Background—The mechanism of ECG changes and arrhythmogenesis in Brugada syndrome (BS) patients is unknown.

Methods and Results—A BS patient without clinically detected cardiac structural abnormalities underwent cardiac transplantation for intolerable numbers of implantable cardioverter/defibrillator discharges. The patient’s explanted heart was studied electrophysiologically and histopathologically. Whole-cell currents were measured in HEK293 cells expressing wild-type or mutated sodium channels from the patient. The right ventricular outflow tract (RVOT) endocardium showed activation slowing and was the origin of ventricular fibrillation without a transmural repolarization gradient. Conduction restitution was abnormal in the RVOT but normal in the left ventricle. Right ventricular hypertrophy and fibrosis with epicardial fatty infiltration were present. HEK293 cells expressing a G1935S mutation in the gene encoding the cardiac sodium channel exhibited enhanced slow inactivation compared with wild-type channels. Computer simulations demonstrated that conduction slowing in the RVOT might have been the cause of the ECG changes.

Conclusions—In this patient with BS, conduction slowing based on interstitial fibrosis, but not transmural repolarization differences, caused the ECG signs and was the origin of ventricular fibrillation. (Circulation. 2005;112:2769-2777.)

Key Words: arrhythmia • ion channels • genes • repolarization • conduction

Right precordial ST-segment elevation in the absence of cardiac structural abnormalities is associated with a high incidence of sudden cardiac death and a mutant SCN5A gene encoding the cardiac sodium channel.1,2 This collection of symptoms and signs, Brugada syndrome (BS),1 has a familial occurrence, and in ∼30% of cases, a mutant SCN5A gene has been demonstrated.1,2 The ECG changes may involve (incomplete) right bundle branch block and J-point elevation in the right precordial leads and are sometimes progressive and associated with other conduction defects.1,3

A decrease in sodium current superimposed on the intrinsic transmural differences in the transient outward current supposedly causes a shortening of the subepicardial action potential in the right ventricular outflow tract (RVOT).2,4 This would explain the ST-segment changes and the susceptibility of these hearts to arrhythmias based on phase 2 reentry.2,8 with a premature beat originating from the RVOT subepicardium.2,4 Intact intercellular coupling, however, may attenuate large transmural differences in action potential duration (APD).6 In addition, SCN5A mutations have been reported in a minority of BS patients. There is evidence that an alternative mechanism involving locally delayed conduction is operative in at least some BS patients.3,7–10 The mechanism of the ECG changes as well as the arrhythmogenic mechanism in patients with right precordial ST-segment elevation associated ventricular fibrillation (VF), therefore, still needs to be clarified.

In this study, we describe the clinical, electrophysiological, histological, and genetic characteristics of the heart from a patient diagnosed with BS who underwent cardiac transplantation because of an intolerably high incidence of VF.11 Our study shows that in the heart of this patient, no appreciable

transmural differences in APD occurred and that VF was caused by RV conduction delay.

Methods
This study was performed in accordance with the Declaration of Helsinki and with written, informed consent of the patient.

Genetic Study
Genomic DNA was extracted from lymphocytes according to standard protocols. The entire coding region and the proximal promoter region of SCN5A (including 2.1 kb upstream of exon 1 [170 bp and noncoding]) and the proximal 439 bp of intron 1 were screened for mutations.12 Sequence analysis results were validated by restriction enzyme digestion with MspAI I (the recognition site for this enzyme was abolished). A population of 150 asymptomatic subjects with the same ethnic background was similarly screened for this mutation.

