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# Electrophysiologic Characterization and Postnatal Development of Ventricular Pre-Excitation in a Mouse Model of Cardiac Hypertrophy and Wolff-Parkinson-White Syndrome

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**OBJECTIVES** We sought to characterize an animal model of the Wolff-Parkinson-White (WPW) syndrome to help elucidate the mechanisms of accessory pathway formation.

**BACKGROUND** Patients with mutations in *PRKAG2* manifest cardiac hypertrophy and ventricular pre-excitation; however, the mechanisms underlying the development and conduction of accessory pathways remain unknown.

**METHODS** We created transgenic mice overexpressing either the Asn488Ile mutant ($TG^{N488I}$) or wild-type ($TG^{WT}$) human *PRKAG2* complementary deoxyribonucleic acid under a cardiac-specific promoter. Both groups of transgenic mice underwent intracardiac electrophysiologic, electrocardiographic (ECG), and histologic analyses.

**RESULTS** On the ECG, ~50% of $TG^{N488I}$ mice displayed sinus bradycardia and features suggestive of pre-excitation, not seen in $TG^{WT}$ mice. The electrophysiologic studies revealed a distinct atrioventricular (AV) connection apart from the AV node, using programmed stimulation. In $TG^{N488I}$ mice with pre-excitation, procainamide blocked bypass tract conduction, whereas adenosine infusion caused AV block in $TG^{WT}$ mice but not $TG^{N488I}$ mice with pre-excitation. Serial ECGs in 16 mice pups revealed no differences at birth. After one week, two of eight $TG^{N488I}$ pups had ECG features of pre-excitation, increasing to seven of eight pups by week 4. By nine weeks, one $TG^{N488I}$ mouse with WPW syndrome lost this phenotype, whereas $TG^{WT}$ pups never developed pre-excitation. Histologic investigation revealed postnatal development of myocardial connections through the annulus fibrosum of the AV valves in young $TG^{N488I}$ but not $TG^{WT}$ mice.

**CONCLUSIONS** Transgenic mice overexpressing the Asn488Ile *PRKAG2* mutation recapitulate an electrophysiologic phenotype similar to humans with this mutation. This includes procainamide-sensitive, adenosine-resistant accessory pathways induced in postnatal life that may rarely disappear later in life. (J Am Coll Cardiol 2003;42:942–51) © 2003 by the American College of Cardiology Foundation

Wolff-Parkinson-White (WPW) syndrome or other supraventricular tachycardias (SVTs) typically occur without obvious monogenic inheritance patterns. However, genetic mutations play a role in the development of some cases of WPW syndrome. Families with WPW syndrome and/or SVT, with or without hypertrophic cardiomyopathy, are described (1). The frequency of SVT in certain congenital heart defects and mitochondrial disorders implicates mutations that disrupt both cardiac structural and electrical system development (2,3). Although most patients with WPW syndrome have structurally normal hearts, a subset exists with ventricular pre-excitation, intraventricular conduction delay, and cardiac hypertrophy with familial occurrence. Recently, Gollob et al. (4) and Arad et al. (5) described families with profound conduction disorders and WPW syndrome with (4,5) and without (6) ventricular hypertrophy. Several missense mutations in *PRKAG2,* the gene for the gamma-2 regulatory subunit of adenosine monophosphate (AMP)-activated protein kinase, were identified. To better understand the molecular and physiologic mechanisms underlying this inherited form of ventricular pre-excitation, transgenic mice were created by overexpressing the wild-type or mutant human *PRKAG2* complementary deoxyribonucleic acid (Asn488Ile) under the cardiac-specific alpha-myosin heavy chain (MHC) promoter (7).

We describe here the natural history, developmental maturation, electrophysiology, and pharmacology of accessory atrioventricular (AV) connections in a murine model that recapitulates the clinical profile from which the mutation was derived. Our data provide evidence that an adenosine-resistant, procainamide-sensitive AV connection, apart from the normal AV node-His pathway, is present in

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**Abbreviations and Acronyms**

AMP = adenosine monophosphate
AV = atrioventricular
CCh = carbamyl choline
EPS = electrophysiologic study
ERP = effective refractory period
HBE = His-bundle electrogram
MHC = myosin heavy chain
SVT = supraventricular tachycardia
VA = ventriculo-atrial
WPW = Wolff-Parkinson-White

mice carrying the mutant transgene, and these accessory AV connections manifest in postnatal life.

## METHODS

**Animals.** Creation of transgenic mice was recently described (7). Surface electrocardiograms (ECGs) were obtained for 28 transgenic mutant *PRKAG2* ($TG^{N488I}$) mice (4 to 16 weeks old), 13 age-matched transgenic wild-type *PRKAG2* ($TG^{WT}$) mice, and 13 control nontransgenic mice. A subset (n = 26) underwent in vivo electrophysiologic studies (EPS) with pharmacologic testing. A group of 10 older $TG^{N488I}$ mice (9 to 15 months old) and 10 littermate controls also underwent ECG and EPS. Finally, serial ECGs were obtained weekly from birth to 12 weeks in a cohort of 16 mice from two litters. Mice were inbred in an FVB background and are genetically equivalent. All protocols conformed to the Association for the Assessment and Accreditation of Laboratory Animal Care and the Children's Hospital Animal Care and Use Committee.

**Preprocedural preparation.** Protocols for the in vivo mouse EPS have been described in detail (8,9). Mice were anesthetized with pentobarbital (0.033 mg/kg intraperitoneally), and multilead ECGs were obtained using subcutaneous electrodes. A jugular vein cutdown was performed, and an octapolar 2F electrode catheter (CIBer mouse-EP; NuMED, Inc., Hopkinton, New York) was placed in the right atrium and ventricle under electrographic guidance to confirm the catheter position.

**EPS.** In vivo EPS were performed in 26 mice (17 $TG^{N488I}$ and 9 $TG^{WT}$ mice). Standard pacing protocols were used to assess atrial and ventricular conduction, refractoriness, and arrhythmia inducibility (8,9). The ECG channels were filtered between 0.5 and 250 Hz, and intracardiac electrograms were filtered between 5 and 400 Hz. The analog signal was digitized with 12-bit precision at a sampling rate of 2 kHz. Recording of a triphasic His-bundle electrogram (HBE), a fixed distance from the ventricular electrogram at baseline, was accomplished using simultaneous multielectrodes and persistent catheter manipulation (9). The ECG intervals were measured in six limb leads and precordial V leads by two observers who were blinded to the genotype. To assess the presence of an accessory AV connection, adenosine (0.5 μg/g intravenously [IV]) was administered during steady-rate atrial pacing, followed by ventricular pacing. Isoproterenol was administered (1 ng/g IV) and the EPS repeated after a 25% heart rate increase. Procainamide was administered (30 μg/g IV) to assess the effects of increased refractoriness and slowed conduction upon the accessory AV connection and arrhythmia inducibility. In the older group of mice, after baseline electrophysiologic parameters were recorded, carbamyl choline (CCh) was administered (50 ng/g IP). The atrial pacing protocol was repeated 5 min after CCh to assess muscarinic modulation and attempt atrial fibrillation induction to measure accessory pathway conduction characteristics (10).

