Abbreviations and Acronyms
CABG = coronary artery bypass grafting
ITA = internal thoracic artery
LAD = left anterior descending artery
LCX = left circumflex artery
RCA = right coronary artery

Despite the various graft configurations that have previously been reported,[10-12] the optimal strategy for graft arrangement remains unknown. Because an excellent early graft patency rate can be highly expected when arterial graft materials are exclusively used, the patency rate of the bypass grafts might not necessarily be useful for evaluation and comparison of the graft arrangements.

The objectives of this study were to delineate the effect of the graft flow in the sequential and composite arterial grafts on the late graft patency and to establish the optimal strategy for the graft arrangement of the arterial conduits for minimizing competitive and reverse flow.

Materials and Methods

The coronary angiograms of 570 patients who underwent off-pump coronary revascularization with single or bilateral ITA grafts and the radial artery without aortic manipulation between December 2000 and June 2005 were reviewed. There were 475 men and 95 women with a mean age of 66.0 ± 9.3 years (Table 1), and all patients provided written informed consent. These patients were consecutive after eliminating those who had a bypass of the saphenous vein, gastroepiploic artery, or inferior epigastric artery; underwent no early postoperative coronary angiography; or had bypass grafting in an individual fashion only. During the same period, off-pump CABG was performed for 821 patients. Early coronary angiography was performed for all 570 patients at about 2 weeks after the operation. The native coronary artery stenosis and the graft patency were independently evaluated by cardiologists. The degrees of stenosis in the precise measurement of the luminal diameter were graded as 51% to 75%, 76% to 90%, and 91% to 100%. The maximal severity of stenosis was recorded for all coronary branches.

TABLE 1. Baseline characteristics

No. of patients	570
Age (y)	66.0 ± 9.3
Male/female sex	475/95
Hypertension	301 (52.8%)
Hyperlipidemia	270 (48.9%)
Diabetes	218 (38.2%)
Left ventricular end-diastolic volume index (mL/m^2)	86.2 ± 29.7
Left ventricular ejection fraction (%)	47.8 ± 11.9
Total distal anastomoses	2083
Distal anastomoses per patient	3.65 ± 0.95
Bypass conduits used	830
Individual in situ ITA	151
Individual composite I-graft	28
Composite Y-graft	358
Composite I-graft	173
Composite K-graft	63
In situ ITA sequential	57

ITA, Internal thoracic artery.

TABLE 2. Concept of flow grading system

	Grade			
	A	B	C	O
Flow direction	Antegrade	Competitive	Reverse	No-flow
Patency	Patent	Patent	Patent	Occluded
Function	Functioning	Functioning	Nonfunction	Nonfunction
Durability	Durable (?)	(?)	(?)	No

A, Antegrade; *B*, competitive; *C*, reverse; *O*, occlusion.

The definitions of terms in the present study are as follows. An *in situ ITA graft* is an ITA that was divided only at its distal portion. A *composite graft* is a bypass conduit consisting of one in situ graft and a free graft anastomosed to it (in end-to-end, end-to-side, or side-to-side fashion). A combination of Y-grafts, K-grafts, and I-grafts and the individual conduit were used in this study. An *individual bypass* was defined as a conduit having one distal anastomosis and one in situ graft. This included an in situ graft that was extended by a free graft and bypassed to one target coronary branch. A bypass conduit having one in situ graft and 2 or more distal anastomoses, such as a sequential graft or a composite Y-graft (or K-graft), was defined as *nonindividual*.

Flow Grading

The concept of determining grading of the graft flow focused on 2 factors: (1) the function as a blood supply to the ischemic myocardium and (2) the possibility of graft failure in the future (Table 2). A patent graft meant that the graft had a complete continuity of the graft lumen in the overall length from the subclavian artery to the anastomotic site with the coronary branch, irrespective of the flow direction. When the continuity of the graft lumen from an in situ ITA graft to the anastomosis with the target coronary branch was interrupted at any level, it was defined as grade O (occlusion), which was regarded as a no-flow situation with closure of the lumen of the bypass graft. Grade A was defined as a situation in which antegrade graft flow (ie, from the in situ graft to the target coronary branch) was found in most of the multiplane ITA angiography. Grade B (competitive) was defined as a situation in which the target vessel was barely opacified from the ITA graft injection and the bypass graft was filled by retrograde flow from the native coronary injection. In the worst of multiplane angiography, the contrast medium from the in situ ITA did not surely reach the target branch. Grade C (reverse) was defined as a situation in which the distal anastomotic site was not opacified from the ITA graft injection at all but was filled clearly by retrograde flow from the native coronary injection. The difference between grades B and C was whether the contrast medium from the in situ ITA finally reached the target branch in the best frame of multiplane examinations. Grades C and O meant that the bypass

TABLE 3. Early angiographic results

	Characteristics of coronary branches	No. of anastomoses	Grade			
			A (%)	B (%)	C (%)	O (%)
Location	LAD main trunk	574	541 (94.3)	17 (3.0)	11 (1.9)	5 (0.9)
	Diagonal	314	296 (94.3)	7 (2.2)	7 (2.2)	4 (1.3)
	LCX	646	587 (90.9)	15 (2.3)	35 (5.4)	9 (1.4)
	RCA	549	477 (86.9)	22 (4.0)	38 (6.9)	12 (2.2)
Stenosis	51%-75%	957	815 (85.2)	53 (5.5)	76 (7.9)	13 (1.4)
	76%-90%	553	521 (94.2)	8 (1.4)	15 (2.7)	9 (1.6)
	91%-100%	573	565 (98.6)	0	0	8 (1.4)
Overall	—	2083	1901 (91.3)	61 (2.9)	91 (4.4)	30 (1.4)

A, Antegrade; *B*, competitive; *C*, reverse; *O*, occlusion; *LAD*, left anterior descending artery; *LCX*, left circumflex artery; *RCA*, right coronary artery.

graft did not contribute to the increase of coronary perfusion in the grafted territory. Grade B bypass grafts probably contributed to the coronary perfusion, but the durability of graft patency was considered uncertain because the retrograde flow from the native coronary branch was almost comparable with that of grade C. The flow grade was recorded for each target coronary branch.

Graft Selection and Strategy

The details of our standard technique and pharmacologic management were reported previously.[13] The bilateral ITAs were preferably used for patients aged less than 75 years with neither severe chronic obstructive pulmonary disease nor diabetes requiring insulin therapy for improvement of the late outcome.[11,14-16] All of the ITA grafts in the present series had a luminal diameter of 1.5 mm or larger. In the side-to-side anastomosis we made a longitudinal incision of approximately 6 to 10 mm in the native coronary artery and arterial graft to achieve a sufficient luminal size without turbulence. The angle of placement of the graft was adjusted to save the graft length and avoid kinking.

The various configurations of the bypass conduits used in our patients are listed in Table 1. The arrangement of the bypass conduits was determined primarily on the basis of the special relationship of the target coronary arteries. Since March 2003, we have introduced our current strategy. Our current graft arrangement consisted of the left ITA to LAD grafting concomitant with an I-graft of the right ITA and radial artery to the left circumflex artery (LCX) and right coronary artery (RCA) in a sequential fashion. In addition, we selected appropriate orientation (clockwise or counter clockwise) to avoid bypass grafting to a coronary branch with 51% to 75% stenosis at the end of the I-graft as much as possible because the terminal end of the conduit was commonly associated with reverse flow.[13,17] Before March 2003, the I-graft was used only in a counterclockwise orientation for all patients.

Early Angiography of 2083 Coronary Branches

To determine the factors that predicted the grade non-A bypass grafts in the early angiography, we collected detailed data regarding the target coronary branch, the bypass conduit, and anastomotic fashion. The variables in the univariate analysis included the territory of the target coronary branch (LAD, LCX, or RCA), the diameter (1.0, 1.25, 1.5, or 2.0 mm, as determined by the intracoronary shunt used), the severity of the native coronary stenosis (51% to 75% or 76% to 100%), the kind of graft material (in situ ITA, free ITA, or radial artery), the type of conduit (in situ ITA, Y-graft, K-graft, or I-graft), the number of distal anastomoses of the conduit (3 or less, or 4 or more), and the type of anastomoses (end-to-side or side-to-side).

Analysis of Clinical Outcome in 570 Patients

We examined the effects of the bilateral in situ ITA grafts, total distal anastomotic sites, vessel disease, presence (or absence) of bypass graft grade non-A in the early angiography, and day of the operation in the period of our current strategy of graft arrangement. The mean follow-up period was 22 ± 16 months.

Statistical Analysis

The continuous variables are expressed as the mean values ± standard deviation. The univariate and multivariate analyses were performed by using the logistic regression method. The Kaplan-Meier method was used to determine the actuarial graft patency rate. Cox regression analysis was used to examine the significance of the clinical and angiographic variables in predicting the cardiac event–free time.

Results

The results of analysis of 2083 anastomoses are shown in Table 3. The overall early patency rate was 2053 (98.6%) of 2083. Sixty-one (2.9%) bypasses were graded B, 91 (4.4%) were graded C, and 1901 (91.2%) were graded A.

