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### Article

Myotonic dystrophy (DM) is the most common form of muscular dystrophy and is caused by expansion of a CTG trinucleotide repeat on human chromosome 19. Patients with DM develop atrioventricular conduction disturbances, the principal cardiac manifestation of this disease. The etiology of the pathophysiological changes observed in DM has yet to be resolved. Haploinsufficiency of myotonic dystrophy protein kinase (DMPK), DM locus-associated homeodomain protein (DMAHP) and/or titration of RNA-binding proteins by expanded CUG sequences have been hypothesized to underlie the multi-system defects observed in DM. Using an *in vivo* murine electrophysiology study, we show that cardiac conduction is exquisitely sensitive to DMPK gene dosage. DMPK<sup>-/-</sup> mice develop cardiac conduction defects which include first-, second-, and third-degree atrioventricular (A–V) block. Our results demonstrate that the A–V node and the His-Purkinje regions of the conduction system are specifically compromised by DMPK loss. Importantly, DMPK<sup>+/-</sup> mice develop first-degree heart block, a conduction defect strikingly similar to that observed in DM patients. These results demonstrate that DMPK dosage is a critical element modulating cardiac conduction integrity and conclusively link haploinsufficiency of DMPK with cardiac disease in myotonic dystrophy.

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# DMPK dosage alterations result in atrioventricular conduction abnormalities in a mouse myotonic dystrophy model

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PUBLICATION

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Myotonic dystrophy (DM) is the most common form of muscular dystrophy and is caused by expansion of a CTG trinucleotide repeat on human chromosome 19. Patients with DM develop atrioventricular conduction disturbances, the principal cardiac manifestation of this disease. The etiology of the pathophysiological changes observed in DM has yet to be resolved. Haploinsufficiency of myotonic dystrophy protein kinase (DMPK), DM locus-associated homeodomain protein (DMAHP) and/or titration of RNA-binding proteins by expanded CUG sequences have been hypothesized to underlie the multi-system defects observed in DM. Using an *in vivo* murine electrophysiology study, we show that cardiac conduction is exquisitely sensitive to DMPK gene dosage. DMPK<sup>-/-</sup> mice develop cardiac conduction defects which include first-, second-, and third-degree atrioventricular (A-V) block. Our results demonstrate that the A-V node and the His-Purkinje regions of the conduction system are specifically compromised by DMPK loss. Importantly, DMPK<sup>+/-</sup> mice develop first-degree heart block, a conduction defect strikingly similar to that observed in DM patients. These results demonstrate that DMPK dosage is a critical element modulating cardiac conduction integrity and conclusively link haploinsufficiency of DMPK with cardiac disease in myotonic dystrophy.

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## Introduction

The disorders of skeletal muscle and cardiac function observed in myotonic dystrophy (DM) occur as a consequence of a CTG repeat expansion (1, 2) located in the 3' untranslated region of a protein kinase, myotonic dystrophy protein kinase (DMPK; 3, 4), on chromosome 19q13.3. The molecular mechanisms by which expanded CTG sequences produce DM pathophysiology remain unresolved, though three models prevail. First, partial loss of DMPK resulting as a consequence of nuclear retention of the mutant DMPK mRNA may contribute to DM (5-8). Second, decreased transcription of a neighboring homeodomain-encoding gene, DMAHP, occurring possibly due to altered chromatin structure near the CTG expansion, may play a role in DM

(9, 10). Lastly, transdominant effects associated with expression of expanded CUG repeats and modulation of splice-site recognition may contribute to DM pathology (11-13).

The primary clinical cardiac manifestation in DM is the development of conduction disturbances, with progressive atrioventricular (A-V) block and bradycardia. Prolonged A-V conduction (first-degree A-V block) is common, and higher-grade A-V block is a significant cause of death (14-17). The His-Purkinje system may also be affected, with bundle branch block and intraventricular conduction delay (18, 19).

To test the hypothesis that partial DMPK loss contributes to DM pathology, we developed a mouse strain lacking functional DMPK (DMPK<sup>-/-</sup>). We have shown that DMPK<sup>-/-</sup> mice develop

late-onset skeletal myopathy as a consequence of abnormal excitation/contraction coupling (20). In this study we demonstrate that DMPK dosage modulates cardiac conduction. On electrocardiogram (ECG), a prolonged P-R interval (first-degree A-V block) was present in adult DMPK<sup>-/-</sup> mice compared to wild-type controls. Electrophysiological evaluation of DMPK<sup>-/-</sup> mice demonstrated more serious conduction disturbances including second and third-degree A-V block. Adult DMPK<sup>+/-</sup> mice also exhibited first-degree A-V block similar to DMPK<sup>-/-</sup> mice. Thus, heterozygous animals have a quantifiable cardiac phenotype reminiscent of DM patients. Our results show that DMPK gene disruption causes A-V conduction abnormalities in both homozygous and heterozygous DMPK-deficient mice, and support loss of DMPK as playing a dominant role in the characteristic DM cardiac phenotype.

