Figures and figure supplements

Potassium dependent rescue of a myopathy with core-like structures in mouse

M Gartz Hanson, et al.
**Figure 1** ENU-induced RyrlAG mutation mimics clinical and pathological features of CCD in heterozygous mice. (A) Missense mutation in exon 93 of Ryrl changes A to G, resulting in substitution of glutamic acid with glycine (E4242G). (B) Average grip strength assayed using vertical digital push–pull strain gauge on Ryrl+/+ and RyrlAG/+ mice at different ages raised on control 0.6% potassium diet. (C) In vivo hanging task determination of upper-body strength of Ryrl+/+ and RyrlAG/+ mice at different ages (B, C, 10 trials/mouse, n = 5 per set). (D, E) H&E staining indicate central nuclei (arrows) in RyrlAG/+(E) but not Ryrl+/+(D) vastus lateralis muscle from 1-year old mice raised on control 0.6% potassium diet. (F, G) NADH-TR staining indicates cores (white arrows) in 1-year old vastus lateralis of RyrlAG/+(F, G) but not Ryrl+/+(H, I) Cytochrome oxidase (COX) staining denotes a decrease in mitochondrial function (white arrows) in vastus lateralis muscle of RyrlAG/+(I) compared to Ryrl+/+(H). Scale bar = 50 μm. (J–L) Transmission electron microscopy of 2-month old RyrlAG/+ soleus muscle. (J, K) Regions of Z line streaming and associated sarcoplasmic disruption and cores (white arrows) of myofibrils. (L) Enlarged T-tubules are present in type I fibers (scale bars: J = 1 μm; K = 2 μm; L = 0.2 μm.).

