Nodoventricular accessory pathways in PRKAG2-dependent familial preexcitation syndrome reveal a disorder in cardiac development

Hanno L. Tan, MD, PhD, Allard C. van der Wal, MD, PhD; Maria E. Campian, MD; Hittjo H. Kruyswijk, MD, PhD; Bram ten Hove Jansen, MD; Dirk-Jan van Doorn, MD; Henk J. Oskam, MD; Anton E Becker, MD, PhD; Arthur A.M. Wilde, MD, PhD

1Heart Failure Research Center and Departments of 2Cardiology and 3Pathology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands, 4Department of Cardiology, Spaarne Hospital, Hoofddorp, The Netherlands, and 5Department of Cardiology, Groene Hart Hospital, Gouda, The Netherlands

Short title: PRKAG2 is linked to nodoventricular pathways

Total word count: 2540

Correspondence:
Hanno L. Tan, MD, PhD
Department of Clinical and Experimental Cardiology
Heart Failure Research Center
Academic Medical Center
Meibergdreef 9
NL - 1105AZ Amsterdam
The Netherlands
Phone 31-20-5663264
Fax 31-20-6975458
Email h.l.tan@amc.nl
Abstract

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 had died prematurely. The R302Q mutation was found in 8 living subjects, and 2 deceased (obligate carriers). Cardiac hypertrophy was found in 7 mutation carriers. ECGs compatible with preexcitation were found in 13 subjects, AV block at varying degrees in 5. All mutation carriers had ECG evidence of preexcitation and/or AV block. Three individuals had high-grade AV block with preexcited conducted beats. Electrophysiologic studies in 3 individuals revealed bypasses with AV node-like properties. Histopathologic studies of one 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, 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.

Key words
preexcitation syndrome, AV block, conduction system, Mahaim fibers, hypertrophic cardiomyopathy
Introduction

Familial preexcitation (Wolff-Parkinson-White) syndrome is linked to mutations in PRKAG2, the gene that encodes the regulatory $\gamma_2$-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’s and Pompe’s disease\(^2\). 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 due to glycogen-laden myocytes\(^3\).

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 colleagues arise from the AV node and insert into the right ventricle (nodoventricular)\(^4\), 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 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 (ECG) and electrophysiologic 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 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. Upon request of the patients, we have included some minor non-relevant changes in the pedigree, as presented in Figure 1, with the aim of making it less recognizable and protect the patients’ and family’s anonymity.

Molecular genetics
Genomic DNA was extracted from lymphocytes using standard procedures. The R302Q mutation in PRKAG2 was confirmed by sequence analysis.

Statement of responsibility
The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree 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 carriership) in 2 deceased. 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 one prematurely deceased (see below), and by echocardiography in 6 living mutation carriers (Table 1).

ECG analysis
ECGs compatible with preexcitation were found in 13 subjects, AV block at varying degrees in 5, and atrial fibrillation in 2. All mutation carriers had ECG 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 three individuals, bypasses with decremental properties were found; in the other three, 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 grams). 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 (PAS) and PAS-diastase (PAS-D), respectively, in order to evaluate the glycogen content of myocardium. In all
sections, the amounts of PAS 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. In order to test the validity of the glycogen stains, we also investigated paraffin embedded myocardial tissue of 3 hearts obtained from patients who died of non-cardiac 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-B).

Since 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 (Figures 3B-C).
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. While these functional derangements seemed unconnected at first, their interdependence was revealed by histopathologic analysis, available in one 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 three 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, e.g., 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 development of the cardiac conduction system and AV node, e.g., 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/months, and p300 prevents apoptosis. AV block may result from conduction slowing due to 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 due to 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\textsuperscript{16}. 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, upon 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 upon histopathologic analysis, although this was absent from patients\textsuperscript{2} and mice\textsuperscript{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\textsuperscript{17} and gene transcription\textsuperscript{8}) are disrupted to different degrees by altered PRKAG2 function associated with the various mutations. While N488I constitutively activates AMPK, the R302Q mutation causes loss-of-function of AMPK\textsuperscript{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 regards to the fact that we found no glycogen accumulation, while previous studies on the R302Q mutation did, we speculate that this is due to species differences, as these previous studies were conducted in mice\textsuperscript{18}.

A clinical implication to follow from our finding that preexcitation in R302Q carriers is supported by true accessory pathways, is that preexcitation may be treated by radiofrequency catheter ablation. Indeed, in R302Q transgenic mice, successful abolishment of preexcitation by such ablation was demonstrated\textsuperscript{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.
Acknowledgements
The authors thank Drs. Irene M. van Langen and Marcel M.A.M. Mannens, Department of Clinical Genetics, Academic Medical Center, Amsterdam, The Netherlands, for genetic analysis, and Dr. Barbara W. van Paassen, Department of Clinical Genetics, Academic Medical Center, Amsterdam, The Netherlands, for constructing Figure 1.

