Fibrosis, Connexin-43, and Conduction Abnormalities in the Brugada Syndrome


Brugada Syndrome

1. **Cardiac Arrhythmia with a distinct ECG pattern**
   - Prolonged PR interval
   - Right Bundle Branch Block (RBBB)
   - ST segment elevation
   - Negative T wave

2. **Hereditary Disorder**
   - Autosomal Dominant

3. **Idiopathic**
   - No evidence of structural disease

**CLINICAL CHARACTERISTICS**

**Prevalence**
- 4% of all SD
- 20% of all idiopathic SD
- Estimated at 1 in 2000
- Family history in 20-30%
- Common in South Asia

**Clinical Presentation**
- Syncope
- Resuscitated SD due to VT/VF
- 80% of adults patients are males
- Many are asymptomatic

**Clinical Diagnosis - ECG**
- J wave >= 2mm
- Negative T wave
- Coved type ST-T configuration
- Gradually descending ST segment

**Management of Brugada Syndrome**
- Implantation of ICD
What causes Brugada Syndrome?

1. Depolarization Theory

2. Repolarization Theory

3. Development Abnormality
JACC paper

Depolarization Theory

1. Fibrosis in the RVOT
   - Atrial enlargement
   - Dilated Cardiomyopathy
   - Spontaneous AF

2. Altered Cx43 Expression
   - Spontaneous and prolonged AF
1. **Study setting and cohorts**

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>Index Presentation</th>
<th>Clinical Abnormality</th>
<th>Cardiac Morphology</th>
<th>Relatives Evaluated</th>
<th>Relatives Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>M</td>
<td>15</td>
<td>SCD in sleep</td>
<td>Diagnosis in relative</td>
<td>Normal</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>B2</td>
<td>M</td>
<td>18</td>
<td>SCD in sleep</td>
<td>Diagnosis in relative</td>
<td>Normal</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>B3</td>
<td>M</td>
<td>19</td>
<td>SCD in sleep</td>
<td>Diagnosis in relative</td>
<td>Normal</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>B4</td>
<td>M</td>
<td>23</td>
<td>SCD with exercise</td>
<td>Diagnosis in relative</td>
<td>Right coronary artery (RCA)</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>B5</td>
<td>M</td>
<td>24</td>
<td>SCD in sleep</td>
<td>Diagnosis in relative</td>
<td>Atrial septal defect</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>B6</td>
<td>M</td>
<td>40</td>
<td>SCD with minimal activity</td>
<td>Diagnosis in relative</td>
<td>Normal</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>

Post-mortem control cohort:
- C1: M 17, RTA, None, Normal
- C2: M 18, RTA, None, Normal
- C3: M 22, Suicide, None, Normal
- C4: M 22, RTA, None, Normal
- C5: M 22, RTA, None, Normal
- C6: M 37, Homicide, None, Normal

In vivo BrS cohort:
- V1: M 48, Multiple syncope, Spontaneous type 1 ECG, Normal
- V2: M 28, Multiple syncope, Asystole-provoked type 1 ECG, Normal
- V3: M 59, VF arrest, Spontaneous type 1 ECG, Normal
- V4: M 29, VF arrest with fever, Spontaneous type 1 ECG, Normal
- V5: M 47, Syncope, Spontaneous type 1 ECG, Normal
- V6: M 27, Multiple syncope, Spontaneous type 1 ECG, Normal

2. **Mutation Analysis**
- SCN5A mutation analysis for in vivo BrS subjects

3. **Specialist Cardiac post-mortem examination**
- Histological examination of tissue sections

4. **Detailed post-mortem RVOT examination**
- Morphometric analysis for collagen/fibrosis
- Confocal microscopy of Cx43 distribution
5 **In vivo open thoracotomy mapping and ablation of RVOT**
- Epicardial RF ablation at sites of late and fractionated EGMs

6 **Biopsy of in vivo substrate sites in RVOT**
- Epicardial samples from sites with abnormal EGMs

7 **Clinical Endpoints**
- Ajmaline test at 6 months

8 **Statistical Analysis**
- Simple and multiple regression analysis
Right precordial ECG Traces from blood relatives of postmortem BrS cases during Ajmaline provocation
**Results**

RVOT Histological Sections Stained for Collagen and Immunoconfocal images of Cx43 expression