DOI: 10.7554/eLife.02923.003
Figure 2. Calcium homeostasis is disrupted in Ryr1AG/ muscle. Fura-2 ratiometric imaging of myoplasmic Ca\(^{2+}\) in 2-month old muscle. (A) Ratiometric analysis of 4-CmC sensitivity in soleus muscles of wild-type (black) and Ryr1AG/ littermates (grey). (B) Average ratiometric analysis of myoplasmic Fura-2 signal normalized to wild-type signal (n above bar represents number of analyzed fibers). (C) Representative fura-2 ratiometric signal of muscle fibers showing percent change after addition of 20 μM thapsigargin (TG) from wild-type (black) and Ryr1AG/ (grey) littermate. (D) TG induced maximum response in muscle fibers (n above bar represents number of analyzed fibers). (E, F) Representation and quantification of Mn\(^{2+}\) quenching of Fura-2 fluorescence ratiometric signal illustrating increased influx of store operated calcium entry in soleus muscle fibers of Ryr1AG/ (grey) muscle fibers compared to wild-type (black) muscle fibers (dash lines represent slope). In (E), arrows indicate when media was introduced with 0 Ca\(^{2+}\) followed by 0.5 mM Mn\(^{2+}\). DOI: 10.7554/eLife.02923.006
Figure 3. Defects in mitochondrial function in Ryr1\(^{AG/+}\) mice. (A–C) Representative isolated fiber with Rhod2 fluorescence of 2-month old Ryr1\(^{AG/+}\) in 3 mM KCl (A) and 1 min (B) and 10 min (C) after application of 1 mM 4-CmC (punctate fluorescence are calcium marks). (D) Quantification of mitochondrial calcium marks visualized with Rhod2-AM from isolated soleus muscle fibers from Ryr1\(^{+/+}\) (black bars) and Ryr1\(^{AG/+}\) (gray bars) mice. (E) Superoxide labeling of isolated muscle fibers from Ryr1\(^{+/+}\) and Ryr1\(^{AG/+}\) mice. (F) Intensity of TMRE labeling of isolated soleus muscle fibers from Ryr1\(^{+/+}\) and Ryr1\(^{AG/+}\) littermates with saponin. The numbers on top of bars in graphs represent number of fibers examined. (G) ATP content from Ryr1\(^{+/+}\) and Ryr1\(^{AG/+}\) isolated soleus muscle from age-matched mice (n = 4 for each age and group). Scale bar = 5 \(\mu\)m. DOI: 10.7554/eLife.02923.007
Figure 4. Detection and compensation of an internal potassium leak in RyR1AG/+ muscle. Fluorescence imaging of PBFJ at 340 nm in Ryr1+/+ (A) and Ryr1AG/+ (B) soleus muscle in 3 mM Ringer’s solutions. (C) Ratiometric potassium imaging obtained at 340 and 380 nm wavelengths provided a ratio of fluorescence in Ryr1+/+ (black) and Ryr1AG/+ (grey) soleus muscle (normalized to Ryr1+/+). (D) Representation of the ratiometric imaging experimental paradigms used in Figure 4 showing bath applications of 3 mM KCl, 7 mM KCl, 0 mM KCl, and 3 mM KCl with 2 μM glibenclamide in Ringer’s solutions. (E) Slope of intracellular K+ fluorescence intensities in experimental conditions. (F, H) Normalized intracellular K+ concentration in Ryr1+/+ (black) and Ryr1AG/+ (grey) soleus muscle in 3 mM KCL (F, H) compared to soleus from contralateral limb in 7 mM KCl Ringer’s solutions (F) or 3 mM KCL plus 2 μM Glibenclamide (H) (muscle was bathed in solutions for 1.5 hr before imaging, n is the number of fibers examined from four mice). (G) Rate of change in PBFJ fluorescence after acute bath application of 2 μM Glibenclamide. Scale bar = 20 μm.
DOI: 10.7554/eLife.02923.008
Figure 4—figure supplement 1. Serum level measurements from Ryr1<sup>+/+</sup> (black bars) and Ryr1<sup>AG/+</sup> (grey bars) in 2- and 6-month old mice. Measurements of serum levels were performed on Ryr1<sup>+/+</sup> (black bars) and Ryr1<sup>AG/+</sup> (grey bars) in 2- and 6-month old mice (n = 6 samples per group per age for Ryr1<sup>+/+</sup> and Ryr1<sup>AG/+</sup>). DOI: 10.7554/eLife.02923.009
Figure 5. Altered activity of K\textsubscript{ATP} channels involved in potassium transport. (A) Western blots of membrane enriched lysate (that lacks mitochondria, peroxisomes, and lysosomes) from 2-month old wild-type and heterozygous Ryr\textsubscript{1AG/+} soleus muscle analyzed for K\textsubscript{ATP}6.1, K\textsubscript{ATP}6.2, and K\textsubscript{IR}2.1. (B) Quantification of protein levels from Western blots of membrane enriched lysates. Each sample was first normalized to its own loading control and then the values from mutant and wild-type on the same blot were compared. Statistical analyses were determined from a minimum of 3 blots and at least two independent samples (n = 4 for each Western). (C) K\textsubscript{ATP} current densities of isolated soleus muscle fibers from 2-month old wild-type and Ryr\textsubscript{1AG/+} littermates with and without glibenclamide. Numbers on top of bars are number of fiber recordings. (D–E) Representative current density recordings with the same tip resistance from Ryr\textsubscript{1++} and Ryr\textsubscript{1AG/+} soleus muscle fibers (E) with and (D) without 2 μM glibenclamide. DOI: 10.7554/eLife.02923.010
