Further insights into inheritable arrhythmia syndromes: Focus on electrocardiograms
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Slow and discontinuous conduction conspire in Brugada syndrome

A right ventricular mapping and stimulation study

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ABSTRACT

BACKGROUND
Brugada syndrome (BrS) is associated with lethal arrhythmias, which are linked to specific ST-segment changes (type-1 BrS-ECG) and the right ventricle (RV). The pathophysiological basis of the arrhythmias and type-1 BrS-ECG is unresolved. We studied the electrophysiological characteristics of the RV endocardium in BrS.

METHODS AND RESULTS
RV endocardial electroanatomical mapping and stimulation studies were performed in controls (n=12) and BrS patients with a type-1 (BrS-1, n=10) or type-2 BrS-ECG (BrS-2, n=12) during the studies. BrS-1 patients had prominent impairment of RV endocardial impulse propagation when compared with controls, as represented by: (1) prolonged activation-duration during sinus rhythm (86±4 versus 65±3 ms), (2) increased electrogram fractionation (1.36±0.04 versus 1.15±0.01 deflections per electrogram), (3) longer electrogram duration (83±3 versus 63±2 ms), (4) activation delays on premature stimulation (longitudinal: 160±26 versus 86±9 ms; transversal: 112±5 versus 58±6 ms), and (5) abnormal transversal conduction velocity restitution (42±8 versus 18±2 ms increase in delay at shortest coupling intervals). Wider and more fractionated electrograms were also found in BrS-2 patients. Repolarization was not different between groups.

CONCLUSIONS
BrS-1 and BrS-2 patients are characterized by wide and fractionated electrograms at the RV endocardium. BrS-1 patients display additional conduction slowing during sinus rhythm and premature stimulation along with abnormal transversal conduction velocity restitution. These patients may thus exhibit a substrate for slow and discontinuous conduction caused by abnormal active membrane processes and electric coupling. Our findings support the emerging notion that BrS is not solely attributable to abnormal electrophysiological properties but requires the conspiring effects of conduction slowing and tissue discontinuities.
INTRODUCTION

Brugada syndrome (BrS) is an inheritable syndrome associated with sudden cardiac death (SCD) by ventricular tachycardia and/or fibrillation (VT/VF). It predominantly affects the right ventricle (RV), as ECG abnormalities are found in right precordial leads, and VT/VF mostly arises in the RV. The signature ECG-change, which is required for the diagnosis and linked to VT/VF, is a coved-type ST-elevation in V1-V3 (type-1 BrS-ECG) and may occur spontaneously. Also, drug challenge with sodium channel blocking drugs (e.g., ajmaline) may lead to the diagnosis when it causes conversion of a normal or type-2 ECG into a type-1 BrS-ECG.

BrS has been regarded a primary electrical disease because gross structural abnormalities are undetectable by clinical imaging. However, the pathophysiological basis of the type-1 BrS-ECG and VT/VF is unresolved. There are two leading hypotheses (reviewed in reference): the depolarization disorder hypothesis, i.e., RV conduction delay, and the repolarization disorder hypothesis, i.e., transmural dispersion of RV action potential morphology. Up to 30% of BrS-patients carry loss-of-function mutations in SCN5A, the gene which encodes the cardiac sodium channel α-subunit that drives impulse conduction. Recently, RV interstitial derangements (myocarditis, cardiomyocyte vacuolization, fibrofatty infiltration) were found in endomyocardial biopsies of BrS-patients. These derangements, even when undetectable by clinical imaging, may result in increased electrical coupling resistance which impairs impulse propagation and act as arrhythmogenic substrate. Accordingly, in the explanted heart of a SCN5A-mutation carrying BrS-patient, we found RV fibrosis, fatty infiltration, conduction slowing and reentrant arrhythmias. The adverse electrophysiological manifestations linked to these interstitial changes were particularly evident by analysis of conduction velocity (CV) restitution. This analysis revealed abnormally strong conduction slowing upon premature stimulation. Thus, CV-restitution analysis may uncover how conduction slowing and increased coupling resistance and/or tissue discontinuities interact in the pathophysiology of BrS.

