** and at the follow-up **(B)**. **C** and **D** correspond to **A** and **B** with outlined leading edges of lumen and external elastic membrane. There is substantial reduction in plaque area observed for the cross-sectional images.

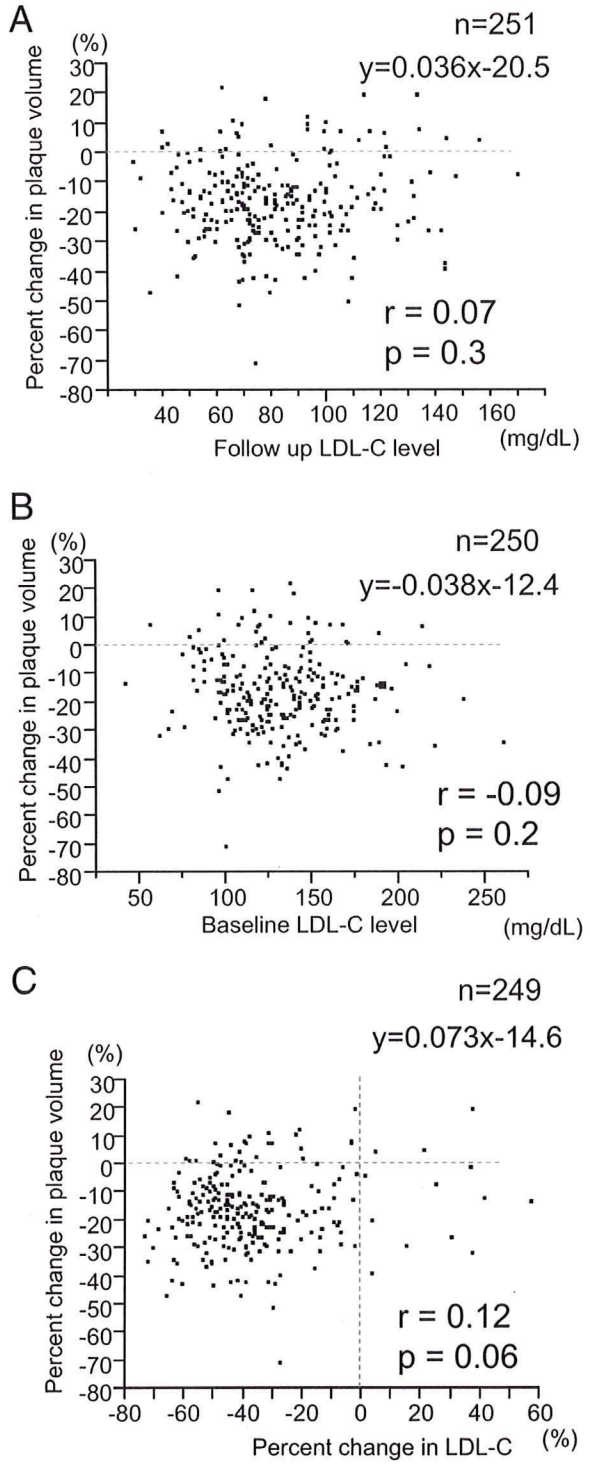

Figure 5 Relationship Between LDL-C and Percent Change in Plaque Volume

(A) Relationship between the follow-up low-density lipoprotein (LDL)-C level and percent change in plaque volume during 8- to 12-month follow-up. **(B)** Relationship between the baseline LDL-C level and percent change in plaque volume during 8- to 12-month follow-up. **(C)** Relationship between percent change in LDL-C and percent change in plaque volume during 8- to 12-month follow-up. There was no or slightly week correlation in these relationships.

Table 4 MACE and Adverse Events

	Pitavastatin (n = 147)	Atorvastatin (n = 149)	p Value
MACE	30 (20.4)	34 (22.8)	0.6
Myocardial Infarction	0	3 (2.0)	0.2‡
Coronary revascularization	30 (20.4)	31 (20.8)	0.9
TLR	16 (10.9)	19 (12.8)	0.6
TVR	9 (6.1)	8 (5.4)	0.8
Other vessel revascularization	8 (5.4)	9 (6.0)	0.8
Death from any cause	0	0	—
Adverse drug reaction	3 (2.0)	3 (2.0)	0.99
Myalgia	0	1 (0.7)	0.99‡
Eczema	2 (1.4)	3 (2.0)	0.99‡
Depression	1 (0.7)	0	0.5‡
Vomiting	1 (0.7)	0	0.5‡
Abnormality of laboratory value	19 (12.9)	17 (11.4)	0.7
AST/ALT*	11 (7.5)	11 (7.4)	0.97
CK†	8 (5.4)	8 (5.4)	0.98
Discontinuation	4 (2.7)	7 (4.7)	0.4
Adverse drug reaction	1 (0.7)	2 (1.3)	0.99‡
Abnormality of laboratory value	3 (2.0)	5 (3.4)	0.7‡
100% adherence	118 (80.3)	110 (73.8)	0.2

Data are expressed as n (%) unless otherwise specified. The chi-square test was used unless otherwise specified. *Upper than 100 (IU/l); in these, causes independent from trial drugs were suspected in 3 cases of the pitavastatin group and 3 of the atorvastatin group respectively. †Upper than 350 (IU/l); in these, causes independent from trial drugs were suspected in 1 cases of the pitavastatin group and 1 of the atorvastatin group respectively. ‡Fisher exact test.

ALT = alanine aminotransferase; AST = aspartate aminotransferase; CK = creatine kinase; MACE = major adverse cardiac events; TLR = target lesion revascularization; TVR = target vessel revascularization.

effects unrelated to LDL-C reduction might be one of the mechanisms of plaque regression. Other pharmacologic and lifestyle interventions applied after the onset of ACS might contribute to the modification of the plaque. In addition, PV regression observed in our study was associated with negative vessel remodeling, which might suggest that nonculprit plaques in patients with ACS were stabilized by statins (21).

Study limitations. The observation of a single plaque in the culprit vessel may not represent the pan-coronary nature of a plaque. Meanwhile, it has been documented that the ACS may represent the pan-coronary process of vulnerable plaque development, suggesting that a single plaque can reflect the general feature of whole coronary artery (19). Another criticism may be that arteries undergoing mechanical interventions were included, which could have affected atheroma measurements. However, IVUS examination for nonculprit vessel in emergent patients with ACS was not possible because of ethical reasons. IVUS might not be appropriate to identify thrombosis. It has been reported that thrombosis can be identified by IVUS with a sensitivity of <50% (22). However, fresh thrombus, which is frequently seen in ACS, can be detected with a true-positive rate of 80% (23). Therefore, meticulous care was taken to exclude thrombus in the present study as strictly as possible with criteria that thrombus in an IVUS image is usually mobile and relatively low echoic, with a uniform texture having

some scintillations, some microchannels, and a soft wavy surface.

Conclusions

Intensive statin therapy with 4 mg/day of pitavastatin or 20 mg/day of atorvastatin in patients with ACS resulted in significant regression of atheroma burden with negative vessel remodeling in a large-scale, multicenter trial using a central IVUS core laboratory in which the noninferiority of pitavastatin to atorvastatin was proved.

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Key Words: acute coronary syndrome ▪ plaque ▪ statins ▪ intravascular ultrasound.

APPENDIX

For a list of JAPAN-ACS Investigators, please see the online version of this article.

Three-Year Outcomes After Sirolimus-Eluting Stent Implantation for Unprotected Left Main Coronary Artery Disease

Insights From the j-Cypher Registry

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Background—Long-term outcomes after stenting of an unprotected left main coronary artery (ULMCA) with drug-eluting stents have not been addressed adequately despite the growing popularity of this procedure.

Methods and Results—j-Cypher is a multicenter prospective registry of consecutive patients undergoing sirolimus-eluting stent implantation in Japan. Among 12 824 patients enrolled in the j-Cypher registry, the unadjusted mortality rate at 3 years was significantly higher in patients with ULMCA stenting (n=582) than in patients without ULMCA stenting (n=12 242; 14.6% versus 9.2%, respectively; $P<0.0001$); however, there was no significant difference between the 2 groups in the adjusted risk of death (hazard ratio 1.23, 95% confidence interval 0.95 to 1.60, $P=0.12$). Among 476 patients whose ULMCA lesions were treated exclusively with a sirolimus-eluting stent, patients with ostial/shaft lesions (n=96) compared with those with bifurcation lesions (n=380) had a significantly lower rate of target-lesion revascularization for the ULMCA lesions (3.6% versus 17.1%, $P=0.005$), with similar cardiac death rates at 3 years (9.8% versus 7.6%, $P=0.41$). Among patients with bifurcation lesions, patients with stenting of both the main and side branches (n=119) had significantly higher rates of cardiac death (12.2% versus 5.5%; $P=0.02$) and target-lesion revascularization (30.9% versus 11.1%; $P<0.0001$) than those with main-branch stenting alone (n=261).

Conclusions—The higher unadjusted mortality rate of patients undergoing ULMCA stenting with a sirolimus-eluting stent did not appear to be related to ULMCA treatment itself but rather to the patients' high-risk profile. Although long-term outcomes in patients with ostial/shaft ULMCA lesions were favorable, outcomes in patients with bifurcation lesions treated with stenting of both the main and side branches appeared unacceptable. **(*Circulation*. 2009;120:1866-1874.)**

Key Words: stents ■ revascularization ■ coronary disease ■ ischemia ■ restenosis

Although coronary artery bypass graft surgery has long been considered the "gold standard" for revascularization of patients with unprotected left main coronary artery (ULMCA) disease, drug-eluting stents (DES) have been used with increasing frequency for the percutaneous coronary intervention (PCI) of ULMCA disease.[1,2] However, recent reports have questioned the long-term safety of DES on the basis of a concern about increased rates of very late stent thrombosis (ST) compared with that found with bare-metal stents (BMS).[3] In patients undergoing ULMCA stenting, stent failure manifesting as restenosis or thrombosis may be associated with a large area of myocardium in jeopardy and subsequent fatal myocardial infarction or sudden death; therefore, the long-term performance of DES in ULMCA disease is considered to be more closely linked to survival outcome than in non-ULMCA disease. To assess long-term outcomes of ULMCA PCI with sirolimus-eluting stents (SES) in the real world, we investigated the 3-year clinical

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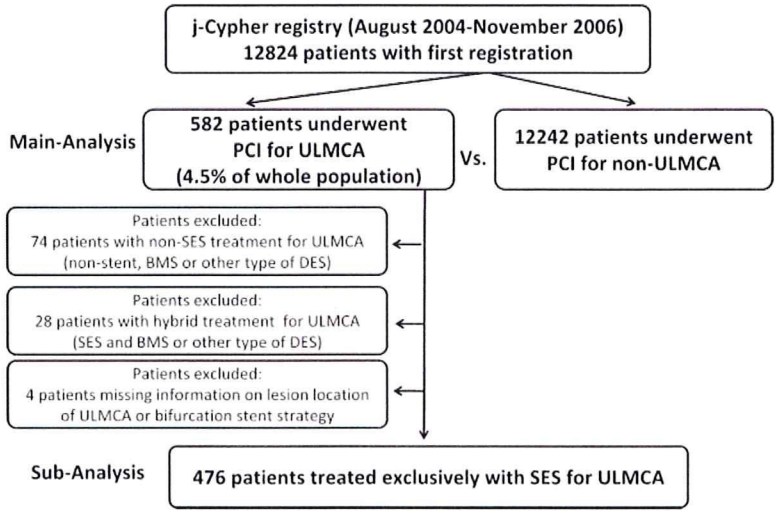


Figure 1. Study flow chart.

outcomes of patients undergoing SES implantation for ULMCA lesions in a large multicenter registry.

