Novel PRKAG2 Mutation Responsible for the Genetic Syndrome of Ventricular Preexcitation and Conduction System Disease With Childhood Onset and Absence of Cardiac Hypertrophy

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Background—We recently reported a mutation in the PRKAG2 gene to be responsible for a familial syndrome of ventricular preexcitation, atrial fibrillation, conduction defects, and cardiac hypertrophy. We now report a novel mutation in PRKAG2 causing Wolff-Parkinson-White syndrome and conduction system disease with onset in childhood and the absence of cardiac hypertrophy.

Methods and Results—DNA was extracted from white blood cells obtained from family members. PRKAG2 exons were amplified by polymerase chain reaction and were screened for mutations by direct sequencing. The genomic organization of the PRKAG2 gene was determined using inter-exon long-range polymerase chain reaction for cDNA sequence not available in the genome database. A missense mutation, Arg531Gly, was identified in all affected individuals but was absent in 150 unrelated individuals. The PRKAG2 gene was determined to consist of 16 exons and is at least 280 kb in size.

Conclusions—We identified a novel mutation (Arg531Gly) in the γ-2 regulatory subunit (PRKAG2) of AMP-activated protein kinase (AMPK) to be responsible for a syndrome associated with ventricular preexcitation and early onset of atrial fibrillation and conduction disease. These observations confirm an important functional role of AMPK in the regulation of ion channels specific to cardiac tissue. The identification of the cardiac ion channel(s) serving as substrate for AMPK not only would provide insight into the molecular basis of atrial fibrillation and heart block but also may suggest targets for the development of more specific therapy for these common rhythm disturbances. (Circulation. 2001;104:3030-3033.)

Key Words: Wolff-Parkinson-White syndrome • genetics • hypertrophy • arrhythmia

The Wolff-Parkinson-White (WPW) syndrome is characterized by electrocardiographic evidence of ventricular preexcitation, which predisposes to supraventricular arrhythmias.1 Tachycardias mediated by accessory atrioventricular connections, the anatomic substrate for WPW, are the most common tachycardias in children <12 years of age.2 WPW as a cause of sudden cardiac death (SCD), presumably as a result of rapidly conducting atrial fibrillation, is well recognized.3 The prevalence of WPW as a cause of SCD remains unknown because histological evidence is seldom sought at autopsy. However, a recent study of SCD victims <35 years of age for whom ECGs were available identified WPW in 10.5% of cases.4 Atrioventricular connections were confirmed by histological examination.4 We recently identified3 2 families with familial ventricular preexcitation associated with a high incidence of atrial fibrillation, conduction defects, and cardiac hypertrophy, and we established the responsible genetic defect to be a mutation in the gene that encodes the γ-2 regulatory subunit (PRKAG2) of AMP-activated protein kinase (AMPK). Paroxysmal atrial fibrillation was particularly frequent, and chronic atrial fibrillation was present in 80% of patients >50 years of age.

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We now report a third, unrelated family, which also has ventricular preexcitation, atrial fibrillation, and conduction defects. The third family has more severe disease, with onset in childhood instead of adolescence. No cardiac hypertrophy is present. DNA analysis of the third family shows the defect to be a novel mutation in PRKAG2. These results also emphasize that AMPK plays a significant role in cardiac development and ion channel regulation.
Methods

Study Cohort

Informed, written consent was obtained from study participants. Subjects were evaluated by a detailed history, physical examination, 12-lead electrocardiography, and 2D echocardiography. Study participants consisted of a proband with 8 family members. Ventricular preexcitation was diagnosed on the basis of a short PR interval (<120 ms) with widened QRS interval (>110 ms) and abnormal initial QRS vector (δ wave). Conduction system disease was diagnosed if evidence of sinus node dysfunction or atrioventricular block was demonstrated on ECG. Twelve-lead ECGs were retrieved from archived medical records when possible.

Mutation Detection and Analysis

Exon-intron boundaries of the protein-encoding sequences of PRKAG2 were identified in the GenBank database, as previously described. For protein-encoding sequences (base pairs 205 to 556) not available by the sequence sampling approach, primers were designed from cDNA sequences and inter-exon long-range polymerase chain reaction (PCR) (Elongase, GIBCO) was used to determine exon-intron boundaries. Intronic primers were designed on the basis of exon-intron boundaries. Genomic DNA fragments were amplified by PCR, and the products were purified using the QIAquick PCR purification kit (QIAGEN). Direct sequencing reactions were performed in both the sense and antisense directions on an ABI PRISM 377 (Perkin-Elmer Applied Biosystems) using big dye chemistry.