**Table 1.** Electrocardiographic Data Summary

| | $TG^{N4881\ +\ BPT}$ (n = 13) | $TG^{N4881\ -\ BPT}$ (n = 15) | $TG^{WT}$ (n = 13) |
|---|---|---|---|
| Age (weeks) | 6.5 ± 2.9 | 7.9 ± 3.6 | 6.8 ± 2.9 |
| SCL (ms) | 260 ± 119* | 174 ± 62 | 197 ± 66 |
| PR (ms) | 9.8 ± 2.7*† | 27.3 ± 3.7 | 30.9 ± 3.7 |
| QRS (ms) | 17.0 ± 2.6*† | 13 ± 2 | 12.9 ± 1.0 |
| JT (ms) | 11.0 ± 5.2 | 9.7 ± 3.0 | 9.5 ± 3.2 |
| QT (ms) | 31.2 ± 7.1*† | 22.7 ± 2.9 | 22.4 ± 3.7 |

*$p < 0.05$ compared with $TG^{N4881\ -\ BPT}$ mice. †$p < 0.05$ compared with $TG^{WT}$ mice. Data are presented as the mean value ± SD.

SCL = sinus cycle length; $TG^{N4881\ +\ BPT}$ = transgenic *PRKAG2* mutant mice with bypass tract; $TG^{N4881\ -\ BPT}$ = transgenic *PRKAG2* mutant mice without bypass tract; $TG^{WT}$ = transgenic *PRKAG2* wild-type mice.

**ECG vector analysis.** Electrocardiographic "delta" wave vector analysis was performed according to a method modified from Arruda et al. (11). Onset of ventricular activation in each ECG lead, determined by two observers, was measured from the onset of the earliest delta wave. The polarity of the delta wave was measured within the first 5 ms of the onset of pre-excitation.

**Histology and morphology.** Hearts were excised from $TG^{N488I}$, $TG^{WT}$, and wild-type mice, washed in Dulbecco's phosphate-buffered saline, arrested in 50 mmol/l KCl, and formalin-fixed (12). Intact hearts were serially sectioned (5 μM) in the sagittal plane and stained with Masson trichrome to allow visualization of the annulus fibrosum of the AV valves. Sections were analyzed on a compound microscope and digitally photographed. Hearts were analyzed by an experienced cardiac pathologist who was blinded to the genotype and ECG.

**Statistical analysis.** Continuous variables, such as ECG intervals and conduction parameters, were measured by two observers and summarized as the mean value ± SD. Mean values for $TG^{N488I}$ mice with and without pre-excitation were compared with control values, using one-way analysis of variance followed by the Scheffé method of multiple comparisons. The Fisher exact test was used for categorical variables (13). A p value $<0.05$ was considered significant.

## RESULTS

**EPS and ECG findings.** The findings of a short PR interval with a wide, slurred QRS complex on the ECG were consistent with manifest ventricular pre-excitation,

