Transgenic Mouse Model of Ventricular Preexcitation and Atrioventricular Reentrant Tachycardia Induced by an AMP-Activated Protein Kinase Loss-of-Function Mutation Responsible for Wolff-Parkinson-White Syndrome

Jasvinder S. Sidhu, MD; Yadavendra S. Rajawat, MD; Tapan G. Rami, MD; Michael H. Gollob, MD; Zhinong Wang, MD, PhD; Ruiyong Yuan, MD; A.J. Marian, MD; Francesco J. DeMayo, MD; Donald Weibacher, MD; George E. Taffet, MD; Joanna K. Davies, PhD; David Carling, PhD; Diras S. Khoury, PhD; Robert Roberts, MD

Background—We identified a gene (PRKAG2) that encodes the γ-2 regulatory subunit of AMP-activated protein kinase (AMPK) with a mutation (Arg302Gln) responsible for familial Wolff-Parkinson-White (WPW) syndrome. The human phenotype consists of ventricular preexcitation, conduction abnormalities, and cardiac hypertrophy.

Methods and Results—To elucidate the molecular basis for the phenotype, transgenic mice were generated by cardiac-restricted expression of the wild-type (TG WT) and mutant (TG R302Q) PRKAG2 gene with the cardiac-specific promoter -myosin heavy chain. ECG recordings and intracardiac electrophysiology studies demonstrated the TG R302Q mice to have ventricular preexcitation (PR interval 10 ± 2 versus 33 ± 5 ms in TG WT, P < 0.05) and a prolonged QRS (20 ± 5 versus 10 ± 1 ms in TG WT, P < 0.05). A distinct AV accessory pathway was confirmed by electrical and pharmacological stimulation and substantiated by induction of orthodromic AV reentrant tachycardia. Enzymatic activity of AMPK in the mutant heart was significantly reduced (0.009 ± 0.003 versus 0.025 ± 0.001 nmol · min⁻¹ · g⁻¹ in nontransgenic mice), presumably owing to the mutation disrupting the AMP binding site. Excessive cardiac glycogen was observed. Hypertrophy was confirmed by increases in heart weight (296 versus 140 mg in TG WT) and ventricular wall thickness.

Conclusions—We have developed a genetic animal model of WPW that expresses a mutation responsible for a familial form of WPW syndrome with a phenotype identical to that of the human, including induction of supraventricular arrhythmia. The defect is due to loss of function of AMPK. Elucidation of the molecular basis should provide insight into development of the cardiac conduction system and accessory pathways. (Circulation. 2005;111:21-29.)

Key Words: Wolff-Parkinson-White syndrome ■ genetics ■ tachycardia

Wolff-Parkinson-White syndrome (WPW) has remained a fascination for the cardiologist, particularly for the electrophysiologist. This is in part because WPW is the sine qua non of reentry as a mechanism responsible for arrhythmias. Reentry predominates as the mechanism for arrhythmias in the atria and ventricles from many different causes. Although the molecular basis for this disorder has remained unknown, extensive anatomic, electrical, and physiological research has been pursued in humans.1 Research in humans has targeted the accessory pathway that bypasses the atrioventricular (AV) node and is responsible for the short PR interval with a slurring of the initial segment of the QRS complex, known as a delta wave. This syndrome is the second most common cause of supraventricular arrhythmias in the Western world and the most common cause in Asian countries such as China.2 Although it is usually associated with symptoms such as palpitations and presyncope, if sustained, it can induce symptoms of heart failure. It is also known to initiate atrial fibrillation, which may deteriorate into sudden death.3 The accessory pathway bypassing the AV node is in itself a curiosity. Several such accessory pathways are present at birth, but they coalesce or are absorbed (apoptosis) over the subsequent weeks or months.4 Thus, the presence of the accessory pathway in adulthood presumably reflects some cardiac developmental abnormality.