## Methods

**Animal care.** A total of 27 male and 20 female adult 129/BS strain mice were studied. The mean age was 63.9 ± 21 weeks, with no differences between genotypes. The average weight was 37.7 ± 9 grams. Another 18 young mice were studied to assess age-related alterations in conduction properties. Death occurred in 6 animals due to procedural complications. Mice were housed in a diurnal facility, in compliance with the American Association for the Accreditation of Laboratory Animal Care and each center's Institutional Animal Care and Use Committee. Mice were anesthetized with intraperitoneal ketamine hydrochloride and pentobarbital (0.033 mg/gm each).

*Electrocardiography and electrophysiology studies.* The mouse electrophysiological study methodology has been previously described in detail (21, 22). A multilead surface electrocardiogram (ECG) was obtained and both epicardial and endocardial surgical techniques were performed. The epicardial approach involved a midline sternotomy for placement of pacing wires on each heart chamber (21). An octapolar mouse electrophysiology catheter (NuMED, Inc., Hopkinton, New York, USA) was placed via jugular vein for pacing and endocardial electrogram recording (22–24). The catheter has precise interelectrode distances, allowing the proximal 2 electrode pairs to pace and record the right atrium, while the distal electrodes lie in the right ventricle for stimulation and signal acquisition. Cardiac rhythm and respirations were monitored, ECG intervals calculated, and standard pacing protocols used to determine the electrophysiologic parameters (21–25). Pharmacological autonomic manipulations were performed using intravenous isoproterenol (1–3 ng/gm) and atropine (1 ng/gm).

*Ambulatory electrocardiogram telemetry.* A subset of 3 DMPK<sup>-/-</sup>, DMPK<sup>+/-</sup>, and DMPK<sup>+/+</sup> mice underwent ambulatory ECG recordings using implantable radiotransmitters (DataSciences International, St. Paul, Minnesota, USA). Following a 48-h stabilization period, ECG recordings were acquired for 5 ± 3 days, including periods of rest and exertion. Each animal underwent a graded exercise test, using a swimming protocol. The mice were placed in a warm bath, and swam for incrementally longer intervals each day, increasing to 9 min on day 10 of exercise. Custom software was designed for automated acquisition, analysis, and computation of heart rate variability parameters. Sympathetic and parasympathetic influences on heart rate regulation were quantified by frequency domain power spectral techniques.

*Data acquisition and analysis.* Surface ECGs and electrogram recordings were acquired on a multichannel amplifier and converted to a digital signal for analysis (MacLab System, AD Instruments, Milford, Massachusetts, USA). Pacing and programmed stimulation was performed with a Bloom DTU-201 stimulator (Fischer Imaging Corp., Denver, Colorado, USA), modified to enable the rapid pacing intervals necessary in mice.

*Statistical analysis.* All continuous variables, such as ECG intervals and cardiac conduction properties, were compared to gender- and age-matched controls, with data presented as the mean ± 1 standard deviation. Statistical analysis included the 2-tailed Student's *t* test; analysis of variance (ANOVA), with Scheffé subgroup testing when appropriate; and analysis of interobserver variability. A *P* value of < 0.05 was considered statistically significant.