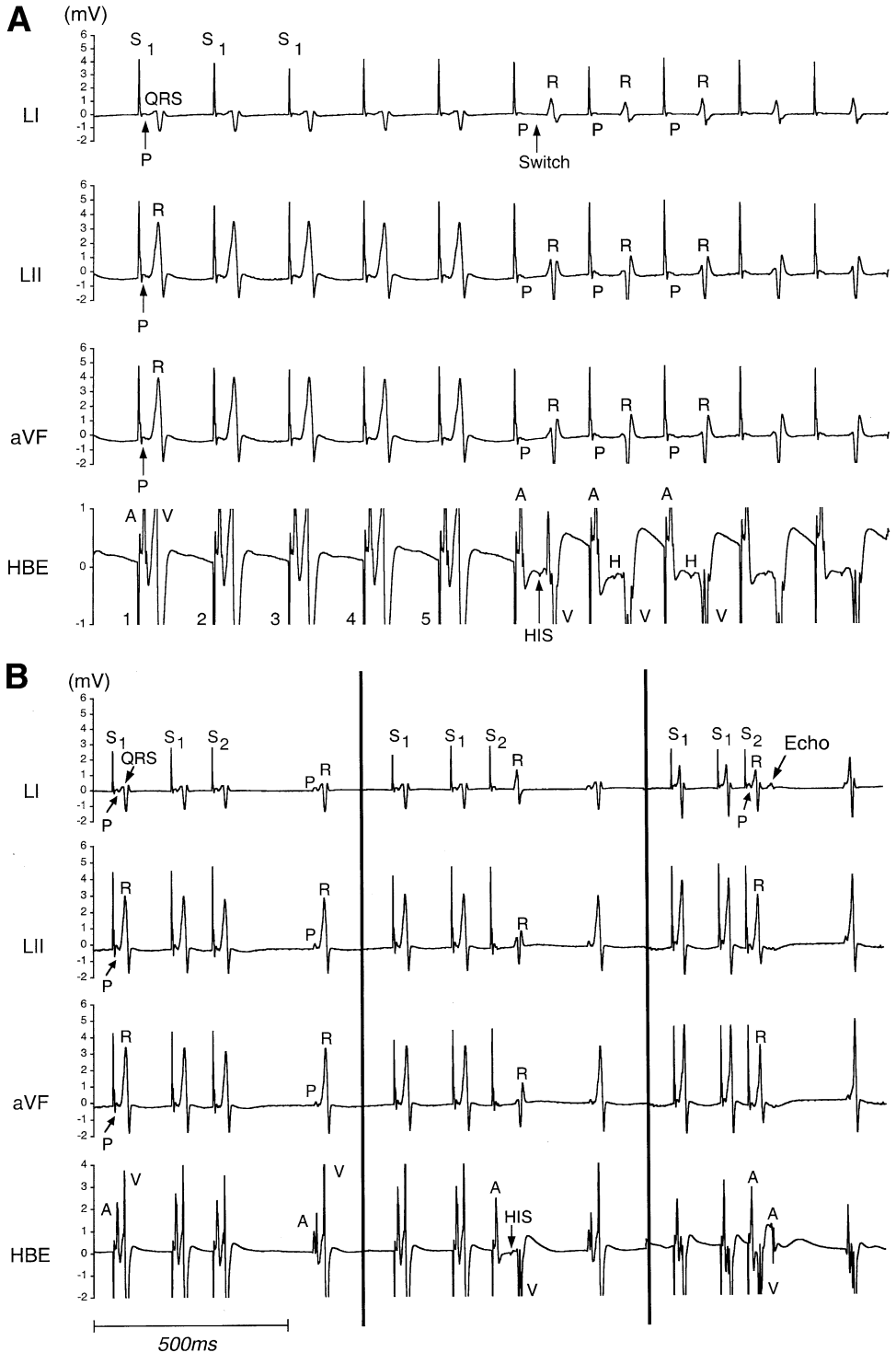


**Figure 1.** Two distinct AV pathways are present in mutant transgenic mice. **(A)** Electrocardiographic leads I, II (LI and LII), and aVF are shown, as well as the His-bundle electrogram (HBE). Atrial pacing at 110 ms with conduction down the bypass tract for the first five beats and fusion of atrial (A) and ventricular (V) intracardiac electrograms on the HBE. The sixth beat **(arrow)** and subsequent beats conduct with a longer PR interval and narrower QRS complex, suggesting block in the bypass tract and conduction down the AV node. Note separation of the atrial and ventricular electrograms on the HBE and the presence of a His potential (HIS). **(B)** The **left panel** displays ECG leads I, II, and aVF, as well as the HBE, and shows a premature atrial extrastimuli ($S_2$) delivered at a coupling interval of 105 ms and a drive train ($S_1$) at 150 ms. All three beats conduct with a short PR interval and wide QRS complex, suggestive of manifest pre-excitation down an accessory AV pathway. The **middle panel** displays the same electrograms, with the premature atrial extrastimulus coupled at 100 ms. The first two beats conduct with a short PR interval and wide QRS complex, but the atrial extrastimulus conducts with a longer PR interval and narrow QRS complex, and the A and V electrograms are separated by a clear HIS. This indicates that the accessory pathway is refractory and conduction is via the AV node. The **right panel** displays the same electrograms in another mouse, with the atrial extrastimuli coupled at 75 ms to the drive train at 150 ms. The first two beats and atrial extrastimuli conduct with a short PR interval and wide QRS complex; on the HBE, there is fusion between the A and V electrograms, suggesting conduction down the accessory AV connection. The last beat is followed by retrograde atrial depolarization (Echo), with a long VA time, suggesting retrograde conduction up the AV node.

**Table 2.** Electrophysiologic Data Summary

| | $TG^{N488I + BPT}$ (n = 8) | $TG^{N488I - BPT}$ (n = 9) | $TG^{WT}$ (n = 9) |
|---|---|---|---|
| Age (weeks) | 7.0 ± 3.7 | 9.9 ± 4.6 | 7.6 ± 3.3 |
| SCL (ms) | 251 ± 50 | 202 ± 40 | 219 ± 59 |
| AH (ms) | | 23.6 ± 5.1 | 26.9 ± 4.6 |
| Hd (ms) | | 4.6 ± 0.7 | 4.9 ± 1.0 |
| HV (ms) | | 9.8 ± 1.7 | 10.8 ± 2.1 |
| AVI (ms) | 9.7 ± 4.1*† | 34.2 ± 6.0 | 37.8 ± 6.0 |
| $AERP_{150}$ (ms) | 41.9 ± 7.0‡ | 38.3 ± 7.5 | 33.9 ± 7.4 |
| $AVERP_{150}$ (ms) | | 106 ± 18.5‡ | 86.1 ± 15.6 |
| $BPERP_{150}$ (ms) | 60.0 ± 7.6 | | |
| AVWBCL (ms) | | 116 ± 13 | 108 ± 19 |
| AV 2:1 (ms) | | 102 ± 14.7 | 88.3 ± 14.8 |
| BPWBCL (ms) | 78.1 ± 11.0 | | |
| BP 2:1 (ms) | 61.3 ± 10.3 | | |
| VAWBCL (ms) | <50 | 119 ± 25 | 125 ± 14 |
| $VERP_{150}$ (ms) | 56.4 ± 11.8†‡ | 55.0 ± 10.4 | 30.6 ± 10.1 |

*$p < 0.05$ compared with $TG^{N488I - BPT}$ mice. †$p < 0.05$ compared with $TG^{WT}$ mice. ‡$p < 0.05$ compared with $TG^{WT}$ mice. Data are presented as the mean value ± SD.

AH = atrio-Hisian interval; Hd = His duration; HV = His-ventricular interval; AVI = atrioventricular interval; $AERP_{150}$ = atrial effective refractory period (ERP) at drive train of 150 ms; $AVERP_{150}$ = atrioventricular ERP at drive train of 150 ms; $BPERP_{150}$ = bypass tract anterograde ERP at drive train of 150 ms; AVWBCL = atrioventricular Wenckebach block cycle length; AV 2:1 = atrioventricular 2:1 block cycle length; BPWBCL = bypass tract anterograde Wenckebach block cycle length; BP 2:1 = bypass tract 2:1 block cycle length; VAWBCL = ventriculoatrial Wenckebach block cycle length; $VERP_{150}$ = ventriculoatrial effective refractory period at drive train of 150 ms; other abbreviations as in Table 1.

present in 8 of 17 $TG^{N488I}$ mice. Based on these initial ECG features, $TG^{N488I}$ mice were classified into two groups: those with apparent pre-excitation ($TG^{N488I+BPT}$) and without pre-excitation ($TG^{N488I-BPT}$). The ECG intervals (Table 1) revealed a slower resting heart rate with a shorter PR interval and wider QRS complex in $TG^{N488I+BPT}$ compared with $TG^{N488I-BPT}$ mice. Heart rates and ECG intervals were similar among $TG^{N488I-BPT}$ and $TG^{WT}$ mice. The ECG and EPS data in $TG^{WT}$ mice are similar to control FVB nontransgenic mice (data not shown).