Results

Clinical Findings

A 3-generation family with 4 affected individuals diagnosed as having Wolff-Parkinson-White syndrome was studied. The proband (II-1 in Figure 1A), a 43-year-old white male, experienced recurrent syncopal events beginning at age 2 and extending through adolescence that were associated with atrial fibrillation and heart rates approaching 230 bpm (Figure 2A and 2B). Ventricular preexcitation on resting ECG was noted at age 2. By age 10, he had undergone 6 electrical cardioversions and continued to have paroxysms of rapid atrial fibrillation and 1:1 atrial flutter. At age 13, paroxysms of atrial fibrillation were followed by ventricular escape rhythms of right bundle branch morphology at rates of 20 to 30 bpm with resultant syncope. A permanent pacemaker was implanted. He has been in chronic atrial fibrillation since age 15 and is currently dependent on ventricular pacing. Since age 33, the patient has required antihypertensive therapy for persistent hypertension. Recent 2D echocardiography showed normal left ventricular (LV) function, interventricular septal thickness of 9 mm, and maximal LV free wall thickness of 11 mm (Figure 2C). LV mass is normal when corrected for...
body surface area. The proband’s father (I-1), diagnosed with Wolff-Parkinson-White syndrome, died suddenly at age 31. The proband’s younger brother (II-3), now 41 years of age, presented with a “seizure” at age 6, which was attributed to a tachycardia associated with Wolff-Parkinson-White syndrome. This patient experienced a clinical course similar to the proband, requiring permanent pacemaker implantation at age 22 because of recurrent syncope in the setting of severe sinus bradycardia and chronotropic incompetence. Two-dimensional echo at age 41 is normal. Interestingly, he has required antihypertensive therapy since age 23. An 8-year-old daughter (III-3) of the proband shows evidence of ventricular preexcitation on ECG but remains asymptomatic.

**Genomic Structure of the PRKAG2 Gene**

We determined that the PRKAG2 cDNA sequence extending from base pairs 205 to 556 encode 2 exons, exon 2 (base pairs 205 to 276) and exon 3 (base pairs 277 to 556), respectively. The splice junctions comply with the ag-gt rule and are as follows: exon 2, 5′-tc ca cc ct gga ac tcgagctcct...ctctct cgc aagagtaagacctt-3′ and exon 3, 5′-tcttc tag gtggacagcc-tttttctgc ag...c tc gtc c...c tc gtc c-3′. The genomic position of exons 1 and 4 to 16 were determined from P1-derived artificial chromosome clones RP11-796I2 (AC074257), RP5-1127D14 (AC006358), RP4-563H24 (AC006966). Analysis of genomic sequence data indicates the PRKAG2 gene is ≥280 kb in size located at 7q36 of chromosome 7.

**Missense Mutation in the PRKAG2 Gene**

A missense mutation, Arg531Gly, in exon 15 of PRKAG2 was identified and shown to be present in all living affected family members. The mutation results from guanine (G) substituted for cytosine (C) at nucleotide 1681 (GenBank accession number AJ249976). This base pair substitution substituted for cytosine (C) at nucleotide 1681 (GenBank accession number AJ249976). This base pair substitution resulted in a glycine (G) at codon 531. Thus, the nucleotide change in the PRKAG2 gene occurred by the following: (1) The mutation was present in all affected family members; (2) the mutation replaces a highly conserved, positively charged arginine residue with a neutrally charged glycine; and (3) the mutation was not present in 150 unrelated normal subjects.

Determination of the intron-exon structure of the PRKAG2 gene showed that it consists of 16 exons and is ≥280 kb in size. Recently, it was determined that a smaller transcription product of PRKAG2 exists as a result of an alternative transcription initiation site in intron 4.6 Thus, this smaller transcript corresponds to exons 5 to 12 of the full-length transcription product. The full-length and truncated transcripts, denoted PRKAG2a and PRKAG2b, respectively, are highly expressed in cardiac tissue.6,7 The previously and currently described mutations are present in exons 7 and 15, and are therefore common to both PRKAG2 transcription products.

AMPK, a serine/threonine kinase, is known to have multiple cellular functions, which may account for the diverse phenotype of ventricular preexcitation, conduction system disease, and cardiac hypertrophy, as has been emphasized in recent studies.5,8 Evidence suggests that AMPK regulates gene transcription,9 and mutations in transcription factors are known to induce congenital heart malformations.10 The mutant AMPK presumably alters atrioventricular septation during cardiogenesis, leading to the presence of accessory atrioventricular fibers responsible for ventricular preexcitation. Atrial fibrillation may occur in ≤15% of sporadic WPW cases.11 The much higher incidence of atrial fibrillation and conduction defects observed in this syndrome suggests that AMPK, through phosphorylation, regulates cardiac ion channels.12 The identification of the cardiac ion channel(s) serving as substrate for AMPK not only would provide insight into the molecular basis of these common rhythm disturbances but also would suggest targets for the development of more specific therapy. This is particularly significant in consideration of the fact that the molecular mechanism for atrial fibrillation from any cause remains elusive. Interestingly, systemic hypertension was present, as was also observed in our previous large kindred. AMPK regulates endothelial nitric oxide synthase (eNOS), a key regulator of blood pressure homeostasis.13 The eNOS mouse knockout model exhibits a phenotype of hypertension and hypertrophy.14 Thus, impaired regulation of eNOS may induce hypertrophy and hypertension.

**Discussion**

We identified a kindred with ventricular preexcitation, rapidly conducting paroxysms of atrial fibrillation and flutter, with progression to high-grade conduction disease. Clinical presentation of symptomatic arrhythmias occurred in early childhood, compared with a clinical presentation in adulthood in the 2 families we have reported previously.5 Despite the early onset of clinical disease, left ventricular hypertrophy was not detected even though affected individuals were in their fifth decade. The absence or variable expression of cardiac hypertrophy in this syndrome may reflect the influence of genetic background. In previous families, we recently demonstrated a mutation in PRKAG2 to be responsible for this clinical syndrome.5 The disease in the present family is caused by a novel mutation (Arg531Gly) in the same gene. The causality of the mutation for the phenotype in this family is confirmed by the following: (1) The mutation was present in all affected family members; (2) the mutation replaces a highly conserved, positively charged arginine residue with a neutrally charged glycine; and (3) the mutation was not present in 150 unrelated normal subjects.

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