Reversibility of \textit{PRKAG2} Glycogen-Storage Cardiomyopathy and Electrophysiological Manifestations

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Background—\textit{PRKAG2} mutations cause glycogen-storage cardiomyopathy, ventricular preexcitation, and conduction system degeneration. A genetic approach that utilizes a binary inducible transgenic system was used to investigate the disease mechanism and to assess preventability and reversibility of disease features in a mouse model of glycogen-storage cardiomyopathy.

Methods and Results—Transgenic (Tg) mice expressing a human N488I \textit{PRKAG2} cDNA under control of the tetracycline-repressible \(\alpha\)-myosin heavy chain promoter underwent echocardiography, ECG, and in vivo electrophysiology studies. Transgene suppression by tetracycline administration caused a reduction in cardiac glycogen content and was initiated either prenatally (Tg\text{OFF(E-8 weeks)}) or at different time points during life (Tg\text{OFF(4–16 weeks)}, Tg\text{OFF(6–20 weeks)}). One group never received tetracycline, expressing transgene throughout life (Tg\text{ON}). Tg\text{ON} mice developed cardiac hypertrophy followed by dilatation, ventricular preexcitation involving multiple accessory pathways, and conduction system disease, including sinus and atrioventricular node dysfunction.

Conclusions—Using an externally modifiable transgenic system, cardiomyopathy, cardiac dysfunction, and electrophysiological disorders were demonstrated to be reversible processes in \textit{PRKAG2} disease. Transgene suppression during early postnatal development prevented the development of accessory electrical pathways but not cardiomyopathy or conduction system degeneration. Taken together, these data provide insight into mechanisms of cardiac \textit{PRKAG2} disease and suggest that glycogen-storage cardiomyopathy can be modulated by lowering glycogen content in the heart. (\textit{Circulation}. 2008;117:144-154.)

Key Words: electrophysiology ■ cardiomyopathy ■ genes ■ glycoproteins ■ Wolff-Parkinson-White syndrome

Mutations in the \textit{PRKAG2} gene, which encodes the regulatory \(\gamma\)-subunit of AMP-activated protein kinase (AMPK), cause glycogen-storage cardiomyopathy associated with ventricular preexcitation and progressive cardiac conduction system (CCS) disease. AMPK, a serine/threonine kinase, is a heterotrimeric complex that consists of catalytic \(-\) and \(\beta\)- and \(\gamma\)-subunits, activated allosterically by rising cellular AMP and by phosphorylation of the \(\alpha\)-subunit by AMPK kinases under conditions of metabolic substrate limitation, hypoxia, exercise, and heat shock. Once activated by a rise in AMP/ATP ratio, AMPK alters enzyme activities in ATP-producing and -consuming pathways and maintains essential homeostatic systems (reviewed in Arad et al and Hardie and Sakamoto). Activation of AMPK during acute low-energy states switches off ATP-consuming pathways, such as glycogen, cholesterol, and fatty acid synthesis, and activates ATP-producing pathways, such as fatty acid oxidation and increased glucose uptake.}

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Mutations in the \(\gamma\)-subunit of AMPK produce human cardiac disease previously classified as hypertrophic cardiomyopathy (HCM) with Wolff-Parkinson-White (WPW) syndrome. Affected individuals develop ventricular hypertrophy and CCS abnormalities, including ventricular preex-
citation and atrial fibrillation, followed later by sinoatrial and atrioventricular (AV) nodal dysfunction, which often necessitates pacemaker implantation.1,2,7 Sudden cardiac death can result from atrial fibrillation with rapid antegrade conduction over the accessory pathway.

Generally, HCM that progresses to dilated cardiomyopathy is uncommon and is associated with diagnosis at a young age and family history.8 Cardiac hypertrophy in patients with PRKAG2 mutations often evolves into a phase characterized by systolic dysfunction and left ventricular dilation, which resembles features of dilated cardiomyopathy, and progresses to heart failure.2,9 This course resembles other glycogen-storage and metabolic cardiomyopathies but is dissimilar from HCM caused by sarcomere gene mutations. Cardiac hypertrophy in PRKAG2 mutations is not due to myocyte disarray and fibrosis, as defines sarcomeric HCM, but instead is characterized by increased intracellular glycogen deposition.