We were surprised to find that the genetic defect responsible for WPW in families we have studied5 was a missense mutation in the gene PRKAG2 that encodes for the γ-2
subunit of AMPK (AMP-activated protein kinase). Subsequently, other investigators have shown the gene to be responsible for WPW in many other families. The functions of AMPK are pleiotropic. Through its action of phosphorylation, it plays a major role in the synthesis and distribution of ATP primarily by regulating carbohydrate and fatty acid metabolism. AMPK mediates many of the actions of leptin and adiponectin and of the oral diabetic drugs, such as metformin. To explore the molecular basis whereby this defect generates a phenotype of WPW and reentrant arrhythmias, we generated a transgenic mouse model by expressing the human mutant PRKAG2 gene. The mouse exhibited a phenotype of preexcitation, inducible orthodromic AV reentrant tachycardia, cardiac hypertrophy, and excessive cardiac glycogen, as observed in the human phenotype. Using this model, we have shown the primary molecular defect is loss of cardiac AMPK activity. This appears to be due to the mutation disrupting the γ2 subunit binding site for AMP, which is required for activation of AMPK activity. The model provides a means to further elucidate the molecular network that leads to development of the accessory pathway (preexcitation), supraventricular arrhythmias, and hypertrophy.

Methods

Transgene Construct

The transgene construct consisted of an α-myosin heavy chain (MHC) promoter, PRKAG2 cDNA, 3′ UTR human growth hormone. The construct was generated by placing an insert (PRKAG2 cDNA) within the cloning site of a cassette that contained the α-MHC promoter and a 3′ UTR human growth hormone. To obtain a PRKAG2 cDNA, human RNA was converted to cDNA and amplified by reverse transcription polymerase chain reaction (RTPCR). A SalI restriction site was attached at each end of the cDNA fragment. Site-directed mutagenesis was used to generate a missense mutation that resulted in a substitution of an arginine for glutamine at residue 302, a mutation responsible for familial WPW in humans. The cDNA was subcloned and sequenced from end to end. Inserts containing mutated PRKAG2 cDNA and wild-type PRKAG2 cDNA were excised with SalI restriction enzymes and reinserted into the α-MHC cassette. PCR and direct sequencing were performed to determine whether the insert was in the correct direction. The transgene construct that contained the α-MHC promoter, PRKAG2 cDNA, and 3′ UTR human growth hormone was then released by BamHI.

Generation of Transgenic Mice

Multiple injections of PRKAG2 cDNA were performed to develop 3 lines of transgenics that expressed the mutant gene, substitution of arginine for glutamine at residue 302 (referred to as TGR302Q mice), and 2 lines of transgenics that expressed the normal human cDNA, referred to as the wild type (TGWT). Linearized transgene was microinjected into fertilized FVB mouse oocytes.

Genotyping

Founders of each transgenic line were identified by PCR screening. The primers, specific for the human PRKAG2 cDNA, in the forward direction were 5′-GGGATCAGGTTTCTCTCCGGCTCCA-3′ and in the reverse direction, 5′-CCATAGTTATTTATATGATGCTATAGT-3′. PCR was performed on the genomic DNA isolated from biopsies of the mice tails with the QIAGEN DNeasy kit, and the product was sequenced.

Transgene Expression

Expression of the transgene in the heart was assessed by RTPCR and Northern blot analysis. Mouse myocardial tissue was homogenized in Trizol reagent and the RNA ethanol precipitated from the homogenate. One-step RTPCR was performed on RNA from TGWT and TGR302Q mice. Primers mentioned previously were used to amplify DNA, and the products were sequenced. Northern blot analysis was performed with a probe specific for the human γ2 subunit cDNA. Ten micrograms of RNA was loaded on denaturing RNA gels, and transferred to a blotting membrane. Membranes were then probed with 32P-labeled human specific probes by the rapid hybridizing technique. The membrane with labeled probe was overlaid with radiographic film and exposed overnight at −70°C.

Myocardial AMPK Enzymatic Activity

Homogenates of heart from TGWT and TGR302Q mice were obtained to isolate AMPK. The AMPK protein was immunoprecipitated from the homogenate with either an antibody specific for the γ2 subunit or an antibody that recognizes both the β-1 and β-2 subunits (pan-β). In each case, the antibodies (γ-2 or pan β-2) cross-react with both human and mouse AMPK. Enzymatic activity was determined in the absence or presence of 0.2 mmol/L AMP as described previously and reported in nmol·min⁻¹·g⁻¹ wet weight.