In the univariate analysis, the end-to-side anastomosis ($P < .0001$), conduit type (Y-graft, $P = .002$; K-graft, $P = .002$; I-graft, $P = .02$), native coronary stenosis of less than 75% ($P < .0001$), location (RCA territory, $P < .0001$; LCX territory, $P = .02$), and graft material (radial artery, $P = .04$) were correlated with grade non-A. In the multivariate analysis, the end-to-side anastomosis ($P < .0001$), 4 or more distal anastomoses of the conduit ($P = .01$), native coronary stenosis of less than 75% ($P < .0001$), and target branch location (RCA territory, $P < .0001$; LCX territory, $P = .02$) significantly correlated with grade non-A (Table 4). Neither the type of the conduit nor the graft material

TABLE 4. Predictors of grade non-A in the early angiography

Variables	Odds ratio	95% CI	*P* value
Univariate analysis			
End-to-side anastomosis	4.51	2.88-7.04	<.0001
Distal anastomoses of conduit >3	1.27	0.92-1.76	.14
Type of conduit, Y-graft (vs in situ ITA)	2.80	1.44-5.43	.002
Type of conduit, K-graft (vs in situ ITA)	3.21	1.52-6.78	.002
Type of conduit, I-graft (vs in situ ITA)	2.30	1.14-4.68	.02
51%-75% stenosis	4.73	3.29-5.80	<.0001
Location, RCA territory (vs LAD territory)	2.51	1.73-3.65	<.0001
Location, LCX territory (vs LAD territory)	1.62	1.10-2.40	.02
Graft material, free ITA (vs in situ ITA)	0.98	0.29-3.28	.97
Graft material, free RA (vs in situ ITA)	1.44	1.02-2.03	.04
Diameter of coronary branch	0.62	0.23-1.69	.35
Multivariate analysis			
End-to-side anastomosis	8.18	4.82-13.87	<.0001
Distal anastomoses of conduit >3	1.73	1.17-2.55	.01
Type of conduit, Y-graft (vs in situ ITA)	1.91	0.91-4.05	.09
Type of conduit, K-graft (vs in situ ITA)	1.71	0.70-4.17	.24
Type of conduit, I-graft (vs in situ ITA)	1.77	0.76-4.14	.19
51%-75% stenosis	6.19	4.22-9.09	<.0001
Location, RCA territory (vs LAD territory)	3.49	1.82-6.69	.0002
Location, LCX territory (vs LAD territory)	3.15	1.71-5.81	.0002
Graft material, free ITA (vs in situ ITA)	0.60	0.15-2.35	.46
Graft material, free RA (vs in situ ITA	0.89	0.47-1.72	.73

CI, Confidence interval; *ITA*, internal thoracic artery; *RCA*, right coronary artery; *LAD*, left anterior descending artery; *LCX*, left circumflex artery; *RA*, radial artery.

anastomosed with the coronary branch correlated with grade non-A.

Intermediate-term Results

In the follow-up period 10 patients died (cardiac death, 8; stroke, 2). Repeated angiography was carried out for 216 bypass grafts in 61 patients, who had some symptoms, including angina, or an ischemic region detected by means of electrocardiography or scintigraphy. Thirty-eight patients underwent percutaneous coronary intervention. The early and late angiographic results of these 216 bypass grafts are shown in Table 5. The patency rate in the late angiography of bypasses that were graded B or C in the early angiography was 7 (28.0%) of 25, whereas that of bypasses graded A was 164 (89.1%) of 184 ($P < .0001$).

TABLE 5. Early and late angiographic results of 216 bypass grafts

Flow grade in early angiography	Bypass grafts	Late angiography		
		Patent	Occluded	Patency rate (%)
A	184	164	20	89.1
B	12	4	8	33.3
C	13	3	10	23.1
O	7	0	7	0.0
Total	216	171	45	79.2

A, Antegrade; *B*, competitive; *C*, reverse; *O*, occlusion.

The actuarial graft patency rates at 3 years were 72.3% for bypasses graded A and 28.6% for bypasses graded B or C ($P < .0001$, Figure 1). There was no significant difference between grades B and C in the actuarial graft patency rate ($P = .20$). The multivariate Cox regression analysis demonstrated that the presence of bypass grafts graded non-A ($P = .007$) was a significant predictor of cardiac events in the intermediate-term outcome, and the period (March 2003-June 2005) was inversely correlated ($P = .008$; odds ratio, 0.32, Table 6).

Discussion

A composite graft, which consists of the left ITA and radial artery, provided total arterial revascularization with an excellent graft patency rate and less incidence of late cardiac events compared with those seen with conventional CABG.[18,19] Various arrangements of the in situ and free arterial grafts have already been practiced and reported.[10-12] Because an excellent early patency rate with less incidence of complications can be highly expected when arterial graft materials are exclusively used, the optimal strategy for graft arrangement remains unknown. For comparison of these graft arrangements and establishment of the optimal strategy, it is necessary to assess this with criteria more specific than "patent" or "occluded."

The angiographic luminal size, which was reported by FitzGibbon and colleagues,[20,21] might not be feasible for evaluation of arterial graft arrangements. At first, the luminal size of the anastomotic site is not precisely measurable in the sequential fashion, especially when the angle of the graft and coronary branch is near 90° or when the contrast medium fills only incompletely because of mixture with the blood flow from the native coronary artery. Additionally,

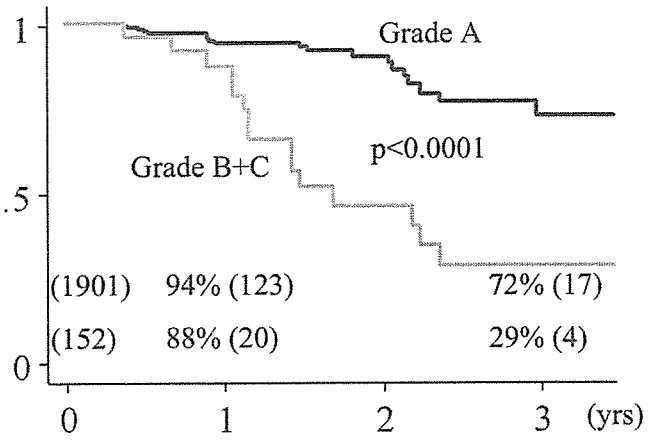

Figure 1. The actuarial graft patency rate.

TABLE 6. Predictors of cardiac events in 570 patients

Variables	Odds ratio	95% CI	*P* value
Univariate analysis			
Total distal anastomoses	1.04	0.77-1.40	.82
Period, March 2003~June 2005	0.30	0.13-0.70	.005
Bilateral in situ ITA	1.10	0.61-1.97	.76
Presence of grade non-A	1.97	1.12-3.45	.02
Vessel disease	1.02	0.61-1.69	.94
Ejection fraction <40%	1.78	0.95-3.30	.07
Hypertension	0.88	0.50-1.55	.66
Hyperlipidemia	1.01	0.63-1.93	.74
Diabetes	0.93	0.72-1.68	.81
Multivariate analysis			
Period, March 2003~June 2005	0.32	0.14-0.74	.008
Presence of grade non-A	1.85	1.05-3.24	.007
Ejection fraction <40%	1.84	0.98-3.40	.055

ITA, Internal thoracic artery.

inadequate surgical maneuvers during the operation can also strongly affect the luminal size as a result of unsuccessful anastomosis or graft kinking. Furthermore, regression of the stenosis and increase or growth of the diameter were relatively common findings in the arterial grafts.[22,23] There were 2 issues associated with insufficient bypass flow in the arterial conduits. These might be potential disadvantages of the strategy with aorta no-touch off-pump CABG using totally arterial grafts. One issue is the subject of bypass function as a blood supply to the myocardial tissue. A bypass graft presenting reverse flow, which means an obviously dominant native coronary flow, will not increase the blood supply to the myocardium in the region of the grafted coronary branch. We previously reported that not only the severity of the native coronary stenosis but also the interactions of the target coronary branches, which were connected with a composite Y-graft or sequential anastomoses, played a crucial role in the occurrence of reverse flow.[13,17] Our current graft arrangement was established to avoid development of graft nonfunction.

The other issue is the thinning or closure of the graft lumen in the postoperative period. If the flow velocity is extremely low, even when its direction is antegrade, the bypass graft might not be durable. Previously, there have been a few studies of early and late angiographies concerning physiologic characteristics of arterial grafts but only in small series.[7-9] Hashimoto and associates[8] reported serial changes of 53 arterial grafts in 38 patients after conventional CABG and demonstrated a significant correlation between "the severity of the native coronary stenosis" and "arterial graft thinning" in the early and follow-up angiographies.

In the present study it was necessary for us to demonstrate the effect of the graft flow in the composite and sequential grafts on graft patency and clinical outcomes and to rationalize the use of the flow grading system in discussing an optimal strategy for graft arrangement because there was no previous report that had been performed to delineate significant correlations between the "bypass flow" in the early angiography and the "graft patency" in the follow-up angiography after totally arterial off-pump CABG with the composite and sequential grafts. Early occlusion caused by a technical problem, which might be the most significant bias, was eliminated by the early angiography. The follow-up period in this study is considered sufficient and suitable for examining the influence of flow condition on the graft patency because physiologic changes in the luminal diameter were found at approximately 14 to 24 months[7-9] or earlier.[22,24] The results of our current study demonstrated that bypass grafts of not only grade C but also grade B were prone to close the graft lumen within the intermediate term. Therefore the flow grading system could be considered suitable and useful for discussing the optimal strategy for graft arrangement of arterial materials.

We found that the significant predictors of grade non-A were native stenosis of 75%, 4 or more distal anastomoses from a single ITA, RCA and LCX territories, and the end of the conduit. The implications of these results were as follows. The sufficient antegrade flow had a favorable effect on the intermediate-term patency of the arterial grafts. When we plan the graft configuration, especially for the multiple coronary branches in the RCA and LCX territories, we have to be conscious of the anticipated graft flow in the created bypass conduit. The most important factor in determining the antegrade flow was the appropriate pressure slope in the bypass conduit, being highest at the proximal portion of the conduit and lowest at the distal end. The Y-graft has 2 ends, and the K-graft has 3 ends, and competitive and reverse flow

was commonly found at the end of the conduit anastomosed with the moderately stenotic branch. To achieve an adequate pressure slope for 2 or 3 ends is less easy than for 1 end of the I-graft. On the other hand, the Y-graft is advantageous in terms of increased flow capacity[25] and availability to distant target branches compared with the I-graft. For the diagonal, LCX, and RCA branches, the Y-graft or K-graft might be preferred when all target branches have severe stenosis, the target diagonal branch is located at the anteroapical portion, or remarkable cardiomegaly exists. Therefore we carefully examine the indications for the Y-graft and K-graft.

Our current arrangement would be one of the simple and useful methods that can be adjusted for each coronary system. The risk of the injury during reoperation in the future is a possible disadvantage of the I-graft in a clockwise orientation. On the contrary, the evident advantage of the I-graft in clockwise orientation is that the total length of the I-graft to the LCX and RCA territories could be minimized compared with that in a counterclockwise orientation. In previous reports the right ITA to the left coronary artery, which also crosses the midline like the clockwise I-graft, is a generally accepted and often recommended procedure of choice.[26] The clockwise I-graft is considered justifiable.

Selection of suitable candidates for this procedure would be a major concern. When graft nonfunction or occlusion at a relatively early period is highly predicted, an alternative strategy, such as aortocoronary bypass, hybrid therapy with drug-eluting stent implantation, and conservation of the arterial graft for the redo operation in the future, might be a reasonable option of choice. In our experience sequential anastomoses with more than 2 moderately stenotic coronary branches were highly associated with flow insufficiency and late occlusion. Aortocoronary bypass would be reasonable because it has higher pressure potential than the in situ ITA.[27]

The present study has several limitations. First, the study is not randomized. Furthermore, the sample size of the late angiography is considered relatively small. The follow-up angiography was performed for 10.7% of the patients who were biased toward clinically evident graft failure. However, all 61 patients underwent both early and late angiographies. Early graft occlusion caused by obviously technical failure, which might be the most significant bias, was eliminated.