A transgenic mouse model carrying the PRKAG2 N488I mutation under the cardiac-specific α-myosin heavy chain (α-MHC) promoter was created and exhibits typical features of the human disease.10 Transgenic mice showed elevated AMPK activity, accumulated large amounts of cardiac glycogen, developed dramatic left ventricular hypertrophy followed by progressive loss of contractile function and dilatation, and exhibited ventricular preexcitation and sinus node dysfunction.10 Ventricular preexcitation developed within the first month of life, and histopathology revealed that the annulus fibrosis, which normally electrically insulates the ventricles from the atria, was disrupted by glycogen-filled myocytes.10,11 Disease manifestations are mediated through increased activity of α2-subunit complexes.12 The aims of the present study were to investigate whether glycogen-storage cardiomyopathy, ventricular preexcitation, and CCS disease in PRKAG2 mutations are preventable and/or reversible by modulation of glycogen content in the heart. Using the tetracycline-controlled transcriptional activator system,13 we provide evidence that reversal of glycogen accumulation restores normal cardiac function and conduction in PRKAG2 cardiomyopathy. Furthermore, in contrast to cardiac hypertrophy and progressive CCS degeneration, ventricular preexcitation can be prevented completely if glycogen accumulation is inhibited during early postnatal development.

Methods

Transgenic Mice

The target transgene is a human PRKAG2 cDNA10 carrying an Asn→Ile mutation (N488I) identified in a large family.1 Wild-type (WT) PRKAG2 transgene was used as a control. To be able to regulate cardiac transgene expression, a tetracycline-suppressible binary α-MHC–driven system was used.13,14 The transactivator protein, tTA, is required for robust expression of the target transgene (sPRKAG2). Competitive binding of tetracycline to tTA suppresses sPRKAG2 transactivation, which enables control of transgene expression by oral drug administration (Figure 1A; Data Supplement Figure IB).

sPRKAG2N488I or sPRKAG2WT transgene DNA was injected into fertilized mouse oocytes. Southern hybridization identified 5 offspring that contained the sPRKAG2N488I transgene and 13 lines with sPRKAG2WT in their genomic DNA. Four had germline transmission of sPRKAG2N488I, which included 2 lines with robust expression and profound phenotype that resembled original PRKAG2N488I mice.10

Genotyping

Offspring of founder mice were genotyped by multiplex polymerase chain reaction with primers specific for PRKAG2 transgene, tTA transgene, and control mouse genomic DNA (Data Supplement Figure IA).

Study Protocol

To suppress the disease-causing transgene, mice were given food containing doxycycline (Dox Diet Sterile, 1 g/kg, Bio-Serv, Frenchtown, NJ) at various time points. Disease preventability was assessed by suppression of the transgene during embryonic and early postnatal development (TgOFF(E-8 weeks), n = 13) by drug administration prenatally (feeding pregnant females throughout pregnancy) and through the first 8 weeks of life (feeding nursing mothers and growing offspring during the first 8 weeks of life).

To assess for disease reversibility, transgene was suppressed depending on onset for distinct disease features. Ventricular preexcitation was present in most TG mice at 4 weeks of age. Therefore, mice were given doxycycline starting at 4 weeks of age (TgOFF(F4–16 weeks), n = 11). To assess reversibility of ventricular wall thickening, which started after the first month, doxycycline was administered from 8 weeks of age (TgOFF(F8–20 weeks), n = 8). To investigate reversibility of cardiac dilation and dysfunction, as well as CCS disease, transgene suppression was started at 20 weeks of age (TgOFF(F20–20 weeks), n = 9) and continued for at least 8 weeks.

Ten sPRKAG2N488I mice never received doxycycline and expressed transgene throughout life, serving as positive controls (TgON). WT mice (n = 16) served as negative controls, of which 7 received lifelong doxycycline treatment. Doxycycline did not influence any electrophysiological or echocardiographic parameters in WT mice (data not shown).

