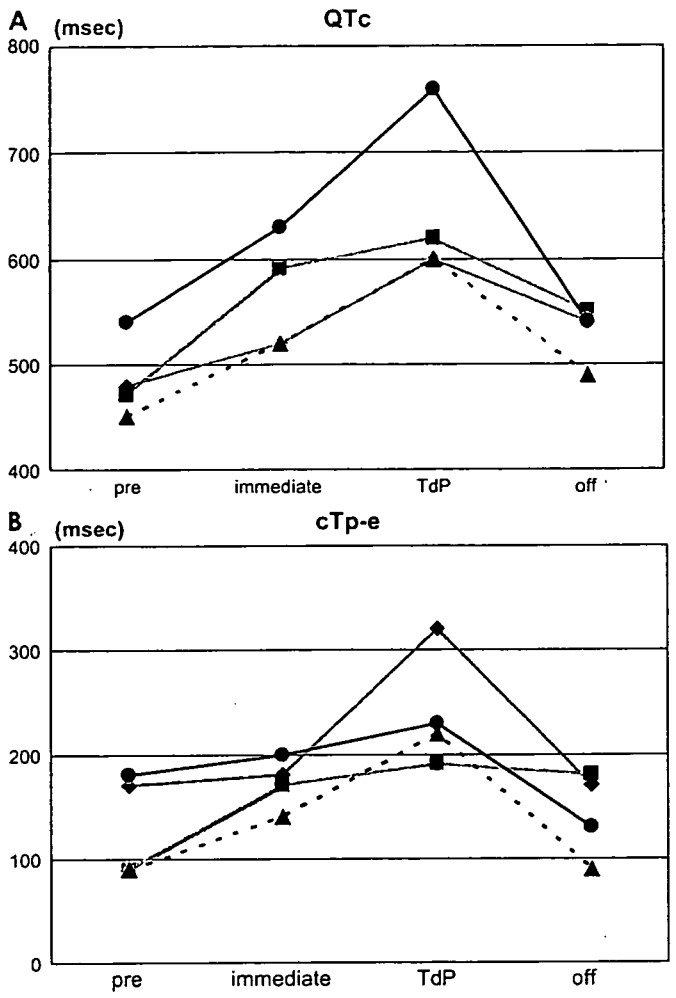

FIGURE 8. Serial changes in QTc and cTp-e in individual patient of TdP group.

proarrhythmia that induces TdP owing to an increased transmural dispersion of repolarization should be taken into account.

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Functional Angiographic Evaluation of Individual, Sequential, and Composite Arterial Grafts

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Background. To help optimize graft arrangement, we examined the effects of target vessel characteristics, conduit type, and interactions between the target vessels on the occurrence of flow reversal or occlusion.

Methods. The postoperative angiograms of 458 patients after total arterial revascularization with an off-pump, no aortic manipulation technique beginning in December 2000 were reviewed. Reverse flow was defined as the lack of opacification of a distal anastomotic site during graft angiography, but clear retrograde graft opacification during native coronary angiography. We analyzed characteristics of the target coronary branches, and bypass conduits. The potential interactions between coronary branches and sequential anastomoses were categorized as those with two 75% stenotic branches (situation 1); one 75% stenotic branch at the end of the graft and a 99% to 100% stenotic branch at the middle of the graft (situation 2); and a composite Y (or K) graft with one end to a 75% stenotic branch and the other to a 99% to 100% stenotic branch (situation 3).

Results. A total of 18 bypasses (1.1%) were occluded while reverse flow was found in 4.5% (74 of 1,627). In a multivariate analysis of the 521 bypass conduits having more than two distal anastomoses, the predictors of reverse flow or occlusion were a right coronary artery target with 75% or less stenosis ($p = 0.006$), more than three distal anastomoses with a conduit ($p = 0.005$), situation 1 ($p = 0.04$), situation 2 ($p < 0.0001$), and situation 3 ($p < 0.0001$).

Conclusions. Interactions between coronary branches and graft arrangement play important roles in flow distribution. Graft arrangement should be adjusted for each patient to minimize reverse flow.

(Ann Thorac Surg 2006;81:807–14)

Off-pump coronary artery bypass grafting (CABG) combined with a "no aortic touch" technique has been accepted as an effective procedure to avoid early morbidity and mortality from neurologic events or aortic injury. A recent prospective randomized study showed that a composite Y graft, consisting of an in-situ internal thoracic artery (ITA) and a radial artery, permitted total arterial revascularization with excellent graft patency, and improved clinical outcomes. Importantly, there were fewer late cardiac events with this technique compared with the conventional technique [1–3].

In these procedures, sequential anastomoses or composite grafts are necessary for multivessel revascularization. However, when two or more distal anastomoses share a single in-situ graft for inflow, there are concerns over the risk of reduced antegrade flow in the in-situ graft and the potential for segmental flow reversal in sequential grafts. Because bypass grafts with reverse flow do not contribute to coronary perfusion, these grafts may be counterproductive. In addition, reduced blood flow in some segments may cause narrowing or occlusion of arterial grafts, because of the tendency for arteries to adapt to flows in their run-off territory [4, 5]. These potential disadvantages to sequential arterial grafting may outweigh the possible reduction in complications for some patients.

