Nodoventricular Accessory Pathways in PRKAG2-Dependent Familial Preexcitation Syndrome Reveal a Disorder in Cardiac Development

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Background—Familial preexcitation syndrome is linked to mutations in PRKAG2. Previous studies on the R302Q mutation have provided evidence for a remarkably high proportion of otherwise rare accessory pathways with atrioventricular (AV) node-like conduction properties (Mahaim fibers). Yet, histopathologic proof is still lacking. We aimed to provide such proof.

Methods and Results—We retrospectively studied the medical records of 17 members of a 5-generation family. Five subjects died prematurely. The R302Q mutation was found in 8 living subjects and 2 deceased subjects (obligate carriers). Cardiac hypertrophy was found in 7 mutation carriers. ECGs compatible with preexcitation were found in 13 subjects and AV block at varying degrees in 5 subjects. All mutation carriers had electrocardiographic evidence of preexcitation, AV block, or both. Three individuals had high-grade AV block with preexcited conducted beats. Electrophysiological studies in 3 individuals revealed bypasses with AV node–like properties. Histopathologic studies of 1 suddenly deceased mutation carrier revealed concentric hypertrophy of the left ventricle with extensive myocardial disarray associated with slight interstitial fibrosis but no lysosomal-bound glycogen. Moreover, there were 3 small nodoventricular tracts (Mahaim fibers) passing through the central fibrous body and connecting the AV node with the working myocardium of the interventricular septum.

Conclusions—Preexcitation associated with the R302Q mutation in PRKAG2 is associated with Mahaim fibers. These findings support the novel insight that PRKAG2 may be involved in the development of the cardiac conduction system. (Circ Arrhythmia Electrophysiol. 2008;1:276-281.)

Key Words: arrhythmia ▪ cardiomyopathy ▪ electrophysiology ▪ Wolff-Parkinson-White syndrome

Familial preexcitation (Wolff-Parkinson-White) syndrome is linked to mutations in PRKAG2, the gene that encodes the regulatory subunit of AMP-activated protein kinase A (AMPK).1 This syndrome is complex, including preexcitation, atrioventricular (AV) block, sinus node dysfunction, cardiac hypertrophy, and sudden death. Most features have been ascribed to the effects of AMPK on glycogen metabolism. The N488I mutation, which causes constitutive AMPK activation,2 is characterized by glycogen-laden cardiomyocytes. Thus, the cardiac hypertrophy phenotype seen in this mutant has similarities with other glycogen storage diseases, such as Danon and Pompe diseases.2 Moreover, the preexcitation phenotype, analyzed using histological studies in N488I transgenic mice, was explained by the finding that the annulus fibrosus, which electrically insulates the atria from the ventricles, had structural disruptions because of glycogen-laden myocytes.3

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Intriguingly, a family with another mutation in PRKAG2 (R302Q) had evidence for a different and unusual type of accessory AV connection. In most (5 of 8) carriers of this mutation who exhibited preexcitation, accessory pathways with decremental (AV node–like) conduction properties were found.1 Accessory pathways with such functional properties are usually termed Mahaim fibers. Although the anomalous connections that were shown histologically by Mahaim and Winston4 arise from the AV node and insert into the right ventricle (nodoventricular), accessory pathways with decremental conduction properties may alternatively connect the lateral right atrium to the apical right ventricle or right bundle branch (atriofascicular).5,6 Mahaim fibers are normally rare. Their abundant presence among R302Q carriers would suggest that this mutation is
associated with developmental derangements of the cardiac conduction system, thereby exposing a hitherto unknown role of \textit{PRKAG2}, namely, an involvement in cardiac development. Yet, histopathologic proof of Mahaim fibers in patients with the R302Q mutation has been lacking. With the aim of providing such proof, we studied a family with the R302Q mutation. We found that histopathologic analysis closely agreed with electrocardiographic and electrophysiological studies (EPS) by providing evidence of multiple Mahaim fibers, which created anomalous connections between the AV node and the ventricular myocardium. These findings foster the novel insight that \textit{PRKAG2} may be involved in the development of the cardiac conduction system.