Screening for known mutations responsible for arrhythmogenic RV dysplasia (ARVD) was performed by direct sequencing of all 14 exons of plakophilin-2 (PKP2); exons 8, 15, 47, and 49 of the cardiac ryanodine receptor (RYR2); and exon 7 of desmoplakin (DSP).13–15

Cellular Electrophysiological Studies
Mutant SCN5A cDNA was prepared as described previously.16 To express the mutant or wild-type (WT) SCN5A, HEK293 cells were cotransfected with 0.5 μg of the WT or mutant α-subunit cDNA and 0.5 μg of the hβ1-subunit cDNA (kindly provided by A. L. George, Vanderbilt University, Memphis, Tenn) with the use of lipofectamine (Gibco BRL, Life Technologies). HEK293 cells were selected for measurements.

Sodium currents were measured with the whole-cell, patch-clamp technique (Axopatch200B amplifier, Axon Instruments) at 20°C and 37°C. Bath solution contained (in mmol/L) NaCl 140, KCl 4.7, CaCl2 1.8, MgCl2 2.0, NaHCO3 4.3, Na2HPO4 1.4, glucose 11, and HEPES 16.8, with pH adjusted to 7.4 (NaOH). Patch electrodes (resistance, 1.4 to 1.6 MΩ) were filled with pipette solution (in mmol/L: CsCl 1, CsF 110, NaCl 10, EGTA 11, MgCl2 1, NaATP 2, and HEPES 10, with pH adjusted to 7.3 (CsOH)). Potentials were corrected for the liquid junction potential. Series resistance was compensated for 85%. Currents were low-pass filtered (5 kHz) and digitized (30 kHz). A cell was used for 1 type of protocol within 4 minutes after the whole-cell configuration was achieved. Data were excluded when >5% rundown occurred.

Current decay at various potentials was fitted with γ=A1exp(-V/V1/2) + A2exp(-V/V2/2), where τγ and ττ are the fast and slow time constants of activation and the steady state of inactivation, respectively. For the averaged voltage dependence of activation and the steady state of inactivation, data were fitted with a Boltzmann function, γ=1/[1+exp(-(V-V1/2)/K)]-1, with V1/2=the half-maximal voltage and K=the slope factor. To study slow inactivation, the interpulse interval was set to 3 times ττ of recovery.

Electrophysiological Measurements
Mapping Experiments
The following electrode configurations were used: (1) 14 epicardial strips (7 electrodes, 1.5 cm apart, distance between strips of 1.5 to 2 cm); (2) an inflatable left endocardial balloon (64 electrodes, ~1.5 cm apart), inserted through the mitral orifice; (3) an inflatable right endocardial balloon (32 electrodes, ~1.5 cm apart), inserted through the pulmonary valves; (4) an 11×11 electrode matrix (grid constant of 5 mm), applied to either side of the interventricular septum or rolled into a cylinder to the endocardial surface of the RVOT; and (5) 24 needles (0.5-mm diameter) containing 4 electrodes and 12 needles with 2 electrodes (5-mm interelectrode distance) for transmural recording of electrograms. A bipolar stimulus electrode was introduced into the atrioventricular (AV) nodal area.

Monophasic Action Potentials
Monophasic action potentials (MAPs) were recorded with a standard Franz electrode. The signals were amplified and filtered with a MAP amplifier (EP Technology). A tungsten needle (diameter, 100 μm), insulated except for the tip, was used for intramural monophasic recordings.

The electrode signals (reference electrode on the aortic root) were fed into a data-acquisition system. Data (1.8 seconds, 247 channels, sampling frequency of 1 to 4 kHz) were stored on hard disk. Offline analysis involved local activation time (time of maximum dV/dt), and local repolarization time (according to Haws and Lux19) Laplacian electrograms were calculated in areas with conduction slowing to better define local activation time. The normal value (600 ms2) for the area under conduction curves (AUCs) was derived from the study by Kawara et al.21

Histology
After the electrophysiological studies, the heart was placed in formalin. Tissue samples were embedded in paraffin. Five-micron sections were stained with hematoxylin/eosin and picrosirius red stains. The degree of myocardial fibrosis and fat was quantified by computerized morphometry in picrosirius red–stained sections, according to the criteria set by Burke et al.22 Red (fibrous tissue) or white (fat) staining was expressed as a percentage of total myocardial area in ×2 and ×10 objective fields showing representative intramyocardial areas, respectively. Epicardial fat and large perivascular fat areas were excluded. Six fields in 2 different sections of the RVOT, RV lateral wall, interventricular septum, and left ventricular (LV) lateral wall were analyzed on digitized images (ImagePro, Media Cybernetics).