The direction of flow in a graft is dependent upon the differential pressures in the bypass conduit and the coronary branches, rather than the graft type per se. In a bypass conduit with two or more distal anastomoses, such as sequential or composite grafts, the determinants of flow distribution are more complex than that in an individual graft. For individual bypasses, postoperative evaluations have assessed the graft type, target coronary characteristics, and degrees of angiographic patency. This type of analysis, however, is not sufficient for various configurations of bypass conduits to several coronary branches [6]. In the present study, we made the assumption that the optimal graft arrangement would maximize antegrade bypass flow. Thus, our angiographic evaluation was aimed at determining the dominant flow direction in each segment of a bypass graft, as well as the anatomic patency of the graft overall. Multivariate analyses were performed, including as variables the possible configurations of both the conduits and the target coronary branches.

Patients and Methods

Definitions of the terms used in this study are as follows. An in-situ graft is either an ITA or right gastroepiploic artery, which was divided only at its distal portion. A

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Table 1. Baseline Patient Characteristics

Characteristic	
Number of patients	458
Age (years)	65.6 ± 9.3
Male/female	380/78
Hypertension	234 (51.1%)
Hyperlipidemia	228 (49.8%)
Diabetes mellitus	176 (38.4%)
Left ventricular end-diastolic volume index (mL/m^2)	88.2 ± 31.0
Left ventricular ejection fraction (%)	48.1 ± 12.1
Total distal anastomoses	1627
Distal anastomoses per patient	3.55 ± 0.94

composite graft is a bypass conduit consisting of one in-situ graft and a free graft anastomosed to it (either end to side, side to side, or end to end). A combination of composite Y grafts, K grafts, and sequential composite grafts were used in this study. An individual bypass was defined as a bypass consisting of one in-situ graft with one distal anastomosis. That includes straight composite grafts where a linear extension of the ITA was made with the radial artery, but was limited to one distal anastomosis. A bypass conduit having one in-situ graft and two or more distal anastomoses, such as a sequential graft or a composite Y (or K) graft, was not individual.

A patent graft was defined as one with complete continuity of the lumen for its entire length from the origin of the in-situ graft to the anastomosis with a coronary branch, irrespective of the flow direction. When the continuity of a graft lumen was interrupted at any level, or when a bypass graft was not visualized by either native coronary angiography or graft angiography, that was defined as an occlusion, which was regarded as a no-flow situation with closure of the lumen. Reverse flow was defined as a situation in which at least one of the distal anastomotic sites was not opacified from the graft injection, but did fill clearly by retrograde flow from the native coronary injection. 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 in a given patient was defined as the proportion of functioning bypasses to the total number of bypassed vessels. The functioning rate for bypass conduits was defined as the proportion of entirely patent conduits without reverse flow to the total number of bypass conduits.

Patients

The coronary angiograms of 458 patients who underwent off-pump CABG using only arterial grafts with avoidance of aortic manipulation between December 2000 and March 2004 were reviewed. There were 380 men and 78 women with a mean age of 65.6 ± 9.3 years (Table 1). Patients who failed to complete postoperative coronary angiography, had individual coronary grafts only, or had one or more saphenous vein grafts were excluded. Coronary and graft angiography was performed at a median of 14 days after surgery. The angiograms were independently evaluated by cardiologists. The severity of stenosis was determined in each coronary branch. Stenoses were grouped as 51% to 75%, 76% to 90%, and 91% to 100% by a precise measurement of the luminal diameter and labeled as 75%, 90%, and 99% to 100%, respectively, for purposes of statistical analysis. The grade of maximal stenosis was recorded for each target coronary branch.

In our 458 patients, there were 1,627 distal anastomoses from 643 bypass conduits. Of these, 122 bypass conduits were individual, and 521 bypass conduits had more than two distal anastomoses (Table 2). The average number of distal anastomotic sites was 3.55 ± 0.94 per patient.

Off-Pump CABG Technique and Pharmacologic Management

Our standard technique of off-pump CABG has been reported previously [7]. We routinely assessed the ITA and the subclavian artery by angiography, computed tomography, or magnetic resonance imaging before operation. An adequately sized ITA after harvest was ensured by insertion of 1.5-mm flexible probe, and all of the ITA grafts in this series had a luminal diameter of 1.5 mm or more. While suturing the anastomosis, the surgical field was maintained by an intracoronary shunt (Anastaflo; Edward Lifescience, Irvine, California [for 1.5-mm and 2.0-mm vessels], or Clearview; Medtronic, Minneapolis, Minnesota [for 1.0-mm and 1.25-mm vessels]). The size of the intracoronary shunt was recorded as the diameter of the target coronary branch. Technical adequacy of each anastomosis was confirmed by flow mea-

Table 2. Bypass Conduits Used

Distal Anastomoses	Number
Individual	122
In-situ ITA	92
In-situ ITA + RA extension	24
In-situ GEA	6
Nonindividual	521
Conduits with one branch (Y graft)	307
In-situ ITA + free RA	288
In-situ ITA + free ITA	16
In-situ ITA + free GEA	2
In-situ ITA + free IEA	1
Conduits with two branches (K graft)	64
In-situ ITA + free RA	63
In-situ ITA + free ITA	1
Conduits with no branch (straight)	150
In-situ ITA + free RA extension	98
In-situ ITA + free GEA extension	2
In-situ ITA sequential	43
In-situ GEA sequential	7

GEA = gastroepiploic artery; IEA = inferior epigastric artery; ITA = internal thoracic artery; RA = radial artery.