## Results

*Adult DMPK<sup>+/-</sup> and DMPK<sup>-/-</sup> mice exhibit first-degree A–V block.* ECG analysis was obtained on mature DMPK<sup>-/-</sup> mice (*n* = 17) and wild-type control animals (*n* = 17) at 6–7 months and again at 17–19 months of age. Additionally, to assess the effects of DMPK haploinsufficiency, 10 DMPK<sup>+/-</sup> animals were studied at 16–19 months of age. The mean sinus cycle length (beat-to-beat heart rate), P-wave duration (atrial conduction time), P–R interval (atrial and A–V nodal conduction time), QRS interval (ventricular depolarization time), J–T interval (ventricular repolarization time), and Q–T interval (surrogate of action potential duration) were measured (Table 1). The average resting sinus cycle length was 156.9 ± 40.3 milliseconds (heart rate 404.8 ± 94.6 beats per minute [bpm]) in DMPK<sup>-/-</sup> mice, 152 ± 34.3 milliseconds (heart rate 413.1 ± 93.7 bpm) in heterozygotes, and 148.2 ± 30.8 milliseconds (heart rate 421.7 ± 88.4 bpm) in wild-type controls. (*P* > 0.05 for all measurements.) Normal sinus rhythm was present in all three groups, implying that sinoatrial node function was not impaired. Both the DMPK<sup>-/-</sup> and the DMPK<sup>+/-</sup> mice had similarly prolonged atrioventricular conduction times when compared to the wild-type controls. On ECG, a prolonged P–R interval was seen in all adult homozygous DMPK<sup>-/-</sup> mice (mean ± SD = 48 ± 7 milliseconds) and in all 10 heterozygous DMPK<sup>+/-</sup> mice (mean P–R = 48 ± 8 milliseconds), compared with wild-type mice (mean P–R = 34 ± 5 milliseconds) (Fig. 1a). (*P* < 0.001 for all measurements.) Basal heart rates (reflecting sinus node automaticity) and other ECG intervals (QRS, J–T, Q–T, Q–Tc, and J–Tc [c indicates interval corrected for heart rate]) in DMPK-mutant homozygotes and heterozygotes were not significantly different from wild-type controls (Table 1). Intraventricular conduction abnormalities,

appreciable on the ECG as either right or left bundle branch block or nonspecific intraventricular conduction delay, were present in 22% of mutant mice, but were also apparent in 12% of wild-type mice (*P* = 0.07). DMPK-deficient mice had P–R prolongation with a normal P-wave duration and QRS interval, indicating A–V node dysfunction. A lack of conduction delay through the working atrial muscle and ventricular myocardium suggests specific action at the level of the specialized conduction tissue.

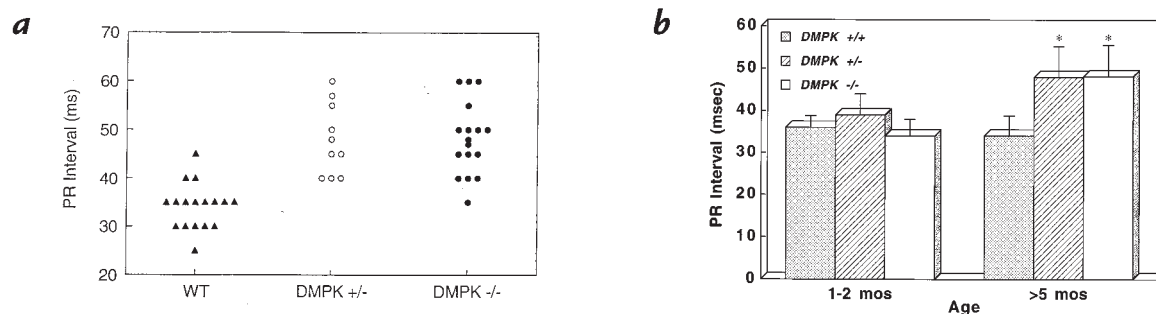
*DMPK<sup>-/-</sup> mice manifest severe A–V conduction disturbances including second- and third-degree A–V block.* Sinus node, atrial, A–V and ventricular conduction properties and refractoriness were further studied in DMPK<sup>-/-</sup>, DMPK<sup>+/-</sup>, and DMPK<sup>+/+</sup> mice using an *in vivo* electrophysiologic study (Table 2). Rate-corrected sinus node recovery time (CSNRT) was evaluated for the indirect determination of sinus node function. CSNRT measurements were more varied among DMPK<sup>-/-</sup> mice; however, the mean CSNRT was similar among all three groups (*P* > 0.05), implying normal intrinsic sinus node function. Programmed atrial stimulation was performed to assess A–V nodal physiology (Fig. 2). DMPK<sup>-/-</sup> mice manifested more severe A–V conduction disturbances during atrial pacing (Fig. 2a). Five of 17 DMPK<sup>-/-</sup> mice had second-degree A–V block (Fig. 2b), and 2 of 17 DMPK<sup>-/-</sup> mice had third-degree A–V block (Fig. 2c). None of the DMPK<sup>+/-</sup> or DMPK<sup>+/+</sup> mice had any higher-grade atrial or A–V dysfunction (Table 3). These results show that DMPK loss results in A–V conduction delay and demonstrate a critical role for DMPK in A–V node function.