To demonstrate the presence of an additional AV connection and elucidate the electrophysiologic effects of the *N488I* mutation, intracardiac EPS were performed. Atrial pacing and programmed stimulation could induce accessory pathway block, resulting in conduction down the AV node, identified by HBE potentials during accessory pathway refractoriness (Fig. 1). The EPS also established that AV intervals were shorter in $TG^{N488I+BPT}$ mice than in $TG^{N488I-BPT}$ and $TG^{WT}$ mice (Table 2). The AV node effective refractory period (ERP) was longer in $TG^{N488I-BPT}$ mice and the ventricular ERP was longer in both $TG^{N488I+BPT}$ and $TG^{N488I-BPT}$ mice than in $TG^{WT}$ mice. Retrograde conduction up the accessory connection was robust at baseline, with 1:1 ventriculo-atrial (VA) conduction at cycle lengths of <50 ms in all $TG^{N488I+BPT}$ mice. However, procainamide induced retrograde accessory pathway block with ventricular pacing at >100 ms, shifting retrograde conduction up the AV node (Fig. 2). Pacing and programmed stimulation, with or without isoproterenol, did not induce SVT, although re-entrant echo beats could be provoked.

**Pharmacologic findings.** To further confirm the presence of a separate AV connection in $TG^{N488I}$ mice, we observed the effect of procainamide and adenosine on the ECG intervals and AV conduction. Intravenous procainamide prolonged the PR interval and narrowed the QRS complex in six of eight $TG^{N488I+BPT}$ mice (Fig. 3A). Procainamide lengthened the AV Wenckebach cycle length in these six $TG^{N488I+BPT}$ mice (85 ± 8 ms vs. 142 ± 13 ms, $p < 0.001$). Intravenous adenosine during atrial pacing resulted in AV block in six of six $TG^{WT}$ mice but zero of six $TG^{N488I+BPT}$ mice (Fig. 3B).

**Neonatal development of pre-excitation.** To investigate the natural history of pre-excitation induced by the *PRKAG2* mutation, we performed serial ECG analysis in a cohort of 16 neonatal mice. Immediately after birth, no pups showed evidence of pre-excitation, but by the first postnatal week, two of eight $TG^{N488I}$ pups displayed pre-excitation. This increased to seven (88%) of eight $TG^{N488I}$ pups by postnatal week 4 (Fig. 4). None of the eight wild-type pups showed pre-excitation through 12 weeks. By week 9, one $TG^{N488I+BPT}$ mouse lost pre-excitation on the ECG, confirmed by EPS (Fig. 5).

By employing ECG algorithms for localizing human accessory pathways (11), 10 of 12 $TG^{N488I+BPT}$ mice appeared to develop anteroseptal accessory AV connections; one was posteroseptal and one was anterolateral by delta wave vector analysis (Fig. 6A). We attempted to directly identify the anatomic location of the bypass tracts by comparing histologic findings on hearts from 1- and 2.5-week-old $TG^{N488I}$, $TG^{WT}$, and wild-type mice. Two animals from each cohort underwent ECG recording; then, the hearts were removed and serially sectioned. Histologic assessment revealed myocardial connections through the annulus fibrosis of the AV valves of both 2.5-week-old $TG^{N488I}$ mice, but not in the other mice analyzed. Atrioventricular connections were present in the right anteroseptal region of hearts from both $TG^{N488I}$ mice with WPW syndrome at 2.5 weeks of age, concordant with the ECG vector analysis (Fig. 6B). Histologically, these connections resembled ventricular muscle and appeared similar to those described in humans (14). No discernable AV connections were identified in the hearts of one-week-old $TG^{N488I}$ or $TG^{WT}$ mice and wild-type animals at either age.

**Electrophysiologic assessment in older $TG^{N488I}$ mice.** A separate group of 10 older $TG^{N488I}$ and wild-type mice underwent EPS. Of these, 7 of 10 $TG^{N488I}$ mice manifested pre-excitation, and all 10 had intact AV conduction. However, the sinus cycle length was slower in preexcited $TG^{N488I}$ mice (338 ± 52 ms, $p < 0.001$) compared with non–pre-excited older mice (226 ± 48 ms) or younger preexcited $TG^{N488I}$ mice (251 ± 50 ms). Spontaneous, nonsustained SVT, atrial bigeminy, marked sinus bradycardia, and pauses up to 1.6 s were seen in older $TG^{N488I}$ mice. Atrioventricular node and accessory pathway conduction characteristics were similar between 9- to 14-month-old and 4- to 16-week-old $TG^{N488I}$ mice.