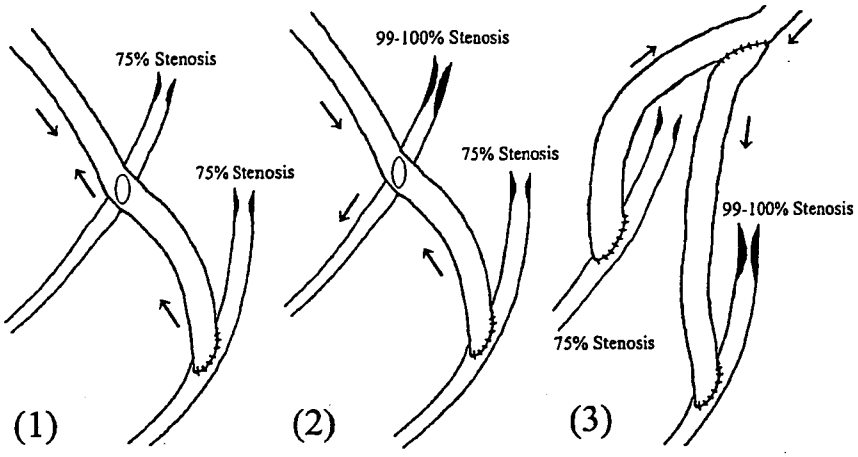

Fig 1. In situation 1, a bypass conduit had sequential anastomoses to more than two coronary branches each with 75% or less stenosis. In situation 2, a bypass conduit had sequential anastomoses to one coronary branch with 75% or less stenosis located at the distal end of the conduit and a more proximal anastomosis to a coronary branch with 99% to 100% stenosis. Presence of the severely stenotic branch may provoke reverse flow at the end of the conduit. In situation 3, one end of a composite Y (or K) graft was connected to a 75% stenotic branch and the other end to a 99% to 100% stenotic branch. The antegrade flow from the in-situ graft and the retrograde flow from the 75% stenotic branch can be directed to the severely stenotic branch. Arrows indicate direction of flow.

surements using a transit time Doppler (BF2004; Medistim AS, Oslo, Norway).

Graft Selection and Strategy

The choice of bilateral or unilateral ITA was based on a consideration of the potential operative risks. Bilateral ITAs were preferentially used for patients less than 75 years of age who maintained an active lifestyle and did not have either severe chronic obstructive pulmonary disease or diabetes mellitus requiring insulin therapy. This choice was based on studies showing that bilateral ITAs provide more abundant flow than a single ITA [8], and improve late outcomes after surgery [9]. For elderly patients, we used at least one ITA to bypass the left anterior descending artery (LAD), and the radial artery was our first choice for a free graft. We harvested only a single radial artery from the nondominant forearm. One radial artery was divided into two pieces when necessary. The gastroepiploic artery was harvested in patients who had significant cardiomegaly, a subclavian artery stenosis, or inadequate ulnar collateral flow.

We utilized the various bypass conduits as summarized in Table 2. The arrangement of the grafts was determined primarily by the spatial relationships of the target coronary arteries.

Analysis 1: Bypass Grafting to 1,627 Coronary Branches

We first conducted a univariate analysis of the data for bypass conduits and target coronaries for all 1,627 anastomoses to determine those factors that predicted a functioning graft at 2 weeks postoperatively. Variables in the univariate analysis included the territory of the target coronary distribution (divided into LAD, left circumflex [LCX], or right coronary artery [RCA]); the severity of the native coronary stenosis (75%, or 90% or greater); the diameter of the target coronary (1.0 mm, 1.25 mm, 1.5 mm, or 2.0 mm as determined by the shunt used); the type of bypass graft (ITA, or other); the number of branches for the bypass conduit (composite Y graft = 1, composite K graft = 2, or straight conduit = 0); the number of distal anastomoses for the bypass conduit (more than 3, or 3 or fewer); and the type of anastomosis (end to side, or side to side).

Analysis 2: 521 Conduits With Two or More Distal Anastomoses

Data for the 521 bypass conduits with two or more distal anastomoses were evaluated separately. Variables assessed by univariate analysis included the number of distal anastomoses (three or fewer, or more than three); the number of revascularized territories (one, two, or three territories), the number of branches for the bypass conduit (composite Y graft = 1, composite K graft = 2, or straight conduit = 0); and the presence (or absence) of a coronary branch with 75% stenosis in the LAD, LCX, or RCA territory.