*The severity of conduction disturbances increases as a function of age in DMPK<sup>-/-</sup> mice.* There were no significant differences in conduction parameters on surface ECG or intracardiac recordings

**Table 1**  
Electrocardiographic data summary

	P–R	QRS	J–T	Q–T	Q–Tc	J–Tc
DMPK <sup>+/+</sup>	34.1 ± 4.75	29.9 ± 6.14	52 ± 15	82 ± 15	214.3 ± 25.6	136.0 ± 30.69
DMPK <sup>+/-</sup>	48.0 ± 7.8	33.6 ± 5.8	50 ± 9.4	84 ± 11	217 ± 23.1	130.4 ± 22.15
DMPK <sup>-/-</sup>	48.2 ± 7.4	29.1 ± 6.59	52 ± 16	81 ± 17	205.2 ± 24.5	130.6 ± 29.26

The resting surface ECG intervals for wild-type (DMPK<sup>+/+</sup>), heterozygote (DMPK<sup>+/-</sup>), and homozygote (DMPK<sup>-/-</sup>) mice are tabulated. The P–R interval was longer in both DMPK<sup>+/-</sup> and DMPK<sup>-/-</sup> mice, compared with DMPK<sup>+/+</sup> controls. The QRS, J–T, Q–T, and rate-corrected Q–T (QTc) and J–T (J–Tc) were not significantly different between groups. ECG, electrocardiogram; DMPK, myotonic dystrophy protein kinase.



**Figure 1**

PR intervals of DMPK-mutant and wild-type mice. (a) Scatter diagram of individual P-R intervals on resting electrocardiograms from mature homozygote DMPK<sup>-/-</sup>, heterozygote DMPK<sup>+/-</sup>, and wild-type DMPK<sup>+/+</sup> mice. The majority of adult mutant mice had a P-R interval > 35 ms, whereas the majority of wild-type mice had a P-R interval ≤ 35 ms. The mean P-R interval was significantly longer in both heterozygote and homozygote adult mice, compared with control mice ( $P < 0.05$ ). (b) Mean (± standard deviation) P-R interval (in ms) from two distinct age groups of DMPK<sup>-/-</sup>, DMPK<sup>+/-</sup>, and DMPK<sup>+/+</sup> mice. There was no significant difference in mean P-R interval among the 1–2-month-old mice. The DMPK-deficient mice over 5 months old had a significantly prolonged mean P-R duration. WT, wild type.

among the younger (1–2-month-old) DMPK<sup>-/-</sup>, DMPK<sup>+/-</sup>, and DMPK<sup>+/+</sup> mice. The mean P-R interval was similar in each genotype (see Fig. 1b). There was a trend towards an increase in P-R intervals with age, such that P-R intervals greater than 50 milliseconds were not observed in the 6-month-old DMPK<sup>-/-</sup> mice, and manifested only at 16–17 months of age in DMPK<sup>-/-</sup> mice. Additionally, second- and third-degree heart block occurred at a higher frequency in 16–17-month-olds and primarily in mice with a P-R interval ≥ 45 milliseconds. Thus, the P-R duration might be used to predict the occurrence of more serious A-V conduction disorders over time in these mice. This developmental progression of A-V block and the correlation of severe conduction disorders with the length of the P-R interval is reminiscent of DM patients.

*Ambulation and exercise testing did not lead to an increase in conduction disorders.* Implantable telemetry ECG recorders were surgically placed in three mice from each group and ECG tracings

obtained at rest and during exercise. During monitoring, prolonged P-R intervals were clearly evident in the homozygous and heterozygous mice (Fig. 2d). However, there was no further progression of higher-grade A-V block noted during rest, ambulation, or graded exercise testing. None of the animals had any documented ectopy, arrhythmias or sudden death during exercise. The telemetered mean heart rate and heart rate variability parameters were statistically similar among all three genotypes (Table 4).

*Neither sympatholytic nor cholinergic effects are primarily responsible for progressive A-V block in DMPK mutant mice.* Programmed stimulation and pacing protocols were performed at baseline and with sympathomimetic stimulation. Central isoproterenol (1–3 ng/gm) was administered to a subgroup of 11 DMPK<sup>+/-</sup> or DMPK<sup>-/-</sup> mice, and control mice. This treatment increased heart rates 20–25%, shortened conduction intervals, and reduced refractoriness. However, catecholaminergic stimula-

tion did not positively or negatively affect A-V conduction parameters in DMPK-deficient mice differentially compared with controls. These results indicate that sympatholytic effects are not directly implicated in the A-V block seen in DMPK mutant mice. Following isoproterenol, the mice were administered a single dose of intravenous atropine (1 ng/gm). This shortened sinus cycle length by 14 ± 6%, without changing P-R duration or significantly shortening atrioventricular conduction times measured during atrial pacing. This data shows a lack of anticholinergic reversal of A-V block, and implies that delayed A-V conduction in DMPK-deficient mice is not secondary to vagal actions.