Histopathology

Mouse hearts were removed, weighed, and fixed in 10% formalin. Total body weight was recorded, and heart weight was expressed as a ratio. One-micrometer sections of the hearts were prepared and stained with hematoxylin and eosin stain or periodic acid Schiff (PAS). PAS staining was used to determine whether there was deposition of glycogen.

Markers of Hypertrophy

Molecular markers (atrial natriuretic peptide and brain natriuretic peptide) known to be expressed during cardiac hypertrophy were assessed by Northern blot analysis. Analysis was performed on myocardial samples from TGR302Q, TGWT, and nontransgenic mice.

Echocardiography

Mice were anesthetized by isoflurane inhalation (isoflurane 1.5%, oxygen 98.5%). Limbs were attached to electrodes for ECG monitoring. M-mode echocardiograms of the left ventricle were recorded at the tip of the mitral valve apparatus with a 5- to 7.5-MHz transducer. Septal and posterior wall thickness, left ventricular end-systolic diameter (ESD), and left ventricular end-diastolic diameter (EDD) were measured, and percent fractional shortening was calculated as (ESD − ESD)/EDD as described previously.

Electrocardiography and Electrophysiology Studies

For ECG screening, mice were anesthetized by intraperitoneal injection (15 mL/kg) of a mixture of sodium pentobarbital (6.61 mg/mL), sodium chloride (4.13 mg/mL), and ethanol (0.18 mL/mL). A body-surface ECG (leads I, II, and III) was recorded in 134 TGR302Q mice starting at 35±16 days of age and in 41 TGWT mice at 88±3 days of age. The ECG was filtered between 0.5 and 500 Hz and sampled at 1 kHz (model MP100, BIOPAC Systems).

To conduct electrophysiology studies, mice were anesthetized, and 6 limb leads were continuously recorded. An 8-electrode lumen catheter (1.1F, model EPR-801, Millar Instruments, or 2F, model CIBer, NuMED) was inserted through the jugular vein and advanced to the tip of the mitral valve apparatus with a 5- to 7.5-MHz transducer. Septal and posterior wall thickness, left ventricular end-systolic diameter (ESD), and left ventricular end-diastolic diameter (EDD) were measured, and percent fractional shortening was calculated as (ESD − ESD)/EDD as described previously.

Electrical stimulation was applied through the catheter electrodes with an external stimulator (model S8800; AstroMed). After a period of baseline rhythm, decremental atrial pacing was applied whereby the cycle length was repeatedly shortened by 10 ms, starting from a pacing cycle length of 20 ms less than the intrinsic sinus cycle length, until continuous atrial capture could not be maintained. After 10 seconds of pacing at each cycle length, sinus node recovery time

...
was determined as the longest pause from the last paced atrial depolarization to the first sinus return cycle. Atrial pacing cycle lengths resulting in Wenckebach and 2:1 AV conduction were also noted. Subsequently, refractory periods were determined by delivering an 8-stimulus drive train (S1) at a cycle length of 100 ms followed by a premature stimulus (S2) that was progressively decremented in 10-ms intervals. The effective refractory period was defined as the longest S1-S2 coupling interval that failed to generate a propagated beat with S2 in 2 separate attempts. Inducibility of arrhythmias was investigated by delivering 2 extrastimuli (S2 and S3) that were programmed at progressively shorter intervals after a drive train (S1). Procainamide was administered intravenously (30 mg/kg) to further confirm the presence of accessory pathways.

Statistical Analysis
Continuous variables were expressed as mean±SD. Differences between TG<sub>R302Q</sub> and TG<sub>wt</sub> mice were compared by Student’s t test. ANOVA was applied for multiple comparisons.