Second, the quality of the target branch, the amount of myocardium, peripheral vascular resistance in the myocardial tissue, and flow demands can also have important roles in the coronary perfusion. However, we do not have reliable methods for quantifying each of these factors.

The third limitation is regarding the capacity of the ITA graft. The margin of the pressure potential of the in situ ITA might also play an important role in the occurrence of competitive and reverse flow.[28] However, there is no alternative graft material for the ITA graft.

The fourth limitation might be the subject of the reproducibility of the flow grading system. Grades O and C are relatively easy to designate. Assigning grade B might be less so. Grade B probably includes both insufficient graft flow because of the strength of the native coronary flow and because of poor vascularity with high resistance in the severely impaired myocardium. Although no bypass graft might be required for the latter, we could not separately predict the insufficient antegrade flow caused by the critically damaged vasculature. In the present series there was no anastomotic stenosis, which restricted the blood flow and caused grade B bypass flow. In spite of these factors, the flow grade and angiographic data were prospectively collected and significantly correlated with the graft patency and clinical outcome. We therefore believe that the results of this study at least imply meaningful suggestions for establishing an optimal strategy for graft arrangement in the future.

In conclusion, the flow grading system was considered feasible as a criterion used for evaluation and comparison of the graft arrangements. Because the sufficient antegrade flow had a favorable effect on the durable patency of the arterial grafts, graft arrangement should be adjusted for each patient's coronary system to minimize competitive and reverse flow and to enhance the advantage of the arterial materials.

We thank Dr Yoshiko Kada for her assistance with the statistical analysis.

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European Journal of Cardio-thoracic Surgery 31 (2007) 276–282

EUROPEAN JOURNAL OF CARDIO-THORACIC SURGERY

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Graft design strategies with optimum antegrade bypass flow in total arterial off-pump coronary artery bypass

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Received 21 August 2006; received in revised form 20 October 2006; accepted 14 November 2006; Available online 20 December 2006

Abstract

Objective: In arterial conduits, graft flow is one of the major determinants of long-term patency. We sought to delineate the effect of strategy for graft arrangement and design to three-vessel disease by evaluation of the dominant flow direction in each segment of a bypass graft. **Materials and methods:** We reviewed coronary angiograms of 1571 bypass grafts in 395 patients who underwent total arterial off-pump coronary revascularization without aortic manipulation for three-vessel disease since December 2000. The graft flow graded as A (antegrade), B (competitive), C (reverse), and O (no flow = occlusion). The current arrangement and design has been introduced since March 2003, and consists of the in-situ left internal thoracic artery (ITA) to the anterior descending artery and the composite I-graft of the right ITA and radial artery to the left circumflex (LCX) and right coronary artery (RCA) territories. Either clockwise or counterclockwise orientation, the I-graft was chosen to achieve a sufficient antegrade flow. Group I consisted of 181 patients with a single in-situ ITA as a composite Y-graft. Group II consisted of 214 patients with bilateral in-situ ITAs, which subdivided into Subgroup II-A consisted of 80 patients with bilateral in-situ ITAs until February 2003, and Subgroup II-B consisted of 134 patients with bilateral in-situ ITAs since March 2003. **Results:** The number of distal anastomoses was 3.52 ± 0.63 in Group I, and 4.36 ± 0.83 in Group II, respectively ($p < 0.0001$). The overall graft patency rate was 98.6% (1549/1571), and there was no significance different between the groups. The rate of grade A in Group II was 863/933 (92.5%) and was significantly higher ($p = 0.049$) than that of Group I 572/638 (89.7%). The rate of functioning bypass in Subgroup II-B was (95.8%) 568/593, and was significantly higher ($p = 0.03$) than that in Subgroup II-A (92.4%) 314/340. In Subgroup II-B, 233/268 (86.9%) of the conduits had completely grade A bypass flow, and this ratio was significantly higher ($p = 0.04$) than that in Subgroup II-A (79.4%) 127/160. **Conclusion:** Usage of bilateral ITAs and selecting the orientation of the I-graft to LCX and RCA branches provide maximal distal anastomotic sites with satisfactory graft patency rate, and simultaneously minimized the incidence of reverse and competitive flow.

Keywords: Off-pump; CABG; Arterial graft; Angiography

1. Introduction

Coronary artery revascularization was first performed on beating hearts [1]. Off-pump CABG combined with a no-aortic-touch technique has been accepted as an effective procedure to avoid the neurologic and aortic complications, and to reduce the operative risk. A composite graft using in-situ and free grafts is necessary for complete revascularization in patients with multi-vessels disease, and the arterial graft is commonly used because of its beneficial characteristics in terms of expectancy of both graft patency and improved late outcome [2].

In the arterial graft, circumstances of the blood flow in the graft lumen may be an important determinant for the durable patency. It has been reported that occlusion or string sign in the arterial graft is closely correlated with the insufficiency of the bypass flow, which represents competitive and reverse flow. It can occur either when the pressure capacity of the bypass graft is not enough; or the intraluminal pressure in the native coronary artery is relatively high due to the moderate stenosis of the native coronary artery. The previous study showed that reverse flow in the non-individual conduit had a significant correlation with the presence of moderately stenotic right coronary artery (RCA) and more than four target coronary branches for a single in-situ internal thoracic artery (ITA) [3]. In addition, the management of a coronary branch with critical stenosis and the strategy for the graft arrangement play essential roles for blood flow distribution [4].

The objectives of this study were (1) to compare the bypass flow in different bypass graft configurations for complete revascularization of the three-vessel territories

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1010-7940/$ – see front matter
doi:10.1016/j.ejcts.2006.11.025

using the flow grading system and (2) to evaluate the different strategy for graft arrangement and design.

2. Materials and methods

2.1. Study population

Between December 2000 and June 2005, 395 consecutive patients (male; 321, female; 74, mean age; 66.1 ± 9.1 years) underwent off-pump complete revascularization for three-vessel disease with arterial grafts (Table 1). Patients were excluded if they (i) had a bypass graft of the in-situ gastroepiploic artery or the saphenous vein, (ii) had individual grafts only, or (iii) did not undergo postoperative coronary angiography.

All patients underwent pre- and 2-week postoperative coronary angiographies, which were evaluated by the cardiologist for the native coronary artery stenosis and the graft patency, respectively.

The evaluation of graft patency is based on the concept of flow grading system (Table 2). A patent graft meant that the graft had a complete continuity of the graft lumen in the overall length from the subclavian artery to the target coronary branch, irrespective of the flow direction.

Grade O; (occlusion) was defined as the continuity of the graft lumen was interrupted at any level until the target coronary branch.

Grade A; means that antegrade graft flow was found in most of the multi-plane ITA angiography. Grade B; (competitive flow) defined as a situation in which the target vessel was slightly opacified from the ITA injection, and the bypass graft did fill by retrograde flow from the native coronary artery injection. Grade C; (reverse flow) the distal anastomotic sit was not opacified from the ITA graft injection at all, but it did fill clearly by retrograde flow from the native coronary artery injection. Flow grade was recorded for each target coronary branch. Any bypass graft graded as occluded or having reverse flow was considered not functioning because it did not contribute to coronary perfusion and relief of ischemia in the target region. A patent bypass without reverse flow was graded as functioning, and the rate of functioning grafts was defined as the proportion of functioning bypass grafts to the total number of bypass grafts. The definition of terms used in the present study is as follow. The in-situ ITA graft is an ITA, which was divided only at its distal portion. A composite graft is a bypass conduit consisting of one in-situ graft and a free graft anastomosed to it (in an end to end, end to side, or side to side fashion). An individual bypass was defined as a bypass conduit having one distal anastomoses and one in-situ graft. This included the straight composite grafts; i.e. I-graft, to one target coronary branch. A bypass conduit having two or more distal anastomosis, such as a sequential, Y-, or K-graft, was defined as non-individual.

Table 1
Baseline characteristics

	Group I (single ITA)	Group II (bilateral ITAs)	*p* value, Group S versus B
No. of patients	181	214	
Age (years)	70.3 ± 7.3	62.5 ± 9.0	<0.0001
Male/female	140/41	181/33	0.07
Hypertension	106 (59%)	108 (50%)	0.11
Hyperlipidemia	89 (49%)	117(55%)	0.28
Diabetes	76 (42%)	84 (39%)	0.58
End-diastolic volume index of LV (ml/m^2)	84.4 ± 23.8	89.4 ± 33.3	<0.0001
Ejection fraction of LV (%)	48.0 ± 10.8	46.0 ± 12.9	0.005
Total distal anastomoses	638	933	
Bypass conduits used			
Individual (target banch = 1)	0	142	
Non-individual (target branches>2)	181	286	
In situ ITA sequential	0	36	
Composite Y-graft	160	88	
Composite K-graft	21	15	
Composite I-graft	0	147	
Total	181	428	

ITA; internal thoracic artery LV; left ventricle.

Table 2
Concept of flow grading

	Flow grade			
	A	B	C	O
Flow direction	Antegrade	Competitive	Reverse	No-flow
Patency	Patent	Patent	Patent	Occluded
Function	Functioning	Functioning	Non-function	Non-function
Durability	Yes	No	No	No

Table 3
Baseline characteristics

	Subgroup II-A (~Feb. 2003)	Subgroup II-B (Mar. 2003~)	p value, Group B-1 versus B-2
No. of patients	80	134	
Age (years)	60.6 ± 8.2	63.6 ± 9.4	0.02
Male/Female	68/12	113/21	0.90
Hypertension	43 (54%)	65 (49%)	0.46
Hyperlipidemia	49 (61%)	68(51%)	0.14
Diabetes	32 (40%)	52 (39%)	0.86
End-diastolic volume index of LV (ml/m^2)	92.9 ± 35.1	87.7 ± 32.0	0.27
Ejection fraction of LV (%)	46.5 ± 13.9	45.6 ± 12.2	0.67
Total distal anastomoses	340	593	
Bypass conduits used	160 (100%)	268 (100%)	
Individual (target banch = 1)	44 (27.5%)	98 (36.6%)	0.053
In situ ITA	27 (16.9%)	93 (34.7%)	<0.0001
Composite I-graft	17 (10.6%)	5 (1.9%)	<0.0001
Non-individual (target branches >1)	116(72.5%)	170 (63.4%)	0.053
In situ ITA sequential	10 (6.3%)	26 (9.7%)	0.21
Composite Y-graft	49 (30.6%)	39 (14.6%)	<0.0001
Composite K-graft	13(8.1%)	2 (0.7%)	<0.0001
Composite I-graft	44 (27.5%)	103 (38.4%)	0.02
ITA-RA-LCX-RCA (clockwise)	0	67 (25.0%)	<0.0001
ITA-RA-RCA-LCX (counterclockwise)	44 (27.5%)	36 (13.4%)	0.0003

ITA; internal thoracic artery LV; left ventricle LCX; left circumflex artery RA; radial artery RCA; right coronary artery.