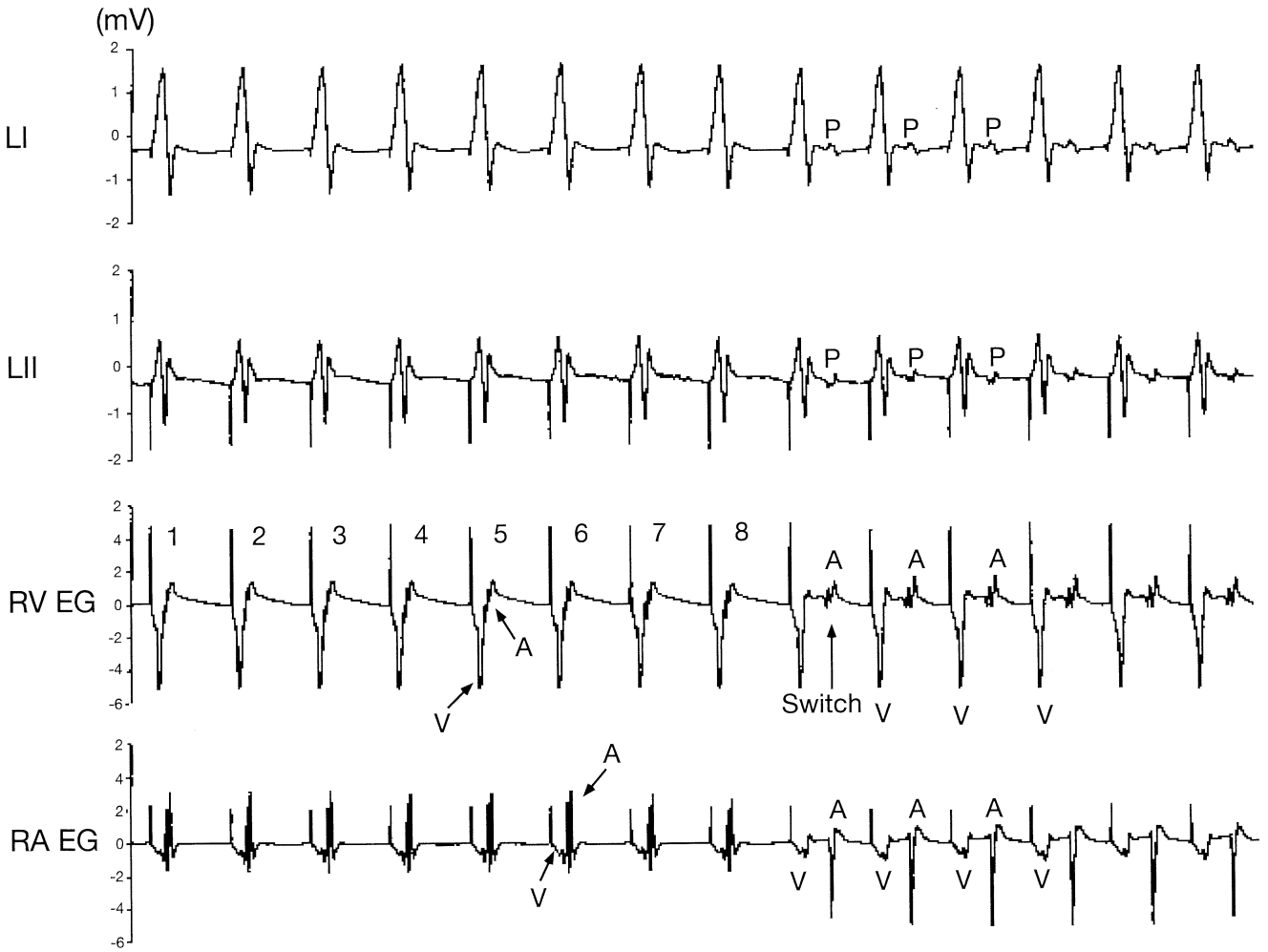


**Figure 2.** Retrograde conduction in mutant transgenic mice. Electrocardiographic leads I and II (LI and LII) are shown with right ventricular (RV EG) and right atrial electrograms (RA EG). Ventricular pacing at 115 ms in the presence of procainamide, with retrograde conduction up the bypass tract for the first eight beats and fusion of atrial (A) and ventricular (V) electrograms on both the RV EG and RA EG. The ninth beat **(arrow)** and subsequent beats conduct with a longer RP interval, revealing retrograde block in the bypass tract and conduction up the AV node. Note there is now separation of the A and V electrograms on both intracardiac electrograms.

Carbamyl choline was administered and the atrial pacing protocol repeated in an attempt to induce atrial fibrillation and assess accessory pathway conduction (10). The CCh dose utilized, determined by a dose-response curve (data not shown), led to a 20% heart rate decrease and shortening of atrial refractoriness (atrial $ERP_{150} = 55 \pm 9$ ms before CCh vs. $43 \pm 8$ ms after CCh, $p < 0.05$). A stable heart rate was observed within 5 min of intraperitoneal CCh and maintained for 30 min, considered effective cholinergic stimulation. However, atrial pacing did not provoke atrial fibrillation in any $TG^{N488I}$ mice; thus, anterograde accessory pathway conduction during atrial fibrillation could not be assessed.

## DISCUSSION

A missense mutation in *PRKAG2* induced accessory AV connections in the murine heart. Several lines of evidence support the formation of accessory AV connections in this transgenic model. Programmed electrical stimulation in $TG^{N488I}$ mice switched the ECG pattern to produce a longer PR interval and narrow QRS complex, with a distinct His potential on the HBE recording, from one with a short PR interval and a wide QRS complex with AV fusion on the HBE. This suggests that the extrastimuli are blocked in the accessory connection and conduct through the AV node. Procainamide produced similar ECG and intracardiac effects, with block induced in the accessory AV connection and conduction transferred to the AV node. Further evidence for an accessory AV connection is demonstrated by continued AV conduction with adenosine in pre-excited $TG^{N488I}$ mice, but adenosine-sensitive AV block in $TG^{WT}$ mice.

This transgenic mouse model recapitulates many phenotypic characteristics of human familial WPW syndrome. Pre-excitation was present in 23% of patients in a family from which the *N488I* mutation was derived (5). The EPS performed in four individuals from another *PRKAG2* family showed seven accessory pathways at various locations (15). Similarly, 14 of 28 $TG^{N488I}$ mice had accessory AV connections with variable anatomic location. In $TG^{N488I}$ mice,