The aim of the present study was to further investigate the electrophysiological characteristics of RV endocardium in BrS-patients. We hypothesized that the type-1 BrS-ECG and associated arrhythmias are based on impaired impulse propagation which may be linked to increased coupling resistance. Furthermore, BrS-patients may have a different arrhythmogenic substrate at the time when they have a type-1 BrS-ECG than at periods when they do not. Accordingly, we conducted electrophysiological studies in BrS-patients when they had a type-1 BrS-ECG or when they had a type-2 BrS-ECG, and in controls, to assess RV electrogram characteristics during sinus rhythm (endocardial mapping) and during premature stimulation (CV-restitution).


METHODS

Patients

In this prospective single-center study, 34 patients were included: 22 BrS-patients and 12 controls. The diagnosis BrS was made according to the consensus criteria, and required documentation of a type-1 BrS-ECG, either spontaneously present or provoked by sodium channel blocking drugs in conjunction with either: documented VT/VF, family history of SCD <45 years old, coved-type ECGs in family members, inducibility of VT, syncope, or nocturnal agonal respiration. BrS-patients were considered to be strongly affected, i.e., with a documented spontaneous type-1 BrS-ECG, a history of aborted SCD and/or documented or suspected VTs (syncope or multiple near-syncopes not otherwise explained). Controls needed a normal ECG, no apparent structural heart disease, no history of (near)syncope or aborted SCD, no family history of SCD, and underwent electrophysiological study for supraventricular tachyarrhythmias (atrioventricular nodal reentrant tachycardia [n=5], atrial tachycardia [n=1], concealed atrioventricular bypass [n=1] or lone atrial fibrillation [n=5]). All patients who fulfilled these criteria were eligible for the study and were asked to participate. All patients who agreed and provided written informed consent (approved by the institutional review board) were included.

Because of the variable nature of the BrS-ECG, BrS-patients were classified according to their ECG at the time of the studies. Consequently, three patient groups were studied: 1) “BrS-1 patients”: BrS-patients with a type-1 BrS-ECG during the studies (n=10); 2) “BrS-2 patients”: BrS-patients with a type-2 BrS-ECG during the studies (n=12, these patients had thus exhibited a type-1 BrS-ECG at some previous point); and 3) “controls” (n=12), (Table 1).

To study RV endocardial characteristics during sinus rhythm, RV electroanatomical mapping was performed in 9 BrS-1 patients, 10 BrS-2 patients, and 9 controls. In 2 of the 9 BrS-1 patients, the type-1 BrS-ECG was induced by ajmaline challenge according to the consensus criteria (they had a type-2 BrS-ECG at baseline but had previously shown a spontaneous type-1 BrS-ECG), and a stable type-1 BrS-ECG was maintained by continuous ajmaline infusion. To study dynamic changes imposed by premature stimuli (CV-restitution), RV stimulation was conducted in 5 BrS-1 patients, 9 BrS-2 patients, and 5 controls. When patients and treating physicians agreed to both mapping and stimulation studies, mapping studies were performed first because the stimulation studies carried with them the risk of VT/VF induction.

Cardiac MRI, including delayed gadolinium-enhanced imaging, was performed in all but 4 BrS-patients. MRI was not available before ICD-implantation/catheterization in 3 BrS-patients, and was refused by one BrS-patient. Echocardiography was performed in these BrS-patients and also in all controls. Molecular genetic analysis of SCN5A was performed in all BrS-patients. Patients were not sedated and all anti-arrhythmic drugs were discontinued.
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CARTO mapping

Electroanatomical mapping of the complete endocardial RV was performed during sinus rhythm with CARTO (Biosense-Webster, USA) and a locatable mapping catheter (Navistar 4mm/8F, Biosense-Webster, USA). Care was taken to perform endocardial mapping at random throughout the RV.