Clinical Perspective on p 1874

Methods

Study Design and Patient Population

The study design and patient enrollment for the j-Cypher registry has been described in detail elsewhere.[4] In brief, the j-Cypher registry is a physician-directed prospective multicenter registry in Japan enrolling consecutive patients undergoing SES implantation. From August 2004 to November 2006, 12 824 patients were enrolled in the j-Cypher registry for the first time, and 10 784 patients were treated exclusively with SES. The recommended antiplatelet regimen was aspirin (≥81 mg/d) indefinitely and a thienopyridine (200 mg of ticlopidine or 75 mg of clopidogrel daily) for at least 3 months. The duration of dual-antiplatelet therapy was left to the discretion of each attending physician.

The relevant review boards in all 37 participating centers approved the study protocol. Written informed consent was obtained from all patients enrolled.

Among the 12 824 patients enrolled in the j-Cypher registry, 582 underwent PCI for ULMCA disease (ULMCA group), and 12 242 patients underwent PCI for non-ULMCA lesions only (non-ULMCA group; Figure 1). Baseline characteristics, clinical outcomes, and causes of death in the ULMCA group were compared with those in the non-ULMCA group. Patients undergoing PCI for protected left main coronary artery lesions (n=101) were included in the non-ULMCA group.

Subgroup analysis was also conducted in 476 patients whose ULMCA lesion was treated exclusively by SES (Figure 1). Clinical outcomes in the subgroup study population were analyzed according to lesion location (ostial/shaft or bifurcation), stenting strategy (bifurcation 1-stent strategy or bifurcation 2-stent strategy), and number of diseased vessels other than ULMCA.

Definitions

The left main coronary artery was defined as "unprotected" when no surgical grafts to the left coronary system were patent. Renal insufficiency was defined as estimated glomerular filtration rate <30 $mL \cdot min^{-1} \cdot 1.73\ m^{-2}$ according to the Modification of Diet in Renal Disease study equation modified for Japanese patients.[5] Coronary angiographic parameters were assessed in each participating center either by visual assessment or by quantitative angiographic measurement. Bifurcation lesion was defined as that which involved a side branch ≥2.2 mm in diameter. Bifurcation 2-stent treatment was defined as stenting of both the main and side branches and 1-stent treatment as stenting of the main branch alone. When stenting for the side-branch ostium (circumflex in the vast majority of the cases) was performed before stenting of the main branch, the procedure was regarded as an elective 2-stent strategy. When stenting for the side-branch ostium was performed after stenting of the main branch, the procedure was regarded as a provisional 2-stent strategy.

During follow-up, death was regarded as cardiac in origin unless obvious noncardiac causes could be identified. Any death during the index hospitalization was regarded as cardiac death. Myocardial infarction was adjudicated according to the definition in the Arterial Revascularization Therapy Study.[6] ST was defined according to the Academic Research Consortium definition.[7] Both Academic Research Consortium "definite ST" and "definite/probable ST" on a patient basis were used as the end points for ST. Definite ST of the ULMCA lesion was also assessed separately. Target-lesion revascularization (TLR) was defined as either PCI or coronary artery bypass graft surgery due to restenosis or thrombosis of the target lesion that included the proximal and distal edge segments and the ostium of the side branches.

Statistical Analysis

Categorical variables are presented as counts and/or percentages and were compared with the χ^2 test. Continuous variables were expressed as mean±SD unless otherwise indicated. Continuous variables were compared with the Student t test, ANOVA, or Wilcoxon rank sum test on the basis of their distribution. Cumulative incidences of adverse events were estimated by the Kaplan–Meier method, and curves were compared with the log-rank test.

We used the Cox proportional hazard model to identify risk factors for end points such as death, cardiac death, and TLR. Proportional hazard assumptions were assessed by the plot of log (time) versus log [−log (survival)] stratified by risk factor variables. All variables in Table 1 were used as candidates for risk factors, and we selected those with $P<0.10$. We then conducted a backward-selection procedure on the multivariable Cox proportional hazard model with all selected risk factors and identified the independent risk factors with $P<0.05$. Lastly, we added ULMCA intervention as a risk factor and developed the final model.

We conducted a similar backward selection for multivariable Cox proportional hazard models to assess the effect of lesion location and bifurcation stenting strategy on all-cause death, cardiac death, or TLR in the subgroup of patients with ULMCA stenting. Then, we reached the final model with independent risk factors and bifurcation lesion or 2-stent strategy.

Adjusted survival curves were drawn for the 2 groups of patients with or without ULMCA stenting by use of the Cox proportional hazard model in conjunction with methods described by Ghali et al,[8] with adjustment for the above-mentioned variables selected by the backward-selection procedure.

Table 1. Baseline Characteristics of Patients With or Without ULMCA Stenting

	ULMCA (n=582)	Non-ULMCA (n=12 242)	P
Age, y	70.7±10.6	68.3±10.2	<0.0001
Age ≥75 y	39.5	29.9	<0.0001
Male	72.7	75.4	0.14
Emergent procedure	20.5	14.8	0.0003
Presence of shock	3.4	1.3	<0.0001
Acute myocardial infarction	12.5	12.2	0.75
Unstable angina	18.4	12.4	<0.0001
Hypertension	74.6	73.5	0.56
Diabetes mellitus	42.6	41.4	0.58
Diabetes (insulin use)	12.0	9.3	0.029
Current smoking	15.1	20.6	0.0013
Estimated GFR, mL · min^{-1} · 1.73 mm^{-2}	55±24	59±23	<0.0001
Renal insufficiency	15.3	10.0	<0.0001
Dialysis	7.4	5.2	0.029
Ejection fraction	56±15	58±13	0.0061
Heart failure	24.1	13.5	<0.0001
EuroSCORE	5.8±3.6	4.6±3.1	<0.0001
EuroSCORE ≥6	46.6	33.2	<0.0001
Bifurcation lesion	81.4	24.0	<0.0001
Bifurcation: 2-stent treatment	29.6	4.6	<0.0001
No. of treated vessels	1.9±0.8	1.3±0.5	<0.0001
Prior PCI	43.3	46.6	0.13
Prior CABG	7.9	7.1	0.45
Prior myocardial infarction	25.1	27.3	0.25
History of stroke	13.2	9.3	0.0029
Extracardiac arteriopathy	13.4	11.8	0.24
Extent of coronary artery disease			
Left main only	6.9	0.0	
1 Vessel	23.9	47.8	
2 Vessel	40.0	30.2	<0.0001
3 Vessel	21.3	14.9	
Post-CABG	7.9	7.1	
Total stent length, mm	56.3±37.2	41.8±27.6	<0.0001
No. of implanted stents	2.6±1.6	1.9±1.6	<0.0001
Intravascular ultrasound use	63.4	46.7	<0.0001

GFR indicates glomerular filtration rate; CABG, coronary artery bypass grafting.

Values are percentages or mean±SD.

Probability was significant at a level of <0.05. All statistical tests were 2-tailed. Statistical analyses were conducted by a physician (M. Toyofuku) and by an independent statistician (T.M.) with the use of JMP5.1.1 (SAS Institute Inc, Cary, NC) and SAS 9.1 (SAS Institute Inc) software. The study sponsor was not involved in the study design; the collection, analysis, and interpretation of data; the writing of the report; or the decision to submit the article for publication.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Table 2. Unadjusted Event Rates in Patients With or Without ULMCA Stenting Through 3 Years

	Events, Incidence (%)		
	ULMCA (n=582)	Non-ULMCA (n=12 242)	P
Total deaths	76 (14.6)	911 (9.2)	<0.0001
Cardiac deaths	39 (7.3)	450 (4.5)	<0.0001
Sudden deaths	9 (1.6)	155 (1.6)	0.50
Myocardial infarction	17 (3.7)	338 (3.4)	0.76
Stroke	23 (5.1)	404 (4.1)	0.31
Definite/probable ST	13 (2.5)	128 (1.3)	0.0059
Definite ST	10 (2.0)	111 (1.2)	0.040
TLR	73 (14.8)	1439 (13.8)	0.33
CABG	9 (1.7)	181 (1.9)	0.81
Any revascularization	180 (35.9)	3407 (32.2)	0.022

CABG indicates coronary artery bypass grafting.

Results

Baseline and Procedural Characteristics

Patients in the ULMCA group were significantly older and sicker than those in the non-ULMCA group, as reflected by the higher incidences of stroke, heart failure, renal insufficiency, unstable angina, shock, and bifurcation disease (Table 1). However, 53% of the patients in the ULMCA group had a EuroSCORE <6, and the procedures were performed electively in 79% of the patients.

Aspirin and thienopyridines were prescribed in 98.7% and 99.2% of patients with ULMCA stenting and 97.8% and 98.6% of patients with non-ULMCA stenting, respectively (online-only Data Supplement Table I). The proportion of patients with dual-antiplatelet therapy longer than 1 year was significantly greater in the ULMCA group than in the non-ULMCA group (73% versus 62%, $P<0.0001$).