RNA Assessment

Cardiac transgene expression was assessed by Northern blot analysis as described previously13 with 5 μg of total cardiac RNA per gel lane and probing with 32P-labeled PRKAG2-specific probes and GAPDH probes for standardization. Band intensities were quantified by densitometry with the ImageQuant Phosphoimager software.

Protein Analyses

Protein extracts and Western blots were performed as described previously16 with antibodies specific for the y2-AMPK subunit (Cell Signaling Technology, Danvers, Mass; 1:1000) and GAPDH as internal control (Chemicon International, Temecula, Calif; 1:20,000). Horseradish peroxidase–conjugated secondary antibody was used for chemiluminescence detection. Proteins levels were measured with National Institutes of Health ImageJ software (available at http://rsb.info.nih.gov/).

Glycogen Determination

Glycogen content was determined by perchloric acid extraction and Aspergillus niger amylglucosidase digestion followed by measurement of the amount of glucose released.16 Glucose levels were determined with a glucose oxidase kit (Sigma-Aldrich, St Louis, Mo).

Histopathology

Mouse hearts were fixed and stained as described previously.15 For measurement of the amount of glucose released.16 Glucose levels were determined with a glucose oxidase kit (Sigma-Aldrich, St Louis, Mo).

Echocardiography

Echocardiography was performed with an Agilent Sonos 4500 ultrasound machine (Hewlett-Packard, Palo Alto, Calif) and a
12-MHz linear-array transducer as described previously in unanesthetized mice.

**ECG and Electrophysiological Studies**

Measurements of ECG and electrophysiological parameters and standardized electrophysiological testing to assess atrial, AV, and ventricular conduction times were performed as described previously. ECG intervals were measured in 6 limb leads and 3 precordial leads by 2 observers blinded to genotype. Ventricular preexcitation was diagnosed on the basis of a short PR (≤15 ms) with widened QRS (>17 ms) interval. To confirm the presence of an accessory AV connection, adenosine (0.5 mg/g IV) was administered during steady-rate atrial pacing followed by ventricular pacing. CCS disease was diagnosed if evidence of sinus node dysfunction or AV block was demonstrated on ambulatory telemetry (Holter) recordings.

**Optical Mapping**

High-resolution optical mapping of ventricular activation in Langendorff-perfused hearts and calculation of conduction velocities were performed as described previously. No pharmacological or mechanical manipulations were used to limit motion.

**Statistical Analysis**

All data are presented as mean±SD. Comparisons between groups were made with Student t test for pairwise comparisons and ANOVA for multiple comparisons, followed by the Bonferroni post hoc test. Categorical variables were compared with χ² test.

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

**Results**

**RNA and Protein Expression**

RNA quantification by Northern analysis showed that doxycycline administration resulted in a decrease of transgene expression in TgOFF mice and that discontinuation of dietary doxycycline caused transgene reexpression to revert to expression levels similar to those of TgON mice (Data Supplement Figure IB). Western blot analyses demonstrated robust AMPK-γ2 expression in TgON mice and almost undetectable levels in TgOFF mice (Data Supplement Figure IC).
Cardiac Glycogen Content, Morphology, and Function

Transgene suppression resulted in reduced purple staining (which indicated glycogen) and a smaller amount of vacuolated myocytes on periodic acid Schiff–stained transverse heart cuts in TgOFF (Figure 1D and 1E) compared with TgON (Figure 1C) mice. Compared with WT mice, quantified myocardial glycogen content was increased 30-fold in TgON mice (n = 6; 56.8 ± 30.3 μg glycogen/mg heart tissue). Transgene suppression caused a significant decrease in TgOFF mice (TgOFF-E-8 weeks: n = 4; TgOFF-E-16 weeks: n = 3; 18.1 ± 11.4 μg glycogen/mg heart tissue; P = 0.01 TgON versus TgOFF). Heart/body weight ratio of adult TgON mice was more than twice that of WT mice (TgON mice 0.010 ± 0.002 versus WT 0.004 ± 0.001, P < 0.001), and transgene suppression resulted in a 30% decrease (heart/body weight ratio in TgON mice 0.010 ± 0.002 versus 0.007 ± 0.001 in TgOFF mice, P < 0.001).