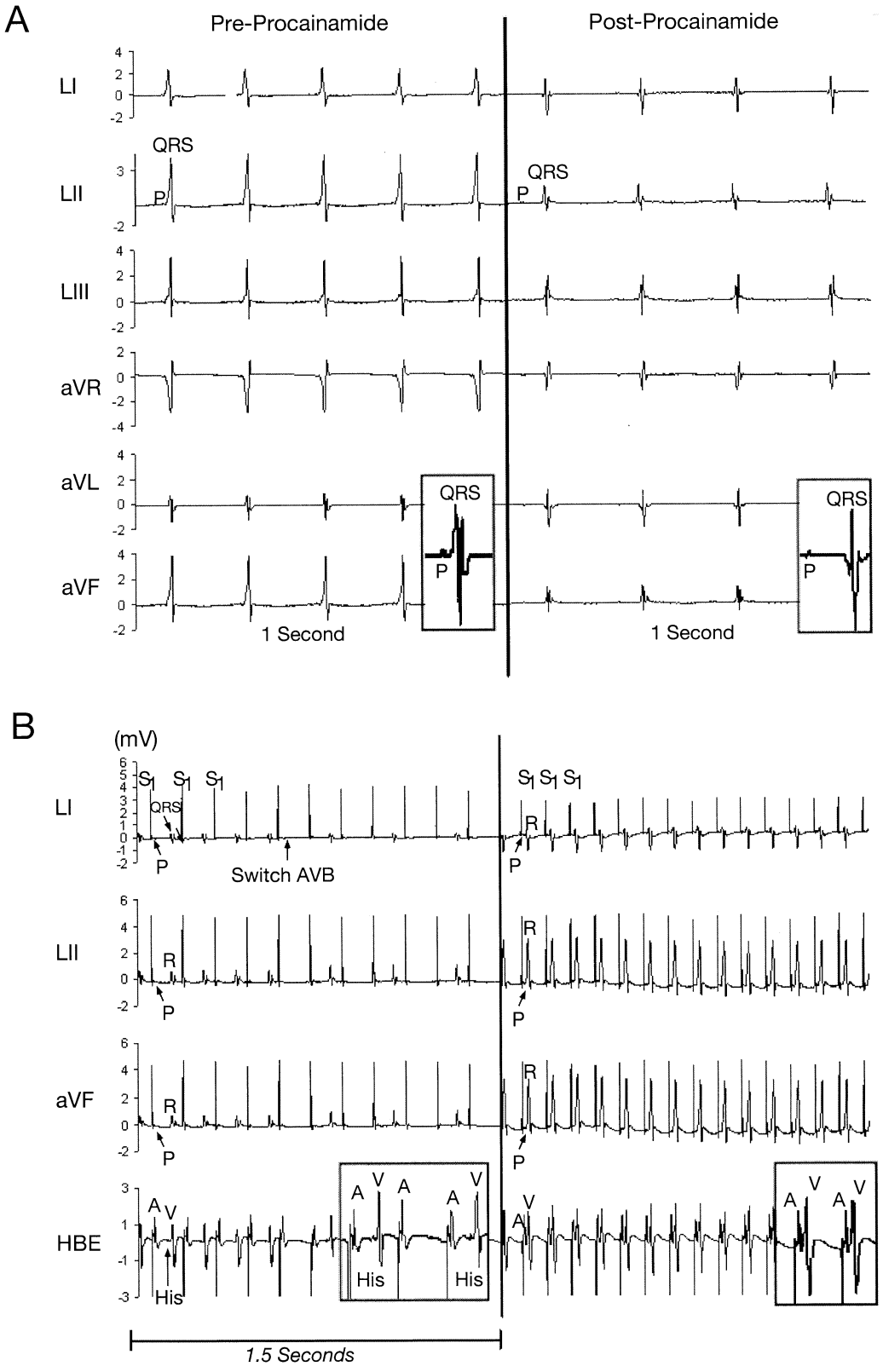


**Figure 3.** Pharmacologic effects on pre-excitation in mutant transgenic mice. **(A)** The **left panel** shows a six-lead electrocardiogram (ECG) at baseline, with a short PR interval and wide, initially slurred QRS complex. The **right panel** shows the same six-lead ECG 2 min after procainamide infusion, which lengthens the PR interval and narrows the QRS complex. The initial positive deflection of the QRS complex in leads I, II, III (LI, LII, and LIII), and aVF in the **left panel** suggests an anteroseptal accessory AV pathway. **(B)** In the **left panel**, surface ECG leads I, II, and aVF, as well as the HBE, from a wild-type mouse are displayed. Atrial pacing at 130 ms initially conducts 1:1 to the ventricles; however, adenosine infusion **(arrow)** produces AV block with 2:1 conduction to the ventricles. In the **right panel**, the same ECG leads, along with the HBE, are displayed from a $TG^{N488I}$ mouse. Atrial pacing at 100 ms conducts 1:1 to the ventricles, despite the presence of adenosine. A = atrial electrogram; AVB = atrioventricular block; His = His potential; V = ventricular electrogram.

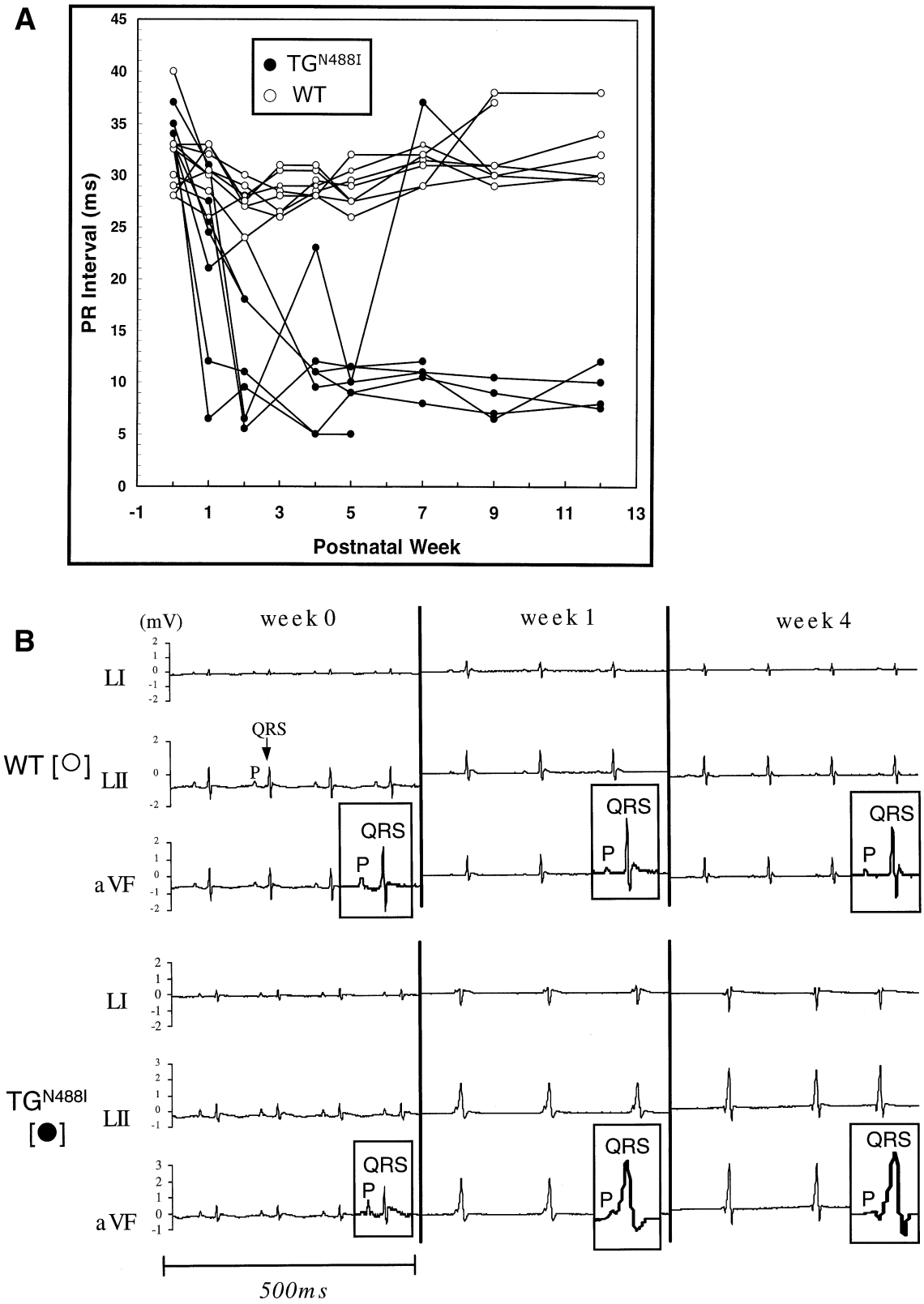


**Figure 4.** Time course of developing pre-excitation in mutant transgenic mice. **(A)** The PR intervals from a cohort of 16 mice pups are plotted over 12 weeks. At birth (week 0), all pups have normal PR intervals without pre-excitation. By week 1, two of eight transgene positive mice **(solid circles)** develop short PR intervals and pre-excitation, increasing to seven mice by week 4. No transgene-negative mice **(open circles)** had short PR intervals or pre-excitation. **(B)** The **top portion** shows electrocardiogram (ECG) leads I, II (LI and LII), and aVF from a $TG^{WT}$ mouse. The PR interval remains distinct and unchanged from birth through four weeks. The **bottom portion** displays the same ECG leads from a $TG^{N488I}$ mouse. At week 0, the PR interval is similar to that of the $TG^{WT}$ mouse. By week 1, a short PR and wide QRS complex develop, suggestive of ventricular pre-excitation. At week 4, the PR interval remains short, with a wide QRS complex, sinus bradycardia, and sinus arrhythmia.