The design and arrangement of the bypass conduits were primarily determined by the operative risk and special relationship of the target sites (Tables 1 and 3). Group I consisted of 181 patients with single in situ ITA graft as Y- or K- graft. In Group II, 214 patients had bilateral in situ ITA in the combination of individual, Y-, K- or I-graft. Group II was divided into two subgroups by the date of surgery, because the current standard strategy has been introduced in March 2003, aiming at preventing high-risk situations of reverse and competitive flow [4]. Subgroup II-A consisted of 80 patients until February 2003, and Subgroup II-B consisted of 133 patients between March 2003 and June 2005 (Table 3). In the standard technique in Subgroup II-B, one in-situ ITA, usually the left, supplies to the left anterior descending artery (LAD) territory and an I-graft of the contra lateral ITA, usually the right, and the radial artery to the circumflex (LCX) and the right coronary artery (RCA) in a clockwise orientation, which meant a side to side anastomosis with the LCX branch and an end to side anastomosis to the RCA branch (Fig. 1). The counter-clockwise orientation was occasionally chosen to avoid grafting to the RCA branch with 75% stenosis at the end of the conduit, because reverse flow is commonly found at the distal end of the conduit [4]. In Subgroup II-A, the I-graft was used only in a counterclockwise orientation for the safety of redo operation in the future (Table 3). When the bypass conduits had grade A bypass flow to all target coronary branches, we considered that the design of the bypass conduit was successful.

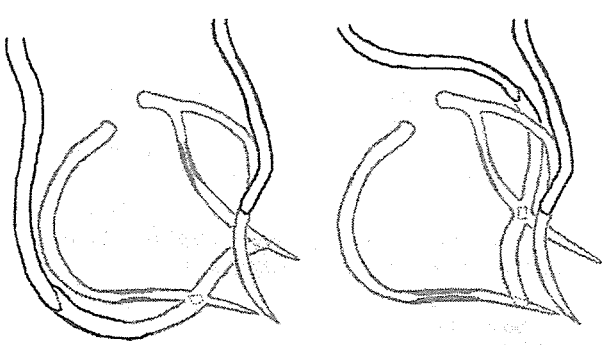

Fig. 1. A composite I-graft in the clockwise (right) and counterclockwise orientation (left).

Through a standard median sternotomy, the pericardial cavity was widely opened and deep pericardial sutures were placed for traction. Heparin was administered and activated coagulation time was maintained at more than 300 s until completion of anastomosis. In the present study, the ITA was harvested using either conventional (combined with vein and fascia), or semiskeletonized (partially combined with vein), or skeletonized technique [5]. All the distal portion of ITA grafts were larger than 1.5 mm in diameter assessed by insertion of 1.5 mm flexible probe. Allen's test was routinely performed before harvesting the radial artery and capillary refilling of the palm within 10 s was judged as negative [6]. Irrespectively to patient's age, the radial artery of non-dominant forearm was harvested by using an ultrasonic scalpel, treated with a papaverin hydrochloride solution [7], and was divided into two pieces when necessary. In the side-to-side anastomosis, a longitudinal arteriotomy of 6–10 mm was performed on both native coronary artery and arterial graft, and it was long enough for anastomosis without turbulence. The angle of the graft placement was adjusted to 0–90 degree to save the length and avoid kinking.

The distal anastomoses were carried out while stabilizing the coronary vessels using Octopus II+ or III stabilizer (Medtronic, Minneapolis, MN) and a retract-O-tape (Quest Medical, Inc., Allen, TX) was placed for temporary proximal occlusion. The surgical field was maintained by CO_2 blower and an intracoronary shunt; Anastsflo (Edwards Lifesciences, Irvine, CA) for coronary artery of 1.5 and 2.0 mm in diameter, Clearview (Medtronic, Minneapolis, MN) for coronary artery 1.25 and 1.0 mm in diameter, was used.

Continuous infusion of diltiazem was started during the operation and continued until oral medication was started, usually on the first postoperative day. It was terminated and replaced by nicardipine hydrochloride if sufficient heart rate could not be obtained. In the intensive care unit, heparin was administrated continuously for 24 h, and replaced by oral administration of aspirin.

2.2. Statistical analysis

The continuous variables are expressed as mean ± standard deviation, and compared by the unpaired Student's *t*-test between the two groups. The data of two independent groups were compared by Fisher's exact probability test. The differences in the outcomes were considered statistically significant at a probability value <0.05.

3. Results

The over graft patency rate was 98.6% (1549/1571). The number of distal anastomoses in Group II was 4.36 ± 0.83, and it was significantly greater than in Group I (3.52 ± 0.63) ($p < 0.0001$) (Table 2). In Group I, 572 (89.7%) bypass were grade A, whereas 20 (3.1%), 36 (5.6%), and 10 (1.6%) were grade B, C and O, respectively. The graft patency rate in Group II was 98.7% (921/933), and was comparable with that in Group I (98.4%) (628/638). However, the rate of grade A bypass flow in Group II was 92.5% (863/933), and was significantly higher than that of in Group I (89.7%) (572/638) ($p = 0.049$). In Group I, the number of bypass conduits of grade A bypass flow to all target coronary branches was 122/181 (67.4%), and was significantly less than that in Group II (360/428) (84.1%) ($p < 0.0001$) (Table 4).

In the comparison of Subgroup II-A to Subgroup II-B, there was no significant difference in the graft patency rate (Table 5). However, the functioning rate in Subgroup II-B was (95.8%) (568/593) and was significantly higher than that in Subgroup II-A (92.4%) (314/340) ($p = 0.03$). In Subgroup II-B, the number of bypass conduits with the grade A bypass flow to all target coronary branches was (233/268) (86.9%), and was significantly higher than that in Subgroup II-A (127/160) (79.4%) ($p = 0.04$) (Fig. 2).

The characteristics of the native coronary branches according to anatomical location and the grade of coronary stenosis, LAD and diagonal had 92.9 and 95.9% grade A from the total number of anastomoses, respectively. The grade A flow of the graft performed for 91–100% stenosis of native coronary branches was 98.8%, while in 76–90% coronary stenosis the grafts had grade A flow in 92.9% and when the grade of coronary stenosis is 51–75%, the graft grade A was 86.0% (Table 6).

Table 4
Angiographic results

	Group I (single ITA)	Group II (bilateral IT As)	*p* value
No. of patients	181	214	
Distal anastomoses	3.52 ± 0.63	4.36 ± 0.83	<0.0001
Flow grade			
A	572 (89.7%)	863 (92.5%)	
B	20(3.1%)	19(2.0%)	
C	36 (5.6%)	39 (4.2%)	
O	10(1.6%)	12(1.3%)	
Total	638	933	
Antegrade flow rate			
A	572 (89.7%)	863 (92.5%)	0.049
Functioning rate			
A + B	592(92.8%)	882 (94.5%)	0.16
Patency rate			
A + B + C	628 (98.4%)	921 (98.7%)	0.64
Bypass conduits	181	428	
Flow grade in the bypass conduits			
A only	122 (67.4%)	360(84.1%)	<0.0001
Non-A (+)	59 (32.6%)	68 (15.9%)	

ITA; internal thoracic artery LV; left ventricle non-A; grade B, C, or O.

Table 5
Angiographic results

	Subgroup II-A (~Feb. 2003)	Subgroup II-B (Mar. 2003~)	*p* value
No. of patients	80	134	
Distal anastomoses	4.25 ± 0.83	4.42 ± 0.83	0.07
Flow grade			
A	307 (90.3%)	556 (93.8%)	
B	7(2.1%)	12 (2.0%)	
C	19 (5.6%)	20 (3.4%)	
O	7(2.1%)	5 (0.8%)	
Total	340	593	
Antegrade flow rate			
A	307 (90.3%)	556 (93.8%)	0.05
Functioning rate			
A + B	314(92.4%)	568 (95.8%)	0.03
Patency rate			
A + B + C	333 (97.9%)	588 (99.2%)	0.11
Bypass conduits	160	268	
Flow grade in the bypass conduits			
A only	127 (79.4%)	233 (86.9%)	0.04
Non-A (+)	33 (20.6%)	35 (13.1%)	

IT A; internal thoracic artery LV; left ventricle non-A; grade B, C, or O.

4. Discussion

Avoidance of cardiopulmonary bypass and manipulation of the aorta can decrease the incidence of preoperative complications. In this aorta no-touch technique, the usage of the in-situ ITA graft is almost essential and it provides a favorable long-term survival with an excellent graft patency because it has a lower incidence of atherosclerotic graft disease than the saphenous vine graft [2,8]. In previous reports, the early and midterm graft patency rates of the radial artery were equivalent to those of the ITA [9–11]. The radial artery in the composite graft may provide a better durability than that proximally anastomosed to the ascending aorta because the exposure to an excessive pressure of the aorta and wall stress and the mismatch of the wall thickness can be avoided [11,12].