In addition, we examined the effect of potential interactions between target coronary branches that were connected to each other by a composite or sequential graft. Based on these preliminary analyses, we hypothesized three situations at high risk for occlusion or reversal flow states as follows (Fig 1). The first is a graft with sequential anastomoses to more than two coronary branches each with 75% or less stenosis (situation 1). The second was a graft with a sequential anastomosis to one coronary branch with 75% or less stenosis located at the distal end of the graft and a more proximal anastomosis to a coronary branch with 99% to 100% stenosis (situation 2). The third was when one end of a composite Y (or K) graft was connected to a 75% stenotic branch and the other end to a 99% to 100% stenotic branch (situation 3). The presence or absence of these situations in each bypass conduit was entered into the analysis.

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Table 3. Graft Function as Assessed by Angiography (Reverse Flow / Occlusion / Anastomoses)

Number of *Distal Anastomoses per Bypass Conduit*	1	2	3	4~	2	3	4~	
End-to-Side / Side-to-Side	*End-to-Side*				*Side-to-Side*			Total Functioning Rate (%)
Overall								
LAD								
LAD branch	0/0/86	1/0/113	2/2/170	9/1/85	0/0/4	0/0/2	—	12/3/460 96.7
Diagonal branch	0/1/2	0/0/31	1/0/39	6/0/48	1/0/35	0/0/53	0/0/35	8/1/243 96.3
LCX	0/0/4	4/3/73	11/1/85	5/0/20	0/0/16	6/1/142	1/0/162	27/5/502 93.6
RCA	0/0/30	3/2/51	9/3/133	14/3/99	0/0/55	1/1/27	0/0/27	27/9/422 91.5
Total	0/1/122	8/5/268	23/6/427	34/4/252	1/0/110	7/2/224	1/0/224	74/18/1627 94.3
Functioning rate (%)	99.2[a]	95.1[b]	93.2[c]	84.9[d]	99.1	96.0	99.6	
75% stenosis								
LAD								
LAD branch	0/0/43	1/0/41	1/1/84	5/0/40	0/0/1	0/0/2	—	7/1/211 96.2
Diagonal branch	0/0/1	0/0/12	1/0/16	5/0/24	1/0/23	0/0/27	0/0/21	7/0/124 94.4
LCX	0/0/2	4/1/39	10/1/44	3/0/12	0/0/10	6/0/71	1/0/82	24/2/260 90.0
RCA	0/0/15	3/1/21	8/2/42	11/1/32	0/0/20	1/0/9	0/0/8	23/4/147 81.6
Total	0/0/61	8/2/113	20/4/186	24/1/108	1/0/54	7/0/109	1/0/111	61/7/742 90.8
Functioning rate (%)	100[e]	91.2[f]	87.1[g]	76.9[h]	98.1	93.6	99.1	
90, 99–100% stenosis								
Total	0/1/61	0/3/155	3/2/241	10/3/144	0/0/56	0/2/115	0/0/113	13/11/885 97.3
Functioning rate (%)	98.4[i]	98.1[j]	97.9[k]	91.0[l]	100	98.3	100	

[a] versus [b] $p = 0.07$, [b] versus [c] $p = 0.33$, [c] versus [d] $p = 0.0008$. [e] versus [f] $p = 0.02$, [f] versus [g] $p = 0.34$, [g] versus [h] $p = 0.03$. [i] versus [j] $p = 0.99$, [j] versus [k] $p = 0.99$, [k] versus [l] $p = 0.002$.

LAD = left anterior descending; LCX = left circumflex artery; RCA = right coronary artery.

Statistical Analysis

Continuous variables are expressed as the mean values ± SD. Data between two independent groups were compared by Fisher's exact probability test. We considered both reverse flow and occlusion to be equivalent adverse outcomes, and used the existence of a functioning graft at 2 weeks postoperatively as our primary outcome measure. Multivariate analyses were performed using the logistic regression method. A cut-off p value of 0.20 in a univariate analysis was used to select variables for inclusion in multivariate models. Differences in outcomes were considered statistically significant at p value less than 0.05.

Results

Analysis 1

Results of the analysis of all 1,627 anastomoses are shown in Table 3. Since 18 bypasses (1.1%) were occluded and reverse flow was found in 74 bypasses (4.5%), a total of 1,535 (94.3%) bypasses were functioning. The functioning rate in the LAD territory was 96.7% (679 of 703), and was significantly higher than the 93.6% (470 of 502; $p = 0.02$) of grafts found to be functioning in the LCX territory, or the 91.5% (386 of 422; $p = 0.0003$) in the RCA territory (Table 3).

In the univariate analysis, neither the diameter of the coronary branch nor the conduit type correlated with graft function. In the multivariate analysis, an end-to-side anastomosis ($p < 0.0001$), a 75% or less stenosis of the target branch ($p < 0.0001$), a graft to the RCA ($p = 0.002$) or LCX territory ($p = 0.0003$), and more than three distal anastomoses for the conduit ($p = 0.0006$) correlated significantly with graft function (Table 4).