### Methods

**Clinical Data**

In this retrospective analysis, we studied the medical records, collected between 1955 and 2007, of all 17 members of a 5-generation Dutch family of whom records were available (Table). The study was approved by the institutional review committee, and the subjects gave informed consent. On request of the patients, we have included some minor nonrelevant changes in the pedigree, as presented in Figure 1, with the aim of making it less recognizable and protecting the patients’ and family’s anonymity.

**Molecular Genetics**

Genomic DNA was extracted from lymphocytes using standard procedures. The R302Q mutation in \textit{PRKAG2} was confirmed by sequence analysis.

### Table 1. Clinical and Electrocardiographic Data

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age at Analysis, years</th>
<th>R302Q Carrier</th>
<th>Preexcitation</th>
<th>AV Block</th>
<th>Pacemaker</th>
<th>Hypertrophy</th>
<th>Electrophysiological Study</th>
<th>Symptoms</th>
<th>Follow-Up</th>
</tr>
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<tbody>
<tr>
<td>I-1</td>
<td>F</td>
<td>37</td>
<td>NP</td>
<td>? (no ECG)</td>
<td>? (no ECG)</td>
<td>–</td>
<td>NP</td>
<td>NP</td>
<td>sd (37)</td>
<td>–</td>
</tr>
<tr>
<td>II-2</td>
<td>M</td>
<td>42</td>
<td>Obligate</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>NP</td>
<td>NP</td>
<td>sd (42)</td>
<td>–</td>
</tr>
<tr>
<td>II-4</td>
<td>M</td>
<td>30</td>
<td>NP</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>NP</td>
<td>NP</td>
<td>sd (30)</td>
<td>–</td>
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<td>II-5</td>
<td>F</td>
<td>38</td>
<td>NP</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>NP</td>
<td>NP</td>
<td>sd (38)</td>
<td>–</td>
</tr>
<tr>
<td>II-6</td>
<td>F</td>
<td>38</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Mahaim bypass</td>
<td>pt</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>III-4</td>
<td>M</td>
<td>36</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<td>III-8</td>
<td>F</td>
<td>32</td>
<td>Obligate</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>Mahaim bypass</td>
<td>pt/sd (42)</td>
<td>–</td>
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<td>–</td>
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<td>–</td>
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<td>–</td>
<td>NP</td>
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<tr>
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<td>32</td>
<td>NP</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>No bypass</td>
<td>pt</td>
<td>–</td>
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<td>F</td>
<td>28</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>NP</td>
<td>No bypass</td>
<td>–</td>
<td>–</td>
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<tr>
<td>IV-5</td>
<td>F</td>
<td>25</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>NP</td>
<td>pt</td>
<td>+</td>
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<tr>
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<td>M</td>
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<td>+</td>
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<td>+</td>
<td>+</td>
<td>NP</td>
<td>pt</td>
<td>+</td>
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<td>F</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>IV-9</td>
<td>M</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>NP</td>
<td>–</td>
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<td>–</td>
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<td>11</td>
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<td>+</td>
<td>–</td>
<td>–</td>
<td>No bypass</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>IV-11</td>
<td>M</td>
<td>26</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>NP</td>
<td>–</td>
<td>NP</td>
<td>–</td>
</tr>
</tbody>
</table>

+ indicates present; –, absent; F, female; M, male; NP, not performed or no echocardiography; pt, paroxysmal tachycardia; sd, sudden death (age in years).
Statement of Responsibility
The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agreed to the manuscript as written.

Results
Clinical and Genetic Analysis
The outcome of clinical and genetic analyses is summarized in Figure 1. Five subjects had died prematurely and suddenly (aged 30, 37, 38, 42, and 42 years). The R302Q mutation in PRKAG2 was found in 8 living subjects and inferred (obligate carriernship) in 2 deceased subjects. It was previously reported that this gene variant was not found in 300 chromosomes from control subjects selected from the general population. Cardiac hypertrophy was found by postmortem analysis in 1 prematurely deceased (see below) and by echocardiography in 6 living mutation carriers (Table).

