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# Supplemental Data

# A Congenital Muscular Dystrophy with Mitochondrial Structural Abnormalities Caused by Defective De Novo Phosphatidylcholine Biosynthesis

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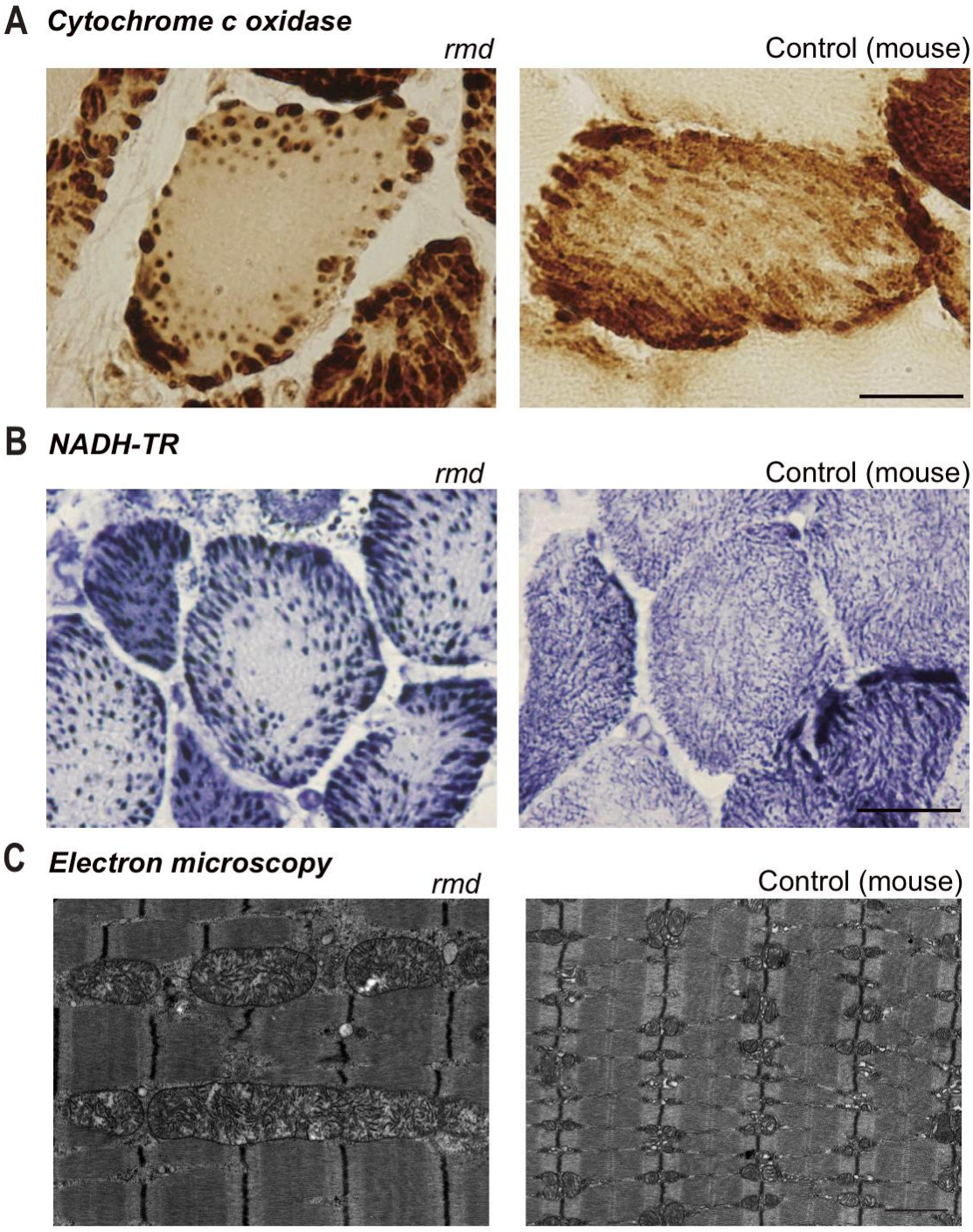
**Figure S1. Muscle pathology of *rmd* mouse and control littermate.**




**Supplementary Fig. 1**

**Figure S1. Muscle pathology of *rmd* mouse and control littermate.**

Cross sections of muscle fiber from *rmd* and control mice. Similar to the patients, *rmd* mice exhibit enlarged mitochondria at the periphery with central areas devoid of mitochondria on cytochrome c oxidase staining (a). Scale bar, 20 μm. On NADH-TR staining, the intermyofibrillar network is preserved even in the central areas that are devoid of mitochondria (b). Scale bar, 20 μm. Electron microscopy reveals enlarged mitochondria with prominent cristae (c). Scale bar, 1 μm.