On echocardiography, TgON mice developed prominent left ventricular wall thickening and septal hypertrophy compared with age-matched WT mice (Figure 2A) by 8 weeks of age. Transgene suppression reversed this process and caused a decrease of ventricular wall thickness to near-normal values in TgOFF-E-20 weeks mice. Beginning at 16 weeks of age, TgON mice showed left ventricular chamber enlargement (Figure 2B) and progressive systolic dysfunction (Figure 2C). Transgene suppression after that time significantly ameliorated myocardial dysfunction in TgOFF-E-20 weeks mice (Figure 2B and 2C). Myocardial wall thickening, dysfunction, and dilatation were delayed but not prevented by transgene suppression during early development in TgOFF-E-8 weeks mice (Figure 2B and 2C).

Electrophysiological Manifestations

Surface ECG showed ventricular preexcitation with shortened PR intervals and prolonged, slurred-upstroke QRS complexes in 50% to 60% of adult TgON mice, starting at ~4 weeks of age (Figure 2D; Data Supplement Table I). Distinct PR and AV morphology on ECG and intracardiac recordings, respectively, as well as optical mapping on ex vivo Langendorff-perfused hearts, suggested the presence of multiple accessory electrical pathways (Figure 3A and 3C). In normal hearts, epicardial activation appeared first at the apex before proceeding to the ventricular free wall (Figure 3B). Electrical activity on TgON hearts appeared near the base in addition to normal activation at the apex and often spread in a sleeve-like fashion from the atria to the ventricles, without clear localization (Figure 3C). Although optical mapping data were obtained from epicardial surfaces, the pattern was consistent with the activation wave front crossing the annulus fibrosus at multiple sites.

Pathological analysis revealed that TgON mice developed severe hypertrophic changes that resulted in a prominent bulge of the upper anterior wall adjacent to the pulmonary outflow tract. Morphologically, there was a marked septal
thickening posterior to the aortic valve. In this region, control (Figure 4A) or 
PRKAG2 WT (data not shown10) mice showed a continuous thin fibrous layer (annulus fibrosis) that electrically insulated the muscle of the atrial and ventricular septum. This fibrous layer, stained blue with Masson trichrome, was rendered discontinuous by vacuolated myocytes in TgON mice (Figure 4B). Disruption was demonstrated on several sections (Data Supplement Figure II), which provides evidence that this was indeed annulus disruption and not misinterpretation of the 3D topography due to changes in orientation related to the sectioning plane.

Suppression of the transgene prenatally and throughout the initial 8 weeks of life in Tg OFF(E-8 weeks) mice prevented preexcitation, even if sPRKAG2 N488I was expressed for >12 weeks later in life (Figure 2D; Data Supplement Table I). Intracardiac electrophysiology studies revealed clear His recordings in all TgOFF(E-8 weeks) mice (even after transgene reexpression), which suggests that electrical activity traveled through the normal AV conduction system (Figure 5A). Adenosine caused AV block in these mice, which further argues against the presence of accessory pathways (Figure 5C). Optical mapping on Langendorff-perfused Tg OFF(E-8 weeks) hearts showed normal elliptical anisotropic activation patterns (Figure 5B). Histology of the AV junction showed a somewhat hypertrophic septum, but the annulus fibrosis was completely formed and intact, in the near absence of interrupting vacuolated myocytes (Figure 4C). After transgene reexpression, Tg OFF(E-8 weeks) hearts displayed more vacuolated myocytes, but the annulus fibrous remained intact.