A reference locator pad under the patient’s back was used for spatial reference, and ECG lead II as time reference. Signals were analyzed off-line by at least two investigators using specially developed software\(^\text{12}\) on Matlab R2006b (MathWorks, USA). The investigators were blinded to catheter location and consensus was required for each electrogram. Only sinus node driven beats were included. Electrograms were excluded if their technical quality was insufficient or if catheter-induced right bundle branch block or extrasystoles occurred. We obtained 3 groups of RV electrogram properties: 1) activation, 2) fractionation and duration, and 3) repolarization. Three-dimensional reconstructions of these measurements, using the spatial information enclosed within CARTO, were studied. For this purpose, 4 regions within the RV were defined: RV-outflow tract (RVOT, upper 25% of RV), RV-apex (lower 25% of RV), RV-free wall, and RV-septum.

To study activation, we assessed activation-time (AT), defined as the interval (ms) from onset of QRS in lead II to the steepest negative dV/dt (≤-0.04 V/sec) of the intrinsic deflection in the endocardial unipolar electrogram.\(^\text{13}\) To avoid selection of remote unipolar components, the timing of the steepest negative dV/dt in the unipolar electrogram had to correspond with a positive or negative deflection in the bipolar electrogram (Figure 1).\(^\text{13}\)

<table>
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<tr>
<th>Table 1</th>
<th>Group characteristics</th>
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<tr>
<td></td>
<td>BrS-1 (n=10)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>46±3</td>
</tr>
<tr>
<td>Male</td>
<td>10 (100)</td>
</tr>
<tr>
<td>Syncope or aborted SCD</td>
<td>3 (30)</td>
</tr>
<tr>
<td>Non-sustained ventricular tachycardia</td>
<td>1 (10)</td>
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<tr>
<td>Multiple episodes of near syncope</td>
<td>4 (40)</td>
</tr>
<tr>
<td>Palpitations</td>
<td>2 (20)</td>
</tr>
<tr>
<td>Family history of BrS/SCD</td>
<td>5 (50)</td>
</tr>
<tr>
<td>SCN5A mutation present</td>
<td>6 (60)</td>
</tr>
</tbody>
</table>

Data are mean±standard error or No. (%) of patients. BrS indicates Brugada syndrome; NA, not analyzed; SCD, sudden cardiac death.

for at least 5 half-lives. Studies in controls were performed after successful radiofrequency ablation.

\(^{12}\) Specially developed software for electroanatomical mapping.

\(^{13}\) Definition of activation-time (AT) for electroanatomical mapping.
was defined as the interval (ms) between the earliest AT of any electrogram (activation-start) and the latest AT of any electrogram (activation-end). In the three-dimensional model of activation (Figure 2), we assessed the presence of conduction block.

Fractionation of electrograms was defined as the presence of ≥2 intrinsic deflections with a $dV/dt \leq -0.04$ V/sec, separated by ≥10ms, in the unipolar signal with corresponding deflections in the bipolar electrogram (Figure 1). Fractionation was expressed as mean number of intrinsic deflections per electrogram. Thus, fractionation index=1 denotes no fractionation in any complex. Electrogram duration was measured in the bipolar signal as the interval between the first and last deflection, and expressed as mean bipolar electrogram duration (ms). The spatial distribution of these parameters was studied in the three-dimensional reconstructions.

To study repolarization, we assessed local activation recovery interval (ARI) and repolarization time (RT). ARI, a measure of action potential duration, was defined as the interval (ms) between AT and the end of recovery; the latter was determined in the unipolar signal as the largest positive $dV/dt$ of the T-wave (Figure 1). RT was defined as AT+ARI (ms). Electrograms with flat or distorted T-waves were excluded from repolarization analysis. Mean values of ARI and RT in each patient were used to compare global differences in ARI and RT. Standard deviations of ARI and RT were used to compare dispersion in ARI and RT.