Outcomes of Patients With or Without ULMCA Stenting

The median follow-up intervals were 942 days in the ULMCA group and 924 days in the non-ULMCA group, with complete 1-year follow-up in 97.0% and 96.5% ($P=0.62$) of patients, respectively. The crude rates of all-cause death and cardiac death up to 3 years were significantly higher in patients with ULMCA disease (Table 2; Figure 2; online-only Data Supplement Table II). Significant risk factors for all-cause death by multivariable Cox proportional hazard model included age >75 years, male gender, shock, heart failure, renal insufficiency, extracoronary arteriopathy, triple-vessel disease, diabetes mellitus, no statin use, absence of intravascular ultrasound guidance, and EuroSCORE ≥6 (online-only Data Supplement Table III). After adjustment for these confounders, there were no significant differences between the ULMCA group and the non-ULMCA group for all-cause death risk (hazard ratio 1.23, 95% confidence interval 0.95 to 1.60, $P=0.12$) or cardiac death risk (hazard ratio 1.10, 95% confidence interval 0.71 to 1.63, $P=0.64$). The adjusted 3-year survival rates were not different between the 2 groups (92.6% versus 93.9%, $P=0.12$; Figure 2).

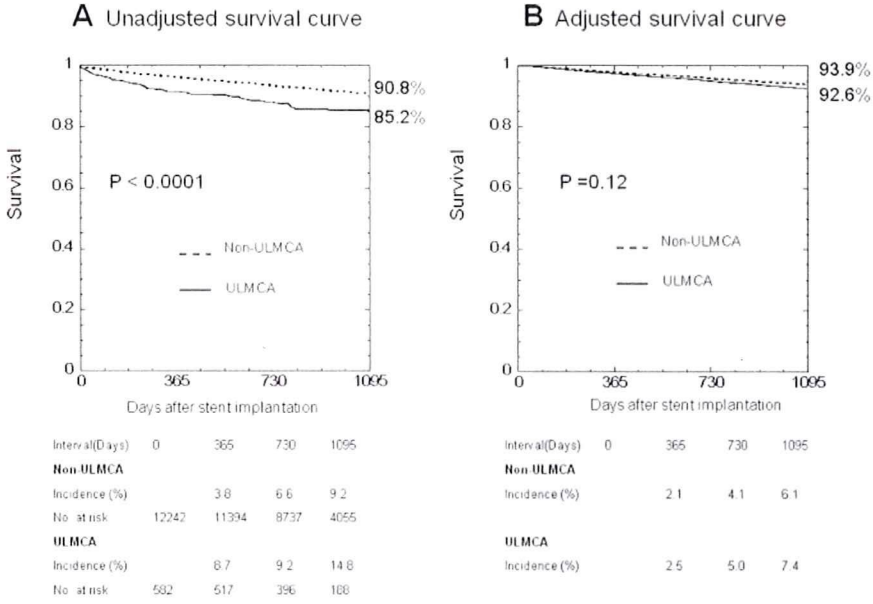


Figure 2. Unadjusted (A) and adjusted (B) survival rate in patients with or without ULMCA stenting.

Distributions of the causes of death were comparable between patients with and without ULMCA treatment (Table 3). There was no excess of patients with documented ventricular fibrillation and/or sudden death in the ULMCA group.

Rates of ST (both definite and definite/probable) were significantly higher in the ULMCA group than in the non-ULMCA group; however, the incidence of definite ST of the ULMCA lesion was relatively low (0.9% at 3 years). Furthermore, the cumulative incidence of myocardial infarction was not different between the ULMCA group and the non-ULMCA group. Angiograms and details of 4 patients with definite ST of ULMCA lesions treated exclusively by SES are shown in Figure 3 and online-only Data Supplement Table IV. No ST occurred in the main body of the ULMCA.

The rate of stroke was not different between the 2 groups with and without ULMCA treatment. The rate of TLR also was not different between the 2 groups, although the rate of any repeated revascularization was slightly but significantly higher in the ULMCA group than in the non-ULMCA group.

Table 3. Causes of Death in Patients With or Without ULMCA Stenting

	ULMCA Group (n=582)	Non-ULMCA Group (n=12 242)	*P*
No. of deaths	76	911	
Documented VF/sudden death	11 (14.5)	152 (16.7)	0.35
Heart failure	14 (18.4)	144 (15.8)	
Acute myocardial infarction	8 (10.5)	50 (5.5)	
Bleeding	0 (0.0)	9 (1.0)	
Stroke	4 (5.3)	85 (9.3)	
Noncardiovascular causes	39 (51.3)	471 (51.7)	

VF indicates ventricular fibrillation.
Values are n (%).

Clinical Outcomes According to Left Main Lesion Location

Among 476 patients whose ULMCA lesions were treated exclusively with SES, 96 (20%) had ostial/shaft left main lesions, whereas 380 patients (80%) had distal left main bifurcation lesions. With regard to lesion and procedural characteristics, patients with ostial/shaft lesions had less calcification, shorter stent length, and higher maximal balloon inflation pressure (Table 4).

Clinical outcomes were markedly favorable in the ostial/shaft group (Table 5; online-only Data Supplement Table V). There was no definite ST of the ULMCA lesion among 97 patients in the ostial/shaft group. The incidence of TLR at 3 years in that group was remarkably lower than in the bifurcation group. (3.6% versus 17.1%, $P=0.0047$; Figure 4). After adjustment for dialysis, small diameter (<3 mm), and diabetes mellitus, the hazard ratio for TLR with ostial/shaft lesions was 0.22 (0.07 to 0.70, $P=0.011$).

There was no difference in the rates of cardiac death between the 2 groups (Figure 4). After adjustment for confounders of heart failure, renal insufficiency, shock, elderly age, and intravascular ultrasound guidance, the hazard ratio for ostial/shaft lesions was 1.10 (95% confidence interval 0.60 to 2.00, $P=0.76$) for all-cause death and 1.40 (0.65 to 3.04, $P=0.39$) for cardiac death.

Clinical Outcomes According to Left Main Bifurcation Stenting Strategies and Extent of Coronary Artery Disease

Among 380 patients treated for ULMCA bifurcation lesions, 261 (69%) underwent bifurcation 1-stent procedures, whereas 119 patients (31%) were treated with 2-stent procedures (Table 6). Among 119 patients with 2-stent procedures, elective and provisional 2-stent strategies were adopted in 99 and 20 patients, respectively. Compared with patients undergoing bifurcation 1-stent procedure, patients with 2-stent

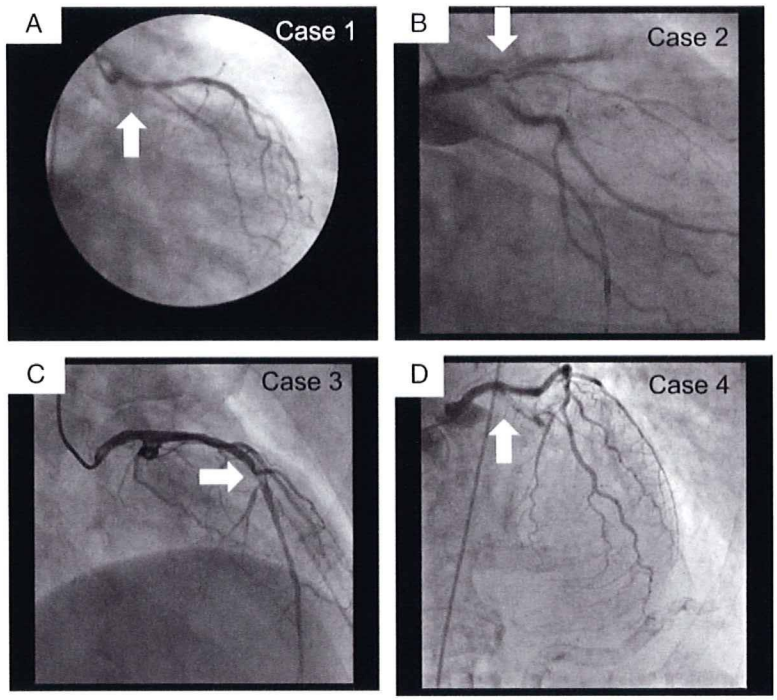


Figure 3. Angiograms of 4 cases of ST after left main coronary artery stenting.

procedures had more severe stenosis of the origin of the circumflex and larger vessel diameter of the circumflex.

Patients in the 2-stent group showed a trend toward a higher incidence of all-cause death and a significantly higher rate of cardiac death than patients in the 1-stent group (Table 7; Figure 5; online-only Data Supplement Table VI). After adjustment for confounders, the hazard ratio of bifurcation 2-stent treatment was 1.64 (95% confidence interval 0.93 to 2.90, $P=0.088$) for all-cause death and 2.78 (1.27 to 6.05, $P=0.010$) for cardiac death. The prevalence of documented ventricular fibrillation or sudden cardiac death among patients who died throughout the 3-year follow-up period was 4 (19%) of 21 patients in the bifurcation 2-stent group and 2 (6.7%) of 30 patients in the bifurcation 1-stent group. The rate of definite ST in the ULMCA lesion also tended to be higher in patients with bifurcation 2-stent treatment than in patients with bifurcation 1-stent treatment (2.8% versus 0.4%; $P=0.050$).

Furthermore, the rate of TLR in the 2-stent group was markedly higher than that in the 1-stent group (30.9% versus 11.1%, $P<0.0001$; Figure 5). After adjustment for confounders, the hazard ratio for TLR with bifurcation 2-stent treatment was 3.17 (95% confidence interval 1.82 to 5.52, $P<0.0001$). When analyzed according to the number of diseased vessels other than the left main coronary artery, the rates of cardiac death, definite/probable ST and any revascularization were significantly higher in patients with ULMCA plus 3-vessel disease (Table 8).

Table 4. Baseline and Procedure Characteristics in Patients With ULMCA Stenting According to Lesion Location

	Ostial/Shaft (n=96)	Bifurcation (n=380)	*P*
Age ≥75 y	46.9	38.7	0.16
Shock	5.2	3.7	0.56
Heart failure	25.0	26.1	0.90
Renal insufficiency	14.6	15.3	0.87
De novo lesion	83.3	78.7	0.39
In-stent restenosis	11.5	9.2	0.56
Calcification (severe)	4.2	14.7	0.0033
Intravascular ultrasound guidance	60.6	65.0	0.47
Total stent length per lesion, mm	16.6±6.8	29.7±13.9	<0.0001
Nominal stent diameter, mm	3.29±0.27	3.30±0.28	0.84
Maximum balloon pressure, atm	20.0±3.0	18.8±3.2	0.0013

Values are percentages or mean±SD.