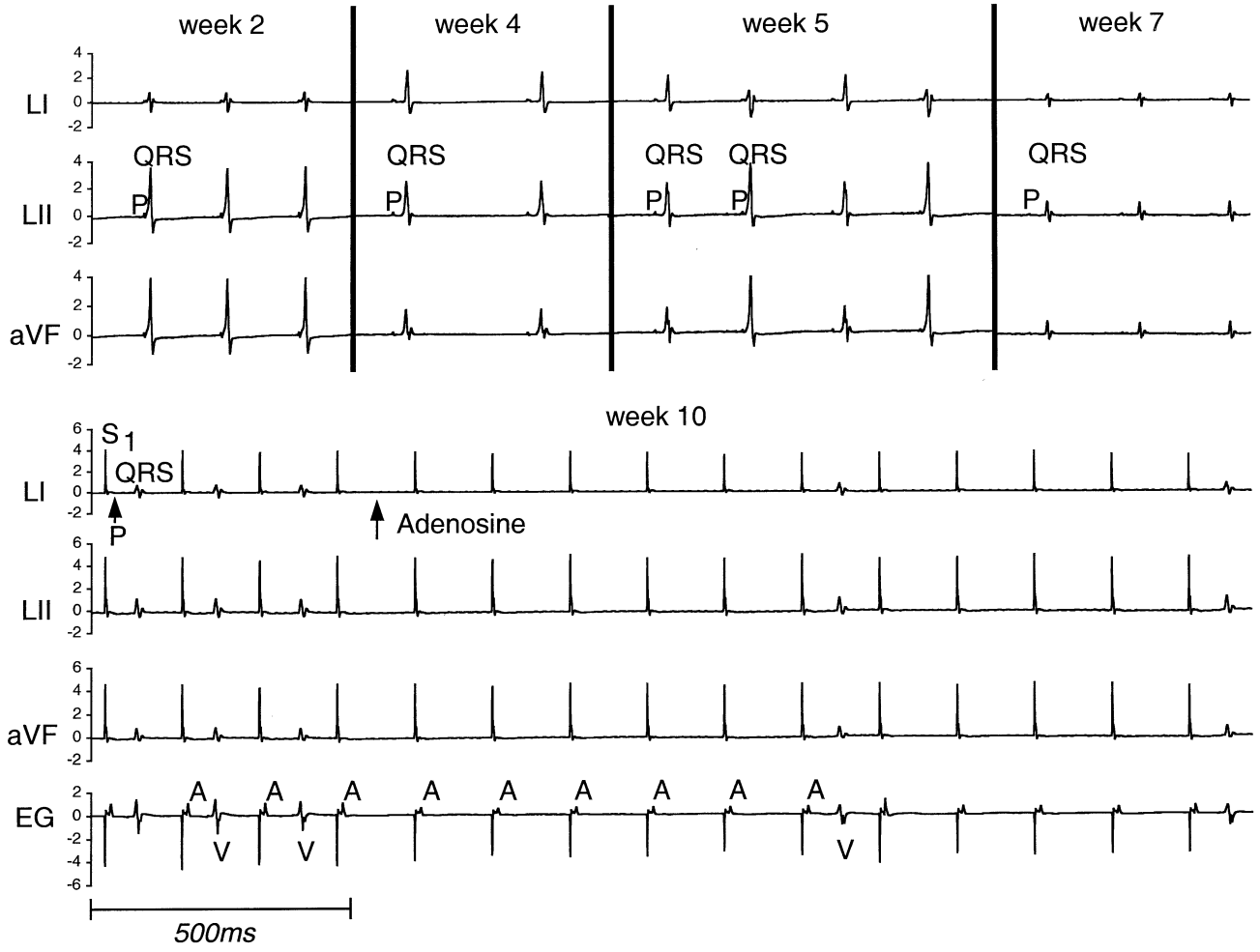


**Figure 5.** Loss of ventricular pre-excitation in a mutant mouse. **(Top panel)** Electrocardiographic leads I, II (LI and LII), and aVF displayed from a $TG^{N488I}$ mouse. An electrocardiogram (ECG) pattern of pre-excitation is evident at postnatal week 2. At week 4, the phenotype was lost, but intermittent pre-excitation returned in week 5. By week 7, the phenotype was completely lost, with a normal ECG. **(Bottom panel)** Electrocardiographic leads are displayed, along with the HBE (EG). At week 10, adenosine infusion **(arrow)** during atrial burst pacing at 150 ms results in AV block with a slow ventricular response. A = atrial electrogram; V = ventricular electrogram.

AV connections were localized using vector analysis of the initial QRS complex deflection, extrapolating ECG algorithms derived from humans (11). Although these algorithms were not designed for analysis of mouse ECGs, they appear to localize the AV connections. In several $TG^{N488I}$ mouse hearts, accessory AV connections anatomically correlated with the surface ECG "delta" wave vector axis (Fig. 6). Other electrophysiologic characteristics that this mouse model recapitulates include sinus bradycardia and AV conduction disorders. Clinically, 31% of patients with the *N488I* mutation developed sinus bradycardia; the sinus cycle length of $TG^{N488I+BPT}$ mice was longer than that of $TG^{WT}$ mice. Also, 8% of these patients developed AV block and 15% required pacemakers (5). Although complete AV block was not observed, AV node refractoriness was significantly longer in $TG^{N488I}$ versus $TG^{WT}$ mice.