ECG Analysis
ECGs compatible with preexcitation were found in 13 subjects, AV block at varying degrees in 5 subjects, and atrial fibrillation in 2 subjects. All mutation carriers had electrocardiographic evidence of preexcitation and/or AV block. Three individuals exhibited the highly unusual pattern of high-grade AV block with preexcited conducted beats (Figure 2). This indicates that AV conduction over the bypasses was impaired, because if the bypasses would function normally and complete AV nodal block were present, bypass conduction would become fully manifest and support 1:1 AV conduction at resting heart rates. The finding that bypass conduction failed at the same time as AV node conduction suggests that the bypasses had AV node–like properties. This notion was supported by EPS in 6 mutation carriers, all with ECG signs of preexcitation. In 3 individuals, bypasses with decremental properties were found; in the other 3, no bypasses were found. EPS also provided evidence that there was AV nodal conduction, because clear His bundle potentials were recorded. Radiofrequency catheter ablation of the bypasses was not attempted.

Histopathologic Analysis
One patient (patient III-8) sustained witnessed sudden death at age 42. At autopsy, her heart showed concentric hypertrophy of the left ventricle (weight, 540 g). Histologically, there was extensive myocardial disarray associated with slight interstitial fibrosis, observed almost circumferentially with a midzonal distribution (~20% of left ventricular mass estimated in 2 transverse slices of the heart; Figure 3A). Several tissue blocks taken throughout the left ventricle were histochemically stained with periodic acid-Schiff and periodic acid-Schiff-diastase to evaluate the glycogen content of myocardium. In all sections, the amounts of periodic acid-Schiff positive and diastase digestible material (indicating glycogen in red) were low. Further investigation of samples of formalin-fixed myocardium at the ultrastructural level (transmission electron microscopy) revealed no lysosomal-bound glycogen. To test the validity of the glycogen stains, we also investigated paraffin-embedded myocardial tissue of 3 hearts obtained from patients who died of noncardiac disease, following the same staining procedure. This showed that the staining pattern and amounts of intracytoplasmatic glycogen in our patient were approximately the same as those of the 3 control hearts (Figure 4A and 4B).

Because previous EPS of the patient had indicated a Mahaim-type bypass with proximal insertion at the AV node, this region was studied in serially cut sections. This procedure confirmed the presence of 3 small nodoventricular tracts passing through the central fibrous body, thus connecting the AV node with the working myocardium of the interventricular septum (Figure 3B and 3C).

Discussion
This family with the R302Q mutation in PRKAG2 had ECG and EPS evidence of a remarkably large number of bypasses with AV node–like properties. Moreover, there was a high prevalence of AV block. Although these functional derangements seemed unconnected at first, their interdependence was revealed by histopathologic analysis, available in 1 patient,
which showed multiple nodoventricular bypasses that originated in the proximal AV node. With this particular anatomy, the highly unusual pattern of high-grade AV block with preexcited conducted beats may be simply explained by the fact that block in the proximal AV node must also result in failure of conduction into the accessory pathways, because these pathways lie directly distally to it. Yet, in previous studies on this mutation, bypasses with AV node–like properties were not always reported. Similarly, we found no bypasses during EPS in 3 mutation carriers with ECG signs of preexcitation. These disparities may be explained by the fact that such bypasses are difficult to find during EPS because of their peculiar anatomy and functional properties. In any case, the presence of the otherwise rare nodoventricular bypasses supports the novel insight that the R302Q mutation in PRKAG2 may disrupt cardiac development. This observation would be consistent with the role of AMPK in gene transcription and previous evidence for cardiac developmental disorders resulting from aberrant transcription factors (eg, NKX2.5). How true accessory pathways and AV block arise in R302Q carriers is a matter of speculation. AMPK may modulate development of the cardiac conduction system and AV node, as it phosphorylates the transcriptional coactivator p300, which interacts with transcription factors involved in the development of the cardiac conduction system and AV node (eg, NKX2.5). Moreover, p300 dysregulation may result in remnant accessory pathways, because such pathways are present at birth, but removed by apoptosis over the subsequent weeks or months, and p300 prevents apoptosis. AV block may result from conduction slowing because of sodium channel dysfunction, as AMPK increases sodium current density. Thus, impaired AMPK function secondary to loss of function in PRKAG2 caused by the R302Q mutation results in reduced sodium current and conduction slowing.