**Stimulation studies**

CV-restitution was assessed with the use of a quadripolar pacing catheter (Evaluator, 6F, St.Jude Medical, USA), a decapolar mapping catheter (MarinrCS, 7F, Medtronic, USA; 5 electrode pairs separated by 2mm, with 5mm spacing between pairs), and the Prucka-Cardiolab (GE Medical Systems, USA). To account for possible anisotropy in propagation at shorter coupling intervals (CI), we studied CV-restitution in two directions, which we estimated to be either parallel or perpendicular to the general RV endocardial fiber orientation and
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activation front (longitudinal and transversal CV-restitution, respectively). Longitudinal CV-restitution was assessed by positioning the mapping catheter longitudinally over the RV-free wall with the tip pointing toward the RVOT, and stimulating with the pacing catheter from the RV-apex (Figure 3). We assessed transversal CV-restitution by positioning the mapping catheter circumferentially in the mid-RV free wall (Figure 3), and stimulating from its distal electrode pair. We paced at twice diastolic threshold using a drive train of 8 stimuli (S1) at a basic cycle length (BCL) of 500ms, followed by a single premature stimulus (S2). The CI of S2 was reduced in steps of 10ms from 400ms to 200ms or until the ventricular effective refractory period (VERP). Bipolar electrograms from the 5 electrode pairs were assessed for paced activation-time (paced-AT), defined as the interval (ms) from the stimulus to the intrinsic deflection (Figure 4). Paced-ATs were assessed off-line by at least two investigators and consensus was required for each electrogram. Electrograms were excluded if their technical quality was insufficient or if fusion beats or spontaneous ventricular extrasystoles interfered with the drive train.

Each endocardial CV-restitution curve was described by 4 parameters (Figure 4): 1) paced-AT at BCL; 2) onset paced-AT increase (ms), i.e., the longest CI at which paced-AT exceeded paced-AT at BCL by 5ms and continued to increase; 3) mean paced-AT increase (ms), i.e., the mean difference for all electrode pairs between paced-AT at BCL and maximum

**Figure 2** CARTO mapping

Typical 3-dimensional reconstructions in antero-posterior view of activation, fractionation, and electrogram duration of a BrS-1 patient (panels A, C, and E), and a control (panels B, D, and F). There is pronounced conduction slowing and increased electrogram fractionation and width in the BrS-1 patient. BrS indicates Brugada syndrome.
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The representation of CV-restitution at the body surface was described by 3 comparable parameters for paced-QRS duration in lead II: 1) paced-QRS at BCL (ms); 2) onset paced-QRS increase (ms), i.e., the longest CI at which paced-QRS duration exceeded paced-QRS duration at BCL by 5ms and continued to increase; 3) paced-QRS increase (ms), i.e., the difference between paced-QRS duration at BCL and the maximal paced-QRS duration.

Statistical analysis
Statistical analyses were performed in SPSS 15.0. Variables were compared by one-way analysis of variance (ANOVA) using a Tukey-HSD or Games-Howell multiple comparisons post-hoc test depending on equality of variances (Levene’s test). Categorical variables were compared by a Fisher’s exact or Chi-square test when appropriate.

Catheter positioning for stimulation studies. Upper panels: recording of longitudinal propagation during stimulation: mapping catheter (arrow) along long axis of the right ventricular (RV) free wall with tip pointing toward the RV outflow tract, pacing catheter (arrow) in RV-apex. Lower panels show recording of transversal propagation during stimulation: mapping catheter circumferentially in mid-RV; stimulation conducted from distal electrode pair. LAO indicates left anterior oblique; RAO, right anterior oblique.