Computer Simulations
We used a computer model to calculate the precordial ECG morphology based on local endocardial and epicardial APDs and activation times.23

Statistical Analysis
The results are expressed as mean±SEM. Cellular electrophysiological data were tested with the unpaired Student t test or 2-way repeated-measures ANOVA, followed by the Holm-Sidak post hoc test, where appropriate. Conduction curves were compared with the Kruskal-Wallis or the Mann-Whitney rank-sum test, where appropriate. P<0.05 was accepted as the level of statistical significance.

Results
Patient Data
The patient (male), aged 34 years, was successfully resuscitated from VF in 1992. His brother had died suddenly at the age of 32 years (no records available). His mother had a BS ECG sign without provocation tests, whereas his father had not. Neither of his 2 sons nor his brother’s 2 sons had signs of BS. Subsequent work-up revealed no structural abnormalities: both coronary angiography and left and right ventriculography results were normal. Echocardiography examination was normal, except for mild tricuspid regurgitation and a slightly enlarged RV diameter (29 mm) from 1999 onward. A
procainamide provocation test (in 1992) increased the ST-segment elevation from 2.5 to 3.5 mm in his precordial ECG leads (Figure 1). Based on these clinical findings, it was concluded that the patient had BS. During an electrophysiological study, a rapid ventricular tachycardia was induced (3 ventricular premature beats) that promptly evolved into VF. He received an implantable cardioverter/defibrillator (ICD).

During follow-up, lead V1 developed QRS widening (160 ms, particularly in its terminal portion) and a negative T wave (at the end of 2001; Figure 1).

The patient subsequently experienced many VF episodes that were terminated by the ICD and were resistant to antiarrhythmic drugs. The incidence of delivered shocks (up to 129 appropriate shocks in 5 months in 2001) became intolerable. Cardiac transplantation was conducted in February 2002.11

Genetic Data and Cellular Electrophysiology

The patient was heterozygous for a nucleotide substitution in codon 1935 (numbering from the initiation codon of cDNA NM_198056), resulting in substitution of glycine by serine (G1935S) within the C terminus of the channel protein. This mutation was absent in 150 control individuals. No known mutations were found in ARVD genes PKP2, RYR2, or DSP. The patient’s mother declined genetic screening.

Figure 2A shows the current-voltage relation of the peak sodium current (normalized to cell size) recorded from HEK293 cells expressing WT or mutant sodium channels. Current density and voltage dependence were similar in WT and mutant channels. Also, there were no statistically significant differences between WT and mutant channels in the

![Figure 1](https://example.com/figure1.png)  
**Figure 1.** ECGs. Left, ECG recorded in 1992 before and after provocation with procainamide. Right, ECG in 2001. Bars=200 ms, 1 mV.

![Figure 2](https://example.com/figure2.png)  
**Figure 2.** Biophysical properties of sodium channels. A, Current-voltage relation (mean±SEM) in HEK293 cells expressing WT or G1935S channels. Peak current was normalized to cell size. B, Slow inactivation of WT and G1935S channels. The interpulse interval was 3 times τ of recovery. The fraction of channels that slowly inactivated was significantly larger in G1935S than in WT (*repeated-measures ANOVA). Inserts above the x axis illustrate voltage protocols. Abbreviations are as defined in text.
time constants (fast or slow) of recovery from inactivation and in $V_{1/2}$ and $K$ of steady-state inactivation (not shown). The fraction of channels that slowly inactivated, however, was significantly larger in G1935S than in WT (Figure 2B). At 20°C, G1935S mutant and WT sodium channels were not significantly different in any of these parameters.