Results
Genotype and Expression of PRKAG2 in Transgenic Mice
One hundred forty mice were transgene positive in the 3 mutant lines, and 50 mice were transgene positive in the 2 wild-type lines. Expression of the transgene was confirmed at the RNA level by Northern analysis (Figure 1). The mouse heart was assessed for protein expression by determining AMPK enzymatic activity in TG<sub>wt</sub>, TG<sub>R302Q</sub>, and nontransgenic mice as indicated subsequently (Figure 2).

Histology of Transgenic Mice
TG<sub>wt</sub> mice, TG<sub>R302Q</sub> mice, and controlled nontransgenic mice were matched for gender and age to compare cardiac mass. The average body weight was similar (28, 24, and 26 g for mutant, wild-type, and nontransgenic mice, respectively). In contrast, the weight of the mutant hearts averaged 296.67 mg, which was twice that of the wild-type mice (140 mg, \( P<0.001 \)) and nearly 3 times that of the nontransgenic mice (117 mg, \( P<0.001 \)). Gross appearance of the hearts appeared normal except for the increase in size of the TG<sub>wt</sub> and TG<sub>R302Q</sub>. Hematoxylin-and-eosin stains showed that myocytes of the TG<sub>R302Q</sub> and TG<sub>wt</sub> mice exhibited multiple nuclei with great variation in size, in keeping with hypertrophy. These changes were observed consistently throughout the right and left ventricles of the wild-type and mutant mice. In the mutant mice, large vacuoles were abundant throughout the myocardium of the right and left ventricles. PAS staining confirmed the vacuoles were filled with PAS-positive material indicating glycogen (Figures 3 and 4). Myocytes containing glycogen were abundant in the myocardium of the right and left ventricles of the mutant mice but were not present in wild-type or nontransgenic mice. Histology of the myocardium was normal in nontransgenic mice. In the mutant mice, muscle bundles that extended from the atrium to the ventricle in the region of the fibrous body were observed, but it remains to be determined whether these are the accessory pathways responsible for electrical conduction bypassing the AV node.

Myocardial AMPK Enzymatic Activity
Enzymatic activity of AMPK in the heart was assessed after immunoprecipitation of the protein. After immunoprecipitation with anti-\( \gamma \)-2 antibodies, myocardial AMPK activity averaged 0.002±0.001 nmol · min<sup>-1</sup> · g<sup>-1</sup> wet weight in TG<sub>R302Q</sub> mice, 0.009±0.003 nmol · min<sup>-1</sup> · g<sup>-1</sup> wet weight in TG<sub>wt</sub> mice, and 0.025±0.02 nmol · min<sup>-1</sup> · g<sup>-1</sup> wet weight in nontransgenic mice (Figure 2). AMP had no significant effect on AMPK activity under these conditions, similar to a previous study. Thus, TG<sub>R302Q</sub> mice enzymatic activity was only 22% of that of transgenic wild type and <4% of that of nontransgenic mice, which is in keeping with loss of function of AMPK. Despite the presence of AMP, enzymatic activity did not increase significantly. This is in keeping with the mutation disrupting the \( \gamma \)-2 subunit binding site for AMP. To determine total AMPK activity in the heart extracts, AMPK complexes were immunoprecipitated with an antibody that recognizes both \( \beta \)-1 and \( \beta \)-2 (Figure 2). With this assay, TG<sub>R302Q</sub> mice enzymatic activity was 17% of that of TG<sub>wt</sub> (0.005±0.003 versus 0.029±0.019 nmol · min<sup>-1</sup> · g<sup>-1</sup>) and
5% of that of nontransgenic mice (0.094±0.058 nmol · min⁻¹ · g⁻¹). Relative levels of AMPK activity in the hearts were almost identical to those obtained with the γ-2 antibodies, and these results demonstrate that the γ-2 mutation results in a loss of function of AMPK.

Markers of Cardiac Hypertrophy
Densitometry shows a 2-fold increase of brain natriuretic peptide present in mutant mice over that in nontransgenic and transgenic wild-type mice. There was no increase in atrial natriuretic peptide observed in either the mutant or transgenic wild-type mice over that in the nontransgenic mice. There was no correlation with gender.