Transgene suppression beginning after 4 weeks of life altered the properties of the electrical accessory pathways in Tg OFF(4–16 weeks) mice: After 8 weeks of transgene suppression, the percentage of mutant mice with clear signs of preexcitation declined from ~50% before transgene suppression to 11% (Figure 2D; Data Supplement Table I; P<0.001). However, some Tg OFF(4–16 weeks) mice showed preexcited beats on atrial pacing, which suggests the presence of concealed pathways (Figure 6C). Histologically, the annulus fibrosis of Tg OFF(4–16 weeks) mice still appeared disrupted and unorganized, but vacuoles disappeared in the septum and the left ventricular wall (Figure 4D). Vacuolated myocytes were still present in the right ventricle. The same histological picture was observed in transgenic sPRKAG2 N488I mice that had no tTA transgene and is attributable to constitutive α-MHC promoter activity, which is higher in the right than in the left ventricular wall (Figure 4D). In contrast, transgene suppression after 8 weeks of life did not alter electrical properties of accessory pathways or cause loss of preexcitation on surface ECG in any Tg OFF(E-8 weeks) mouse (Figure 2D). Therefore, the time point of transgene suppression is critical for the reversibility of ventricular preexcitation.

Transgene reexpression after 16 weeks of age had the following effect in Tg OFF(4–16 weeks) mice: Most of the Tg OFF(4–16 weeks) mice
that demonstrated ventricular preexcitation on surface ECG before doxycycline administration redeveloped either clear preexcitation (Figure 6A) or shortened PR interval with transgene reexpression. Three mice remained without preexcitation on surface ECG after transgene reexpression (Figure 6B), but preexcited beats were induced by atrial pacing (Figure 6C) or adenosine (data not shown). Histopathology of the annulus fibrosis resembled that seen before transgene suppression, with abundant vacuolated myocytes in the annulus fibrosis and ventricular free walls.

We hypothesized that glycogen content in cardiomyocytes affects conduction velocity and thereby modifies conduction through the accessory connections. Optical mapping of ex vivo Langendorff-perfused hearts demonstrated that conduction velocity was similar between TgON (left ventricle: minimum conduction velocity 0.68±0.05 m/s, maximum 1.01±0.06 m/s, ratio 1.49±0.11; right ventricle: minimum conduction velocity 0.69±0.10 m/s, maximum 1.08±0.10 m/s, ratio 1.62±0.08) and TgOFF (left ventricle: minimum conduction velocity 0.58±0.06 m/s, maximum 0.93±0.06 m/s, ratio 1.81±0.33; right ventricle: minimum conduction velocity 0.66±0.14 m/s, maximum 0.91±0.15 m/s, ratio 1.41±0.09; P>0.2, TgON versus TgOFF) mice. Cardiac conduction velocity within the specialized conduction system was not assessed by this method.

Because progressive conduction system disease has been described in patients with PRKAG2 mutations, CCS function was evaluated in 20-to-32-week-old TgON mice by Holter recordings and in vivo intracardiac electrophysiological studies. By this age, TgON mice developed conduction system disease that included sinus and AV nodal abnormalities (Table; Figure 3D). Their heart rates were slower than those of age-matched WT mice, and 30% of TgON mice displayed sinoatrial exit block on baseline recordings (Table). AV conduction properties were ~50% prolonged in TgON mice compared with WT mice (Table), and 1 of 8 TgON mice had intermittent complete AV block on baseline Holter recordings (Figure 3D). Frequent supraventricular tachycardia was present in half of the TgON mice at this age (Figure 3D). Early postnatal transgene suppression followed by transgene reexpression did not prevent the development of sinoatrial and AV nodal disease in TgOFF(E-8 weeks) mice. Heart rates of 28-week-old TgOFF(E-8 weeks) mice were significantly slower than those of WT mice and were comparable to rates of TgON mice. Sinoatrial exit block was seen in approximately one third of TgOFF(E-8 weeks) mice, and intermittent complete AV block was present in half of all TgOFF(E-8 weeks) mice, comparable to TgON mice (Figure 5D). However, no supraventricular tachycardia was seen in old TgOFF(E-8 weeks) mice, consistent with the absence of accessory pathways, as shown in the Table.