<table>
<thead>
<tr>
<th><strong>Epicardium</strong></th>
<th><strong>Epicardial Fat Layer</strong></th>
<th><strong>Epicardial Myocardium</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>B1</td>
<td>C1</td>
</tr>
<tr>
<td>Interstitial Collagen</td>
<td>Replacement Collagen</td>
<td>Longitudinal Connexin43</td>
</tr>
<tr>
<td>A2</td>
<td>B2</td>
<td>A4</td>
</tr>
<tr>
<td>Enface Connexin43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A5</td>
<td>B5</td>
<td></td>
</tr>
</tbody>
</table>
Results

Scatterplot of Collagen and Cx43 in RVOT epicardium

Table 2: Univariable and Multivariate Regression Analysis of Proportional Collagen Content, as Evaluated by Morphometric Analysis of PSR Staining in BrS Cases Versus Control Hearts

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariable Analysis</th>
<th>Multivariate Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>p Value</td>
</tr>
<tr>
<td>Disease</td>
<td>1.42 (1.06-1.90)</td>
<td>0.024</td>
</tr>
<tr>
<td>RV</td>
<td>1.66 (1.11-2.50)</td>
<td>0.019</td>
</tr>
<tr>
<td>RVOT</td>
<td>1.98 (1.34-2.91)</td>
<td>0.003</td>
</tr>
<tr>
<td>Endo</td>
<td>1.00 N/A</td>
<td>1.00 N/A</td>
</tr>
<tr>
<td>Mid</td>
<td>1.27 (1.02-1.58)</td>
<td>0.033</td>
</tr>
<tr>
<td>Epi</td>
<td>2.00 (1.46-2.73)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

BrS = Brugada syndrome; CI = confidence interval; Endo = endocardium; Epi = epicardium; LV = left ventricle; Mid = mid-myocardium; OR = odds ratio; PSR = picrosirius red; RV = right ventricle; RVOT = right ventricular outflow tract.

Table 3: Multivariable Regression Analysis of Proportional Connexin43 Content in BrS Post-Mortem Cases Versus Control Hearts

<table>
<thead>
<tr>
<th>Variable</th>
<th>BrS vs. Control Hearts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease</td>
<td>0.59 (0.44-0.79)</td>
</tr>
<tr>
<td>Endocardium</td>
<td>1.00</td>
</tr>
<tr>
<td>Mid-myocardium</td>
<td>0.97 (0.64-1.49)</td>
</tr>
<tr>
<td>Epicardium</td>
<td>1.16 (0.76-1.78)</td>
</tr>
<tr>
<td>Disease (corrected for collagen)</td>
<td>0.58 (0.36-0.96)</td>
</tr>
</tbody>
</table>

Expression according to zone and after correction for collagen content is also shown. Abbreviations as in Table 2.
1. Increased levels of myocardial fibrosis
   - Increased collagen content in all ventricular walls
   - Fibrosis both epicardial and intramyocardial noted in the RVOT associated with late potentials
   - Fibrosis in all cases of BrS, whether they harbored SCN5A mutation or not

2. Cx43 expression diminished in BrS compared with control
   - Changes at the intercalated discs may cause electrical uncoupling and reported in mice model

3. Epicardial ablation at sites of late potentials in RVOT terminated BrS with type-I ECG
   - Suppression of VT/VF episodes due to open thoracotomy catheter ablation

4. Depolarization vs Repolarization theory for BrS pathogenesis
   - Conduction delay in RVOT in BrS in vivo reinforced by other human studies
   - Correlation between conduction delay and fibrosis (histopathology), fragmented electrograms
1. Biased Population
   - Symptomatic BrS cases

2. Etiology of death in post-mortem cases
   - No previous ECG evidence
   - Diagnosis based on BrS in blood relatives
<table>
<thead>
<tr>
<th><strong>What did they do?</strong></th>
<th><strong>What did they find?</strong></th>
</tr>
</thead>
</table>
| Investigated substrate (collagen, fibrosis, Cx43) underlying BrS at post-mortem, and in vivo | - BrS associated with **increased collagen, fibrosis and reduced Cx43 expression** in the RVOT  
- Myocardial abnormal potentials collocate with fibrosis in RVOT |

<table>
<thead>
<tr>
<th><strong>What’s next?</strong></th>
<th><strong>What does it mean?</strong></th>
</tr>
</thead>
</table>
| - Quantification of fibrosis and gap junction proteins for risk stratification  
- Identify predictors and determinants of the structural abnormalities | - **Depolarization theory** is the likely pathogenesis path of BrS  
- Ablation in RVOT can reverse BrS ECG signature |