On planning the configuration and design of the arterial grafts in off-pump CABG without aortic manipulation, consciousness for the anticipated direction of bypass flow

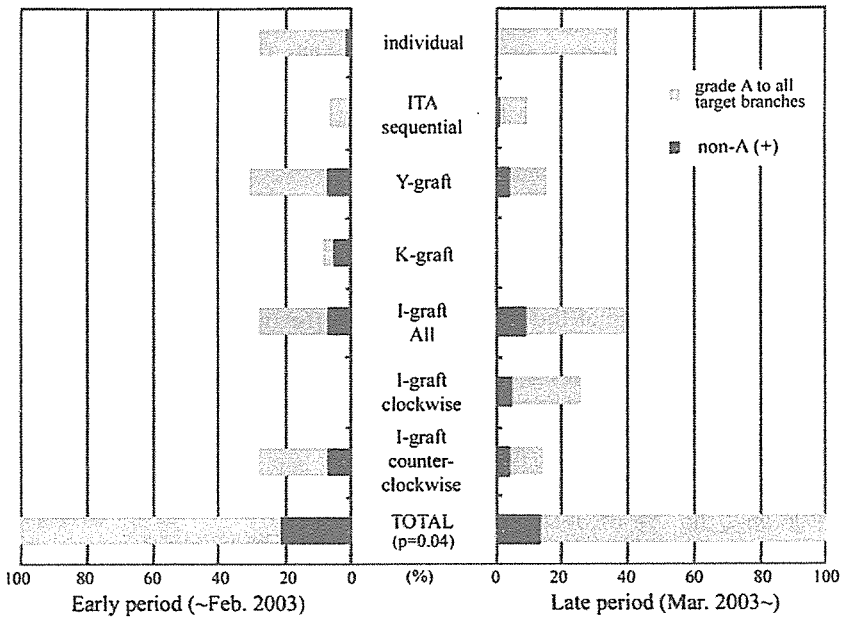

Fig. 2. Comparison of the proportion of bypass conduits used in the early and the late periods, and the rate of the conduits having only grade A, in various configurations.

may be mandatory. It was reported previously that reverse flow in the non-individual graft have significant correlations with the moderately stenotic RCA and the number of distal anastomotic sites of the composite graft [3]. Interaction of the coronary branches, which were connected to each other by a sequential or composite graft, and management of the coronary branches with moderate and severe stenoses play crucial roles in the occurrence of competitive and reveres flow. On the contrary, the graft material and size of the target branch does not correlate with the direction of bypass flow [4]. Additionaly, the patency rate of the graft with sufficient antegrade bypass flow was significantly higher than those of the graft presenting reverse and competitive flow, and the bypass grafts graded B or C were prone to close the graft lumen in the intermediate term [13,14]. It is generally believed that the long-term graft patency can be highly expected whenever arterial materials are exclusively used. However, the arterial graft without a sufficient antegrade flow has no obvious advantage regarding the long-term patency rate, as compared with the venous graft. A composite graft allows total arterial revascularization with an excellent graft patency rate and a lower incidence of cardiac events, especially for patient with atherosclerosis of the ascending aorta [15,16]. Although different arrangements and designs of the in-situ and free arterial grafts have already been reported [8,17], no optimal strategy for graft arrangement has been established yet. The decision of the configuration actually depends on the surgeon's preference or the custom of each group.

FitzGibbon et al. examined the venous conduits angiographically using the grading system of the luminal size at the narrowest portion, and the intimal irregularity as well. [18,19]. This grading system predicted the late atherosclerotic graft occlusion, which is considered as a major determinant of the long-term patency of the venous grafts. However, in the ITA graft, atherosclerosis hardly developed [20,21]. Additionally, the luminal size of the anastomotic site is not precisely measurable in the sequential fashion,

Table 6
Early angiographic results

Characteristics of coronary branches	Number of anastomoses	Grade			
		A (%)	B (%)	C (%)	O (%)
Location					
LAD main trunk	397	369 (92.9)	14 (3.5)	10 (2.5)	4 (1.0)
Diagonal	196	188 (95.9)	2 (1.0)	5 (2.6)	1 (0.5)
LCX	461	424 (92.0)	6 (1.3)	26 (5.6)	5(1.1)
RCA	517	454 (87.8)	17 (3.3)	34 (6.6)	12 (2.3)
Stenosis (%)					
51–75	727	625 (86.0)	31 (4.3)	61 (8.4)	10 (1.4)
76–90	410	381 (92.9)	8 (2.0)	14 (3.4)	7 (1.7)
91–100	434	429 (98.8)	0	0	5 (1.2)
Overall	1571	1435 (91.3)	39 (2.5)	75 (4.8)	22 (1.4)

LAD; left anterior discending artery, LCX; left circumflex artery, RCA; right coronary artery.

especially when the angle of the graft and coronary branch is near to 90 degrees, or when the contrast medium dose fills only incompletely due to mixture with the blood flow from the native coronary artery. Furthermore, although inadequate surgical maneuvers during the operation strongly affect the luminal size by unsuccessful anastomosis or graft kinking, regression of the stenosis and the increase or growth of the diameter were relatively common finding in the arterial grafts [22]. Thus, we consider that the angiographic luminal size or graft patency may be not relevant for pure comparison of graft arrangement and design of the arterial conduits.

In the present study, the configuration and design of the arterial graft were compared not only by classifying the anatomical patency or occlusion, but also by the dominant flow direction in the arterial composite and sequential grafts. Although there was no significant difference in the graft patency rate among the groups, the use of bilateral ITAs enabled more distal anastomosis with reduced competitive and reverse flow. We considered that the appropriate pressure slope in each segment of the bypass conduit should be higher at the proximal than that at the end of the conduit to achieve an antegrade flow. The anastomosis of the bypass conduit end with a moderately stenotic coronary branch is unfavorable in most cases. Thus, the composite I-graft is useful because the target coronary branch at the end of the conduit can be chosen by determining its orientation. In the composite Y-graft, the adequate pressure slope to the both ends should be made and the indication for Y or K graft should be more carefully decided. On the other hand, the Y-graft has an advantageous in terms of increased flow capacity [23] and availability to the distant target branches, as compared with the I-graft. For the diagonal, LCX, and RCA branches, the Y- or K-graft is preferred when all target branches have severe stenosis, target diagonal branch is located at the anteroapical portion, or remarkable cardiomegaly exists. Also, selection of suitable candidates for this procedure is a major concern.

Since the bypass grafts with reverse flow do not contribute to the coronary perfusion in the grafted territory, the efficacy of CABG may be unpromising, even when the bypass graft is anatomically patent. When non-functioning or occluded graft is highly predicted at early period, an alternative therapeutic strategy should be considered such as hybrid therapy with drug eluting stent implantation for conservation of the arterial grafts for the future redo operation.

In previous study, sequential anastomoses with more than two moderately stenotic coronary branches including one at the end of the conduit were highly associated with flow insufficiency and late occlusion. Although the gastroepiploic artery is an option of choice for the RCA and LCX territories, it is unsuitable for moderately stenotic coronary branch because its pressure potential is inferior to that of the in-situ ITA [24,25].

This study has some limitations as it is not randomized. Peripheral vascular resistance in the myocardial tissue also has an important role in the coronary perfusion. Grade B flow probably includes insufficient graft flows due to both strength of the native coronary flow and poor vascularity with high resistance in the severely impaired myocardium. Although no bypass graft may be required for the latter, we could not predict the insufficient antegrade flow caused by the critically damage vasculature.

5. Conclusion

The use of bilateral ITAs significantly increased the bypass grafts with sufficient antegrade flow, and the I-graft to LCX and RCA branches with avoidance of anastomosis with moderately stenotic coronary branch at the end of the graft was effective for reduction of reverse and competitive flow by selecting its orientation. Flow consciousness in the graft arrangement and design may be a major concern to confirm the advantage of the arterial material and complete revascularization when the arterial grafts are exclusively used.

Acknowledgement

We thank Dr Yoshiko Kada for her assistance with the statistical analysis.

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Does an Overlap Syndrome Really Exist Between Brugada Syndrome and Progressive Cardiac Conduction Defect (Lenegre Syndrome)?

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Editorial Comment

The Brugada syndrome is characterized by coved-type ST-segment elevation in the right precordial electrocardiographic leads (V1–V3) (a so-called Type 1 ECG) and a high incidence of sudden death due to ventricular fibrillation (VF) in patients without structural heart disease.[1,2] The prevalence of Brugada syndrome is estimated to be 5 per 10,000 inhabitants in Thailand.[3] Type 1 Brugada ECG without symptoms, i.e., asymptomatic Brugada syndrome, is commoner with a prevalence of 12 per 10,000 inhabitants in Japan.[4,5] More than 80–90% of patients affected by Brugada syndrome are males,[4,6] although males and females are expected to inherit the defective gene equally.

Experimental studies employing arterially perfused canine right ventricular wedge preparations, which have been mainly conducted by Antzelevitch and his group, have elucidated the cellular and molecular basis for typical ST-segment elevation and subsequent VF.[7] An accentuated transient outward current (I_{to})-mediated action potential (AP) notch and subsequent loss of the AP dome in the epicardial cells, but not in the endocardial cells, of the right ventricle gives rise to a transmural voltage gradient, producing coved-type ST-segment elevation in the ECG. Heterogeneous loss of the AP dome in the restricted epicardial area creates a marked epicardial dispersion of repolarization, giving rise to premature beats caused by phase 2 reentry which can precipitate nonsustained polymorphic ventricular tachycardia (VT) or VF.[8,9]

Evidence of conduction abnormality has accumulated in patients with Brugada syndrome. The original report by Brugada and Brugada published in 1992 included right bundle branch block (RBBB) pattern as one of the ECG characteristics of this syndrome.[1] Although RBBB is now believed not to be necessary for definitive diagnosis, Brugada patients have a higher incidence of complete or incomplete RBBB than the normal population. Widening of P wave and QRS duration, and prolongation of the PQ interval and HV interval, all of which represent conduction abnormality, are often observed in patients with Brugada syndrome.[10] Smits and coworkers reported greater prolongation of PQ interval in Brugada patients with *SCN5A* mutations than in those without *SCN5A* mutations.[11] Approximately 60–70% of patients with Brugada syndrome show late potentials (LPs) detected by a signal-averaged electrocardiogram.[12,13]

Several phenotypes other than Brugada syndrome have been reported to result from *SCN5A* mutations such as the LQT3 form of the congenital long QT syndrome (LQTS), cardiac conduction defect (Lenegre syndrome), atrial standstill, and atrioventricular block. Interestingly, patients with a specific *SCN5A* mutation share multiple phenotypes, thus creating a category of overlapping phenotype. Bezzina and coworkers have reported a large family affected with a specific insertion mutation, 1795insD, in which family members showed both LQT3 and the Brugada phenotype.[14] Priori et al. demonstrated that flecainide, a class IC sodium channel blocker, unmasked Brugada phenotype in 6 of 13 patients with the LQT3 syndrome.[15] Kyndt et al. described a large French family with a specific *SCN5A* missense mutation, G1406R, in which phenotypes of both the Brugada syndrome and the Lenegre syndrome were observed.[16]

Probst et al. suggest a more common association of conduction abnormalities in the *SCN5A*-related Brugada patients in this issue of *Journal of Cardiovascular Electrophysiology*.[17] They have identified intraventricular conduction defects in 59 (76%) of 78 *SCN5A* mutation carriers recruited from 16 Brugada families, while baseline spontaneous ST-segment elevation was seen only in 28 (36%) mutation carriers. They suggest that *SCN5A* Brugada syndrome-type mutation carriers exhibit various degrees of progressive cardiac conduction defects similar to the Lenegre syndrome, and therefore need clinical and ECG follow-up. These data raise the question as to whether an overlap syndrome really exists between the Brugada syndrome and progressive cardiac conduction defect (Lenegre syndrome), especially in *SCN5A* mutation carriers.