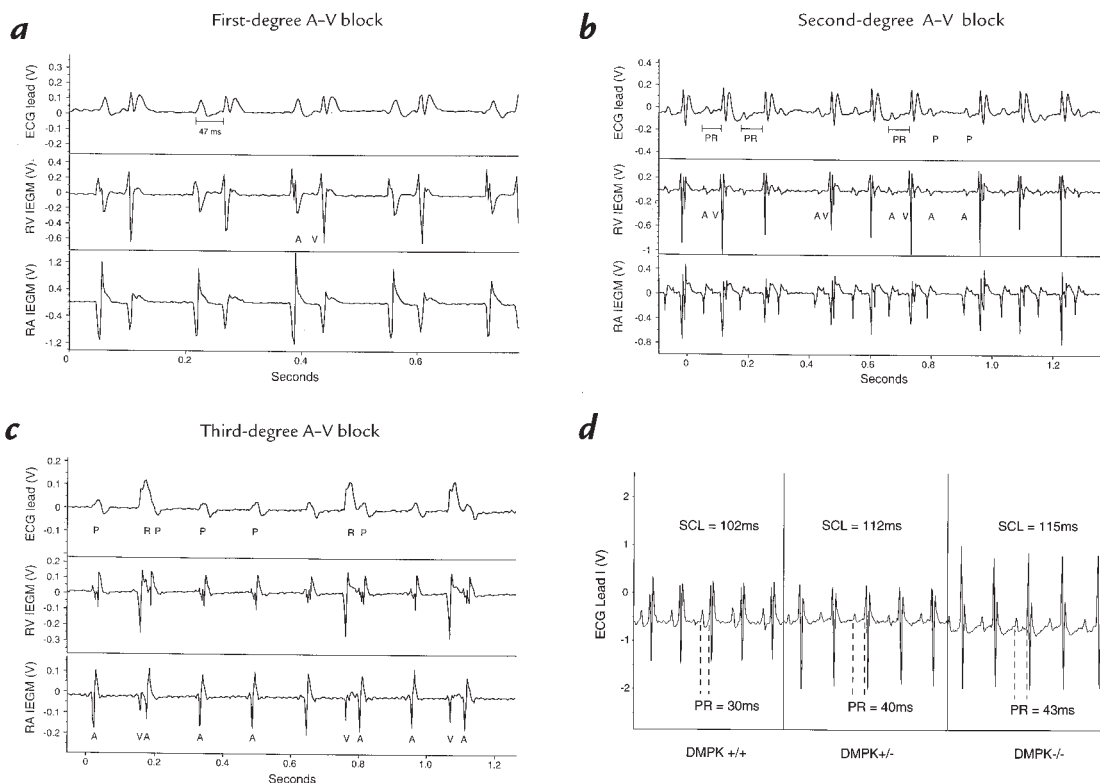
*Gross structural differences were not observed in DMPK<sup>+/-</sup> and DMPK<sup>-/-</sup> hearts.* Histological cardiac evaluation was performed on serial sections, with particular focus on the A-V node. Sections were stained with hematoxylin-eosin and Gomori trichrome to determine if structural alterations and/or increased

**Table 2**

Electrophysiologic data summary

	SNRT	CSNRT	AP WENK	AP 2:1	AVERP	AERP	MIN VAC	VERP
DMPK <sup>+/+</sup>	152.1 ± 57.8	15.7 ± 15.4	73.3 ± 16.3	60 ± 14.1	53 ± 19.7	48 ± 14.8	91 ± 18.9	63 ± 7.6
DMPK <sup>+/-</sup>	226.9 ± 67.4	33.8 ± 35.4	103.8 ± 16	86.2 ± 15.1	62 ± 8.3	40 ± 10	112 ± 20	66 ± 8.9
DMPK <sup>-/-</sup>	221.2 ± 165	66.3 ± 110.8	88.6 ± 35.5	77.3 ± 32.9	60 ± 12	54 ± 8.9	98.7 ± 24.7	59 ± 31.1