The presence of a linear relationship between endocardial and body surface (ECG) CV-restitution curves was estimated with Linear regression and Pearson’s correlations. A two-sided significance level of 5% defined a statistically significantly difference between groups. Continuous variables are presented as mean±standard error. The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

RESULTS

Baseline characteristics
Age and gender distributions were similar between groups. BrS-1 and BrS-2 patients had similar histories (Table 1). Cardiac imaging detected no gross structural abnormality in any

Figure 3 Catheter positioning

Catheter positioning for stimulation studies. Upper panels: recording of longitudinal propagation during stimulation: mapping catheter (arrow) along long axis of the right ventricular (RV) free wall with tip pointing toward the RV outflow tract, pacing catheter (arrow) in RV-apex. Lower panels show recording of transversal propagation during stimulation: mapping catheter circumferentially in mid-RV; stimulation conducted from distal electrode pair. LAO indicates left anterior oblique; RAO, right anterior oblique.
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Patient. The proportion of patients with a SCN5A-mutation was larger among BrS-1 patients than among BrS-2 patients, but this difference was not statistically significant. Three BrS-1 patients had a SCN5A frameshift mutation leading to a truncated protein while three other BrS-1 patients had a missense mutation. One BrS-2 patient had a frameshift mutation leading to a truncated protein while another had a missense mutation.

**Sinus rhythm: Activation**

CARTO maps contained 81±6 mapping positions on average and ajmaline was used in two BrS-1 patients. In all patients, activation started in the lower septum/apex and subsequently diverged towards the tricuspid annulus and RVOT (Figure 2 panel A,B). No apparent conduction block was observed in any patient. Yet, activation-duration was significantly longer in BrS-1 patients compared to controls (86 vs. 65ms), and activation-end was delayed (117 vs. 81ms) (Table 2). In contrast, BrS-2 patients and controls had similar activation-durations and activation-ends. In the two BrS-1 patients in whom activation mapping was performed before

![Figure 4 Conduction velocity](image)

and after a type-1 BrS-ECG was induced by ajmaline infusion, activation-duration increased by 20ms on average when their type-2 BrS-ECG turned into a type-1 BrS-ECG.

**Sinus rhythm: Electrogram fractionation and duration**

Electrograms were more fractionated in both BrS-1 and BrS-2 patients compared to controls (Table 2, fractionation index 19% higher on average). Additionally, electrograms were wider in BrS-1 and BrS-2 patients (Table 2, 83 and 76 vs. 63ms). Figure 2 shows a typical example of the spatial distribution of electrogram fractionation in a BrS-1 patient and in a control (panel C,D). Fractionation increased by 9% on average in the two patients in whom a type-2 BrS-ECG converted into a type-1 BrS-ECG during ajmaline infusion. In BrS-patients, fractionated electrograms often appeared to cluster in various RV regions. However, the localization of fractionation differed between patients and there were no overall differences between regions. Electrogram duration showed a distribution in the RV which often mirrored the distribution of fractionation with clustering of widest electrograms in different RV regions (Figure 2 panel E,F). Electrogram duration increased by 13ms on average in the two BrS-patients who received ajmaline. Both fractionation and electrogram duration were not affected by the presence/absence of a SCN5A-mutation.

**Sinus rhythm: Repolarization**

Mean heart rate, ARI and RT did not differ between BrS-patients and controls, nor did dispersion of ARI or RT (Table 2). In both patients who received ajmaline, conversion of their type-2 BrS-ECG to a type-1 BrS-ECG was associated with reductions in mean ARI (24ms) and RT (10ms) on average, but no change in ARI or RT dispersion (-0.6ms and +3.7ms,
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RV stimulation: Conduction and CV-restitution

Stimulation thresholds were within normal limits (<1V) in all patients. Delivering premature stimuli at increasingly shorter CIs evoked an increase in paced-AT and paced-QRS at shortest CIs. A typical CV-restitution pattern of a BrS-1 patient is shown in Figure 4 panel C and D. Endocardial and ECG CV-restitution curves were clearly related in all patients (Figure 4 panel E, F). This relation was as follows for longitudinal propagation: mean slope=0.92±0.06, range Pearson-R=0.825-0.977. For transversal propagation: mean slope=1.19±0.15, range Pearson-R=0.44-0.95.