Table 5. Outcomes After ULMCA Stenting According to Lesion Location Through 3 Years

	Events, Incidence (%)		
	Ostial/Shaft (n=96)	Bifurcation (n=380)	*P*
Total deaths	14 (14.9)	51 (15.1)	0.66
Cardiac deaths	9 (9.8)	27 (7.6)	0.41
Sudden deaths	3 (3.5)	5 (1.4)	0.20
Myocardial infarction	1 (1.2)	13 (4.5)	0.24
Stroke	7 (10.7)	13 (4.2)	0.070
Definite/probable ST	1 (1.0)	10 (3.0)	0.38
Definite ST	0	8 (2.5)	0.17
Definite ST (ULMCA)	0	4 (1.1)	0.33
TLR	3 (3.6)	55 (17.1)	0.0047
CABG	1 (1.2)	5 (1.5)	0.88
Any revascularization	21 (25.9)	124 (37.9)	0.11

CABG indicates coronary artery bypass grafting.
Values are n (%).

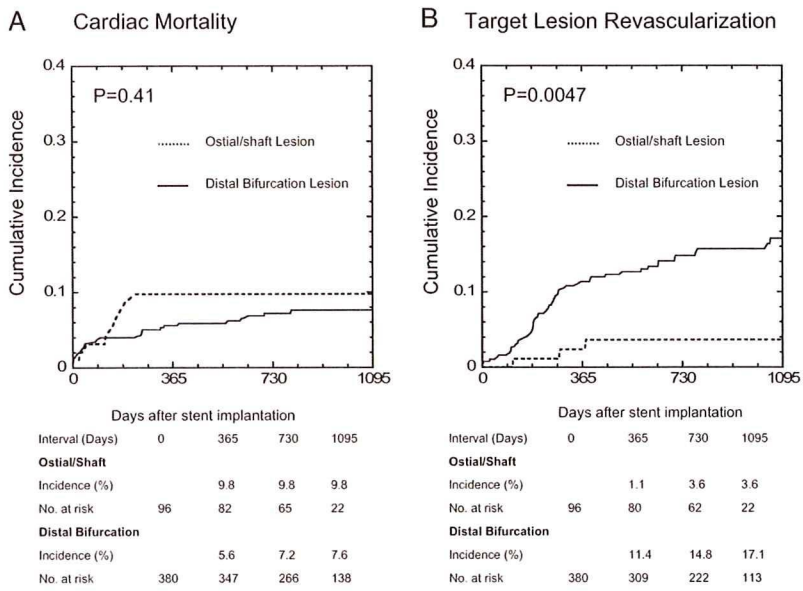


Figure 4. Kaplan–Meier curves for cumulative incidences of cardiac death (A) and TLR (B) among patients treated for ULMCA according to lesion location.

Discussion

The main findings of this study are as follows: (1) PCI for ULMCA lesions was relatively safe, with adjusted mortality rates similar to those with non-ULMCA lesions, which indicates that fatal events due to stent failure manifesting as thrombosis or restenosis were rare; (2) SES implantation in ostial/shaft left main lesions was associated with an excellent 3-year TLR rate; and (3) bifurcation 2-stent treatment was associated with significantly higher rates of cardiac death and TLR.

Table 6. Baseline and Procedure Characteristics in Patients With ULMCA Stenting According to Bifurcation Stenting Strategy

	One-Stent Bifurcation (n=261)	Two-Stent Bifurcation (n=119)	*P*
Age ≥75 y	38.1	39.5	0.82
Shock	4.2	2.5	0.56
Heart failure	26.4	25.2	0.90
Renal insufficiency	13.8	18.5	0.28
De novo lesion	79.7	76.5	0.48
In-stent restenosis	7.3	13.5	0.058
Calcification (severe)	12.3	20.2	0.060
Intravascular ultrasound guidance	67.1	60.5	0.25
Final kissing balloon technique	74.7	93.3	<0.0001
Total stent length per lesion, mm	24.1±10.7	42.0±12.3	<0.0001
Nominal stent diameter(main branch), mm	3.30±0.29	3.29±0.27	0.70
Nominal stent diameter (side branch), mm	. . .	2.91±0.38	. . .
Max balloon pressure, atm	18.9±3.3	18.6±3.0	0.51
50% Stenosis of ostial CX	34	85	<0.0001
Vessel diameter of CX, mm	2.66±0.52	2.81±0.61	0.02

CX indicates circumflex coronary artery.
Values are percentages or mean±SD.

Mortality Rate After ULMCA Stenting

In patients with ULMCA disease, surgical revascularization has significantly improved the survival rate compared with medical management.[9] Therefore, PCI in patients with ULMCA disease should demonstrate at least comparable mortality rates to those with surgical revascularization, if PCI is a clinically acceptable alternative.

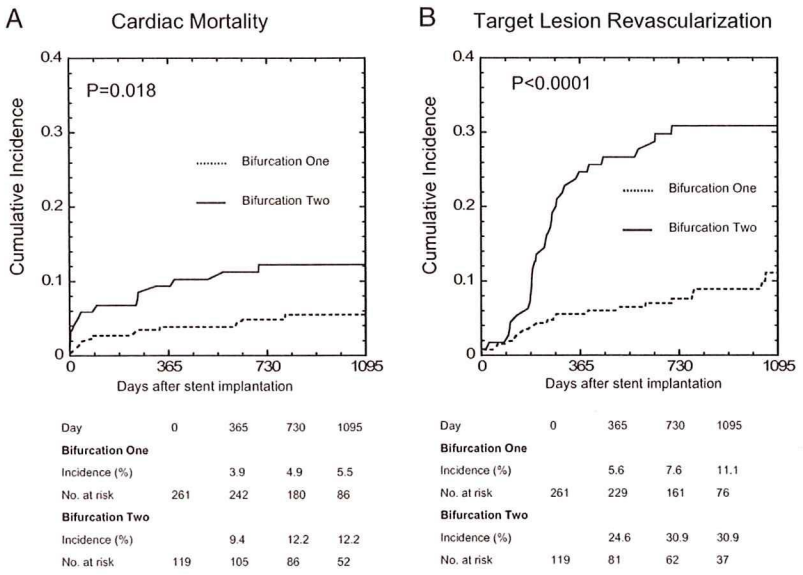
Recent reports comparing percutaneous and surgical revascularization for ULMCA disease have shown similar survival rates between the 2 therapies.[10,11] Likewise, 1-year results of the SYNTAX (Synergy Between PCI With TAXUS and Cardiac Surgery) trial showed comparable mortality rates after PCI with DES or coronary artery bypass graft.[12]

In the present study, the crude mortality rate was 1.7% and 14.6% at 30 days and 3 years, respectively. The 30-day mortality rate was comparable to that in a recent systematic review of ULMCA stenting with DES[13] and current benchmarks with coronary artery bypass graft for ULMCA[14,15];

Table 7. Outcomes After ULMCA Stenting According to Bifurcation Stenting Strategies Through 3 Years

	Events, Incidence (%)		
	One-Stent Bifurcation (n=261)	Two-Stent Bifurcation (n=119)	*P*
Total death	30 (13.4)	21 (18.8)	0.12
Cardiac deaths	13 (5.5)	14 (12.2)	0.018
Sudden deaths	2 (0.8)	3 (2.7)	0.15
Myocardial infarction	8 (4.5)	5 (4.7)	0.58
Stroke	10 (4.7)	3 (3.0)	0.53
Definite/probable ST	3 (1.5)	7 (6.3)	0.0076
Definite ST	3 (1.5)	5 (4.7)	0.054
Definite ST (ULMCA)	1 (0.4)	3 (2.8)	0.050
TLR	22 (11.1)	33 (30.9)	<0.0001
CABG	2 (0.9)	3 (0.3)	0.15
Any revascularization	77 (35.5)	47 (44.0)	0.017

CABG indicates coronary artery bypass grafting.



Day	0	365	730	1095
Bifurcation One				
Incidence (%)		3.9	4.9	5.5
No. at risk	261	242	180	86
Bifurcation Two				
Incidence (%)		9.4	12.2	12.2
No. at risk	119	105	86	52

Day	0	365	730	1095
Bifurcation One				
Incidence (%)		5.6	7.6	11.1
No. at risk	261	229	161	76
Bifurcation Two				
Incidence (%)		24.6	30.9	30.9
No. at risk	119	81	62	37

Figure 5. Kaplan–Meier curves for cumulative incidences of cardiac death (A) and TLR (B) among patients treated for ULMCA according to distal bifurcation stenting strategy.

however, the late mortality rates appeared to be higher in the present series. The prevalence of comorbidities in the present study population may explain the relatively high late mortality rates. Indeed, the 1-year mortality rate of 4.2% in low-risk patients (EuroSCORE <6) in the present study was similar to the 4.8% seen in the low-risk patients in the systematic review mentioned above.[13] Moreover, after adjustment for confounders, there was no significant difference in the risk of all-cause death at 3 years between the 2 groups of patients with or without ULMCA stenting. These results suggest that fatal events were secondary to clinical presentation and comorbidities rather than to the performance of the implanted device. This finding is consistent with recent reports[11,16,17] that the occurrence of fatal events owing to stent failure such as thrombosis or restenosis after ULMCA stenting is rare.

Stent Thrombosis

The present study population included high-risk conditions for ST, such as renal failure, heart failure, diabetes mellitus, and bifurcation lesions; however, the incidence of definite ST of the ULMCA lesion was relatively low (0.9% at 3 years). Moreover, no ST occurred in the main body of the ULMCA. This result is consistent with some reports that have suggested that the incidence of definite ST after ULMCA stenting is relatively lower than in other lesion subsets.[16,17] However, the ST rate tended to be higher with the bifurcation 2-stent strategy than with the bifurcation 1-stent strategy.