Funding sources
Dr. Tan was supported by the Royal Netherlands Academy of Arts and Sciences (KNAW) and the Netherlands Organization for Scientific Research (NWO, grant ZonMW-Vici 918.86.616).

Disclosures
None.
References


Legends

Figure 1
Pedigree. +, carrier of R302Q mutation; -, non-carrier of R302Q mutation; ?, DNA analysis or clinical investigation not conducted; obl, obligate carrier; sd, sudden death (at age in years).

Figure 2
ECG (25 mm/s, 10 mm/mV) of patient 5 showing high-grade AV block with preexcited conducted beats. ECGs shown are from one 12-lead ECG, but the various leads are not all recorded simultaneously.

Figure 3
A. Detail of ventricular septal myocardium showing interweaving and whorling of hypertrophied cardiomyocytes associated with fibrosis (arrows), characteristic for myocardial disarray (Elastic van Gieson stain, bar = 125 μm).
B. Nadoventricular connection (asterisks); AVN= atrioventricular node; F= fibrous tissue of central fibrous body; VM= ventricular myocardium (Elastic van Gieson stain, bar = 1250 μm).
C. Detail of nodoventricular connection (asterisk) at the site of contact with ventricular myocardium (Haematoxylin & Eosin stain, bar = 250 μm).

Figure 4
Glycogen deposition in myocardium.
A. High magnification of myocardium of control patients showing discrete clusters of small bright red staining droplets (arrow) representing glycogen deposits.
B. Myocardium of the patient at same magnification showing similar sized intracellular glycogen deposits (arrow) at approximately the same amount as in Figure 4A. (PAS stains, bar = 60 μm).
Table. 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>Electrophysiologic study</th>
<th>Symptoms</th>
<th>Follow-up</th>
</tr>
</thead>
<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>
</tr>
<tr>
<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>III-2</td>
<td>f</td>
<td>38</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>American Heart Association</td>
<td>Mahaim bypass</td>
<td>pt</td>
<td>+</td>
</tr>
<tr>
<td>III-4</td>
<td>m</td>
<td>36</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Septal bypass</td>
<td>pt</td>
<td>+</td>
<td></td>
</tr>
<tr>
<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>
</tr>
<tr>
<td>III-9</td>
<td>m</td>
<td>43</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NP</td>
<td>NP</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>IV-1</td>
<td>f</td>
<td>31</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NP</td>
<td>NP</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>IV-2</td>
<td>m</td>
<td>32</td>
<td>NP</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>no bypass</td>
<td>pt</td>
<td></td>
</tr>
<tr>
<td>IV-4</td>
<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>
</tr>
<tr>
<td>IV-5</td>
<td>f</td>
<td>25</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NP</td>
<td>pt</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>IV-6</td>
<td>m</td>
<td>27</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NP</td>
<td>pt</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>IV-8</td>
<td>f</td>
<td>20</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>NP</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>IV-9</td>
<td>m</td>
<td>22</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NP</td>
<td>NP</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>IV-10</td>
<td>m</td>
<td>11</td>
<td>+</td>
<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>NP</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

+=present; -=absent; f=female; m=male; NP=not performed or no echocardiography; pt=paroxysmal tachycardia; sd=sudden death (age, years)
Nodoventricular Accessory Pathways in PRKAG2-dependent Familial Preexcitation Syndrome
Reveal a Disorder in Cardiac Development
Hanno L. Tan, Allard van der Wal, Maria Campian, Hittjo Kruyswijk, Bram ten Hove Jansen,
Dirk-Jan van Doorn, Henk Oskam, Anton Becker and Arthur Wilde

Circ Arrhythm Electrophysiol. published online September 12, 2008;
Circulation: Arrhythmia and Electrophysiology is published by the American Heart Association, 7272 Greenville Avenue,
Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-3149. Online ISSN: 1941-3084

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circep.ahajournals.org/content/early/2008/09/12/CIRCEP.108.782862

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in
Circulation: Arrhythmia and Electrophysiology can be obtained via RightsLink, a service of the Copyright Clearance
Center, not the Editorial Office. Once the online version of the published article for which permission is being
requested is located, click Request Permissions in the middle column of the Web page under Services. Further
information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Arrhythmia and Electrophysiology is online at:
http://circep.ahajournals.org//subscriptions/