However, the relationship of the cardiac conduction defects detected in the majority of Brugada patients to the pathophysiological mechanism of VF and risk stratification in patients with Brugada syndrome seems to be clinically more important. Even though this syndrome is a monogenic inherited disorder, the Brugada syndrome typically manifests during adulthood, with a mean age of sudden death of 41 ± 15 years.[4] Cardiac conduction defects in Brugada patients, which gradually progress with age, may contribute to the pathogenesis of VF and to the late onset of first cardiac events in patients with Brugada syndrome. In other words, the mechanism of coved-type ST-segment elevation and the

J Cardiovasc Electrophysiol, Vol. 17, pp. 276-278, March 2006.

Dr. W Shimizu was supported by the Hoansha Research Foundation, Japan Research Foundation for Clinical Pharmacology, Ministry of Education, Culture, Sports, Science and Technology Leading Project for Biosimulation, and health sciences research grants (H17 – Research on Human Genome, Tissue Engineering – 009) from the Ministry of Health, Labour and Welfare, Japan.

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doi: 10.1111/j.1540-8167.2006.00406.x

first ventricular premature beat initiating VF can be explained by the accentuated I_{to}-mediated AP notch and heterogeneous loss of the AP dome in the epicardial cells as well as subsequent phase 2 reentry between the epicardial cells.[7] However, some degree of ventricular conduction abnormalities may be required to perpetuate polymorphic VT or to maintain VF.

Aiba and coworkers recently conducted a high-resolution optical mapping in an experimental Brugada model employing a canine right ventricular wedge preparation, which allowed a detailed measurement of cellular repolarization and depolarization in the epicardial and endocardial surfaces.[9] Their data suggested that the initiating phase 2 reentry-induced ventricular premature beats originated from the epicardial area with a steep gradient of ventricular repolarization time due to heterogeneous loss of the AP dome. In contrast, wave break appeared at sites of delayed epicardial conduction during the first few reentrant waves, which was closely associated with VF susceptibility, suggesting that conduction abnormalities contribute to the maintenance of VF in the Brugada condition. Kanda et al. reported that the inducibility of VF by ventricular programmed electrical stimulation was related to the severity of conduction abnormalities, such as longer QRS or HV intervals, and a higher incidence of RBBB or LPs in patients with symptomatic Brugada syndrome,[18] also indicating the role of conduction abnormalities in the maintenance of VF. Because most cardiac events occur during sleep in patients with Brugada syndrome, sustained VF or nonsustained polymorphic VT lasting more than 10 or 20 seconds is required to produce symptoms, i.e., sudden cardiac death, syncope, or nocturnal agonal respiration. The usefulness of programmed electrical stimulation to stratify risk of subsequent cardiac events is still controversial in patients with symptomatic or asymptomatic Brugada syndrome.[18-21] Brugada et al. suggested that inducibility of VT/VF is a strong indicator of subsequent cardiac events in both symptomatic and asymptomatic patients.[19] However, Priori et al.,[20] Kanda et al.,[18] and Eckardt et al.[21] failed to find an association between VF inducibility and new cardiac events or recurrence of VT/VF. If the progressive cardiac conduction defects often observed in patients with Brugada syndrome are really linked to VF maintenance, progressive conduction parameters such as QRS widening, LPs, or inducibility of VF by programmed electrical stimulation may still have a potential to predict new or subsequent cardiac events. A much larger patient population with similar patient characteristics and stimulation protocol, and a longer follow-up period are required to make a definitive conclusion regarding the predicting value of conduction parameters for new or further cardiac events.

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Common Sodium Channel Promoter Haplotype in Asian Subjects Underlies Variability in Cardiac Conduction

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Background—Reduced cardiac sodium current slows conduction and renders the heart susceptible to ventricular fibrillation. Loss of function mutations in *SCN5A*, encoding the cardiac sodium channel, are one cause of the Brugada syndrome, associated with slow conduction and a high incidence of ventricular fibrillation, especially in Asians. In this study, we tested the hypothesis that an *SCN5A* promoter polymorphism common in Asians modulates variability in cardiac conduction.

Methods and Results—Resequencing 2.8 kb of *SCN5A* promoter identified a haplotype variant consisting of 6 polymorphisms in near-complete linkage disequilibrium that occurred at an allele frequency of 22% in Asian subjects and was absent in whites and blacks. Reporter activity of this variant haplotype, designated HapB, in cardiomyocytes was reduced 62% compared with wild-type haplotype ($P=0.006$). The relationship between *SCN5A* promoter haplotype and PR and QRS durations, indexes of conduction velocity, was then analyzed in a cohort of 71 Japanese Brugada syndrome subjects without *SCN5A* mutations and in 102 Japanese control subjects. In both groups, PR and QRS durations were significantly longer in HapB individuals ($P\leq0.002$) with a gene-dose effect. In addition, up to 28% and 48% of variability in PR and QRS durations, respectively, were attributable to this haplotype. The extent of QRS widening during challenge with sodium channel blockers, known to be arrhythmogenic in Brugada syndrome and other settings, was also genotype dependent ($P=0.002$).

Conclusions—These data demonstrate that genetically determined variable sodium channel transcription occurs in the human heart and is associated with variable conduction velocity, an important contributor to arrhythmia susceptibility. (***Circulation***. **2006;113:338-344.**)

Key Words: arrhythmia ■ conduction ■ death, sudden ■ genetics ■ ion channels

Sudden cardiac death (SCD) accounts for 20% of all mortality in Western countries.[1] One key determinant of normal excitation and conduction of the cardiac impulse is the cardiac sodium channel, responsible for rapid depolarization in most cardiomyocytes. Reduced sodium current predisposes to SCD. For example, although sodium channel blockers have been used for antiarrhythmic therapy, the Cardiac Arrhythmia Suppression Trial (CAST) showed that these agents increase the incidence of SCD.[2] Loss of function mutations in *SCN5A*, the cardiac sodium channel gene, causes ≈20% of cases of the Brugada syndrome, which is associated with a high risk of SCD.[3] Furthermore, there is evidence that such sodium channel mutations also may lead to enhanced fibrosis in myocardial tissue.[4,5]

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The overall hypothesis underlying the work presented here is that variability in regulation of sodium channel expression contributes to interindividual variability in cardiac conduction and consequently can be considered a candidate modulator of arrhythmia susceptibility, especially in the presence of other stressors such as drugs or acute myocardial ischemia.[6] As a first step in testing this hypothesis, we cloned and characterized the proximal promoter region of *SCN5A* and identified multiple cis-acting elements regulating gene expression.[7] We report here identification of an ethnic-specific, common *SCN5A* promoter variant that modulates PR and QRS durations, indexes of cardiac conduction.

Received August 3, 2005; revision received September 15, 2005; accepted October 11, 2005.

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Guest Editor for this article was Douglas P. Zipes, MD.

The online-only Data Supplement can be found at http://circ.ahajournals.org/cgi/content/full/CIRCULATIONAHA.105.580811/DC1.

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Circulation **is available at http://www.circulationaha.org** **DOI: 10.1161/CIRCULATIONAHA.105.580811**

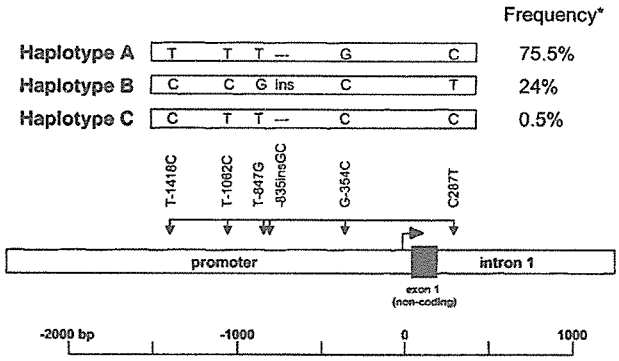

Figure 1. Haplotypes identified in the cardiac sodium channel gene (*SCN5A*) promoter. Nucleotide variations are indicated by their position relative to the major transcription initiation site (+1),[7] with the most frequent nucleotide given below and the least frequent nucleotide given above the position. *Frequency in the Japanese (control) population.

Methods

Identification of Polymorphisms

Resequencing 2.8 kb of the *SCN5A* promoter region in a single individual of Asian origin identified him as a homozygote for 6 DNA polymorphisms in the region: T-1418C, T-1062C, T-847G, −835insGC, G-354C, and C287T (Figure 1). The resequenced region encompassed positions −2190 to 613, relative to the major transcription initiation site[7] of the *SCN5A* promoter, including 2.2 kb upstream of exon 1, exon 1 (which is 173 bp and noncoding), and the proximal 439 bp of intron 1. The fragment was amplified by long and accurate polymerase chain reaction (PCR; TaKaRa kit) with primers F1 and R1 (Data Supplement Table I; see http://circ.ahajournals.org/cgi/content/full/CIRCULATIONAHA.105.580811/DC1). Further studies described below established that these polymorphisms were common and in near-total linkage disequilibrium, thereby identifying 2 common haplotype blocks, designated HapA and HapB. We also detected a third combination of polymorphisms, designated HapC, in <1% of subjects. In addition to the study populations, 150 white and 100 black individuals were tested for these haplotypes.

Functional Analysis

Generation of Constructs

The 2.8-kb fragment described above was amplified from genomic DNA of HapA- and HapB-homozygous individuals. These fragments were cloned into the pGEM-T Easy vector (Promega), and inserts were subsequently subcloned into the pGL3-basic vector (Promega), which contains the firefly luciferase coding sequence, to generate *SCN5A* promoter–luciferase fusion constructs for reporter assays. These constructs were designated pGL3-Hap A and pGL3-Hap B.