To evaluate whether CCS disease was reversible by lowering the glycogen content in TgOFF(E-8 weeks) mice, transgene was suppressed at 20 weeks, and mice were studied at 28 weeks. Overall, CCS function improved in TgOFF(E>20 weeks) mice after transgene suppression: TgOFF(E>20 weeks) mice did not have sinoatrial block, AV block, or supraventricular tachycardia (Table). Furthermore, AV conduction properties during electrophysiological studies were significantly faster in TgOFF(E>20 weeks).
than in TgON mice (Table). Mean heart rates were comparable to those of TgON mice and lower than WT mice, which suggests that CCS function had not been restored completely (Table).

Discussion

A transgenic mouse carrying a modifiable PRKAG2 N488I mutation serves as a model to elucidate the dynamics of evolution and reversibility of glycogen-associated cardiomyopathy, ventricular preexcitation, and CCS degeneration. The present data indicate that glycogen-storage cardiomyopathy and CCS degeneration associated with PRKAG2 mutation are reversible by lowering of the cardiac glycogen content. We furthermore show that development of accessory electrical pathways can be prevented by inhibition of glycogen accumulation during early postnatal development.

Tetracycline-Controlled Transcriptional Regulation System

An externally modifiable transgenic system based on the tetracycline-controlled transcriptional regulator was reengineered for cardiac-specific expression in mice and provides reliable transgene expression regulation. The present data demonstrate that tetracycline administration effectively suppressed PRKAG2 transgene expression (Data Supplement Figure IB). However, cardiac glycogen content in mutant hearts under tetracycline treatment was still higher than in WT hearts, because of some constitutive promoter activity. Transgene RNA levels were slightly lower when the transgene was reexpressed later in life, and there were fewer vacuolated myocytes after reexpression compared with untreated mutant hearts. TgOFF(E-8 weeks) mice represent the group with transgene suppression occurring during early postnatal development. Tetracycline was started prenatally to achieve adequate tissue levels at birth, because the MHC promoter is not significantly expressed until shortly after birth, when there is an isoform switch from to MHC in mice.

Glycogen-Associated Cardiomyopathy

Molecular studies of patients with clinical features of HCM but without sarcomere-protein gene defects have led to identification of other genetic causes of cardiac hypertrophy, including PRKAG2, GAL, and LAMP2. The glycogen-storage cardiomyopathy produced by PRKAG2 and LAMP2 mutations resembles HCM but is distinguished by electrophysiological abnormalities, particularly preexcitation. PRKAG2 mutations cause myocyte hypertrophy by stimulating glycogen-filled vacuoles but cause neither myocyte disarray nor interstitial fibrosis, as occur with sarcomeric mutations. The mechanisms that lead to glycogen accumulation in transgenic N488I hearts remain unclear. Glycogen accumulation may occur through increased glucose uptake combined with increased fatty acid utilization. The results of the present study show, however, that glycogen accumulation is reversible by decreasing AMPK activity due to N488I trans-
gene suppression. After N488I transgene suppression, sPRKAG2N488I mice may utilize excess glycogen to restore normal levels, similar to their ability to use accumulated glycogen as energy to increase cardiac function during exercise.12

There are several possibilities to explain how increased AMPK activity might cause cardiomyopathy and cardiac dysfunction. A cell metabolism defect due to failure to conserve ATP could impair myocyte contractile function and induce hypertrophic responses through disordered metabolism,24 but we previously showed that the N488I mutation does not perturb resting cellular energy levels in the heart.12 In the present study, transgene suppression reversed cardiac hypertrophy and ameliorated cardiac dysfunction by normalizing glycogen content. These results illustrate that the functional impairment in transgenic N488I mice is more likely due to cardiac glycogen deposition than to energy depletion.12

Ventricular Preexcitation

The second disease feature shown to be preventable and reversible by normalization of cell metabolism in the present model is the presence of abnormal electrical connections (accessory pathways) between the atria and ventricles. Interestingly, ventricular preexcitation was only reversible if transgene suppression occurred in juvenile mice (at 4 weeks of life) and not in adult mice (transgene suppression at 8 weeks of life). This finding suggests that the treatment time point is important in preventing and reversing ventricular preexcitation in PRKAG2 disease.