Echocardiographic Assessment
Echocardiography was performed in 15 wild-type, 12 mutant, and 11 nontransgenic mice (Table). The hearts of the TG$_{R302Q}$ and TG$_{WT}$ mice were hypertrophied, with posterior wall thickness averaging 0.13 cm for TG$_{WT}$ mice (age 2 months) and 0.12 in the TG$_{R302Q}$ transgenic mice (age 2 months) compared with 0.09 for nontransgenics (P<0.001). There was no significant difference between posterior wall thickness of TG$_{WT}$ and TG$_{R302Q}$ mice. There was an increased end-diastolic diameter and end-systolic diameter in both TG$_{WT}$ and TG$_{R302Q}$ mice with decreased systolic function.

Phenotype Characterization by Electrocardiography and Electrophysiology
An ECG (Figure 5) identified 21 TG$_{R302Q}$ mice manifesting ventricular preexcitation at 51±29 days of age, as evidenced by a short PR interval (10±2 versus 33±5 ms in TG$_{WT}$; P<0.001) and wide QRS (20±5 versus 10±1 ms in TG$_{WT}$; P<0.001). Figure 6 summarizes the distribution of 134 TG$_{R302Q}$ mice with and without ventricular preexcitation according to age. ECG recordings were repeated in 12 TG$_{R302Q}$ mice with manifest ventricular preexcitation, and maintenance of preexcitation was confirmed in all mice during observation up to 225 days of age.

An electrophysiology study was performed on 12 TG$_{R302Q}$ mice with manifest ventricular preexcitation and on 11 TG$_{WT}$ mice. TG$_{R302Q}$ mice showed significant prolongation in the sinus cycle length (176±41 versus 118±20 ms in TG$_{WT}$; P<0.001), whereas sinus node recovery times were similar (43±27 versus 45±16 ms in TG$_{WT}$; P=NS).

Evidence of an accessory pathway outside the AV node in TG$_{R302Q}$ mice was confirmed by atrial programmed electrical
stimulation. Decremental atrial pacing resulted in fixed AV conduction in TGR302Q mice until accessory pathway refractoriness was reached, whereas TGWT mice exhibited normal AV nodal response characterized by gradual prolongation in AV conduction. During atrial extrastimulation (Figure 7), blockade of the accessory pathway by a premature stimulus was accompanied by a prolonged PR interval (29 ± 8 ms) and a narrow QRS duration (12 ± 2 ms), which is consistent with normal conduction over the AV node. The accessory pathway refractory period was 76 ± 9 ms, and the AV node refractory period in TGR302Q mice was 44 ± 9 ms (53 ± 11 ms in TGWT, P = NS). The longest atrial pacing cycle length in TGR302Q mice that resulted in AV nodal Wenckebach periodicity was 70 ± 5 ms (71 ± 8 ms in TGWT, P = NS) and the longest that resulted in AV nodal 2:1 conduction was 58 ± 8 ms (61 ± 9 ms in TGWT, P = NS). The atrial effective refractory period in TGR302Q mice was 43 ± 8 ms (45 ± 10 ms in TGWT, P = NS).

Retrograde VA conduction over the accessory pathway was confirmed in TGR302Q mice by decremental ventricular pacing, which resulted in fixed VA conduction. Ventricular programmed electrical stimulation showed that the ventricular refractory period was significantly prolonged in TGR302Q mice (63 ± 6 versus 44 ± 15 ms in TGWT mice, P = 0.024).

### Induction of Orthodromic AV Reentrant Tachycardia

Atrial electrical stimulation induced supraventricular tachycardia in 6 of 12 TGR302Q mice and was consistent with orthodromic AV reentrant tachycardia characterized by antegrade conduction via the AV node and retrograde conduction over the accessory pathway (Figure 8). Only single reentrant beats could be induced in 4 other TGR302Q mice. Reentrant beats or tachycardia could not be induced in 2 TGWT mice. Induced tachycardia duration ranged from 2 to 64 beats, with an average cycle length of 69 ± 2 ms. The VA interval during tachycardia (18 ± 4 ms) was similar to that during ventricular pacing (19 ± 5 ms). Reentrant tachycardia could not be induced by ventricular electrical stimulation. Reentrant single beats or tachycardia could not be induced in TGWT mice.