**Activation Mapping**

Figure 3 shows a set of activation maps during stimulation (cycle length, 600 ms) from the AV nodal area. The figure shows a normal rapid spread of activation of the left septal endocardium and of the LV. In the RVOT, crowding of isochrones indicates slowed conduction. The entire RV wall
endocardium and epicardium) was activated relatively late. The latest activation occurred at 154 ms, commensurate with the QRS duration of a recent ECG (Figure 1).

VF was induced with 2 premature stimuli (from the AV nodal area). Figure 3 (lower panels) shows the activation patterns of the last stimulated beat (with more conduction delay than the basic beat, top panels) and the first beat of VF. It originated in the RVOT endocardium (320 ms), followed by RV epicardial breakthrough 50 ms later (stars in lower right panels). RVOT endocardial electrograms demonstrated multiple deflections.

Because the apparent focal origin of VF might have been caused by the low spatial resolution of the electrode grid used, a high-resolution electrode array was introduced into the orifice of the pulmonary artery with its lateral margins meeting, and programmed stimulation at a basic cycle length (BCL) of 430 ms was performed. A single premature stimulus (from the middle of the sheet) induced VF. Figure 4 shows the activation maps after S1 and S2 and the first beats of VF. Crowding of isochrones in the activation map of S2 occurred in the RVOT free wall.

A complex pattern of reentrant activations started VF in the endocardium of the RVOT free wall with 2:1 block to the remainder of the area. Three reentrant loops can be discerned (lower right panel), the last of which proceeded toward A. The tissue under electrodes A–C showed fractionation during the last paced basic beat (S1).

Activation on either side of the interventricular septum was also mapped in detail with the same 11x11 electrode array (stimulation at the AV nodal area). Early endocardial RV activation was recorded after 46 ms and early LV activation after 36 ms. At 8 RV endocardial electrode sites, Purkinje
spikes preceding ventricular activation were recorded (not shown), indicating the absence of right bundle branch block.

Restitution of Propagation
Restitution of propagation was measured at a BCL of 600 ms with the 11×11 electrode grid in the RVOT. The activation patterns (Figure 5) show smooth isochrones after S1 and crowding of isochrones after S2 (coupling interval, 245 ms). Conduction curves are shown for the 4 marked electrode positions. The conduction curve recorded from site C was abnormal, with activation delay occurring at a relatively long coupling interval. Conduction curves from sites A and D did not show the abrupt increase in conduction time, but activation delay increased at a coupling interval of 340 ms. Note that sites A, C, and D but not B show double potentials. Figure 5 (lower right panel) shows 4 representative conduction curves of the LV myocardium (stimulation of the LV apex).

Conduction curves were constructed for all LV and RV endocardial and epicardial recording sites during stimulation (BCL, 600 ms) from the apex. The activation time difference with a basic beat was quantified for each coupling interval, and the AUC was calculated (gray areas in Figure 5). The LV AUC (78 sites) was $377\pm21$ ms$^2$, and the RV AUC was $655\pm65$ ms$^2$ ($n=64$, $P<0.001$, Mann-Whitney test). No statistical differences were observed between endocardial and epicardial sites (at either the LV or RV). The RV AUC was significantly larger and the LV AUC significantly smaller than the critical value for normal conduction curves.$^{21}$

Repolarization
Endocardial and epicardial RVOT MAP duration at 50% and 80% of repolarization was measured after pacing (from the AV node) at various cycle lengths (Figure 6A). Several minutes were allowed for equilibration after a change of cycle length. The largest endocardial-epicardial difference in APD$50$ was $\approx20$ ms, obtained during pacing at 1500 ms (top right panel). The figure also includes data obtained from an intramural (subepicardial) RVOT tungsten needle from which MAPs were obtained.