Analysis 2

Results of the analysis of the 521 bypass conduits having more than two distal anastomoses are shown in Table 5. Reverse flow or occlusion was found in 74 conduits (14.2%) and 17 conduits (3.7%), respectively. The functioning rate for bypass conduits to a single-vessel territory was 96.8% (91 of 94) and was significantly higher than that of bypass conduits to two-vessel territories, which was only 82.3% (190 of 231, $p = 0.0003$; Table 5). For bypass conduits to the LCX and RCA territories (whether or not a diagonal branch was also grafted) the functioning rate for straight conduits was 80.2%, which was significantly higher than that of composite Y or K grafts (57.6%; $p = 0.02$).

The multivariate analysis demonstrated that more than three distal anastomoses ($p = 0.005$), a stenosis of 75% or less in an RCA territory branch ($p = 0.006$), and any of the interactions between the coronary branches in situation 1 ($p = 0.04$), situation 2 ($p < 0.0001$), and situation 3 ($p < 0.0001$) had significant effects on the occurrence of reverse flow or occlusion in the conduit (Table 6).

Table 4. Predictors of Graft Nonfunction in 1,627 Anastomoses

Variables	Odds Ratio	95% CI	*p* Value
Univariate analysis			
End-to-side anastomosis	4.065	(2.147–7.698)	<0.0001
Distal anastomoses of conduit > 3	1.849	(1.205–2.837)	0.005
Number of branches of conduit	1.431	(1.036–1.977)	0.03
75% stenosis	3.610	(2.242–5.812)	<0.0001
Location, RCA territory (versus LAD territory)	2.639	(1.551–4.489)	0.0003
Location, LCX territory (versus LAD territory)	1.926	(1.120–3.313)	0.02
Graft material, ITA (versus the others[a])	0.666	(0.416–1.067)	0.09
Diameter of coronary branch	0.422	(0.106–1.683)	0.22
Multivariate analysis			
End-to-side anastomosis	8.064	(3.861–16.840)	<0.0001
Distal anastomoses of conduit > 3	2.465	(1.469–4.134)	0.0006
Number of branches of conduit	1.061	(0.729–1.545)	0.76
75% stenosis	4.316	(2.629–7.087)	<0.0001
Location, RCA territory (versus LAD territory)	3.570	(1.606–7.935)	0.002
Location, LCX territory (versus LAD territory)	4.008	(1.878–8.553)	0.0003
Graft material, ITA (versus the others[a])	1.062	(0.511–2.209)	0.87

[a] The others included radial artery, GEA, and IEA.

CI = confidence interval; LAD = left anterior descending artery; LCX = left circumflex artery; RCA = right coronary artery; ITA = internal thoracic artery.

Comment

The use of an in-situ ITA, especially as a composite Y graft to three-vessel territories may result in an increased risk of functional insufficiency of the graft, with hypoperfusion or even reversal of flow in some segments of the graft. Ventricular hypertrophy, a large LAD (greater than 2.5 mm), a small diameter of the ITA, technical problems with the anastomosis to the LAD, interruption of an old vein graft and replacement by in-situ ITA, and baseline left ventricular dysfunction have all been reported to be predictors of a hypoperfusion syndrome [10, 11].

The functional adequacy of a bypass conduit is determined by two factors: its flow capacity, and its pressure capacity. In previous reports, construction of a composite Y graft has been shown to increase free flow in a single ITA pedicle by 75% and leave a sufficient flow reserve for the graft after it is connected to its target distribution [12, 13]. In addition, follow-up studies have shown rapid growth of the in-situ ITA composite Y graft to produce increases in the amount of graft flow [14].

For predicting the dominant direction of blood flow in a given segment of a graft, the pressure differential between the graft segment and the native coronary will outweigh the overall flow capacity of the graft. Interest-

Table 5. Graft Function in 521 Bypass Conduits: Reverse Flow (+) / Occlusion (+) / No. of Conduits (Functioning Rate %)

	Shape of Bypass Conduit						
Revascularized Territories	Y or K Graft		Straight		*p* Value	Total	
One-vessel territory	0/1/33	(97.0)	2/0/61	(96.7)	0.99	2/1/94	(96.8)
LAD	0/1/33	(97.0)	1/0/37	(97.3)	0.99		
LCX	—	—	0/0/4	(100)	—		
RCA	—	—	1/0/20	(95.0)	—		
Two-vessel territories	22/4/158	(83.5)	12/3/73	(79.5)	0.46	34/7/231	(82.3)
LAD + LCX	14/2/109	(85.3)	0/0/2	(100)	0.99		
LAD + RCA	5/1/40	(85.0)	0/0/1	(100)	0.99		
LCX + RCA	3/1/9	(55.6)	12/3/70	(78.6)	0.21		
Three-vessel territories							
LAD + LCX + RCA	37/8/180	(75.0)	1/1/16	(87.5)	0.36	38/9/196	(76.0)
Diagonal branch + LCX + RCA	*9/1/24*	*(58.3)*	*1/1/16*	*(87.5)*	*0.08*		
LCX + RCA ± diagonal	*12/2/33*	*(57.6)*	*13/4/86*	*(80.2)*	*0.02*		
Total	59/13/371	(80.6)	15/4/150	(87.3)	0.07	74/17/521	(82.5)

LAD = left anterior descending artery; LCX = left circumflex artery; RCA = right coronary artery.