Reporter Activity

Reporter activity was assayed in neonatal mouse cardiomyocytes and in Chinese hamster ovary cells as described in detail previously.[7] In brief, 1 μg pGL3-Hap A or pGL3-Hap B was transfected into neonatal mouse cardiomyocytes or Chinese hamster ovary cells. In each experiment, 0.05 μg pRL-TK plasmid (Promega) encoding Renilla luciferase was cotransfected to normalize for experimental variability caused by differences in cell viability or transfection efficiency. Luminescence was measured 48 hours after transfection with the Dual-Luciferase Reporter Assay System (Promega). The pGL3-basic (promoterless) plasmid was tested in each experiment; its activity level served as the baseline.

Study Participants

Participants in the clinical study were ascertained at the National Cardiovascular Center (Osaka, Japan). All protocols (including molecular screening) were reviewed and approved by the Ethical Review Committee of the National Cardiovascular Center, and informed consent was obtained from all individuals.

The control population consisted of 102 subjects drawn from mutation-negative relatives in congenital long-QT syndrome families in which the causative mutation had been identified. Only 1 person was drawn from each family. There were 67 male and 35 female subjects ranging from 9 to 69 years of age; mean age was 40±14 years (mean±SD).

The Brugada syndrome population included 80 patients diagnosed with Brugada syndrome, defined as type 1 "coved" ST-segment elevation in V_1 through V_3 (spontaneous in 70 patients, induced by sodium channel blocker in 10 patients).[8] In all patients, physical examination, chest roentgenogram, laboratory values, echocardiography with wall motion analysis, and Doppler screening excluded structural heart disease. Aborted cardiac arrest or ventricular fibrillation (VF) was documented in 30 patients, syncope was identified in 20, and 30 were asymptomatic. All patients had previously been screened for *SCN5A* coding region mutations, and a mutation had been identified in 9 patients. The patient group included 76 male and 4 female subjects ranging from 1 to 76 years of age (mean±SD, 47±16 years).

ECG Phenotypes

ECGs were assessed by an investigator (W.S.) who was blinded to age, gender, and genetic and clinical information. Phenotypes assessed included RR interval, PR interval measured in lead II (PR_{II}), QRS interval measured in leads V_1 (QRS_{V1}) and V_6 (QRS_{V6}), ST amplitude at J point (ST_J), and ST amplitude at 80 ms after the end of the QRS (ST_{80}).

The effects of intravenous administration of sodium channel blockers on these ECG parameters were examined in 49 of 80 Brugada syndrome patients. Pilsicainide (maximum 1 mg/kg at a rate of 0.1 mg · kg^{-1} · min^{-1}) was used in 37 patients, flecainide (maximum 2 mg/kg at a rate of 0.2 mg · kg^{-1} · min^{-1}) was used in 9 patients, and disopyramide (maximum 2 mg/kg at a rate of 0.2 mg · kg^{-1} · min^{-1}) was used in 3 patients.

Genotyping

Genomic DNA was prepared from blood leukocytes. Genotyping for the T-1418C and T-1062C single nucleotide polymorphisms (SNPs) was performed by restriction fragment length polymorphism analysis after PCR amplification with *Ear*I and *Hae*III, respectively. PCR primers used to amplify the 161-bp fragment encompassing the T-1418C SNP were F2 and R2, and those used to amplify the 123-bp fragment encompassing the T-1062C SNP were F3 and R3 (Data Supplement Table II). Genotyping for the other 4 polymorphisms (T-847G, 835insGC, G-354C, and C287T) was done by DNA resequencing of both strands. PCR primers used to amplify the 638-bp fragment encompassing the T-847G, 835insGC, and G-354C polymorphisms were F4 and R4; those used to amplify the 599-bp fragment encompassing the C287T polymorphism were F5 and R5.

Statistical Analysis

Using the individual genotypes for the 6 polymorphisms, we estimated haplotype frequencies using an E-M algorithm.[9] The haplotype frequencies were used to calculate the probabilities of the haplotype pairs compatible with the genotype combinations of the multiple heterozygous patients using Bayes' theorem. Observed haplotype pair frequencies were compared with those expected under Hardy-Weinberg equilibrium in the Brugada syndrome population and control population separately with a χ^2 test. To compare haplotype pair frequencies among Brugada syndrome patients and control subjects, Fisher's exact test was used.

All quantitative phenotypes were normally distributed, and data are expressed as mean±SD. Continuous ECG phenotypes were compared between *SCN5A* mutation–negative Brugada syndrome patients, *SCN5A* mutation–positive Brugada syndrome patients, and control subjects by ANOVA adjusted for age and gender, followed by a post hoc test for pairwise comparisons. Student *t* tests were used

to compare the after-drug-challenge continuous ECG phenotypes between *SCN5A* mutation–negative and –positive Brugada syndrome patients. Correlations between quantitative phenotypes before and after sodium channel blockade are expressed as Pearson correlation coefficients (r). For comparison of the proportion of male subjects, Fisher's exact test was used.

The effect of haplotype pairs on the continuous ECG phenotypes was tested in the Brugada syndrome patients and control subjects separately by ANOVA with adjustment for age and gender. The 9 *SCN5A* mutation–positive Brugada syndrome patients were treated as a separate category (7 HapA/HapA homozygotes, 2 HapA/HapB heterozygotes, pooled). The 2 individuals with the rare HapC variant (1 patient from each group) were excluded from analyses. In all analyses, the proportion of variance attributable to the haplotype pair (R^2) was calculated and corrected for the effects of age and gender.

Differences in reporter gene expression activity between HapA and HapB were examined for statistical significance with Student's t test. Throughout, values of $P<0.05$ were interpreted as being significant. All statistical analyses were done with SAS software (version 9, SAS Institute).

Multiple Testing

When a Bonferroni correction for the 24 statistical models is used to compare the continuous ECG phenotypes, the significance level for the overall probability values is 0.002. Similarly, the Bonferroni-corrected significance levels for the pairwise comparisons between 3 and 4 groups is 0.017 and 0.008, respectively.

Results

Haplotypes

The 6 polymorphisms were in near-complete linkage disequilibrium, with only 2 (similar) discordant haplotypes (of 364; <1%), each occurring in 1 subject from each population. We designated HapA as containing all common alleles and HapB as containing all minor alleles (Figure 1). The discordant haplotype was designated HapC. The estimated frequencies of HapA, HapB, and HapC were 0.755, 0.240, and 0.005 in the control subjects and 0.782, 0.211, and 0.007 in the *SCN5A* mutation–negative Brugada syndrome patients, respectively. Haplotype distributions were in Hardy-Weinberg equilibrium ($P>0.05$) in both populations. No significant difference in haplotype frequencies was observed between the Brugada syndrome group and the control subjects. The haplotypes were absent in white and black samples.

Functional Analysis

In cardiomyocytes, reporter activity of HapB was markedly reduced, by 62%, compared with HapA: 5.5±0.4 (mean±SE) versus 14.5±2.8 (normalized activity units; n=9 each; $P=0.006$; Figure 2). A similar trend was seen in the noncardiac cells: 2.7±0.3 versus 3.6±0.3 (n=13 each; $P=0.04$; Figure 2).

Phenotypic Characteristics of the Control and Brugada Syndrome Patient Populations

The decreased reporter activity for HapB suggested that individuals carrying this promoter haplotype would display ECG-detectable conduction slowing. Accordingly, the relationships between genotype and ECG intervals were evaluated in the control and Brugada syndrome populations.

ECG data are shown in Table 1. As expected, Brugada syndrome patients had significantly longer conduction intervals (PR_{II}, QRS_{V1}, QRS_{V6}) and greater ST-segment elevation

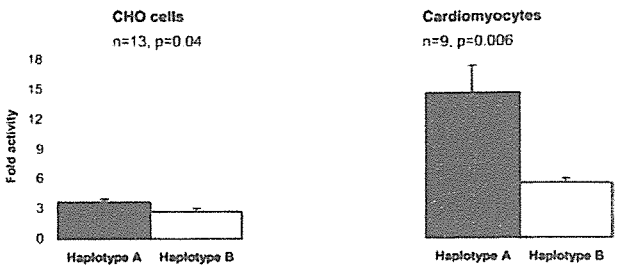

Figure 2. Reporter activity of *SCN5A* promoter haplotypes A and B. Firefly luciferase expression levels, which report the activities of the inserted *SCN5A* sequence, were divided by coexpressed Renilla luciferase activities and expressed as relative luciferase units.[7] Data are presented as mean±SE (vs empty vector). CHO indicates Chinese hamster ovary.

(ST_J, ST_{80}) compared with control subjects. Heart rate was not significantly different between the 2 populations. In addition, we found differences between *SCN5A* mutation–positive and *SCN5A* mutation–negative Brugada syndrome patients similar to those previously reported[10]: Mutation-positive subjects had significantly longer baseline PR and QRS intervals and longer RR intervals. Data on the subset of Brugada syndrome patients who underwent drug challenge are presented in Table 2. For all ECG parameters investigated, highly significant ($P<0.0001$) correlations were present between measures before and after drug challenge (Table 2). As previously reported, *SCN5A* mutation–positive patients displayed longer PR and QRS intervals after challenge with sodium channel blockers compared with *SCN5A* mutation–negative patients.[10]

Haplotype Pair Effects

PR and QRS durations were significantly longer in HapB individuals in both study populations (Brugada syndrome and control subjects: $P\leq0.002$ for PR_{II}; $P<0.0001$ for QRS_{V1} and QRS_{V6}; Figure 3). In the control population, PR_{II}, QRS_{V1}, and QRS_{V6} intervals showed a gene-dose effect, being longest in HapB homozygotes, intermediate in HapA/HapB heterozygotes, and shortest in HapA homozygotes. A similar pattern was observed in the *SCN5A* mutation–negative Brugada syndrome patient group. As discussed earlier, these analyses excluded data in the 2 individuals with HapC. PR_{II}, QRS_{V1}, and QRS_{V6} means (±SD) per haplotype group for the 2 populations are listed in the Data Supplement Table II. Both the overall and pairwise probability values were highly statistically significant even after correction for multiple testing.

The amount of variance (R^2) in PR and QRS intervals explained by the haplotype pair after correction for age and gender is shown in Table 3. As can be seen, a significant proportion of variance in PR and QRS intervals, both at baseline (both groups) and after drug challenge (Brugada syndrome group), was attributable to the haplotype. No significant association was found between haplotype and RR, ST_J, and ST_{80} in either population (data not shown).