Our data contrast sharply with previous findings in the N488I mutation, in which the most prominent feature was cardiac hypertrophy, which was explained by intracellular glycogen accumulation. Moreover, preexcitation in N488I transgenic mice was explained by structural disruptions of the annulus fibrosus because of glycogen-laden myocytes. Support for a causal relation between glycogen accumulation and preexcitation was provided by an elegant study in a mouse model in which N488I mutant transgene expression could be suppressed by doxycycline administration. Transgene suppression (if started at a sufficiently young age) resulted in reduction in glycogen accumulation. This was associated with a reduction in the proportion of mice that exhibited preexcitation. Conversely, on discontinuation of transgene suppression, preexcitation redeveloped. These studies also revealed that transgene suppression throughout embryonic development and during the first 8 weeks after birth prevented preexcitation. Of interest, discontinuation of transgene suppression after 8 weeks did not result in the emergence of preexcitation, despite the occurrence of vacuolated myocytes, a sign of glycogen accumulation. This finding suggests that normal functioning of PRKAG2 during embryonic development and the first weeks after birth ensures robust development of the annulus fibrosus and prevents development of preexcitation later in life. This observation would seem to support our proposal that PRKAG2 is involved in cardiac development. In any case, the overall findings of that study are consistent with the notion that preexcitation in N488I
carriers and N488I transgenic mice results from the loss of fibrous separation between atrial and ventricular myocardium, secondary to glycogen loading, rather than from true accessory pathways. In contrast, we did find true accessory pathways. Conversely, we were unable to detect glycogen accumulation, although we expected to find it, based on the existing literature. We have, therefore, conducted electron microscopy to confirm that glycogen accumulation was truly absent. Moreover, we found clear myocardial disarray on histopathologic analysis, although this was absent from patients\(^2\) and mice\(^3\) with the N488I mutation. The phenotypic disparities between the R302Q mutant and the N488I mutant are unexplained. It is speculated that they may be based on the fact that the distinct effects of PRKAG2 (cellular energy control\(^1\) and gene transcription\(^8\)) are disrupted to different degrees by altered PRKAG2 function associated with the various mutations. Although N488I constitutively activates AMPK, the R302Q mutation causes loss of function of AMPK.\(^15\) We cannot rule out that our patient III-8 had, in addition to the R302Q mutation, another mutation in a sarcomere protein-encoding gene that is associated with a typical form of hypertrophic cardiomyopathy (with myocardial disarray). There was, however, no DNA available to test this possibility. With regard to the fact that we found no glycogen accumulation, whereas previous studies on the R302Q mutation did, we speculate that this is due to species differences, as these previous studies were conducted in mice.\(^18\)

A clinical implication to follow from our finding that preexcitation in R302Q carriers is supported by radiofrequency catheter ablation. Indeed, in R302Q transgenic mice, successful abolishment of preexcitation by such ablation was demonstrated.\(^19\) Also of clinical relevance, the high prevalence of AV block and sudden death at a young age indicate that pacemaker implantation must be considered in R302Q carriers.

In summary, our findings support the view that preexcitation based on the R302Q mutation in PRKAG2 is associated with Mahaim fibers, and they foster the novel insight that PRKAG2 may be involved in the development of the cardiac conduction system.

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None.

**References**


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**CLINICAL PERSPECTIVE**

Familial preexcitation syndrome is linked to mutations in *PRKAG2*. The phenotypic effects may be mutation specific. By conducting clinical, electrocardiographic, electrophysiological, and histopathologic studies in a family with the R302Q mutation, we found evidence that this mutation is associated with a high prevalence of sudden death with atrioventricular block and preexcitation based on nodoventricular accessory pathways. These findings indicate that pacemaker implantation should be considered in carriers of this mutation. We also show that catheter ablation may effectively abolish preexcitation. The high prevalence of the otherwise rare nodoventricular pathways supports involvement of *PRKAG2* in the development of the cardiac conduction system.
Nodoventricular Accessory Pathways in \textit{PRKAG2}-Dependent Familial Preexcitation Syndrome Reveal a Disorder in Cardiac Development


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