The intracardiac electrophysiological parameters are summarized for wild-type (DMPK<sup>+/+</sup>), heterozygote (DMPK<sup>+/-</sup>), and homozygote (DMPK<sup>-/-</sup>) mice. SNRT was measured following 15 seconds of atrial pacing at cycle length 100 ms, as the point from the last paced atrial electrogram to the first return spontaneous sinus beat. CSNRT was calculated as SNRT minus the sinus cycle length. AP WENK and AP 2:1 were measured. AVERP and AERP are maximum non-conducted coupling intervals during programmed atrial stimulation. MIN VAC was assessed as the minimum ventricular pacing cycles conducting 1:1 to the atrium. VERP was determined by programmed ventricular stimulation as the maximum coupling interval not depolarizing the ventricle. SNRT, sinus node recovery time; CSNRT, rate-corrected sinus node recovery time; AP WENK, maximum atrial-paced cycle length causing Wenckebach-type atrioventricular block; AP 2:1, maximum cycle length during decremental burst atrial pacing which conducts every other beat; A-V, atrioventricular; AVERP, effective refractory period of the A-V node–His-Purkinje system; AERP, effective refractory period of the atrium; MIN VAC, retrograde ventriculoatrial conduction; VERP, ventricular effective refractory period.



**Figure 2**  
 Selected examples of ECG and intracardiac electrogram tracings from DMPK<sup>-/-</sup> mice with varying degrees of atrioventricular (A-V) block. (a) First-degree A-V block. The P-R interval on ECG is prolonged to 47 ms, coincident with a long A-V interval on intracardiac recordings from right atrium and ventricle. (b) An example of Mobitz type I second-degree A-V block (Wenckebach phenomenon) in a DMPK<sup>-/-</sup> mouse, with gradual P-R prolongation on surface ECG and A-V prolongation on intracardiac electrograms on each successive cycle until the atrial impulse is not conducted to the ventricle. (c) An example of third degree A-V block in a DMPK<sup>-/-</sup> mouse, with complete dissociation of the P waves from the QRS complexes. (d) Representative composite telemetered ECG samples from 3 different mice during unrestrained, unanesthetized ambulation. (a) A control DMPK<sup>+/+</sup> mouse has an SCL of 102 ms and a P-R interval of 30 ms on the frontal ECG lead. (b) An example of a telemetered ECG from a DMPK<sup>+/-</sup> mouse, with an SCL of 112 ms and a P-R interval of 40 ms. (c) A tracing from a DMPK<sup>-/-</sup> mouse, with an SCL of 115 ms and a P-R interval of 43 ms. IEGM, intracardiac electrogram; RA, right atrium; RV, right ventricle; SCL, sinus cycle length.

fibrosis could account for the conduction defects. No notable increase in either fibrosis or atrophy was detected in working myocardium or conduction system tissue, suggesting that functional deficits associated with DMPK loss and/or structural changes of the conduction system, rather than myocardial tissue changes, may underlie the observed conduction defects. Echocardiographic assessment demonstrated no differences among the three genotypes in cardiac function, chamber dimensions, wall thickness, or ventricular shortening.

### Discussion

The presented work demonstrates that DMPK-deficient mice have distinct cardiac electrophysiological abnormalities specifically affecting atrioventricular conduction. The degree of P-R prolongation on ECG is similar between mice heterozygous and

homozygous for the DMPK mutation. However, DMPK<sup>-/-</sup> mice display more severe disturbances of A-V conduction during electrophysiologic testing. These results indicate that cardiac conduction is sensitive to DMPK dosage and link haploinsufficiency of DMPK with atrioventricular conduction disturbances, which are the predominant feature of cardiac disease in DM. This murine DMPK homologue has a high degree of amino acid homology with DMPK in humans, making it a useful model for studying the cardiac pathophysiology of DMPK inactivation (20).

Abnormal impulse propagation in DMPK-mutant mice may reflect changes in the magnitude of the depolarizing current and/or alterations in the geometry of cell-cell electrical connections. As neither fibrosis nor gross changes in cardiac anatomy were detected, functional alterations in impulse propagation may be the pri-

mary event underlying the conduction disturbances. These data do not rule out subtle changes in cell-cell communication occurring as a consequence of structural changes in the conduction system, alteration of gap junctions, or connexin isoform distribution.

In this study, we show that impulse generation and sinus node automaticity is unaltered by DMPK loss as both heart rate and CSNRT were normal in DMPK mutant mice. Conversely, A-V