Mutations in PRKAG2,7 LAMP2,9 and GAA25 have been identified to cause hereditary forms of preexcitation associated with glycogen-storage cardiomyopathy. These patients have a phenotype that is clearly different from those with isolated WPW syndrome, who typically have structurally normal hearts. Accessory pathways occur in association with other cardiac abnormalities or congenital heart disease in only a small percentage of WPW patients.26

Figure 6. Reversibility of ventricular preexcitation with transgene suppression. A and B, Surface ECG lead I recordings of 2 different TgOFF4–16 week mice at 3 time points. There was a loss of preexcitation on ECG after transgene suppression. C, Intracardiac electrophysiological testing revealed conduction via accessory pathways on atrial pacing in some of these TgOFF4–16 week mice. Transgene reexpression caused reoccurrence of preexcitation in some (A) and no WPW recurrence in others (B). RV indicates right ventricular.
The prevalence of typical WPW is 1.5 to 3 per 1000 persons.27 A higher prevalence of WPW in first-degree relatives of affected individuals suggests a hereditary contribution,28 but no gene defect associated with typical WPW has yet been identified. In particular, no PRKAG2 mutations have yet been found in patients with typical WPW in the absence of glycogen-storage cardiomyopathy.29

Three conditions are required for functional preexcitation: (1) an anatomic substrate, (2) electrical coupling between adjacent ventricular and atrial myocytes, and (3) higher conduction velocity than in the normal conduction system. In human development, accessory pathways are present prenatally but coalesce or are absorbed (apoptosis) during maturation.30 Accessory AV pathways may result from developmental failure to eradicate remnants of AV connections during cardiogenesis. This may be a consequence of disruption of the role of AMPK as a transcription factor regulating an unknown gene.31 AMPK subunits have a close homology with transcription factors involved in regulating genes that encode glucose-metabolizing enzymes.32 The fact that transgene suppression in embryonic and early postnatal life prevented preexcitation in the present study argues for this hypothesis. Another explanation could be that rapidly developing hyper trophy in the growing newborn heart might lead to myocyte swelling disproportionate to the fibrous tissue, thus creating an anatomic substrate for preexcitation. Transgene expression during the first 8 weeks of life, may be insufficient to overcome the constraints of the rigid fibrous ring to create physical communication between enlarging ventricular and atrial myocytes. The hypothesis of fast-occurring myocyte growth during the neonatal period and adolescence might also explain the absence of preexcitation reversibility in mice in which transgene suppression began at 8 weeks of life.

With regard to the second condition, electrical coupling between adjacent ventricular and atrial myocytes, there might be a global or regional difference in connexin expression/activity due to increased AMPK or glycogen. There was no difference in connexin43 and connexin40 levels of total N488i PRKAG2 heart extract (data not shown). In contrast, we could show that glycogen depletion alone abolished electrical signal conduction via accessory pathways without altering the anatomic substrate, mainly via interruption of the annulus fibrosis. This suggests that glycogen accumulation may promote electrical cell coupling, and conversely, glycogen depletion might electrically isolate cell-to-cell contact.

Finally, the presence of glycogen might cause conduction differences to create preexcitation. The 2 major factors that contribute to conduction velocity in both normal and diseased myocardium are cell size and gap junction distribution.33 Glycogen depletion could cause physical shrinkage of the accessory pathway, thereby slowing conduction without abolishing the anatomic substrate for preexcitation. In the present study, conduction velocity was not assessed specifically in the CCS; however, epicardial ventricular conduction velocity was not decreased after glycogen depletion. This was surprising, because expression of the mutant PRKAG2 gene caused a striking increase in intracellular glycogen with concurrent increased ventricular mass, which was reversed with transgene suppression. There are several potential explanations as to why changes in cell size were not accompanied by changes in epicardial conduction velocities. First, the effects of increased intracellular glycogen on gap junction distribution are not known in this model. If gap junctions are either redistributed to the transverse edges of the cell or are not upregulated commensurate with the increase in cell area, increases in conduction velocity may be opposed. Second, given that ion channels are also spatially organized on the cell membrane34 and are concentrated near the gap junctions on the intercalated discs, a redistribution and/or decreased concentration of sodium channels in mutant hearts could lead to decreased sodium current densities35 and concurrent slowing of conduction velocity. Additional molecular and cellular electrophysiology studies are necessary to determine the impact of these factors on maintenance of normal ventricular conduction velocities in this model.