Lesion Location and Bifurcation Stenting Strategy

Experiences with DES implantation for ULMCA showed marked reduction of restenosis rates compared with bare-metal stents.[1,2] Chieffo et al[18] recently showed that SES or paclitaxel-eluting stent implantation in ostial or mid-shaft lesions is associated with an excellent long-term restenosis rate of 0.9%, a finding that is consistent with the 3-year TLR rate of 3.6% seen in the present study. Patients with ostial and mid-shaft left main coronary artery lesions that did not

Table 8. Estimated Event Rates at 3 Years After ULMCA Treatment According to Extent of Coronary Artery Disease

	ULMCA Only (n=40)	ULMCA+SVD (n=113)	ULMCA+DVD (n=188)	ULMCA+TVD (n=93)	ULMCA+CABG (n=42)	*P*
Total death	21.4	11.2	14.0	21.7	9.5	0.15
Cardiac death	2.5	7.3	7.8	15.1	0.0	0.033
Sudden death	0.0	1.9	1.7	3.5	0.0	0.56
Myocardial infarction	4.4	2.1	4.9	5.6	0.0	0.57
Stroke	0.0	8.6	4.4	4.9	8.8	0.40
Definite/probable ST	0.0	0.0	1.9	8.4	2.6	0.0029
Definite ST	0.0	0.0	1.9	5.4	2.6	0.13
Definite ST (ULMCA)	0.0	0.0	0.6	3.6	0.0	0.07
TLR	2.6	7.1	18.7	18.9	15.6	0.027
Any revascularization	25.2	31.3	37.1	48.3	24.5	0.0016

SVD indicates single-vessel disease; DVD, double-vessel disease; TVD, triple-vessel disease; and CABG, coronary artery bypass grafting.

Values are percentages.

require bifurcation treatment appeared to be good candidates for percutaneous treatment with DES.

However, distal left main disease has been reported to be associated with relatively high TLR rates of 13% to 38%, particularly when a bifurcation 2-stent procedure was undertaken.[19,20] In the present study, SES deployment with a bifurcation 2-stent strategy was the strongest predictor of TLR after ULMCA stenting (3-year TLR rate of 30.9%), a finding that is in line with previous reports. The present results also point to an increased incidence of thrombotic events and a significantly higher incidence of cardiac death in patients treated with bifurcation 2-stent strategies, although the statistical power is obviously insufficient to detect differences in the incidences of these hard events. Also, survival analysis of cardiac death could be seen as a competing risk situation, because there are multiple types of death. Use of the Kaplan–Meier method in this situation may result in an overestimation of the true cumulative incidence of cardiac death.[21]

Because the patients with bifurcation 1-stent treatment had relatively favorable outcomes in terms of survival and need for TLR, one should make every effort to finish with main-branch stenting alone when treating left main bifurcation lesions. Also, foreseeing the likelihood of the need for a 2-stent approach appears to be important in selecting patients for ULMCA stenting. The use of DES in patients with distal bifurcation lesions that are likely to require a 2-stent strategy is probably premature. Future innovative solutions are crucial for the percutaneous treatment of left main true bifurcation lesions.

The higher rates of cardiac death and any revascularization in patients with ULMCA plus 3-vessel disease observed in the present study are consistent with the findings from the SYNTAX trial.[12] Therefore, serious consideration must be given to the indication for PCI in this subgroup of patients with the most complex coronary anatomy.

Relative to the selection of revascularization strategies in patients with extensive coronary artery disease, the SYNTAX trial highlighted the lower incidence of stroke after PCI than after coronary artery bypass graft. Although the rate of stroke in the ULMCA group in the present study (2.8% at 1 year) appeared to be higher than in the PCI arm of the SYNTAX trial (0.6% at 1 year), the rates of stroke were similar between the 2 groups with or without ULMCA stenting. The relatively high incidence of stroke in the present study cohort might be related to the high prevalence of stroke at baseline.

Study Limitations

There are several limitations to the present study. First, this was an observational study, and comparison of the clinical outcomes between patients treated for ULMCA and those treated for non-ULMCA disease might be biased even after adjustment for known confounders. Furthermore, the treatment strategy for bifurcation lesions was not based on randomized assignment. Second, TLR events in the present study included both clinically driven and angiographically driven events. The clinical significance of angiographically driven TLR of ULMCA remains unclear. Third, the length of clinical follow-up is still limited. Fourth, although the number of study patients was larger than reported previously, the present study population included heterogeneous patients with a high prevalence of elderly age, renal failure, and heart failure. Cautious interpretation is required to generalize our results. Fifth, we could not incorporate the SYNTAX score, which would have helped us to stratify the risk among patients undergoing ULMCA stenting. Finally, the relatively lower rate of ST (1.3% at 1 year) in the present study than reported in the SYNTAX trial (3.3% at 1 year) might result from the much more complex anatomic characteristics of patients in the SYNTAX trial. Alternatively, the different rates of ST could be derived from differences related to ethnicity. Therefore, it might be difficult to extrapolate the present study results outside Japan.

Conclusions

The higher unadjusted mortality rate in patients undergoing ULMCA stenting did not appear to be related to treatment of ULMCA itself but rather to the high-risk profile of the ULMCA patients treated by PCI in real-world clinical practice. Although long-term outcomes of patients with ostial/shaft ULMCA lesions were favorable, outcomes of patients who underwent bifurcation 2-stent treatments appeared unacceptable.

Appendix

A complete list of investigators and committees of the j-Cypher registry has been published previously.[11]

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Disclosures

Drs Kimura and Isshiki have served as advisory board members and have received honoraria from Cordis Cardiology, Japan, Johnson & Johnson. Dr Mitsudo has received honoraria from Cordis Cardiology, Japan, Johnson & Johnson. The remaining authors report no conflicts.

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CLINICAL PERSPECTIVE

Despite the growing popularity of stenting unprotected left main coronary arteries with drug-eluting stents, long-term outcomes have not been assessed adequately. This large multicenter registry in Japan (the j-Cypher registry) compared the 3-year clinical outcomes of 582 patients undergoing percutaneous coronary intervention for unprotected left main coronary artery (ULMCA) lesions with those of 12 242 patients undergoing percutaneous coronary intervention for non-ULMCA lesions only. The influence of lesion location and bifurcation stenting strategy on clinical outcomes was also assessed in 476 patients whose ULMCA lesions were treated exclusively with sirolimus-eluting stents. The main findings of this study are as follows: (1) Percutaneous coronary intervention for ULMCA lesions was associated with a higher late mortality rate than for lesions located elsewhere, but this finding was mainly related to factors other than the left main being the treatment site; (2) sirolimus-eluting stent implantation in ostial/shaft left main lesions was associated with a better 3-year target-lesion revascularization rate than in distal bifurcation lesions; and (3) patients with ULMCA plus 3-vessel disease had poor long-term outcome in terms of coronary revascularization, stent thrombosis, and cardiac death. Therefore, although ULMCA stenting with a sirolimus-eluting stent is an attractive option, clinical outcomes are less satisfactory in patients who need bifurcation 2-stent treatment or who have extensive coronary artery disease outside the ULMCA. Consideration of the individual patient's risk stratification is important when selecting coronary revascularization strategies in patients with ULMCA disease.

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QUARTERLY FOCUS ISSUE: HEART RHYTHM DISORDERS

D85N, a KCNE1 Polymorphism, Is a Disease-Causing Gene Variant in Long QT Syndrome

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Objectives This study aims to address whether D85N, a KCNE1 polymorphism, is a gene variant that causes long QT syndrome (LQTS) phenotype.

Background KCNE1 encodes the beta-subunit of cardiac voltage-gated K^+ channels and causes LQTS, which is characterized by the prolongation of the QT interval and torsades de pointes, a lethal arrhythmia. D85N, a KCNE1 polymorphism, is known to be a functional variant associated with drug-induced LQTS.

Methods In order to elucidate the prevalence and clinical significance of this polymorphism, we performed genetic screening in 317 LQTS probands. For comparison, we examined its presence in 496 healthy control subjects. We also conducted biophysical assays for the D85N variant in mammalian cells.

Results The allele frequency for D85N carriers was 0.81% in healthy people. In contrast, among LQTS probands, there were 1 homozygous and 23 heterozygous carriers (allele frequency 3.9%). Seven of 23 heterozygous carriers had additional mutations in LQTS-related genes, and 3 female subjects had documented factors predisposing to the symptom. After excluding these probands, the D85N prevalence was significantly higher compared with control subjects (allele frequency 2.1%, $p < 0.05$). In a heterologous expression study with Chinese hamster ovarian cells, KCNE1-D85N was found to exert significant loss-of-function effects on both KCNQ1- and KCNH2-encoded channel currents.

Conclusions The KCNE1-D85N polymorphism was significantly more frequent in our LQTS probands. The functional variant is a disease-causing gene variant of LQTS phenotype that functions by interacting with KCNH2 and KCNQ1. Since its allele frequency was ~1% among control individuals, KCNE1-D85N may be a clinically important genetic variant. (J Am Coll Cardiol 2009;54:812–9) © 2009 by the American College of Cardiology Foundation

Long QT syndrome (LQTS) is a disorder that is characterized by repolarization abnormalities in the heart, leading to torsades de pointes (TdP), syncope, and sudden death. Among the LQTS-related genes identified to date, KCNQ1 and KCNE1 are known to encode the alpha and beta subunits of voltage-gated K^+ channels, which carry I_{Ks}, a slowly activating component of delayed rectifier K^+ current (1,2). KCNE1 is also known to regulate KCNH2 (3), which encodes the Kv11.1 protein, the alpha subunit of rapidly activating delayed rectifier K^+ current (I_{Kr}) (4–6).

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A KCNE1 C-terminal polymorphism, D85N, has been found in the normal population and is known to cause a G-to-A transition at codon 253 (c.253G>A), which leads to the amino acid substitution of aspartic acid for asparagines (7). This has been shown to cause an approximately 50% reduction in KCNQ1-encoded currents in a heterologous expression system using *Xenopus* oocytes (8), although biophysical study data are not available for mammalian cells.

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The allele frequency of the polymorphism is reported to be 0.7% in apparently healthy Asians (7). Paulussen et al. (9) demonstrated in a European population that the allele frequency of D85N was 5% in acquired LQTS patients who experienced TdP as a result of drug administration, but was 0% in the control group. Iwasa et al. (10) reported that the allele frequency was 2% in 100 Japanese cases, but their cohort contained both LQTS patients and normal individuals.

In the present study, we examined the incidence rate of KCNE1-D85N polymorphisms in 317 LQTS probands from unrelated families and 496 control healthy individuals. We identified 23 heterozygous and 1 homozygous probands (allele frequency 3.9%), described the demographics of these index patients, and examined the possibility that the D85N polymorphism is an LQTS-causing genetic variant. We also conducted detailed functional assays of the variant while it was coexpressed with the 2 alpha subunits of cardiac delayed rectifier K^+ channels, KCNQ1 and KCNH2, by using a heterologous expression system involving Chinese hamster ovarian (CHO) cells.