**Table 3**  
 High-grade atrioventricular block

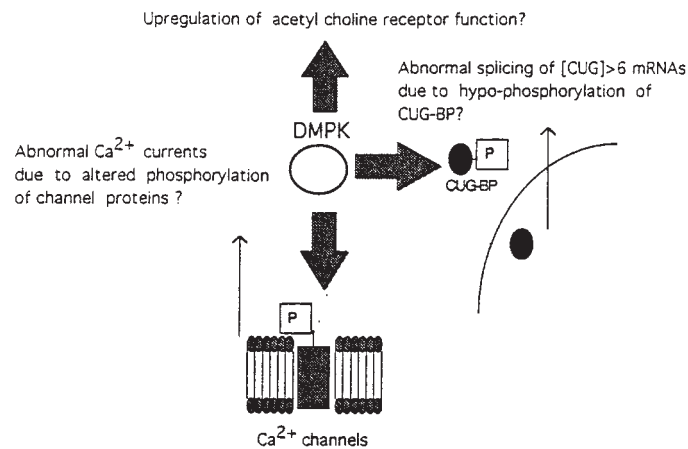
	Second-degree A-V block	Third-degree A-V block
DMPK <sup>+/+</sup>	0	0
DMPK <sup>+/-</sup>	0	0
DMPK <sup>-/-</sup>	5/7	2/17

The murine corollary of first-degree A-V block was seen in the homozygote and heterozygote DMPK mice. Higher grades of A-V block were seen only in DMPK<sup>-/-</sup> mice.

node function was found to be sensitive to DMPK dosage as both the DMPK<sup>-/-</sup> and DMPK<sup>+/-</sup> mice exhibited prolongation of the P-R interval in conjunction with a normal P-wave duration and QRS interval. Further abnormalities of the A-V nodal-His-Purkinje system may lead to the more severe A-V disturbances observed in DMPK<sup>-/-</sup> mice upon atrial pacing.

As the A-V node is a slow-conducting, calcium-channel dependent tissue, it is possible that calcium current (I<sub>Ca</sub>) inhibition could contribute to the prolonged A-V conduction time in DMPK mutant mice. These results are consistent with our previous study of DMPK<sup>-/-</sup> skeletal muscle abnormalities in excitation-contraction coupling occurring downstream of calcium efflux from the sarcoplasmic reticulum, leading to a 30–50% decrease in twitch and tetanic force generation (20). Benders *et al.* have demonstrated that the amplitude of calcium responses in DMPK<sup>-/-</sup> cultured myotubes are reduced by 40% compared to controls (26). Similar reductions in I<sub>Ca</sub> activation or amplitude in the A-V node could contribute to abnormally slow impulse propagation. Less likely is the possibility that alterations in sodium channel function in the fast-conducting tissues of the A-V node and of the His-Purkinje system contribute to the conduction abnormalities, because cardiac voltage-gated sodium channel function is not modulated by DMPK activity when assayed in *Xenopus* oocytes (27). Abnormalities in potassium channel function are also unlikely to significantly contribute to the conduction disturbances, as repolarization was found to occur normally as reflected by normal J-T intervals and lack of repolarization delay or heterogeneity (28).

DMPK loss may alter the phosphorylation status of ion channels, particularly calcium currents, and consequently modulate ion homeostasis in the specialized conduction system. Timchenko *et al.* have demonstrated that recombinant DMPK alters the phosphorylation status of dihydropyridine receptors *in vitro*, with activation of the β subunit (29). The profile of channel function affected by DMPK loss is currently being evaluated in DMPK<sup>-/-</sup> mice both by patch-clamp single cell analysis and by studying the treatment of heart block with drugs which modulate specific ion channel functions. A second



Possible mechanism underlying the conduction disorders observed in DMPK mutant mice

**Figure 3**

Schematic mechanistic model of DMPK mouse A-V conduction abnormalities. The diagram illustrates the possible ionic and cellular interactions and effects of DMPK deficiency on the modulation of cardiac conduction.

DMPK substrate which may play a role is a CUG repeat binding protein (CUG-BP) which has been shown to modulate splice-site recognition (13, 30). It has been previously demonstrated that CUG-BP is a splicing factor that is directly phosphorylated by DMPK, and that both DM patients and DMPK<sup>-/-</sup> mice accumulate hypophosphorylated CUG-BP in cardiac cell nuclei (30). It is unclear whether nuclear accumulation of hypophosphorylated CUG-BP results in abnormal splicing in DMPK<sup>-/-</sup> mice. Alterations in splice-site recognition, if present in DMPK<sup>+/-</sup> and DMPK<sup>-/-</sup> mice, may contribute to the

observed conduction disturbances. Upregulation of acetylcholine receptor function, either directly or indirectly, by DMPK deficiency may also result in prolongation of A-V conduction or higher-grade A-V block. DMPK has been shown to localize at the neuromuscular junction (31), supporting a role for cholinergic receptor modulation. Thus, our data is consistent with DMPK being part of a tightly regulated signaling pathway in which small changes in DMPK dosage result in underphosphorylation of key targets (Fig. 3), resulting in both decreased skeletal force generation and in specific