Atrial premature beats and/or tachycardia were observed spontaneously or after atrial electrical stimulation in 7 (58%) of 12 TGR302Q mice and in 3 (27%) of 11 TGWT mice. Ventricular electrical stimulation did not induce ventricular arrhythmias in either TGR302Q or TGWT mice.

### Pharmacological Blockade

Procainamide blocked the accessory pathway in TGR302Q mice within 3 to 5 minutes of administration. As illustrated in Figure 9, procainamide resulted in sudden prolongation of the PR interval from 11 ± 4 to 27 ± 6 ms and narrowing of the QRS duration from 17 ± 3 to 13 ± 3 ms (n = 8). These sudden ECG changes were not observed in TGWT mice after procainamide administration. Procainamide has been documented in many studies to block conduction in the accessory pathway with relative sparing of AV nodal conduction.14,15

### Discussion

We generated a transgenic mouse that expressed the human PRKAG2 gene containing the mutation R302Q, which is responsible for a phenotype of WPW, conduction system disease, and ventricular hypertrophy with excessive cardiac glycogen. The presence of a functioning accessory pathway bypassing the AV node giving rise to the WPW was demonstrated in TGR302Q mice by the following diagnostic criteria:
baseline ECG recordings exhibited a short PR interval and increased QRS duration with slurred upstroke; (2) atrial pacing blocked the accessory pathway at a long refractory period and was accompanied by immediate prolongation of the PR interval and shortening of the QRS duration (consistent with conduction through the AV node); (3) atrial pacing at a critical cycle length reproducibly provoked AV reentrant beats and tachycardia with antegrade conduction through the AV node and retrograde conduction via the accessory pathway; (4) ventricular pacing resulted in a fixed short VA interval and was similar to the VA interval during reentrant beats or tachycardia (in line with retrograde conduction through the accessory pathway); and (5) procainamide blocked the accessory pathway and abolished ventricular preexcitation, as demonstrated by sudden lengthening of the PR interval and abbreviation of QRS duration (in agreement with normal conduction through the AV node). In keeping with extensive experience,16,17 anesthesia did not affect identification of the accessory pathways. The mutant hearts showed abundant myocardial glycogen. Ventricular hypertrophy was confirmed by a significant increase in weight of the TGwt hearts and more than a doubling of the heart weight in TGR302Q mice. This was confirmed by echocardiographic analysis that showed increased thickness of the posterior wall.

Thus, the phenotype of our animal model is virtually identical to the human phenotype.

AMPK, a metabolic sensor of ATP stores, makes ATP available in response to the cell’s energy demands.9 It inhibits glycogen synthase, which increases the availability of glucose for energy production. Glycogen deposition was markedly increased in the TGR302Q hearts, similar to that observed in the human phenotype.6 A similar increase in cardiac glycogen deposition was observed by Arad et al10 in their transgenic model expressing the N488I missense mutation in the PRKAG2 gene. The present in vivo studies showed minimal cardiac AMPK activity in the transgenic mutant mice, consistent with results of in vitro studies. In vitro studies by Daniel and Carling18 showed marked attenuation or absent enzymatic activity associated with the R302Q mutation. Further in vitro studies by Scott et al19 confirm the mutation R302Q to be associated with loss of enzymatic activity.

Figure 6. Age distribution of TGR302Q mice. Solid bars indicate number of mice exhibiting preexcitation on 3-lead surface ECG.