Methods

Study subjects. Three hundred and seventeen consecutive LQTS probands who showed a prolongation of the QT interval were referred to our laboratory for genetic evaluation and were enrolled in our analysis. The electrocardiogram diagnostic criteria of Keating and Sanguinetti (11) included a corrected QT interval (QTc) of ≥470 ms in asymptomatic individuals and a QTc of >440 ms for male subjects and of >460 ms for female subjects that had 1 or more of the following: 1) stress-related syncope; 2) documented TdP; or 3) a family history of early sudden cardiac death.

The protocol for genetic analysis was approved by our institutional ethics committee and was performed under its guidelines. Informed consent was obtained from all individuals or their guardians before the analysis. The QT intervals were measured from electrocardiographic lead II or an available rhythm strip and were corrected for heart rate according to Bazett's formula. As for the control cohort, we screened the frequency of the D85N polymorphism in 496 randomly selected cases, consisting of healthy volunteers and mutation-negative family members such as probands' spouses. Their QTc were <440 ms for male subjects and <460 ms for female subjects.

Genotyping. Genomic deoxyribonucleic acid (DNA) was isolated from venous blood by use of the QIAamp DNA midikit (Qiagen, Hilden, Germany). Genetic screening for KCNE1-D85N was performed by direct polymerase chain reaction. Other LQTS-related genes, including KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2, and KCNJ2, were assayed by denaturing high-performance liquid chromatography using a WAVE System Model 3500 (Transgenomic, Omaha, Nebraska). Abnormal conformers were amplified by polymerase chain reaction. Sequencing was performed with an ABI PRISM3100 DNA sequencer (Applied Biosystems, Wellesley, Massachusetts).

Abbreviations and Acronyms

CHO = Chinese hamster ovarian
LQTS = long QT syndrome
QTc = corrected QT interval
TdP = torsades de pointes

Site-directed mutagenesis. Complementary deoxyribonucleic acid (cDNA) for human KCNQ1 (GenBank AF000571) and KCNE1 (GenBank M26685) were kindly provided by Dr. J. Barhanin (Institut de Pharmacologie Moléculaire et Cellulaire, CNRS, Valbonne, France). The cDNAs were subcloned into pIRES2-EGFP (for KCNQ1) and pIRES-CD8 (for both wild-type and mutated KCNE1) vectors. cDNA for human KCNH2 (GenBank AF363636) was kindly donated by Dr. M. Sanguinetti (University of Utah, Salt Lake City, Utah). The cDNA was subcloned into a pRc-CMV vector. A KCNE1-D85N variant was constructed using a Quick Change II XL Site-Directed Mutagenesis Kit, according to the manufacturer's instructions (Stratagene, La Jolla, California). Nucleotide sequence analysis was performed on each variant construct before the expression study to confirm their sequences.

Cell transfection. CHO cells were maintained at 37°C in Dulbecco's modified Eagle medium and Ham's F12 nutritional mixture (Gibco-BRL, Rockville, Maryland) containing 10% fetal bovine serum supplemented with 1% penicillin and 1% streptomycin. Wild-type KCNQ1, KCNH2, and wild-type and/or variant KCNE1 clones were expressed transiently in CHO cells using the LipofectAMINE method according to the manufacturer's instructions (Invitrogen, Carlsbad, California).

To identify the cells that were positive for KCNH2 expression, CHO cells were cotransfected with 1 μg of pRc-CMV/KCNH2 vector and 0.5 μg of pEGFP-N1/CMV vector. Forty-eight to 72 h after transfection, green fluorescent protein-positive cells and anti-CD8 antibody-coated bead (Dynabeads CD8, Dynal Biotech, Oslo, Norway) decorated cells were used for the patch-clamp study.

Electrophysiological assays. Whole-cell configuration of patch-clamp techniques was employed to record membrane currents at 37°C with an EPC-8 patch-clamp amplifier (HEKA, Lambrecht, Germany). Pipette resistance ranged from 2.5 to 4 MΩ when filled with the pipette solutions described in the following text. The series resistance was electronically compensated for at 70% to 85%. The extracellular solution contained (mmol/l): 140 NaCl, 0.33 NaH_2PO_4, 5.4 KCl, 1.8 $CaCl_2$, 0.5 $MgCl_2$, 5.5 glucose, and 5 HEPES, and the pH was adjusted to 7.4 with NaOH. The internal (pipette) solution contained (mmol/l): 70 potassium aspartate, 70 KOH, 40 KCl, 10 KH_2PO_4, 1 Mg_2SO_4, 3 Na_2-ATP, 0.1 Li_2-GTP, 5 EGTA, and 5 HEPES, and the pH was adjusted to 7.2 with KOH.

KCNQ1/KCNE1-encoded currents were measured by depolarizing pulses from a holding potential of −90 mV to test potentials between −70 and +50 mV (with a 10-mV step increment), before being repolarized to −50 mV in

order to monitor tail current amplitude. KCNH2/KCNE1-encoded currents were elicited by depolarizing pulses from a holding potential of −80 mV to test potentials between −60 to +50 mV (with a 10-mV step increment), before being repolarized to −60 mV in order to monitor tail current amplitude. Current densities (pA/pF) were calculated for each cell studied, by normalizing peak tail current amplitude to cell capacitance (Cm). The Cm was calculated by fitting a single exponential function to the decay phase of the transient capacitive current in response to ± 5 mV voltage steps (20 ms) from a holding potential of −50 mV. The liquid junction potential between the test solution and the pipette solution was measured as approximately −10 mV and was corrected. Data were collected and analyzed using the Patch master and Igor Pro (WaveMetrics, Lake Oswego, Oregon).

Data analyses. The voltage-dependence of current activation was determined by fitting the normalized tail current (Itail) versus test potential (Vtest) to Boltzmann's function, which is expressed by: $Itail = 1/(1 + \exp[(V_{0.5} - Vt)/k])$, where $V_{0.5}$ is the voltage at which the current is half-activated and k is the slope factor. Time constants for deactivation (τ_{fast} and τ_{slow}) were obtained by fitting a 2-exponential function to the time course of the deactivating tail currents. All data are expressed as mean ± standard error. Statistical comparisons were made using analysis of variance, followed by a *t* test, and differences were considered significant at a value of $p < 0.05$.

Results

Clinical characteristics and genotyping. Of the 496 control volunteers, 8 (mean QTc 420.5 ± 7.5 ms) had heterozygous D85N genotypes (allele frequency 0.81%). In contrast, 23 of the 317 LQTS probands had heterozygous D85N genotypes and 1 (Table 1) (Patient #24) had a homozygous D85N genotype (allele frequency 3.9%). Table 1 and Figure 1 summarize the demographics of the 24 index patients. Their mean age was 34.8 ± 4.4 years, and their mean QTc was 507.9 ± 9.2 ms. Among the D85N-negative cases, we identified 116 probands that were positive for other LQTS-related gene mutations (Fig. 1), and their mean QTc was significantly longer (540.6 ± 6.1 ms) than those of the 24 D85N carriers ($p < 0.05$).

Seven of the 23 heterozygous probands (30%) had other LQTS-related gene variants (KCNQ1 or KCNH2), and 3 female patients (13%; Patients #1, #6, and #10) had documented predisposing factors, such as electrolyte disturbances, QT prolonging drug intake, or bradycardia (Table 1). The allele frequency of the remaining 13 patients (2.1%) was significantly higher than that in the control subjects ($p < 0.05$). Six of these 13 patients (46%) had syncope and/or TdP while 9 of 10 patients (90%) with multiple genetic variants or triggering factors were symptomatic (Fig. 1).

The mean onset age of the 6 symptomatic heterozygous D85N carriers without compromised factors to affect QT

Table 1 Clinical Characteristics of the LQTS Probands Who Carried the KCNE1-D85N Variant

Patient #	Age (F/M)	QTc (ms)	Syncope	TdP	Compound Variant	Underlying Predisposing Triggers
1	36 (F)	540	+	+		Drug (bromocriptine), hypokalemia
2	9 (M)	478	−	−		
3	21 (F)	533	−	+	KCNH2 (a, S706F)*	Drug (amphetamine), hypokalemia
4	42 (F)	650	+	+		
5	51 (F)	490	+	+	KCNH2 (a, E58K)	Sinus bradycardia
6	73 (F)	493	+	−		Drug (disopyramide), sinus bradycardia
7	30 (F)	502	+	−	KCNH2 (G745fs+55X)*	
8	17 (F)	470	−	−		
9	13 (F)	462	+	−	KCNH2 (a, S320L)	
10	41 (F)	490	−	+		Hypomagnesemia
11	73 (F)	608	+	−	KCNQ1 (a, S277L)	Sinus bradycardia
12	75 (F)	494	+	+		
13	17 (M)	500	−	−		
14	13 (F)	512	+	−		
15	57 (M)	472	−	−		
16	53 (M)	462	+	+		
17	17 (F)	520	+	−		
18	22 (F)	472	−	−		
19	13 (F)	522	−	−	KCNH2 (a, R823W)	
20	13 (M)	467	+	−		
21	52 (M)	524	+	+	KCNH2 (a, R948S)*	Drug (minor tranquilizer), hypokalemia
22	11 (M)	491	−	−		
23	51 (M)	493	−	−		
24	39 (F)	545	−	−		

*Novel variant.
LQTS = long QT syndrome; QTc = corrected QT interval; TdP = torsades de pointes.