**Table 4**

Heart rate variability parameters

	DMPK <sup>-/-</sup> n = 21	DMPK <sup>+/-</sup> n = 20	DMPK <sup>+/+</sup> n = 17	P -/- vs. +/+	P -/- vs. +/-	P +/- vs. +/+
RR mean (ms)	108	110	106	0.7	0.7	0.4
RR median (ms)	108	110	106	0.7	0.7	0.4
HR (bpm)	572	555	569	0.9	0.6	0.5
PLF (ms <sup>2</sup> )	87.1	259.7	20.6	0.1	0.5	0.3
PHF (ms <sup>2</sup> )	74.3	97.3	25.9	0.1	0.8	0.3
PLFn	0.38	0.46	0.45	0.5	0.3	0.6
PHFn	0.62	0.54	0.55	0.7	0.5	0.7
PLFn:PHFn	0.61	0.85	0.82	0.8	0.3	0.4

Frequency domain methods were used to quantify sympathetic and parasympathetic influences on heart rate variability. The mean and median RR intervals (in ms) and conversion to heart rate (bpm) were similar among DMPK<sup>-/-</sup>, DMPK<sup>+/-</sup>, and DMPK<sup>+/+</sup> groups. PLF was defined as a range of 0.4–1.5 Hz bandwidth, and PHF as a range of 1.5–4 Hz. Normalized units PLFn and PHFn were obtained as fractional equivalents of total power minus very low frequency power. The ratio PLFn:PHFn is given to determine relative power spectra. There were no significant differences in any of the heart rate variability parameters measured among the 3 genotypes during telemetered ambulatory drug-free ECG acquisition. RR, cycle length between R waves. HR (bpm), heart rate in beats per minute; PLF, low-frequency power; PHF, high-frequency power.

cardiac conduction disorders.

These findings were compared with studies performed on transgenic mice which overexpress DMPK and as a consequence develop hypertrophic cardiomyopathy, a condition which is not characteristic of DM. Importantly, there were no notable abnormalities in *ex vivo* ventricular hemodynamics or ECG measurements in isolated perfused hearts from DMPK overexpressor mice (32). Consequently, our results support the hypothesis that CTG expansion results in decreased DMPK protein levels, leading to key pathological changes. The present data do not preclude the possibility that partial DM locus-associated homeodomain protein (DMAHP) loss may contribute to the observed symptoms by altering transcription of other relevant proteins, such as sodium-potassium pumps or ion channel regulatory genes. As DMAHP has homology to the mouse transcription factor AREC3/six4, which regulates expression of the Na<sup>+</sup>/K<sup>+</sup>-ATPase  $\alpha$ 1 subunit in developing skeletal muscle (33), it is conceivable that haploinsufficiency of DMAHP acts synergistically with DMPK loss to further alter ion homeostasis. Targeted gene disruption experiments should determine the role of partial DMAHP loss in the etiology and spectrum of DM.

In humans with DM, cardiac involvement typically manifests as conduction disturbances, with progressive deterioration in A-V nodal and intraventricular conduction. A clinical study demonstrated progressive ECG abnormalities, with 39% of subjects developing first-degree A-V block and 66% demonstrating intraventricular conduction delays (34). All ECG intervals lengthened with increasing patient age, but the P-R interval in particular had a direct correlation with age. Interestingly, the incidence of cardiac arrhythmic events (including sudden death, complete heart block, atrial fibrillation and syncope) occurred more frequently among patients with a longer baseline P-R interval, and no correlation was seen with any other ECG interval (34). These findings suggest that the P-R interval may be a predictive marker of patients at high risk for future arrhythmic events. Furthermore, correction of first-degree A-V block may prevent the development of more serious conduction disorders later in life. Similarly, in the present study, the P-R interval was

prolonged in both DMPK<sup>-/-</sup> and DMPK<sup>+/-</sup> adult mice, but not in younger DMPK-deficient mice, concordant with the human electrocardiographic phenotype. During *in vivo* electrophysiologic testing, some DMPK<sup>-/-</sup> mice also had higher-grade A-V block without abnormalities in atrial or ventricular conduction properties or refractoriness. The findings from this study suggest the potential for use of the standard ECG and selected use of electrophysiologic studies when P-R prolongation is seen, for assessment of A-V conduction in accordance with this murine DM model. Genetically manipulated DMPK mice could provide a tool for testing the efficacy of medications used to prevent heart block. DMPK replacement strategies currently being developed, similarly to dystrophin deficiency treatments, could be employed to prevent the incidence of cardiac complications related to DMPK insufficiency (35–38). This data might be extrapolated clinically for use in developing therapeutic interventional strategies for cardiac disease in myotonic dystrophy patients.

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