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PRE-CLINICAL RESEARCH

Microtubule-Mediated Misregulation of Junctophilin-2 Underlies T-Tubule Disruptions and Calcium Mishandling in *mdx* Mice

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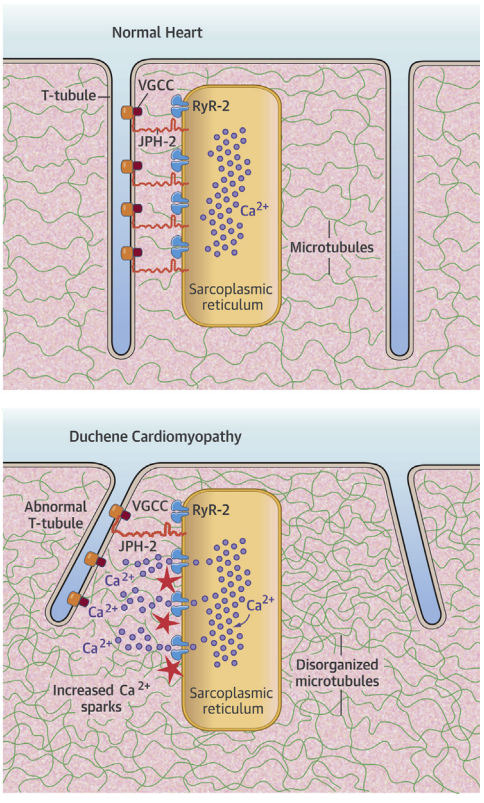
VISUAL ABSTRACT



Prins, K.W. et al. J Am Coll Cardiol Basic Trans Science. 2016; 1(3):122–30.

HIGHLIGHTS

- **Decreased junctophilin-2 levels are associated with cardiac t-tubule derangements in *mdx* mice, the mouse model of Duchenne muscular dystrophy (DMD).**
- **Reduced junctophilin-2 protein levels correlate with increases in total microtubule content in *mdx* hearts.**
- **Colchicine-mediated microtubule depolymerization increases junctophilin-2 protein levels and improves localization patterns which, in turn, are associated with t-tubule reorganization and reduced calcium sparks.**
- **This study identifies microtubule-mediated misregulation of junctophilin-2 as a novel molecular mechanism in Duchenne cardiomyopathy.**

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SUMMARY

Cardiac myocytes from the *mdx* mouse, the mouse model of Duchenne muscular dystrophy, exhibit t-tubule disarray and increased calcium sparks, but a unifying molecular mechanism has not been elucidated. Recently, improper trafficking of junctophilin (JPH)-2 on an altered microtubule network caused t-tubule derangements and calcium mishandling in a pressure-overload heart failure model. *Mdx* cardiac myocytes have microtubule abnormalities, but how this may affect JPH-2, t-tubules, and calcium handling has not been established. Here, we investigated the hypothesis that an inverse relationship between microtubules and JPH-2 underlies t-tubule disruptions and calcium mishandling in *mdx* cardiac myocytes. Confocal microscopy revealed t-tubule disorganization in *mdx* cardiac myocytes. Quantitative Western blot analysis demonstrated JPH-2 was decreased by 75% and showed an inverse hyperbolic relationship with α- and β-tubulin, the individual components of microtubules, in *mdx* hearts. Colchicine-induced microtubule depolymerization normalized JPH-2 protein levels and localization, corrected t-tubule architecture, and reduced calcium sparks. In summary, these results suggest microtubule-mediated misregulation of JPH-2 causes t-tubule derangements and altered calcium handling in *mdx* cardiac myocytes. (J Am Coll Cardiol Basic Trans Science 2016;1:122–30)

Loss of dystrophin causes Duchenne muscular dystrophy (DMD), an X-linked disease characterized by striated muscle dysfunction resulting in a life expectancy of only 20 years to 30 years (1). Heart failure is the cause of death for 20% to 25% of DMD patients (2); therefore research aimed at understanding the molecular and cellular phenotypes underlying the cardiomyopathy of DMD has been conducted. Altered calcium homeostasis marked by increased calcium sparks (3–5) and t-tubule disarray were documented in cardiac myocytes from the dystrophin-deficient *mdx* mouse (6,7), but a unifying molecular mechanism has not been identified.

Junctophilin (JPH)-2, the protein that links the plasma membrane of t-tubules to the ryanodine receptor, is essential for proper t-tubule structure and function (8). Recently, it was reported that JPH-2 mislocalization due to abnormalities in microtubule cytoskeleton caused pathological t-tubule remodeling and abnormal calcium handling in the pressure overload-induced heart failure model (9). However, the relationship between microtubules and JPH-2 in other models of heart failure or cardiomyopathy has not been analyzed. Because previous studies documented microtubule derangements in *mdx* cardiac myocytes (5,10), we tested the hypothesis that microtubule alterations cause JPH-2 misregulation and result in t-tubule disruptions and calcium mishandling in *mdx* mice.

Finally, to investigate the translational aspects of our hypothesis, we examined the cardiomyopathy of *mdx* mice via echocardiography and isoproterenol stress tests as previous studies showed mildly reduced systolic function (11–14) and excessive mortality with isoproterenol administration (10,15,16) in *mdx* mice. Because Zhang et al. (9) and Guo et al. (17) showed improvement in systolic function with normalization of JPH-2, we hypothesized colchicine-induced JPH-2 normalization would reduce the severity of *mdx* cardiomyopathy.

METHODS

MICE. Control C57BL/10 and *mdx* mice were purchased from Jackson Laboratories. All animals were housed and treated following the guidelines set forth by the University of Minnesota Institutional Animal Care and Use Committee.

ANTIBODIES. Polyclonal antibodies for voltage-gated calcium channel (VGCC) (Sigma, Waltham, Massachusetts) and JPH-2 (ThermoScientific) and monoclonal antibodies for α-tubulin (Sigma), β-tubulin (Sigma), and dystrophin (Leica, Buffalo Grove, Illinois) were purchased from the identified vendors. Alexa-Fluor-488 or Alexa-Fluor-568–conjugated anti-rabbit antibodies were purchased from Molecular Probes (Eugene, Oregon). Infrared dye-conjugated anti-mouse and anti-rabbit antibodies were purchased from LICOR Biosciences (Lincoln, Nebraska).

ISOLATION OF CARDIAC MYOCYTES. Isolation of ventricular cardiac myocytes was performed as described previously (18).

T-TUBULE ASSESSMENT. Freshly isolated cardiac myocytes were fixed in 4% paraformaldehyde for 10 min at 37°C, washed with phosphate-buffered saline (PBS) 2 times for 5 min, incubated with AlexaFluor 488

ABBREVIATIONS AND ACRONYMS

DMD = Duchenne muscular dystrophy

JPH-2 = junctophilin-2

PBS = phosphate-buffered saline

SR = Sarcoplasmic reticulum

TT = transverse tubules

VGCC = voltage-gated calcium channel

WT = wild-type