Thirty-six needles with a total of 120 terminals were introduced in the LV and RV walls, including the RVOT. Figure 6B (right) shows RVOT electrograms during pacing (BCL, 600 ms, S2 after 400 ms). Activation recovery intervals were calculated according to Haws and Lux.$^{19}$ Figure 6B shows representative activation recovery intervals at various sites. In the RVOT, the maximum transmural difference in activation recovery interval was 24 ms. Note the absence of ST-T–segment changes.

Histopathology
The heart showed marked hypertrophy of the RV wall (thickness of 12 mm) and focal endocardial fibroelastosis. The tip of the defibrillation catheter was located in the RV apex and was firmly attached to fibrotic endocardium. The LV, heart valves, and
coronary arteries were normal. The RV myocardium showed prominent bundles of hypertrophic cardiomyocytes focally interspersed with mature adipose tissue. This fatty infiltration was particularly evident in sections taken from the RVOT, in which adipose tissue extended subendocardially (Figure 7A). Inflammatory infiltrates were absent. In addition, several areas of fibrosis were present (Figure 7B). The AV node and the proximal bundle branches were normal.

Morphometric analysis (the Table) showed that intramyocardial fibrosis and adipose tissue content were larger in the RVOT and RV lateral wall compared with LV and septal myocardium, whereby RVOT tissue was most severely affected.

**Computer Simulations**

Figure 8 shows ECGs calculated with the ECGSIM program under 2 conditions: (1) localized epicardial conduction delay (left ECG panel, top activation map) and (2) total RV activation delay (right ECG panel, lower activation maps) for which RV activation times from Figure 3 were used. The latter led to ECG changes similar to those in Figure 1 (2001 ECG). When epicardial activation was delayed in 2 epicardial nodes only (left panel), an ECG emerged with a right precordial J-point elevation. The steplike morphology of the calculated ECGs resulted from the wide spacing of the nodes in the model.

**Discussion**

This study describes the genetic, electrophysiological, and morphological cardiac characteristics of an explanted heart of a patient with right precordial ST-segment elevation and multiple episodes of VF without clinically demonstrable structural heart disease, matching the criteria of BS 10 years before transplantation. We have demonstrated a cardiac sodium channel mutation leading to enhanced slow inactivation and discrete interstitial changes in the RV. These changes were associated with overt conduction abnormalities leading to reentry and VF originating in the RVOT subendocardium. Abnormal restitution of propagation, which is associated with alterations in tissue architecture, was present in the RV but not the LV myocardium. Right bundle branch block was absent, and a transmural APD gradient in the RV wall was absent even at long cycle lengths. In a computer model, incorporation of RV conduction delays reproduced the patient’s ECG.

Although the patient was diagnosed with BS, it might be argued that the patient is not truly representative of BS because of the presence of structural abnormalities in the RV and the progressive ECG changes, which favor a diagnosis of ARVD. However, interstitial fibrosis was detected only after explantation of the heart, and progressive ECG changes have been described in BS. So far, the scarce postmortem data available in BS patients have demonstrated abnormal cardiac (micro)anatomy. Nevertheless, the response to procainamide is not specific for BS but may occur in some cases of ARVD.
Not only BS but also some cases of familial cardiomyopathy have been associated with cardiac sodium channel mutations, albeit in a minority of patients. The diagnosis of BS is, however, not dependent on the presence of an SCN5A mutation.

The syndrome has been considered a subliminal form of ARVD. The histopathologic analysis of the heart in our study did show signs that are somewhat in agreement with ARVD, although overt transmural fatty infiltration was absent, excluding classic ARVD. Moreover, features of myocyte degeneration and focal inflammatory infiltrates characteristic for ARVD were absent. Known mutations in the ARVD genes were not found. According to the differential diagnostic criteria, the patient fulfilled most of the criteria for BS. The question is whether the early ECG changes compatible with BS in this patient can be explained by a mild form of conduction delay. Our simulation study showed that localized epicardial conduction delay in the RVOT may reproduce these changes. This is in accordance with the study of Nagase et al. Thus, at least some cases of BS with clinically undetected structural changes (causing the Brugada sign, slowed conduction, and reentrant arrhythmias) resemble a form of ARVD, as has been suggested by Corrado et al.