Table 6. Predictors of Graft Nonfunction in 521 Bypass Conduits

Variables	Odds Ratio	95% CI	*p* Value
Univariate analysis			
Distal anastomoses of conduit > 3	3.287	(2.026–5.334)	<0.0001
No. of branches of the conduit	1.681	(1.160–2.435)	0.006
Revascularized territories	2.006	(1.402–2.872)	0.0001
75% stenosis in LAD territory	0.739	(0.461–1.184)	0.21
75% stenosis in LCX territory	2.195	(1.388–3.470)	0.0008
75% stenosis in RCA territory	3.539	(2.188–5.727)	<0.0001
Interactions between the sequential anastomoses			
End of the graft to 75% stenotic branch + proximal to 75% stenotic branch (situation 1)	2.365	(1.421–3.935)	0.0009
End of the graft to 75% stenotic branch + proximal to 90% stenotic branch	0.737	(0.214–2.547)	0.63
End of the graft to 75% stenotic branch + proximal to 99–100% stenotic branch (situation 2)	6.339	(2.970–13.532)	<0.0001
Interactions in the composite Y (or K) graft			
One end to 75% stenotic branch + the other end to 99%–100% stenotic branch (situation 3)	4.795	(2.781–8.269)	<0.0001
Multivariate analysis			
Distal anastomoses of conduit > 3	2.523	(1.333–4.776)	0.005
No. of branches of the conduit	0.968	(0.601–1.557)	0.89
Revascularized territories	1.270	(0.786–2.052)	0.33
75% stenosis in LCX territory	1.507	(0.825–2.753)	0.18
75% stenosis in RCA territory	2.434	(1.285–4.609)	0.006
End of the graft to 75% stenotic branch + proximal to 75% stenotic branch (situation 1)	2.139	(1.006–4.545)	0.04
End of the graft to 75% stenotic branch + proximal to 99–100% stenotic branch (situation 2)	7.704	(3.076–19.292)	<0.0001
One end to 75% stenotic branch + the other end to 99–100% stenotic branch (situation 3)	6.352	(3.234–12.477)	<0.0001

CI = confidence interval; LAD = left anterior descending artery; LCX = left circumflex artery; RCA = right coronary artery.

ingly, these two factors (pressure and flow) do not always vary proportionally. Diastolic graft pressure is regarded as a significant predictor of bypass flow, because the left ventricular myocardium is perfused exclusively in diastole [15, 16]. In spite of similar flow capacities, diastolic pressures in ITA grafts have been found to be significantly higher than in gastroepiploic artery grafts. This finding has been implicated as a major cause of insufficient antegrade flow in gastroepiploic artery grafts [17, 18].

Royse and colleagues [19] have demonstrated that the cut-off for a stenosis in the target coronary that will leave graft occlusion was higher for a composite Y graft than for a noncomposite in-situ graft. Thus, despite an increased flow capacity, composite Y and K grafts have lower pressure capacities than straight conduits. Pressure capacity of a given conduit may be determined by certain anatomic characteristics of the conduit, such as its shape and length. We hypothesize that the number of branches created in a conduit should correlate with the pressure capacity of the graft, because the Y and K graft has a following relative stenosis. The proximal part of a composite Y graft consists of an in-situ ITA and the distal part is either an ITA or a free radial artery.

In previous studies, the measured diameter of the ITA was reported to be smaller than that of the radial artery: 2.13 mm (1.8 to 2.6 mm) versus 2.75 mm (2.5 to 3.0 mm) [4, 20–22]. Using these size estimates, the ratio of the cross-sectional area of an average radial artery to an ITA would calculate to be 1.67. The ratio of the cross-sectional area of the entire outflow of a composite Y graft to its inflow would be 2.67 (= 1 + 1.67). Thus, at its creation there is a relative stenosis proximal to the radial artery portion of a composite Y graft of 63% (= 1 − 1 / 2.67). Since the outflow of a composite K graft consists of an ITA and two segments of radial artery, a K graft would have an 77% (= 1 − 1 / [1 + 1.67 + 1.67]) stenosis. On the other hand, linear extension of an in-situ ITA with a radial artery would produce only about 40% (= 1 − 1 / 1.67) stenosis, which is not significant. The length of the conduit (ie, the distance from the proximal origin of the ITA to the target coronary anastomosis) may correlate with pressure capacity. It is also true that the middle portion of the conduit has a higher pressure potential than the end of the conduit. In our multivariate analysis, the location of the target branch (ie, RCA territory) and an anastomosis at the end of the conduit were significant predictors of reverse flow or occlusion, whereas creation of branches on the in-situ ITA did not correlate significantly with graft function.