Figure 7. Simultaneous ECG and intracardiac electrograms during atrial stimulation in TGR302Q mouse with ventricular preexcitation. AV conduction propagated through accessory pathway during stimulation drive train (S1) delivered at cycle length of 100 ms. PR interval and QRS duration and morphology during S1 were similar to intrinsic rhythm. Premature extrastimulus (S2) delivered during accessory pathway refractoriness (70 ms after S1) caused AV conduction to proceed only through AV node, as evidenced by prolonged PR interval and narrowed QRS. LRA indicates low right atrial electrogram; HRA, high right atrial electrogram.
suggest the mechanism to be decreased binding to AMP. The γ-2 subunit has 4 copies of a motif of 60 amino acids referred to as the CBS motif and occurs in tandem pairs to form the Bateman domain.\(^{20}\) The Bateman domain is highly conserved across species, and in more than 1000 proteins, it is responsible for binding adenosyl compounds. Mutations in the CBS sequence are known to cause hereditary diseases, including homocystinuria, retinitis pigmentosa, congenital myotonia, epilepsy, hypercalciuric nephrolithiasis, and Bartter’s syndrome.\(^{20}\) All of these mutations are associated with loss of function of AMPK due to decreased affinity of the Bateman domain for AMP. In assays for cardiac AMPK activity, increasing concentrations of AMP were not accompanied by a corresponding increase in enzymatic activity, perhaps in keeping with previous studies that showed that mutations within the CBS domains of γ-2 interfere with AMP binding and activation of AMPK.\(^ {18,19}\) It is also significant that 3 other AMPK mutations known to cause WPW have also been shown in vitro to be associated with loss of enzymatic activity.\(^ {19}\) Thus, the mutation R302Q occurring in a CBS motif prevents binding of AMP to the γ-2 subunit and is responsible for the phenotype of the transgenic animal model for AMPK. In assays for cardiac AMPK activity, increasing concentrations of AMP were not accompanied by a corresponding increase in enzymatic activity, perhaps in keeping with previous studies that showed that mutations within the CBS domains of γ-2 interfere with AMP binding and activation of AMPK.\(^ {18,19}\) It is also significant that 3 other AMPK mutations known to cause WPW have also been shown in vitro to be associated with loss of enzymatic activity.\(^ {19}\) Thus, the mutation R302Q occurring in a CBS motif prevents binding of AMP to the γ-2 subunit and is responsible for the phenotype of the transgenic animal model and the human phenotype in the families we have studied.\(^ {5}\) Arad et al\(^ {10}\) identified a mutation, N488I, in the PRKAG2 gene to be responsible for WPW. This mutation is associated with increased AMPK activity. The phenotype of increased glycogen deposition is surprising because AMPK is well documented to inhibit glycogen synthesis. The authors suggest the mechanism is increased absorption of glucose. The human phenotype due to the mutation R302Q includes cardiac hypertrophy,\(^ {5}\) and reported families with other AMPK mutations have a similar if not higher incidence of ventricular hypertrophy.\(^ {6,7}\) The mechanism for the hypertrophy is most likely compensatory owing to insufficient ATP made available by the defective AMPK protein, as suggested by Doevendans and Wellens\(^ {21}\) and others.\(^ {22,23}\) However, the wild type also exhibited hypertrophy, which makes it less likely to be compensatory, but perhaps there is some energy deficiency even in the wild type due to decreased enzymatic activity of the human protein relative to that of the mouse.

In the mutant mice, but not in the wild-type or nontransgenic mice, we observed muscle bundles that extended from the atrium to the ventricle in the region of the fibrous body. It remains to be determined whether they are the accessory pathways responsible for electrical conduction bypassing the AV node. These are similar to the muscle bundles observed and reported by Arad et al.\(^ {10}\) Detailed pathological studies that involve sectioning of the AV ring and other portions of the conduction system will be required and are ongoing. Any suspected anatomic substrate will require testing for electrical conduction to prove or disprove its candidacy as the accessory pathway.