**Figure S2. CHK activities in recombinant proteins**

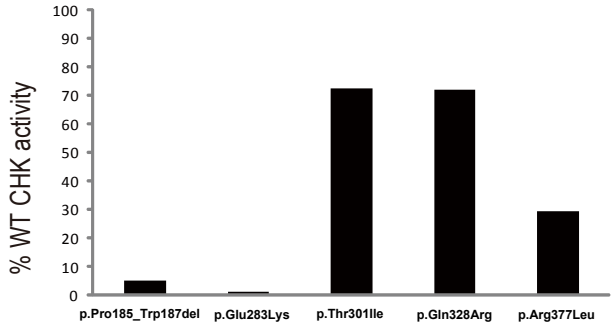



**Figure S2. CHK activities in recombinant proteins**

Four missense substitutions, p.Glu283Lys, p.Thr301Ile, p.Gln328Arg, and p.Arg377Leu, and one three-amino-acid deletion p.Pro185_Trp187del were identified in three patients. Patients 7 and 9 had two homozygous substitutions, p.Glu283Lys and p.Thr301Ile, and p.Pro185_Trp187del and p.Gln328Arg, respectively. Patient 8 was homozygous for the p.Arg377Leu mutation. None of these missense mutations or in-frame deletions was found in 210 control chromosomes. CHK activities in recombinant proteins were compared to wild type CHK-β. CHK-β with missense substitutions p.Pro185_Trp187del, p.Glu283Lys and p.Arg377Leu are decreased more than 30%. In contrast, the CHK activities of p.Thr301Ile and p.Gln328Arg are reduced by only ~25% compared to wild type, suggesting these could be neutral polymorphisms or only mildly hypomorphic mutations. Therefore, homozygous mutations, p.Glu283Lys, p.Arg377Leu and p.Pro185_Trp187del are likely to be causative in Patients 7, 8 and 9, respectively.

**Figure S3. cDNA analysis of patients with c.1031+1G>A mutation**

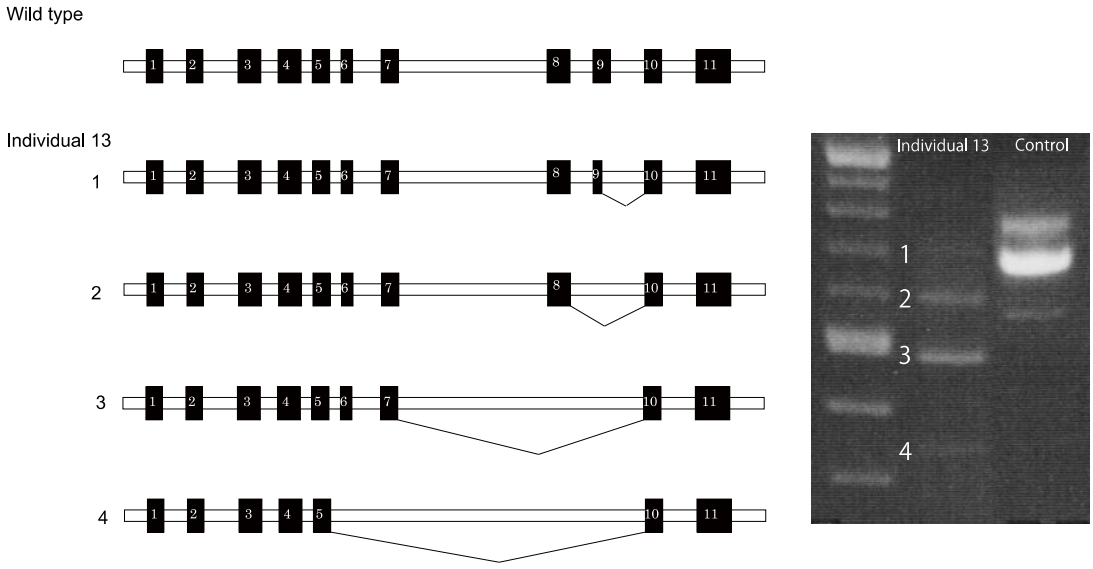



**Figure S3. cDNA anaylsis of patients with c.1031+1G>A mutation**

cDNA analysis from Patient 13 who possesses the mutation c.1031+1G>A shows four kinds of splice variations which truncate important domains for CHK activity. Two variants result in frame-shifts from the beginning and middle of exon 9. Two variants cause skipping of exon 6 to 9 and exons 8 and 9.

**Figure S4. CHK-α expression in muscle.**

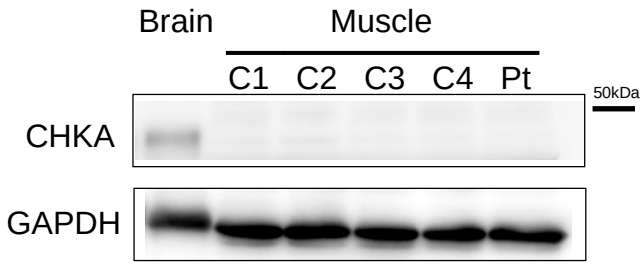



**Figure S4. CHK-α expression in muscle**

Westernblot analysis of CHK-α in human brain and muscle tissue was shown. CHK-α was detected in brain tissue, however, not detected in muscle.

Proteins were extracted from human brain and biopsied muscles and suspended in SDS sample buffer; 125 mM Tris-HCl pH 6.8, 5% β-mercaptoethanol, 2% SDS, 10% glycerol. Extracted proteins (10μg) were separated on acrylamide gels, and then transferred onto PVDF membranes (Millipore). Primary antibody used was rabbit anti-CHKA (ab38290, abcam) and mouse anti-GAPDH (RGM2, Advanced Immuno Chemical). Secondary antibody used was horse radish peroxidase-labeled goat anti-rabbit and anti-mouse antibody (Beckman Coulter). ImageQuant LAS 4000 Mini Biomolecular Imager (GE Healthcare) was used for evaluating bands. C: control, Pt: Individual 4.