The results of this study suggest some strategies for graft arrangement when multiple distal anastomoses are planned with one graft. For the LAD territory, the isolated in-situ ITA to LAD has been widely accepted as the "gold standard" bypass graft, providing both long-term durability and improved survival. For management of the diagonal branch, it is necessary to construct grafts so as not to interfere with the ITA to LAD bypass. The functioning rate for sequential anastomoses with an in-situ ITA to both the diagonal and LAD was satisfactory, even

when both branches had only 75% stenoses, and comparable with that of a composite Y graft to the diagonal and LAD. Therefore, either of these bypasses are equivalent options and the choice should depend upon the relative positions of the planned anastomotic sites to prevent graft kinking beyond the diagonal. Either configuration can help avoid having more than four anastomoses arising from the opposite ITA.

We have several recommendations for sequential grafting of the LCX and RCA territories. First, it is recommended that the number of distal anastomoses for each conduit be kept to less than three, especially when a branch with stenosis of 75% or less is present. As the number of the coronary branch connections increase, the total amount of cross-sectional area in the distal graft increases, and the pressure potential per target vessel decreases. As shown in Table 3, when a conduit had four or more distal anastomoses, even branches with 90% stenoses were associated with reverse flow. Second, end-to-side anastomoses should be minimized. Utilization of straight composite grafts is preferable, because they have only one end whereas composite Y and K grafts have two and three ends, respectively. Third, we have to pay special attention to the management of target branches with 99% to 100% stenoses. As shown in situations 2 and 3, when the middle portion of a graft is connected to a severely stenotic branch, reverse flow can be provoked more distally in the end of the graft. The overall graft arrangement for a given patient should be designed to create a favorable pressure slope from proximal to distal. That can be accomplished by selecting an appropriate orientation (clockwise or counterclockwise), or harvesting another in-situ graft to provide a separate inflow for additional bypass grafts.

A sequential bypass to two coronary branches having moderate stenosis (situation 1) was also associated with reverse flow or occlusion. In our experience, the incidence of reverse flow and occlusion can be reduced by the use of bilateral ITAs. However, harvesting more in-situ grafts to separate inflow sources and avoid multiple sequential anastomoses, or utilizing an aortic connection (which should provide the highest driving pressure), may be reasonable options. However, high pressures can promote atherosclerotic graft disease, and spoil the long-term durability of the graft [23].

A moderately stenotic branch of the RCA as a target vessel was one of the most significant predictors of reverse flow. There is an ongoing controversy regarding the management of a moderately stenotic branch in the RCA territory [5, 19, 24]. In the present study, bypass grafting with a composite arterial graft to an RCA branch in a side-to-side fashion provided a satisfactory patency rate, even when the native coronary stenosis was moderate. These results suggest that arterial composite grafting might be an effective solution for this situation.

The purpose of this study was not to prove the superiority of totally arterial off-pump CABG without aortic manipulation, but to examine strategies for optimal sequential graft arrangement and minimizing the potential disadvantages of this technique. In the present study, occlusion and reverse flow were assessed as a composite outcome variable. The reasons for this were the following: (1) both occlusion and reverse flow are unfavorable results with at least segments of nonfunctioning grafts; (2) it is impossible to distinguish occlusion from reverse flow when flow velocity is extremely low; and (3) as reverse flow mainly affects coronary branches with moderate stenoses, this analysis would not be applicable to bypass grafts to severely stenotic coronaries. The flow demands of the myocardium, the vascular resistance in the supplied coronary bed, and the effect of a phasic delay in flow from the in-situ grafts to composite Y or K-grafts may each play a role in determining the direction of flow through a composite graft. However, we do not have reliable methods for quantifying each of these factors. An additional factor may be the luminal size at the anastomotic site itself. This factor is not precisely measurable, especially when a side-to-side anastomosis is performed at a near 90-degree angle to the coronary (diamond shape) or the anastomotic site is not clearly visualized because of mixed blood flow from the native coronary and the bypass graft. These factors are limitations to this study.

In conclusion, off-pump CABG with all arterial grafts and no aortic manipulation provided satisfactory graft patency in our hands, but the incidence of nonfunctioning grafts was not negligible. Since interactions between the target coronary branches played a distinct role in the occurrence of reverse flow, the arrangement of sequential grafts needs to be adjusted according to each patient's anatomy in order to minimize the occurrence of reverse flow and occlusion.

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Angiographic flow grading and graft arrangement of arterial conduits

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Objective: We sought to delineate the effects of competitive and reverse flow on the intermediate-term patency of arterial conduits and examined graft arrangements for maximizing antegrade bypass flow.

Methods: The angiograms of 2083 bypass grafts in 570 patients who underwent off-pump total arterial revascularization without aortic manipulation since December 2000 were reviewed. The blood flow in the bypass grafts were graded A (antegrade), B (competitive), C (reverse), or O (occlusion). The mean number of distal anastomoses was 3.65 ± 0.94 per patient.