The mechanism whereby loss of AMPK activity induces the accessory pathway that leads to WPW remains uncertain. There is no doubt that the accessory pathway provides the requisite portion of the loop to give rise to reentrant supraventricular tachyarrrhythmias. The supraventricular arrhythmia could be induced and clearly inhibited by blocking the accessory pathway. It is also evident that the mutation is necessary for the WPW phenotype, because it occurs only in...
The supraventricular tachycardia could reflect nodal reentry (AV nodal reentrant tachycardia); however, the evidence strongly suggests the accessory pathway is the mechanism. We have clearly demonstrated there is a functioning accessory pathway. The phenotype is identical to that observed in the families we have studied. In addition, the reentry tachycardia was consistently induced only after antegrade block of the accessory pathway during atrial extrastimulus testing. If AV nodal reentrant tachycardia were inducible, one would expect to see some instances of tachycardia induced before accessory pathway block, and possibly bystander accessory pathway conduction during the tachycardia. These features were never observed, which provides strong evidence that AVRT was the mechanism of the tachycardia. Furthermore, the VA interval during tachycardia (18±4 ms) was similar to that during ventricular pacing, which was fixed and nondecremental (19±5 ms) in mutant mice, in line with retrograde conduction over the accessory pathway. In contrast, in wild-type mice, the VA interval was not fixed, and prolongation (decremental conduction) was observed at longer ventricular pacing cycle lengths.

In humans, there are several accessory pathways available for conduction at birth, but they disappear after a few weeks.\(^4\) It is reasonable to assume the altered metabolic state in some way predisposes to the retention or development of the accessory pathway and subsequent WPW. One postulate is that the accessory pathways normally undergo apoptosis, but lack of AMPK activity prevents it. There is also the possibility that the AMPK mutation induces de novo development of an accessory pathway. In the human phenotype, there is also progressive slowing of AV conduction, which not infrequently requires a pacemaker.\(^5\) The gradual progression of conduction impairment in humans may be reflected in the present mouse model by the slower resting heart rate and occasional atrial arrhythmias observed in the TG\(_{R302Q}\) mouse. The mechanism whereby conduction is impaired remains to be elucidated. The cardiac conduction system is more resistant to ischemia and other injury than myocytes, in part because of increased capacity for generation of ATP from glycolysis.\(^24\) The loss of AMPK activity results in increased glycogen synthesis and less glucose available for generation of ATP. Light et al\(^{25}\) have documented that the AMPK R302Q mutation resulted in slowing of open-state inactivation of the sodium channel and prolongation of action potential duration. This may be the explanation for impaired cardiac conduction. We have thus shown the primary defect is loss of AMPK activity due to a mutation that disturbs the AMP binding site. Studies are now under way, in conjunction with Light et al, to further ascertain why the accessory pathway develops and why other features, such as conduction abnormalities, are associated with loss of AMPK activity. Elucidation of

---

**Figure 9.** Simultaneous ECG and intracardiac electrograms during procainamide administration in TG\(_{R302Q}\) mouse with ventricular preexcitation. Procainamide resulted in sudden block of accessory pathway 3 to 5 minutes after intravenous infusion, as evidenced by spontaneous PR interval prolongation and QRS narrowing. RVA indicates right ventricular apical electrogram; RVB, right ventricular basal electrogram; LRA, low right atrial electrogram; and HRA, high right atrial electrogram.
the molecular network whereby loss of AMPK activity mediates WPW and other consequences has the potential to unravel therapeutic targets not just for the treatment of arrhythmias but also for conduction disorders, including hypertrophy.

Acknowledgments
This work was supported in part by an NIH training grant, HL-07706-10 (Dr. Roberts), SCOR, 5P50-HL54313-09 (Dr. Roberts), Modifier Grant, 5R01-HL068884-03 (Dr. Marian), and Research Grant 5R01-HL068768-03 (Dr. Khoury). We appreciate the administrative assistance of Deborah Graustein and Fran Baas in the preparation of this manuscript and figures. We also thank Dr. Jeffrey Robbins for providing the α-myosin heavy chain promoter.

References
Transgenic Mouse Model of Ventricular Preexcitation and Atrioventricular Reentrant Tachycardia Induced by an AMP-Activated Protein Kinase Loss-of-Function Mutation Responsible for Wolff-Parkinson-White Syndrome


Circulation. 2005;111:21-29; originally published online December 20, 2004;
doi: 10.1161/01.CIR.0000151291.32974.D5

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/111/1/21

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation 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 is online at:
http://circ.ahajournals.org/subscriptions/