Figure 6: Increased potassium diet can rescue muscle strength and reverse the CCD histology and myopathy. (A) Normalized internal potassium concentrations of soleus muscle from 2-month old Ryr1<sup>+/+</sup> and Ryr1<sup>AG+/+</sup> mice fed 0.6% K<sup>+</sup> diet for 4 weeks, then placed on 5.2% K<sup>+</sup> diet or 0.6% diet + enalapril for 4 weeks, and then bath exposed to different extracellular potassium concentrations. (B, C) Average grip strength (B, five trials/mouse and 5 mice/set; p values <0.001) and in vivo hanging task (C, 10 trials/mouse, n = 5 per set; p values <0.001) assayed from 2-month old Ryr1<sup>+/+</sup> (black bar) and Ryr1<sup>AG+/+</sup> (grey bar) mice maintained for 4 weeks on control 0.6% K<sup>+</sup> diet, 5.2% K<sup>+</sup> diet, or 0.6% K<sup>+</sup> diet supplemented with enalapril. (D) Quantification of number of internalized nuclei per 100 myofibers in 12-month old mice or in 2-month old mice maintained for 4 weeks on control 0.6% K<sup>+</sup> diet, 5.2% K<sup>+</sup> diet, or 0.6% K<sup>+</sup> diet supplemented with enalapril (n = 10 per muscle, n = 3 per set of muscles for total of n = 30; p values <0.001). (E–P) Vastus lateralis myofibers from 2-month old Ryr1<sup>+/+</sup> and Ryr1<sup>AG+/+</sup> mice maintained for 4 weeks on control 0.6% K<sup>+</sup> diet, 5.2% K<sup>+</sup> diet, or 0.6% K<sup>+</sup> diet supplemented with enalapril. Cross-sections stained with H&E (left panels) and COX (right panels). Ryr1<sup>AG+/+</sup> mice on 5.2% K diet show increased COX staining and no internalized nuclei, similar to Ryr1<sup>+/+</sup>. These pathological features are still observed in Ryr1<sup>AG+/+</sup> mice on 0.6% K<sup>+</sup> diets. Enalapril increases COX staining but some internalized nuclei are observed, even in Ryr1<sup>+/+</sup>. Scale bar = 50 μm; error bars as standard error of the mean (SEM).
DOI: 10.7554/eLife.02923.012
Figure 6—figure supplement 1. Blood pressure measurements from Ryr1+/+ (black bars) and Ryr1AG/+ (grey bars) in 2-month old mice. Non-invasive measurements of systolic (A) and diastolic (B) blood pressure were performed using a Visitec-2000 tail-cuff apparatus. Measurements were taken for 3 days to acclimatize the animals for a more reproducible blood pressure measurement and then the data were collected on day 4.
DOI: 10.7554/eLife.02923.013
**Figure 7.** Increased potassium diet influences $K_{ATP}$ channel activity and mitochondrial function. (A) Membrane enriched lysate (lacking mitochondria, peroxisomes, and lysosomes) from soleus muscle of 2-month old $Ryr1^{+/+}$ and $Ryr1^{AG/+}$ mice fed 5.2% potassium supplemented diet for 4 weeks analyzed by Western blot for $K_{ATP}6.1$, $K_{ATP}6.2$, and $K_{ATP}2.1$ (* is $p < 0.001$). (B) $K_{ATP}$ current densities of isolated soleus muscle fibers from $Ryr1^{+/+}$ and $Ryr1^{AG/+}$ littermates after 4 weeks on 5.2% K diet compared to 0.6% K diet. (C) ATP content from $Ryr1^{+/+}$ and $Ryr1^{AG/+}$ isolated soleus muscle from mice on 0.6% or 5.2% potassium diet. (D) Quantification of calcium marks visualized with Rhod2-AM from isolated soleus muscle from $Ryr1^{+/+}$ and $Ryr1^{AG/+}$ mice with and without diet therapy. (E) Superoxide labeling of isolated soleus muscle from $Ryr1^{+/+}$ and $Ryr1^{AG/+}$ mice with and without therapy. Error bars as standard error of the mean (SEM). DOI: 10.7554/eLife.02923.014
Figure 8. Inhibition of K<sub>ATP</sub> channels can reverse the histological and myopathic phenotypes. (A, B) Average grip strength (A, five trials/mouse and 5 mice/set; p values <0.001) and in vivo hanging task (B, 10 trials/mouse, n = 5 per set; p values <0.001) assayed from 2-month old Ryr1<sup>−/−</sup> (black bar) and Ryr1<sup>AG/+</sup> (grey bar) mice maintained for 4 weeks on 0.6% K diet and glibenclamide (15 mg/kg/day). (C) ATP content from soleus muscle of Ryr1<sup>−/−</sup> and Ryr1<sup>AG/+</sup> mice after 4 weeks on 0.6% K diet without or with glibenclamide (n = 4 muscles per condition). (D) Quantification of calcium marks visualized with Rhod2-AM from isolated soleus muscle from Ryr1<sup>−/−</sup> and Ryr1<sup>AG/+</sup> mice with and without glibenclamide therapy. (E) Normalized internal potassium concentrations after glibenclamide therapy. (F) K<sub>ATP</sub> current densities of isolated soleus muscle fibers from Ryr1<sup>−/−</sup> and Ryr1<sup>AG/+</sup> littermates after 4 weeks on glibenclamide therapy compared to 0.6% K diet. (G) Quantification of number of internalized nuclei per 100 myofibers in all conditions (n = 10 per Figure 8. Continued on next page
Figure 8. Continued

muscle, n = 3 per set of muscles for total of n = 30; p values <0.001). (H–K) Vastus lateralis myofiber from Ryr1+/- and Ryr1AG/- mice maintained for 4 weeks on glibenclamide. Arrow in Ryr1+/- myofiber (F) shows the rare occurrence of internal nuclei. Cross-sections stained with H&E (left panels) and COX (right panels). Ryr1AG/- mice on glibenclamide show increased COX staining, similar to Ryr1+/. Scale bar = 50 μm; error bars as standard error of the mean (SEM).
DOI: 10.7554/eLife.02923.015
Figure 9. Continued on next page.
Figure 9. Continued

Figure 9. Relative variation of quantitative qRT-PCR of human control RNA and RNA from human muscle biopsies. Whisker plots derived from the data in Table 2 of control muscle (yellow), congenital myopathy (CCD) with a mutation in RyR1 (green), and without a mutation in RyR1 (white). (A) ABCC8, (B) ATPA1, (C) KCNJ2, (D) KCNJ8, (E) KCNJ11, (F) PRKAA1. Values normalized to GAPDH before normalization to pooled control human muscle RNA. DOI: 10.7554/eLife.02923.017