Two hypotheses have been proposed for the explanation of the ECG signs and the pathophysiological consequences of BS: (1) subepicardial reduction of the AP dome and (2) localized conduction delays. Reduction of the sodium current is thought to critically reduce net inward currents (superimposed on the intrinsic transmural differences in the transient outward current) to provoke loss of the AP dome in the former and to amplify conduction delays in the latter hypothesis. A recent study from our institute indirectly supports both hypotheses.

In disagreement with hypothesis 1 were the following: (1) We found that the AP dome of RVOT cells persisted at slow heart rates (30/min), (2) the first beat of VF occurred subendocardially, not subepicardially, and (3) giant local T waves, occurring when large regional differences in APD existed at the site where AP is shortest, were not encountered in this study. In contrast, hypothesis 2 was supported by the observations that conduction slowing in the RVOT occurred, particularly at fast heart rates. These conduction abnormalities increased with shorter coupling intervals, leading to abnormal restitution of conduction in the RV but not the LV wall. Abnormal restitution of conduction is associated with altered tissue architecture. Overall, the aforementioned observations support the activation-delay mechanism. Irrespective of the diagnostic classification of this patient, our study accentuates that right precordial ST-segment elevation can be explained by localized conduction changes and do not necessitate transmural gradients in APD.

Enhanced slow inactivation of the mutated sodium channel extends into diastole and may lead to loss of sodium current during prolonged, rapid cardiac rhythms. Although we did not observe loss of the AP dome in the epicardium (Figure 6), we did document abnormally increased conduction slowing at short coupling intervals in the RV but not in the LV. Activation delay was only observed at RV sites where loss of myocardium and increased fibrosis occurred. The sodium channel mutation found in this patient, therefore, may not have functional consequences in structurally normal myocardium with a high safety factor for propagation, but only in tissue with altered architecture, leading to load mismatch, in combination with prolonged, fast heart rates.

Sodium channel mutations may be causally involved in the changes in myocardial architecture. Cellular structural abnormalities and degenerative changes have been described in patients with an SCN5A mutation. Also, heterozygous SCN5A-knockout mice have increased myocardial fibrosis. Alternatively, the presence of subtle and clinically undetectable structural abnormalities may be unmasked by concurrent loss of sodium channel function (of either pharmacological or genetic origin). This concept gains support from the observation in our patient that the BS ECG abnormalities were only unmasked after procainamide provocation at presentation. As the degenerative changes become more extensive, sodium channel blockade is no longer required to produce the ECG signature of BS. This may be one of the explanations why the proportion of carriers of sodium channel mutations in the BS population is low and why BS manifests itself at middle age.

**Strengths and Limitations**

It cannot be excluded that the presence of the ICD lead or the frequent defibrillation shocks may have influenced local tissue architecture. However, the lead was lodged in the RV apex, far from the site with the most evident structural changes. Because we studied the heart in the Langendorff mode, we may have underestimated the inducibility of arrhythmias occurring in the ejecting heart.

Demonstration of the absence of a transmural gradient in APD was done under conditions where clear ST-segment changes were lacking. However, we have shown that ST-segment elevation may be explained by localized conduction delay in the RVOT only.

Extrapolation of our findings to the physiopathology of BS in general is not possible, based on the observations in a single patient. However, ours is the first study in which combined histopathologic, electrophysiological, and genetic analysis was performed in the same subject.

**Conclusions**

At least in some cases of BS, subtle structural interstitial changes that are undetectable by routine diagnostic procedures may underlie both the ECG changes and the propensity for life-threatening arrhythmias. Our study provides evidence in support of the delayed-activation hypothesis for BS. Localized conduction delay in the RVOT epicardium may provoke the Brugada sign.

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