Drug Challenge and Haplotype

The haplotype pairs were also highly associated with conduction intervals (PR_{II}, QRS_{V1}, QRS_{V6}) after sodium channel

TABLE 1. Baseline ECG Characteristics of the Control and Brugada Syndrome Patient Populations

		Brugada Syndrome Patients			Pairwise Comparison *P*	
	Control Subjects	*SCN5A*$^{-ve}$	*SCN5A*$^{+ve}$	Overall *P*	*SCN5A*$^{-ve}$ vs *SCN5A*$^{+ve}$	*SCN5A*$^{-ve}$ vs Control Subjects
n	102	71	9			
Male, n (%)	67 (66)	67 (94)	9 (100)	<0.0001	1.000	<0.0001
Age, y	40.0±14.2	46.5±16.3	51.1±8.4	0.005	0.376	0.005
RR, ms	925.3±130.0	913.7±134.3	1055.6±154.2	0.012	0.003*	0.572
PR_{II}, ms	162.3±21.8	180.4±20.4	238.9±26.7	<0.0001*	<0.0001*	<0.0001*
QRS_{V1}, ms	93.8±11.8	104.9±19.3	142.2±19.1	<0.0001*	<0.0001*	<0.0001*
QRS_{V6}, ms	87.4±12.4	100.2±19.1	139.4±21.6	<0.0001*	<0.0001*	<0.0001*
ST_J, mV	0.10±0.05	0.30±0.14	0.34±0.18	<0.0001*	0.249	<0.0001*
ST_{80}, mV	0.18±0.10	0.25±0.12	0.24±0.13	0.001*	0.778	0.001*

Values are given as mean±SD.
*Below the Bonferroni-corrected overall or pairwise significance levels (see Multiple Testing).

blockade in 44 *SCN5A* mutation–negative Brugada syndrome patients who underwent drug challenge (for PR_{II}, QRS_{V1}, QRS_{V6}, $P<0.0001$; Figure 3). PR_{II}, QRS_{V1}, and QRS_{V6} means (±SD) per haplotype group are listed in the Data Supplement Table II. Here also, overall and pairwise probability values were highly statistically significant even after correction for multiple testing.

In addition, the extent of QRS widening (ΔQRS) after drug challenge was genotype dependent, and a gene-dose effect was also observed (ΔQRS_{V6}: HapB/HapB=30 ms [mean± SD]; HapA/HapB=24.2±7.9; HapA/HapA=17.8±7.2; $P=0.002$; Figure 4). A similar trend was seen for extent of PR widening (ΔPR) after drug challenge (ΔPR_{II}: HapB/HapB=40 ms; HapA/HapB=33.8±13.2; HapA/HapA=28.6±8.3; $P=0.05$).

Discussion

We demonstrate that a set of 6 *SCN5A* promoter polymorphisms found in Asian subjects are in near-complete linkage disequilibrium, have a significant impact on sodium channel expression in vitro, account for a large proportion of variance in ECG conduction parameters in 2 independent Japanese populations, and represent pharmacogenetic markers predicting variable drug response.

Twin studies have identified strong genetic effects for ECG parameters, including PR and QRS durations.[11–14] Indeed, associations have been reported between ECG parameters and single coding region nonsynonymous (amino acid–changing) SNPs in ion channel genes.[15,16] However, common functional variants in regulatory regions that strongly modulate basal ECG intervals have not previously been identified; 1 preliminary report has suggested an association between a potassium channel promoter polymorphism and QRS axis in women only.[17] Only recently has the concept of tightly linked polymorphisms (constituting a haplotype block) been applied to understanding variability in cardiac electrophysiology. In 1 study, a small degree of variance (<1%) in QT interval in a central European population could be attributed to single SNPs and haplotype blocks in 4 potassium channel genes.[18]

TABLE 2. Clinical Characteristics of the Brugada Syndrome Patients After Sodium Channel Blocker Challenge

	SCN5A^{-ve}	*SCN5A*$^{+ve}$	*P*	*r*, Before and After Sodium Channel Blockade
n	44	5		
Male, n (%)	42 (95)	5 (100)	1.000	
Age, y	46.3±14.8	52.0±5.4	0.397	
aRR, ms	892.3±113.1	956.0±99.4	0.234	0.94
aPR_{II}, ms	209.6±25.1	278.0±35.6	<0.0001*	0.95
$aQRS_{V1}$, ms	124.1±16.1	166.0±17.8	<0.0001*	0.92
$aQRS_{V6}$, ms	119.2±17.1	166.0±17.8	<0.0001*	0.92
aST_J, mV	0.51±0.21	0.78±0.25	0.013	0.84
aST_{80}, mV	0.41±0.17	0.70±0.31	0.109	0.63

Values are given as mean±SD. Pearson correlation coefficients (*r*) observed between measures before and after sodium channel blocker challenge ($P<0.0001$). Mean baseline ECG parameters for the 44 *SCN5A*$^{-ve}$ and 5 *SCN5A*$^{+ve}$ patients (not shown) were very similar to those for the total patient group given in Table 1.
*Below the Bonferroni-corrected overall significance levels (see Multiple Testing).

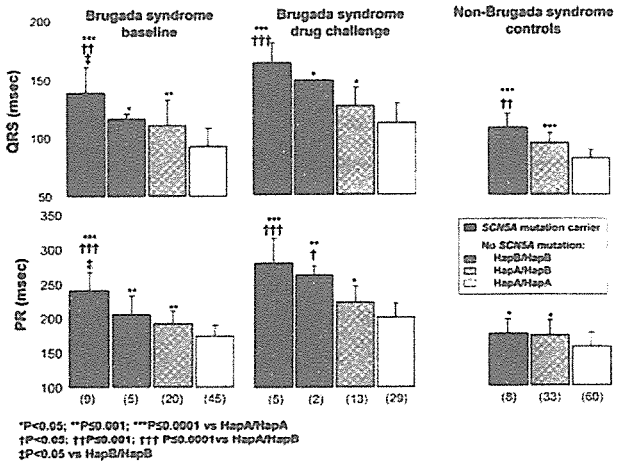

Figure 3. *SCN5A* promoter haplotype effects on durations of QRS_{V6} and PR_{II} in Brugada syndrome patients at baseline and after challenge with sodium channel blocking agents and in non–Brugada syndrome control subjects. Patient numbers are indicated in parentheses. Genotype effects on QRS_{V1} were similar to those on QRS_{V6} because of a high correlation between these 2 parameters (Pearson's coefficient, r=0.96). Data are presented as mean±SD. For Bonferroni-corrected significance levels for pairwise comparisons, refer to the Multiple Testing section in Patients and Methods.

In contrast, the *SCN5A* promoter haplotype we report here explained a remarkable proportion of variance in conduction parameters in the Japanese subjects studied (Table 3). Such associations could arise because the haplotypes studied are, in turn, in linkage disequilibrium with other functionally important variants in regulatory or other regions of the gene. However, in this case, the in vitro functional studies indicate that the effect is attributable to a variant within the haplotype block; at this point, the specific variant mediating this effect has not been identified.

A principal determinant of cardiac conduction in atrial and ventricular muscle is the sodium current; sodium channel blockers prolong PR and QRS durations, an effect also seen with loss of function mutations in *SCN5A*.[3] Critical degrees of conduction slowing represent a final common pathway to VF,[19] so dissection of the genetic determinants of cardiac conduction in the general population is a key step to understanding variable susceptibility to common arrhythmias resulting from conduction slowing, as in myocardial ischemia

TABLE 3. Variance Explained by the Haplotype Pair

	R^2, %		
	Control Subjects	Brugada Syndrome Baseline	Brugada Syndrome Drug Challenge
PR_{II}	12.2	28.4	33.0
QRS_{V1}	47.6	26.4	33.0
QRS_{V6}	48.5	24.9	36.2

or heart failure.[19] Thus, the data we present here implicate the *SCN5A* promoter variant HapB, which slowed conduction in normal subjects and exacerbated conduction slowing in those with Brugada syndrome, as a candidate modulator of variability in risk of SCD. Importantly, imposition of further depression of sodium channel function by administration of sodium channel blocking drugs further exacerbated conduction slowing in a gene-dose-dependent fashion. Studies in large numbers of subjects at risk for SCD are required to further establish the role of this and other regulatory region polymorphisms in modulating that risk.

Differences in disease penetrance and expression have been widely reported in the cardiac sodium and other channelopathies.[20–23] Relatives carrying an *SCN5A* mutation identical to that of the proband may be clinically unaffected,[20] and family members may display different phenotypes, eg, Brugada syndrome or conduction disease.[23] Genetic variants like the one presented here are obvious candidate modulators of this variability in phenotypic expression. Interindividual variability also has been noted in response to pharmacological challenge with sodium channel blockers in Brugada syndrome patients.[20,24] In some patients, even some carrying an *SCN5A* mutation, drug challenge fails to unmask a Brugada syndrome ECG. The significantly greater increases in PR and QRS durations with sodium channel blockade in HapB carriers thus identify variability in expression of the drug target, the sodium channel, as a key mediator of this variable drug effect. It is thus possible that other sodium channel blocker response phenotypes such as the increased mortality with sodium channel blockers in the CAST[2] was determined by variable sodium channel expression. DNA samples from that important clinical trial were not archived, so this question will remain unanswered. More generally, the data

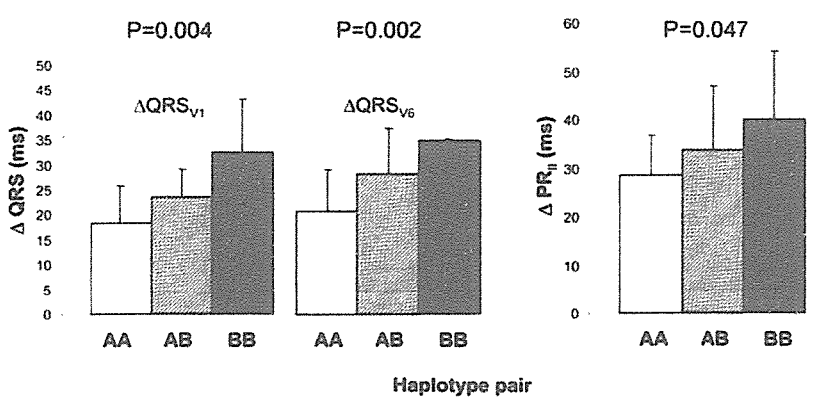

Figure 4. *SCN5A* promoter haplotype effects on extent of QRS (ΔQRS_{V1} and ΔQRS_{V6}) and PR (ΔPR_{II}) widening after sodium channel blockade. AA, n=29; AB, n=13; BB, n=2. Data are presented as mean±SD. The Bonferroni-corrected significance level is 0.002.