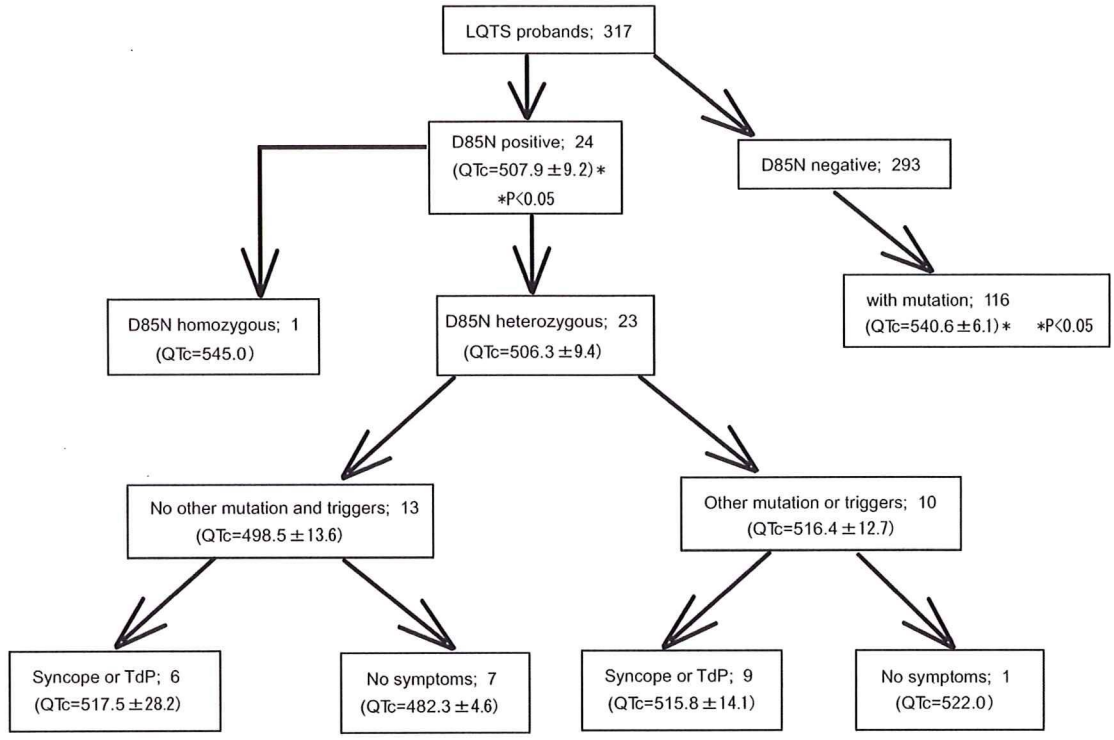

Figure 1 **Summary of LQTS Probands**

LQTS = long QT syndrome; QTc = corrected QT interval; TdP = torsades de pointes.

interval was 35.5 ± 10.4 years. It was significantly older than the mean onset age of the other genotyped symptomatic LQTS patients (21.0 years in our cohort of 94 genotyped LQTS) (Horie M. et al., unpublished data, September 2008). Although the clinical features of KCNE1-D85N–positive probands differed with respect to the QTc and the onset age from those of other genotyped LQTS patients, this variant appeared to be a disease-causing gene variant in congenital LQTS.

Biophysical assays of the genetic variant. KCNE1-D85N WITH KCNQ1. In order to confirm that the D85N is a disease-causing variant, we conducted functional assays using a heterologous expression system with a mammalian cell line (CHO cells). In the first line of experiments, we examined how KCNE1-D85N affected the reconstituted KCNQ1/KCNE1 currents. Figure 2 depicts representative current traces recorded from cells that coexpressed KCNQ1 and wild-type (Fig. 2A-a) or D85N (Fig. 2A-b) KCNE1 (1 μg each). Peak tail current densities measured after repolarization to −50 mV from various test pulses were calculated in individual cells and are plotted as a function of test potential in Figure 2B. Solid circles indicate the mean peak current densities from 21 cells that were transfected with KCNQ1 and wild-type KCNE1; open circles indicate the mean peak current densities from 25 cells that were transfected with KCNQ1 and D85N, and solid triangles indicate the mean peak current densities from 25 cells that were transfected with KCNQ1 alone. D85N reduced the peak tail currents of wild-type KCNQ1/KCNE1-encoded currents by 28% at 0 mV test potential ($p < 0.05$ vs. wild type).

In Figure 2C, peak tail current densities have been normalized using the current densities recorded after a test pulse to +50 mV and are plotted as a function of test potential. Fitting of data plots to Boltzmann's equation yielded $V_{0.5}$ values of −4.36 ± 1.8 mV for the wild type and 0.38 ± 1.7 mV for D85N ($p < 0.05$), suggesting that the KCNE1 variant produced a significantly positive shift in KCNQ1-encoded current activation kinetics (Table 2). The deactivation process of tail currents could be fitted by 2 exponentials, yielding fast and slow time constants. No significant difference with respect to the fast time constants was evident between the wild-type and D85N genotypes; however, slow deactivation was significantly accelerated by coexpression of D85N (Table 2).

KCNE1-D85N WITH KCNH2. In the next line of experiments, we examined how KCNE1 and its D85N variant influence KCNH2-encoded currents. Figures 3A-a and 3A-b depict 2 sets of current traces recorded from CHO cells that had been transfected with KCNH2 plus wild-type or D85N KCNE1 (1 μg each). Peak tail current densities at −60 mV were calculated in the respective cells and are plotted as a function of test potential in Figure 3B. Solid circles and open circles indicate the mean current densities calculated from 23

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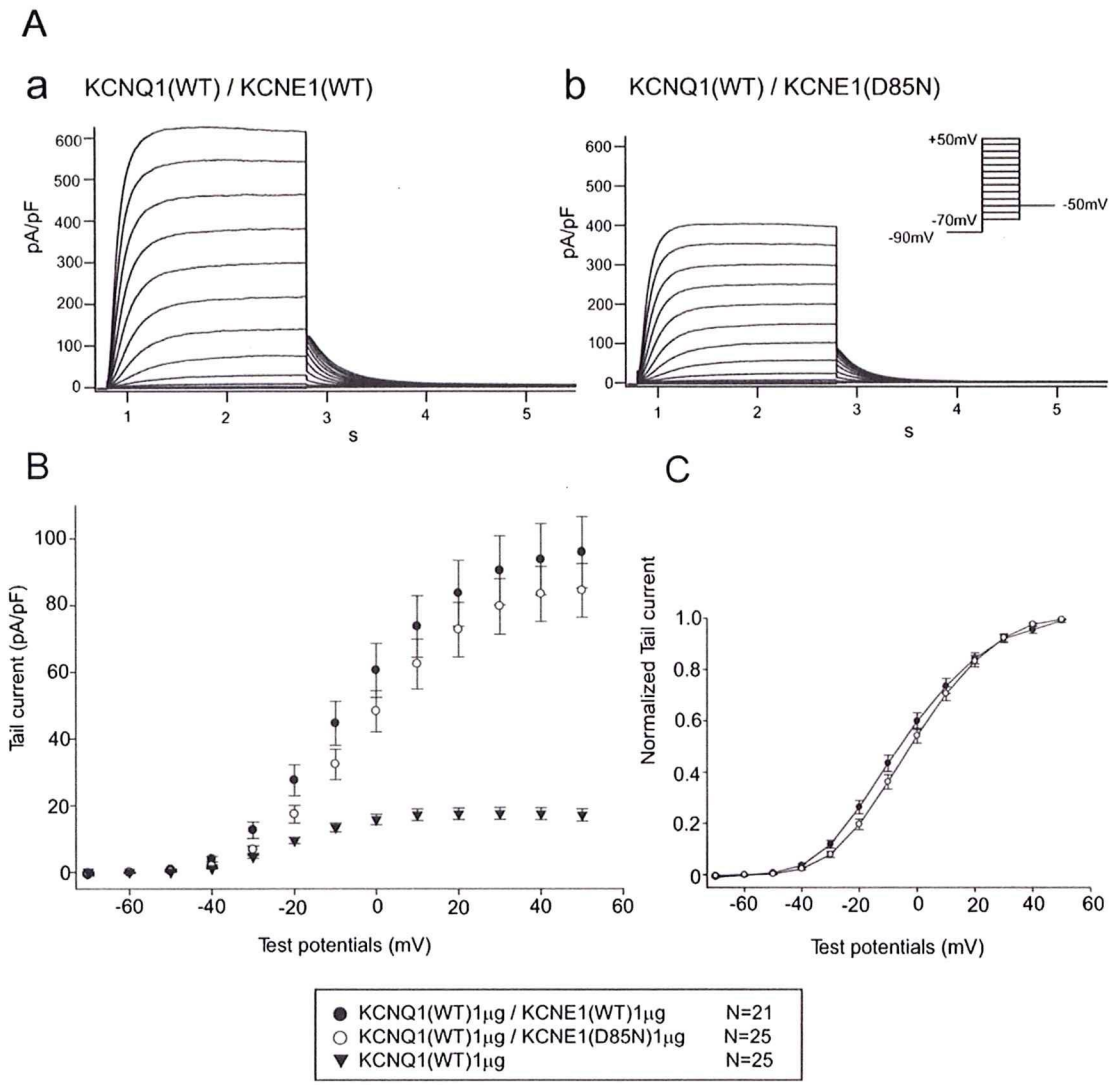

Figure 2 **Functional Expression of KCNQ1 With KCNE1-D85N in Chinese Hamster Ovarian Cells**

(A) Representative current traces of KCNQ1 coexpression with the wild-type (WT) or D85N KCNE1. **(a)** KCNQ1 (1 μg) plus WT KCNE1 (1 μg). **(b)** KCNQ1 (1 μg) plus D85N KCNE1 (1 μg). **(B and C)** Functional consequences of KCNQ1 coexpression with the WT or D85N KCNE1 (**[B]** activation curve; **[C]** normalized activation curve). **Solid circles** indicate the mean peak current densities from 21 cells that have been transfected with KCNQ1 and WT KCNE1 (1 μg of each), and **open circles** indicate the mean peak current densities from 25 cells that have been transfected with KCNQ1 and D85N KCNE1 (1 μg of each). **Solid triangles** show those obtained from 25 cells that were transfected with KCNQ1 alone (1 μg).

and 20 cells, respectively, which were transfected with 1 μg of KCNH2 and 1 μg of wild-type or D85N KCNE1.

D85N reduced the peak tail currents of wild-type KCNH2/KCNE1-encoded currents by 31% to 36% at test potentials between 0 and +50 mV ($p < 0.005$ vs. wild type). Fitting of normalized data to Boltzmann's equation yielded a $V_{0.5}$ of −18.33 ± 0.8 mV for the wild-type KCNH2/KCNE1 and of −22.07 ± 1.6 mV for KCNH2/KCNE1-D85N ($p < 0.05$), suggesting that the KCNE1 variant causes a significantly negative shift of KCNH2/KCNE1-encoded current activation kinetics (Fig. 3C, Table 2). Deactivation of tail currents could be fitted by 2 exponentials, yielding fast and slow time constants. The fast and slow kinetics were not significantly different between the 2 types of KCNH2 channel currents (Table 2).