In line with the observations during sinus rhythm, stimulation at BCL evoked more conduction slowing in BrS-1 patients than in BrS-2 patients or controls, as evidenced by longer paced-AT during both longitudinal and transversal propagation (Table 3; 160 and 112ms for BrS-1 vs. 86 and 58ms for controls, respectively). Accordingly, paced-QRS at BCL was longer in BrS-1 patients than in controls during both longitudinal and transversal propagation (224 and 230ms for BrS-1 vs. 159 and 152ms for controls, respectively). Similar differences in paced-

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<td>BrS-1 (n=5)</td>
</tr>
<tr>
<td>Longitudinal</td>
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<tr>
<td>Paced-activation time at basic cycle length</td>
<td>160±26*</td>
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<td>Onset paced-activation time increase</td>
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<td>Mean paced-activation time increase</td>
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<td>Ventricular effective refractory period</td>
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<td>Paced-QRS at basic cycle length</td>
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<td>Transversal</td>
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<td>Paced-activation time at basic cycle length</td>
<td>112±5*†</td>
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<td>Onset paced-activation time increase</td>
<td>260±23</td>
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<tr>
<td>Mean paced-activation time increase</td>
<td>42±8*†</td>
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<tr>
<td>Ventricular effective refractory period</td>
<td>203±6</td>
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<td>Paced-QRS at basic cycle length</td>
<td>230±11*†</td>
</tr>
<tr>
<td>Onset paced-QRS increase</td>
<td>247±15</td>
</tr>
<tr>
<td>Paced-QRS increase</td>
<td>34±9</td>
</tr>
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</table>

Data are mean±standard error. ANOVA post hoc test: *p<0.05 for BrS-1 vs. control; †p<0.05 for BrS-1 vs. BrS-2; ‡p<0.05 for BrS-2 vs. control.

respectively).
QRS at BCL, albeit smaller, were observed between BrS-2 patients and controls (184ms and 188ms for BrS-2 respectively). BrS-1 patients had abnormal CV-restitution with significantly larger increases in paced-AT than controls during transversal propagation at shortest CIs (42 vs. 18ms). In contrast, there were no differences between groups with respect to VERP, increase in paced-AT for longitudinal propagation, or the onset of paced-AT increase for either longitudinal or transversal propagation. During RV-apex stimulation for longitudinal CV-restitution analysis, VF was induced in one BrS-2 patient.

**DISCUSSION**

This study shows that BrS is associated with wider and more intensively fractionated electrograms at the RV endocardium as evidenced in BrS-patients with either a type-1 or type-2 BrS-ECG. Furthermore, the type-1 BrS-ECG is associated with slow impulse propagation during sinus rhythm and premature stimulation along with abnormal transversal CV-restitution. In contrast, repolarization characteristics were not different between groups. Impairment of impulse propagation in BrS appears to reside particularly in slowing of cell-to-cell transmission between myocardial cells, rather than propagation slowing in the specialized conduction system. This is suggested by more severe delays during RV stimulation (specialized conduction system not involved) than during sinus rhythm (specialized conduction system involved) (Tables 2-3). Activation delays almost doubled in BrS-1 patients during stimulation. These endocardial changes were faithfully reflected on the ECG by increases in the paced QRS-complex duration. A near doubling of activation delay upon stimulation in BrS-1 patients is in accordance with studies in transgenic mice that lack one SCN5A allele. Of interest, BrS-2 patients also had wider QRS-complexes upon premature stimulation suggesting a reduced safety factor for conduction, although they had normal activation-duration during sinus rhythm. In a recent study, BrS-patients with inducible VF were noted to have wider paced QRS-complexes than BrS-patients without inducible VF. Such a relation was not notable in the present study. However, in that study the patients with wider paced QRS-complexes tended to have more ST-elevation. Impulse propagation is determined by both active and passive membrane properties. Abnormal membrane excitability that causes conduction slowing in BrS may follow from reduced sodium current due to reduced sarcolemmal sodium channel density and/or changes in sodium channel function. For instance, delayed recovery from slow inactivation reduces sodium channel availability and reduces sodium current at short CIs. Additionally, increased coupling resistance between cells or changes in fiber orientation may also reduce conduction velocity. In uniformly anisotropic cardiac tissue the resistance to current flow is higher in the direction perpendicular to fiber orientation and is lower parallel to it. Although histological confirmation was lacking in the present study we did not find functional changes to support
the possibility that RV endocardial fiber orientation was grossly different in BrS, as the pattern of activation during sinus rhythm was normal\textsuperscript{17} in all patients (although activation was on average ~30\% slower in BrS-1 patients). Consequently, passive membrane properties that cause impairment of propagation in BrS may be caused by increased coupling resistance, e.g., fibrosis\textsuperscript{7,8} or loss of connexins.\textsuperscript{22} Such findings suggest overlap between BrS and ARVC.\textsuperscript{8,23,24} Of interest, fibrosis and reduced connexin expression may also result from reduced sodium current.\textsuperscript{7,8,18,22}