Results: In the early angiography 91.3% (1901/2083) of the bypasses were grade A. Thirty (1.4%) bypasses were grade O, whereas 2.9% (61/2083) were grade B, and 4.4%(91/2083) were grade C. 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 of the right coronary artery territory ($P < .0001$) and left circumflex artery territory ($P = .02$) significantly correlated with grade non-A. The patency rate in the late angiography of the bypasses graded B or C in the early angiography was 7 (28.0%) of 25, whereas that of the bypasses graded A was 164 (89.1%) of 184 ($P < .0001$). The actuarial graft patency rate of the bypasses graded A was 72.3% at 3 years and was significantly higher than that of the bypasses graded B or C (28.6% at 3 years after surgical intervention, $P < .0001$).

Conclusions: The sufficient antegrade bypass flow had a favorable effect on the graft patency of arterial conduits. The graft arrangement should be adjusted for each patient so as to maximize the antegrade bypass flow and to confirm the advantage of arterial grafts.

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The use of the in situ internal thoracic artery (ITA) graft to the left anterior descending artery (LAD) is widely accepted as a standard strategy that provides long-term patency and improves late survival. The radial artery is also useful as a free graft for coronary artery bypass grafting (CABG). For the radial artery, the graft patency mostly depended on the severity of the native coronary stenosis rather than on the proximal anastomotic site[1,2] and was equivalent to that of ITA, even when the target coronary branch was small in diameter or had severe atherosclerosis.[3,4]

In the bypass conduits having 2 or more distal anastomoses, dominant reversal flow is not quite rare. Because the bypass grafts with reverse flow do not contribute to the coronary perfusion in the grafted territory, the efficacy of CABG might be unpromising, even when the bypass graft is anatomically patent. In addition, as a consequence of inappropriate graft flow, graft failure, such as diffuse narrowing or occlusion, can occur because it has been reported that the arterial materials have shown adaptability of their own diameter to the circumstances of the blood flow in the graft lumen.[5-9]

ACD

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,

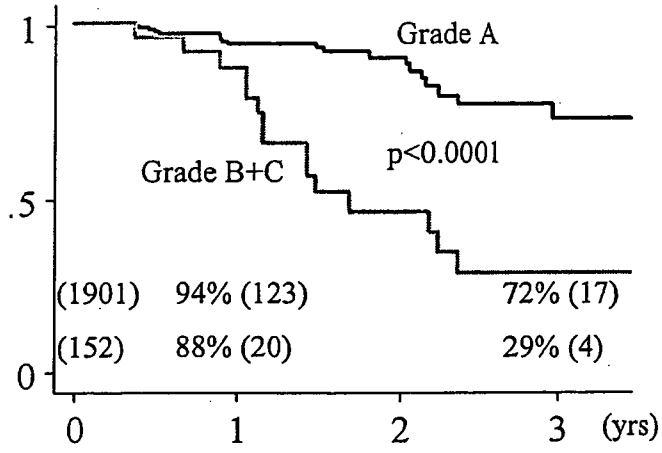

Figure 1. The actuarial graft patency rate.

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.

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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.

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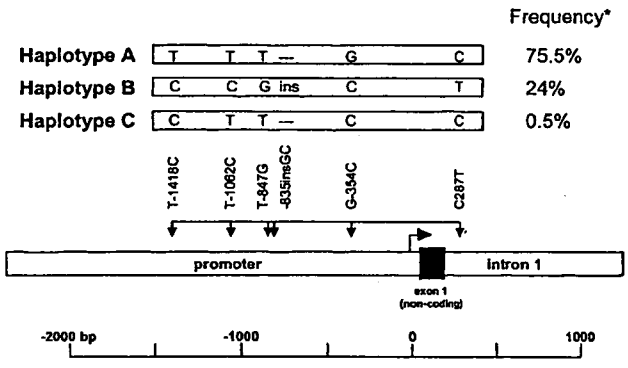

Figure 1. Haplotypes identified in the cardiac sodium channel gene (*SCN5A*) promoter. Nucleotide variations are indicated by their position relative to the major transcription initiation site (+1),[7] with the most frequent nucleotide given below and the least frequent nucleotide given above the position.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

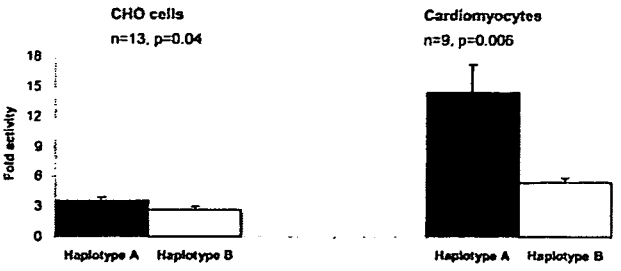

Figure 2. Reporter activity of *SCN5A* promoter haplotypes A and B. Firefly luciferase expression levels, which report the activities of the inserted *SCN5A* sequence, were divided by coexpressed Renilla luciferase activities and expressed as relative luciferase units.[7] Data are presented as mean±SE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