Discussion

The present study demonstrates that the allele frequency of KCNE1-D85N is significantly higher in LQTS patients than in control subjects after excluding cases with compromised factors to prolong QT interval ($p < 0.05$). A biophysical assay of D85N showed that the variant affected both reconstituted I_{Ks} and I_{Kr} channel function, leading to a prolongation of the QTc with D85N working as a disease-causing variant. In a heterologous expression system with *Xenopus* oocytes (8),

Table 2 $V_{0.5}$, Slope Factor k, and τ Deactivation at +20 mV

	n	$V_{0.5}$	k	τ_{fast}	τ_{slow}
KCNQ1 (WT) 1 μg	25	−20.86 ± 1.034	8.223 ± 0.421	0.070 ± 0.005	0.136 ± 0.019
KCNQ1 (WT) 1 μg/KCNE1 (WT) 1 μg	21	−4.364 ± 1.834*	12.724 ± 0.407*	0.145 ± 0.013*	0.586 ± 0.070†
KCNQ1 (WT) 1 μg/KCNE1 (D85N) 1 μg	25	0.382 ± 1.717*‡	12.566 ± 0.429*	0.141 ± 0.013*	0.409 ± 0.050*‡
KCNH2 (WT) 1 μg/KCNE1 (WT) 1 μg	23	−18.326 ± 0.775	7.373 ± 0.289	0.183 ± 0.016	1.077 ± 0.102
KCNH2 (WT) 1 μg/KCNE1 (D85N) 1 μg	20	−22.069 ± 1.560§	7.037 ± 0.389	0.193 ± 0.013	1.258 ± 0.090

*$p < 0.0001$ versus KCNQ1 (wild type [WT]) 1 μg; †$p = 0.0001$ versus KCNQ1 (WT) 1 μg; ‡$p < 0.05$ versus KCNQ1 (WT) 1 μg/KCNE1 (WT) 1 μg; §$p < 0.05$ versus KCNH2 (WT) 1 μg/KCNE1 (WT) 1 μg.

KCNE1-D85N has been reported to cause an approximately 50% reduction in KCNQ1-encoded currents, although data for mammalian cells is not available. In our experiments using CHO cells, D85N significantly reduced KCNQ1-encoded currents by 28% ($p < 0.05$ vs. wild type), although this effect was smaller than that in *Xenopus* oocytes.

When KCNH2 was coexpressed with the wild-type or D85N variant of KCNE1, D85N significantly reduced wild-type KCNH2/KCNE1-encoded currents by 31% to 36% ($p < 0.005$ vs. wild type). Regarding the interaction between KCNE1 and KCNH2, McDonald et al. (3) demonstrated that KCNE1 forms a stable complex with KCNH2 and

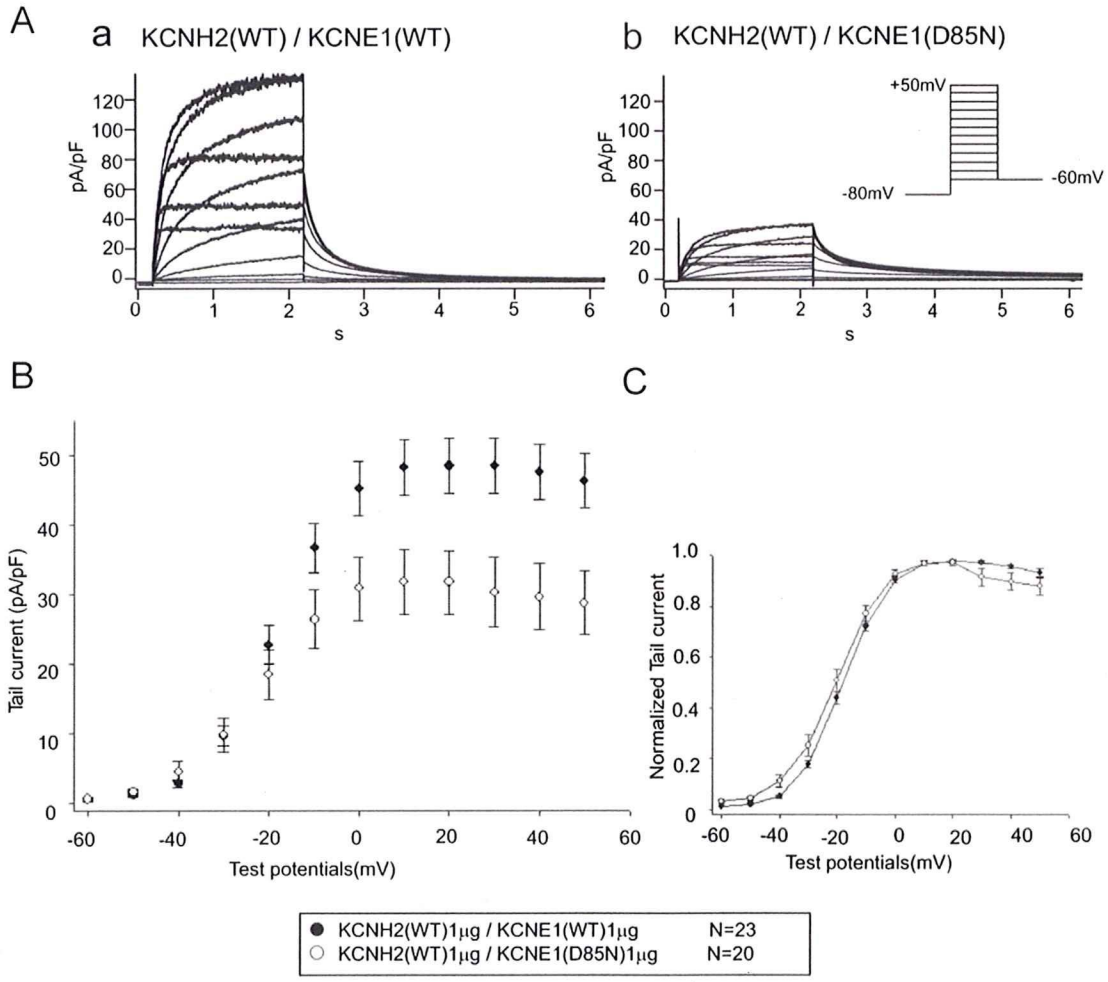

Figure 3 Functional Expression of KCNH2 With KCNE1-D85N in Chinese Hamster Ovarian Cells

(A) Representative current traces of KCNH2 coexpression with the wild-type (WT) or D85N KCNE1. **(a)** KCNH2 (1 μg) plus WT KCNE1 (1 μg). **(b)** KCNH2 (1 μg) plus D85N KCNE1 (1 μg). **(B and C)** Functional consequences of KCNH2 coexpression with the WT or D85N of KCNE1 (**[B]** activation curve; **[C]** normalized activation curve). **Solid circles** indicate data from 23 cells that were transfected with KCNH2 and WT KCNE1 (1 μg of each). **Open circles** indicate data from 20 cells that were transfected with KCNH2 and D85N KCNE1 (1 μg of each).

up-regulates I_{Kr}-like currents by 50% in CHO cells. Bianchi et al. (12) also showed interactions between the KCNE1-D76N mutation and both KCNQ1 and KCNH2 in HEK cells. In atrial tumor myocytes that expressed I_{Kr} alone, Yang et al. (13) demonstrated that antisense oligonucleotides against minK cDNA (KCNE1) significantly reduced the I_{Kr} by ~62%. More recently, Ohno et al. (14) identified a missense KCNE1 mutation, A8V, in a sporadic case of LQTS and reported that the mutation significantly reduced the magnitude of KCNH2- but not KCNQ1-encoded currents.

Collectively, it is of clinical importance that the KCNE1-D85N variant modifies not only KCNQ1- but also KCNH2-coded channel currents. Furthermore, its inhibitory action on KCNH2 was even stronger than that on KCNQ1. The KCNE1-D85N polymorphism may therefore cause phenotypes similar to those observed in type 2 LQTS such as bradycardia (15,16). The deactivation process of I_{Kr} plays a significant role in maintaining the appropriate rate of pacemakers (17) and, therefore, a decreased I_{Kr} will lead to sinus bradycardia. In the present study, 3 D85N carriers (13%) had sinus bradycardia (Table 1).

The mean onset age of 6 symptomatic heterozygous D85N carriers (Table 1) was 35.5 years, and this was significantly older than the mean age of other genotyped symptomatic LQTS patients. Shimizu et al. (18) reported that in 95 Japanese LQT1 patients with transmembrane domain mutations or C-terminal domain mutations, the mean ages of first event were 11 ± 8 years and 13 ± 9 years. Nagaoka et al. (19) also demonstrated that in 118 Japanese LQT2 patients with pore mutations or nonpore mutations, the mean ages of first event were 16 ± 10 years and 20 ± 13 years. In addition, the mean QTc of 13 D85N carriers was prolonged (498.5 ± 13.6 ms) but significantly shorter than that in 116 probands with other LQTS-related gene mutations (541 ms) (Fig. 1). These different phenotypes appear to reflect the fact that D85N causes a milder channel dysfunction than other LQTS mutations, and reveals a "forme fruste" phenotype (20).

The allele frequency of the KCNE1-D85N polymorphism was 0.81% among apparently healthy control individuals. We found only 1 report concerning D85N frequency (0.7%) (7) in control subjects, which showed equivalent results to our study. Based on 2008 healthy French individuals, Gouas et al. (21) demonstrated that the allele frequency of D85N was significantly higher in the 200 subjects with the longest QTc than in those with the shortest QTc (3.1% vs. 0.75%), suggesting that this single nucleotide polymorphism may influence the QTc length in healthy individuals.

LQTS can remain latent or subclinical because of "repolarization reserve" (22), and can become unmasked upon the intake of QT-prolonging drugs. Heterozygous D85N carriers in the control group may be at a potentially higher risk of long QT-related arrhythmias. Assuming that genetic surveys are feasible before drug therapy, D85N carriers may be recommended to avoid the secondary factors that predispose them to further QT prolongation such as QT prolonging drugs (23) and electrolyte disturbances (23–25). It is also clinically useful to search for other variants of long QT-related genes (8,26,27).

Study limitations. In the present study, we screened the mutations that are responsible for LQT1, 2, 3, 5, 6, and 7. Therefore, the comorbidity of other types of LQTS was not completely excluded, although their frequency was quite low. In general, single nucleotide polymorphisms are thought to be nonpathological although some may modify the clinical features of a disease. For example, the KCNH2-K897T polymorphism is a typical genetic modifier that aggravates LQTS phenotypes directly by reducing channel function in association with the KCNH2 mutation A1116V (28). Such a role for KCNE1-D85N was not addressed in this study and warrants further study.

Conclusions

KCNE1-D85N was a highly frequent variant in our LQTS probands and was found to cause loss-of-function effects on both I_{Kr} and I_{Ks} and work as a disease-causing variant. Since its allele frequency was 0.81% among control healthy individuals, KCNE1-D85N may be a clinically important genetic variant.

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