Wide and fractionated electrograms are a common manifestation of increased coupling resistance following separation of myocardial fibers by fibrosis or fat, and indicate that the electrical impulse travels discontinuously.\textsuperscript{13,21,25} Accordingly, we found ~19\% more electrogram fractionation and ~12 to 20ms wider electrograms on average in BrS-patients. Still, discontinuous conduction can also be caused by functional derangements, e.g., functional block and current-to-load mismatch.\textsuperscript{10,11,26} In the present study, this was illustrated by an increase in electrogram duration and fractionation with a decrease of sodium channel availability induced by ajmaline administration. Thus, wider and fractionated electrograms in BrS-patients may be caused by both structural changes and derangements in active membrane properties. Of note, electrograms were rarely as severely fractionated or wide as may be found in the diseased area in myocardial infarction\textsuperscript{21} or ARVC.\textsuperscript{25} However, the mean electrogram duration in BrS-1 and BrS-2 patients was similar to previously reported electrograms in the affected area in ARVC.\textsuperscript{25} Interestingly, there was no correlation between the width or the degree of electrogram fractionation and the presence/absence of a SCN5A-mutation.

Our previous electrophysiological and histological study of the heart of a BrS-patient\textsuperscript{8} indicated that impaired active membrane properties and interstitial changes may be probed by CV-restitution analysis. In that patient, the SCN5A-mutation caused enhanced slow inactivation while interstitial fibrosis and fatty infiltration were also found. These changes resulted in abnormal CV-restitution. Abnormal CV-restitution with a large increase in activation delay at short CIs, as found in the BrS-1 patients, is thought to represent the capacity of the myocardium to support slow conduction and act as an arrhythmic substrate that renders patients susceptible to reentrant arrhythmias.\textsuperscript{11} CV-restitution is dependent on the recovery rate of sodium channels and always exists in cardiac tissue at short diastolic intervals.\textsuperscript{27} Yet, structural changes cause abnormal CV-restitution and are associated with increased coupling resistance and fractionated electrograms.\textsuperscript{9,11} In addition, structural changes may be a prerequisite for abnormal CV-restitution, as shown in a study of transgenic mice which lack one SCN5A allele and had profound conduction slowing at a young age but no electrogram fractionation, structurally normal hearts and normal CV-restitution at that time.\textsuperscript{18} Conversely, conduction slowing does appear to play a role in abnormal CV-restitution in BrS, as BrS-1 and BrS-2 patients had similar electrogram fractionation but only the added presence of sufficient conduction slowing in BrS-1 patients resulted in abnormal CV-
restitution. Thus, unless diastolic intervals are abbreviated due to delay in repolarization, abnormal CV-restitution results from the interplay between conduction slowing and tissue discontinuities. Of note, we did not find an early onset of increase in activation delay (i.e., at long CIs), as typically found in structurally severely disrupted tissue. Accordingly, a modest increase in electrogram fractionation and duration and the lack of gross structural abnormalities in BrS-patients as evidenced cardiac MRI and echocardiography findings, support a relatively mild interstitial derangement in these patients.