Despite firm evidence for the presence of an accessory AV connection and observation of spontaneous, nonsustained SVT in older $TG^{N488I}$ mice, orthodromic AV reciprocating tachycardia could not be induced, even with isoproterenol or procainamide. There are two potential reasons why SVT could not be induced: 1) retrograde conduction up the accessory AV connection is brisk (1:1 VA conduction >1,200 beats/min in all $TG^{N488I+BPT}$ mice); and 2) most had anteroseptal AV connections in close proximity to the His-Purkinje system, which may be particularly germane in the small murine heart. Together, these factors produce a small re-entrant circuit with a rapid transit time, so the His bundle cannot recover from refractoriness and maintain tachycardia, even when bypass tract conduction is slowed. Perhaps with a combination of advancing age, growth of the heart, and slower VA conduction, these factors may be more conducive for initiation and maintenance of SVT. In the present series, mice were studied up to 15 months, whereas the incidence of arrhythmia may increase with age, as in humans with this mutation (5). In fact, we have observed SVT, sinus bradycardia, and pauses in older mice (>50 weeks) by single-lead telemetric ECG, including several

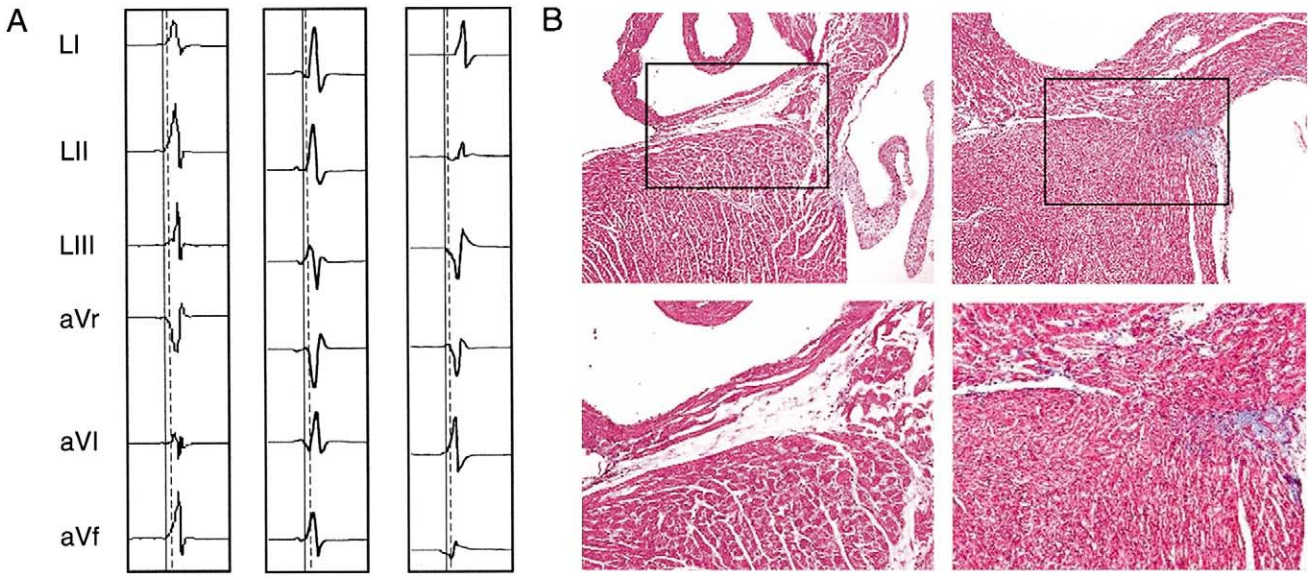


**Figure 6.** Accessory connection localization in mutant transgenic mice. **(A)** Vector analysis of surface six-limb lead ECG recordings from three $TG^{N488I}$ mice. In each panel, the **solid line** is placed at the onset of pre-excitation, and the **dotted line** is placed 5 ms later, intersecting the preexcited waveform. The **left panel** shows a pattern consistent with an anteroseptal AV connection (leads I and aVL and leads II, III, and aVF are positive). The **middle panel** shows a pattern consistent with a left lateral AV connection (leads I and aVL are negative and leads II, III, and aVF are positive), and the **right panel** shows a pattern of a posteroseptal AV connection (lead I is isoelectric, lead aVL is positive, and leads II, III, and aVF are negative). **(B)** Masson trichrome-stained sections (×5) from one-week-old $TG^{N488I}$ **(left panels)** and 2.5-week-old $TG^{N488I}$ **(right panels)** hearts through the right paraseptal area anterior to the aortic outflow tract. The fibrous separation between the atrial and ventricular myocardium is intact in the mutant heart from the one-week-old animal, whereas there is physical contact between the atrial and vacuolated ventricular myocytes from the 2.5-week-old animal in the right anteroseptal region. Bottom inserts magnified ×20.

episodes correlated with sudden bradycardic death (7). However, on EPS, these mice did not have inducible AV re-entrant tachycardia or atrial fibrillation.

Interestingly, during serial ECG recordings in newborns, no $TG^{N488I}$ pups had pre-excitation immediately after birth. After the first postnatal week, 25% of $TG^{N488I}$ mice showed pre-excitation, increasing to 88% by week 4. Because the transgene is under control of the murine alpha-MHC promoter, we did not expect the phenotype to express until postnatal life, as cardiac alpha-MHC expression increases 16-fold during the first postnatal week (16). This provides evidence that the mutant *PRKAG2* gene is responsible for expression of the phenotype, but more intriguingly, the accessory AV connections are induced in postnatal life after completion of cardiac organogenesis. This suggests that constitutively activating AMP kinase mutations induce formation of accessory AV connections, independent of septation and organogenesis. Although AMP kinase is known to regulate ribonucleic acid transcription (17), the mechanism by which these tracts form postnatally remains unknown. However, the anatomic basis for pre-excitation in *PRKAG2* mutants does not appear to be failed resorption of embryonic AV tracts.

In this regard, we saw one *PRKAG2* mutant mouse that developed pre-excitation but lost the phenotype after several weeks. The EPS with adenosine revealed no evidence of accessory pathways, with a relatively short AV node ERP (70 ms) and good anterograde AV node conduction (AV Wenckebach node = 85 ms). These data suggest that ion channel remodeling may have induced concealment of the AV connection in the anterograde direction (by enhancing AV node conduction and slowing accessory tract conduction), rather than physical loss of the AV connection. Another possibility is that late remodeling and fibrosis anatomically altered the accessory connection and affected its conductive properties. The AMP kinase modulates adenosine triphosphate-dependent ionic conductance (18), so accessory pathway conductance may increase by direct effects of AMP kinase on ion channels. These cardiomyocytes accumulate large amounts of cytoplasmic glycogen (4–7), which can absorb water and alter the ionic environment and conductive properties. The accumulation of cardiac glycogen may promote accelerated conduction, as seen in Pompe's disease, or contribute to disruption of the annulus fibrosis, causing a novel acquired form of WPW syndrome. The creation of this animal model allows for molecular and basic electrophysiologic analyses to provide further insight into the mechanisms governing the development and maintenance of accessory AV pathways.

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