**Cardiac Conduction System Disease**

Progressive CCS disease is associated with PRKAG2 mutations and often requires pacemaker implantation.1-27 The gradual progression of conduction impairment in humans is
recapitulated in the present mouse model by slowed sinoatrial and AV nodal conduction properties. CCS cells have a higher glycogen content than working myocytes, but glycogen accumulation obviously disturbs conduction system cell function. Perhaps AMPK, through phosphorylation, regulates cardiac ion channels. Patch-clamp studies on myocytes transfected with the AMPK R302Q mutation showed a slowing of open-state inactivation of the sodium channel and prolongation of the action potential duration.

In the present study, CCS degeneration was a destructive process that occurred later in life and was not preventable by glycogen depletion early in life. However, the lowering of glycogen content by transgene suppression later in life restored AV nodal function and, in part, sinoatrial nodal function. Thus, conduction system disease should not be considered an inevitably destructive process but rather a potentially reversible malfunction due to disturbances in cell metabolism.

Conclusions

Patients with glycogen-storage–associated cardiomyopathy generally have a poor prognosis due to severe cardiac dysfunction and progressive conduction system degeneration. PRKAG2K38 mutant transgene regulation in mice demonstrates that critical developmental timing of glycogen depletion in the heart prevents or reverses cardiac hypertrophy, dysfunction, and conduction system disease in PRKAG2 glycogen-associated cardiomyopathy. In the era of advanced therapeutic approaches such as gene therapy and enzyme replacement, these data imply that myocardial dysfunction and CCS disease in glycogen-storage cardiomyopathy could potentially be treated by modulation of cardiac glycogen content and that early initiation of treatment is critical to prevent the development of accessory pathways. Lowering cardiac glycogen content significantly ameliorates morbidity in PRKAG2 cardiomyopathy. Targeting AMPK could be a viable alternative treatment option to device implantation and heart transplantation for patients who have PRKAG2 mutations with glycogen-storage cardiomyopathy and arrhythmias.

Acknowledgments

We thank Dorothy Branco for technical help in the performance of electrophysiologic studies in vivo mouse studies.

Sources of Funding

The Howard Hughes Medical Institute (Dr C.E. Seidman); National Heart, Lung, and Blood Institute, National Institutes of Health (Dr S.J.G. Seidman and C.E. Seidman); Boston Children’s Heart Foundation and the Sean Roy Johnson Memorial Fund (Dr S.J.G. Seidman and C.E. Seidman); Israel Science Foundation (Dr Arad); and the Reynolds Foundation (Dr J.G. Seidman) supported these studies.

Disclosures

None.

References


**CLINICAL PERSPECTIVE**

Patients with glycogen-storage–associated cardiomyopathy due to PRKAG2 mutations generally have a poor prognosis because of severe cardiac dysfunction, ventricular preexcitation, and progressive conduction system disease. This study used a system that allows regulation of the timing of expression of the PRKAG2N488I mutation in mice during development. Altering the timing of glycogen depletion in the heart prevents or reverses cardiac hypertrophy, cardiac dysfunction, ventricular preexcitation, and conduction system disease in PRKAG2 glycogen-associated cardiomyopathy. In the era of such advanced therapeutic approaches as gene therapy and enzyme replacement, these data imply that myocardial dysfunction and conduction system disease in glycogen-storage cardiomyopathy could potentially be treated by modulation of glycogen content in the heart and that early initiation of treatment is critical to prevent the development of accessory pathways. Lowering cardiac glycogen content significantly ameliorates morbidity in PRKAG2 cardiomyopathy and could eventually be a viable alternative treatment option to device implantation and heart transplantation for patients who have PRKAG2 mutations with glycogen-storage cardiomyopathy and arrhythmias.
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_Circulation_. 2008;117:144-154; originally published online December 24, 2007; doi: 10.1161/CIRCULATIONAHA.107.726752

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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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/117/2/144

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