Abnormal CV-restitution in BrS-1 patients was detected during transverse but not longitudinal propagation. With the placement of the catheters during the stimulation studies based on previous anatomical findings, activation data, and normal activation patterns as shown with CARTO, CV-restitution was studied approximately parallel to the RV endocardial fiber orientation (longitudinal CV-restitution) and perpendicular to the fiber orientation (transversal CV-restitution). Although we lack histological confirmation, the finding that only transversal CV-restitution was abnormal suggests that increased coupling resistance/tissue discontinuities more strongly affect impulse propagation perpendicular to the myocardial fiber orientation than propagation parallel to it. This may be explained by smaller sarcolemmal expression of connexins (which are required for cell-to-cell impulse transmission) and/or more interstitial fibrosis at the long axis of the myocytes than at their short axis as shown previously in dogs and mice. The resulting reduction in conduction capacity may render transversal conduction more likely to slow or fail. Another explanation why we did not observe abnormal longitudinal CV-restitution may relate to the profound conduction slowing observed in BrS-1 patients, particularly at short CIs. A beat evoked by a premature stimulus at a short CI will arrive with great delay at a site distant from where it was evoked, and paradoxically increase the CI of this beat at the distant site. This effect is more likely to occur during RV-apex stimulation (long distance stimulus-electrodes) than during transversal stimulation (short distance stimulus-electrodes). Regardless of the underlying mechanisms, the disparate effects of premature stimulation on longitudinal and transversal propagation will further increase anisotropy, thereby facilitating reentrant excitation and arrhythmias.

In summary, our findings support the emerging notion that BrS is not solely due to abnormal electrophysiological (ionic) properties, but requires the concerted effects of slow and discontinuous conduction in relation to impaired active membrane processes and increased coupling resistance. The observation that most BrS-patients only develop arrhythmias after the third decade of their life may be explained by the requirement of interstitial changes to evolve over time. When this occurs, the effects of reduction in wave length due to conduction slowing are exacerbated by augmented anisotropy and discontinuous conduction due to interstitial changes. This will promote conduction block, reentry and wave break, and may be the elements to cause VT/VF in BrS. In this concept, tissue discontinuities
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are required, and may already be relevant when relatively mild, because they conspire with functional impairments, e.g., loss-of-function SCN5A-mutations with or without mutations in modifying genes and/or environmental stressors such as sodium channel blocking drugs, vagal stimulation or fever.

Limitations
The major limitation of our study is the absence of histological data or immunohistochemistry. These analyses would have the potential to definitely correlate the endocardial electrophysiological findings in these patients with fiber orientation, structural/interstitial derangements, altered distribution of gap junction proteins and/or sodium channels. Another limitation is the relatively small number of studied subjects.

Proof or refutation of an accompanying role of transmural dispersion of RV action potential morphology, as proposed in the repolarization disorder hypothesis, remains elusive as action potential morphology cannot be directly measured in the catheterization laboratory. Still, electrograms in proximity to the epicardial RVOT have been recorded simultaneously with endocardial RVOT electrograms in BrS-patients with the use of a guidewire in the conus branch. That study showed that ARI and RT were longer at RVOT epicardium than at RVOT endocardium after pilsicainide administration and that a delay in activation time occurred. Although this method also has its limitations, it would have been valuable to study electrograms in this RV region. Finally, we cannot address with this study the relationship between (abnormal) CV-restitution and the recently reported steeper ARI restitution at the RV-apex in BrS-patients with inducible VF.

Conclusions
BrS-1 and BrS-2 patients are characterized by wider and more fractionated electrograms at the RV endocardium as compared to controls. BrS-1 patients display additional RV endocardial conduction slowing during sinus rhythm and premature stimulation, along with abnormal conduction velocity restitution. This suggests that these patients exhibit a substrate for slow and discontinuous conduction caused by impairment of active membrane processes and electrical coupling between cells. Our findings support the emerging notion that BrS is not solely due to abnormal electrophysiological properties but requires the conspiring effects of conduction slowing and tissue discontinuities.

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