The influence of a genetic variant in *CCDC78* on *LMNA*-associated skeletal muscle disease

Nathaniel P. Mohar [1,2], Efrem M. Cox [3,4], Emily Adelizzi [1,5], Steven A. Moore [3], Katherine D. Mathews [6], Benjamin W. Darbro *[1,6] and Lori L. Wallrath *[1,2]

Supplementary materials

Table S1. Antibody List.

Antigen	Use	Antibody Type	Source	Product Number	Dilution
Merosin	IHC	Rat monoclonal	Millipore (clone 4H8)	MAB1922	1:100
Embryonic myosin heavy chain (eMHC)	IHC	Mouse monoclonal	Developmental Studies Hybridoma Bank (DSHB)	F1.652	1:100
Lamin A/C (N18)	IHC	Goat polyclonal	Santa Cruz Biotechnology, Inc.	Sc-6215	1:200
Emerin (H12)	IHC	Mouse monoclonal	Santa Cruz Biotechnology, Inc.	Sc-25284	1:500
FG-repeat containing nuclear pore proteins (MAB414)	IHC	Mouse monoclonal	Covance	E11CF00696	1:2,000
CCDC78	IHC	Rabbit polyclonal	ThermoFisher	PA5-44001	1:100
Texas Red-X Phalloidin	IHC	N/A	Invitrogen Molecular Probes	T7471	1:400
Dystrophin	IHC	Mouse monoclonal clone 6A9	Developmental Studies Hybridoma Bank	MANEX50 (6A9)	1:100
P62/SQSTM1	IHC	Mouse monoclonal	R&D Systems	MAB8028-SB	1:200
RyR1	IHC	Mouse monoclonal	Developmental Studies Hybridoma Bank	34C	1:100

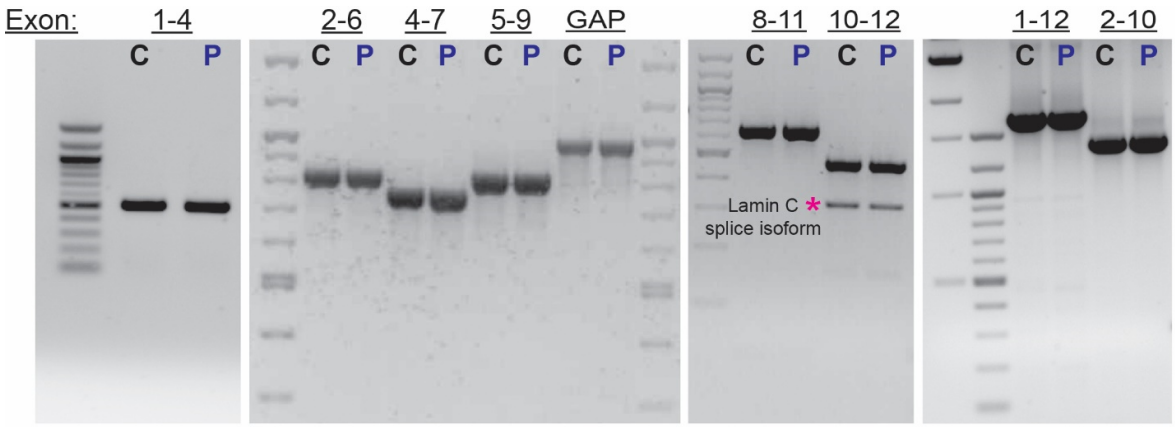

Figure S1. RT-PCR analysis of *LMNA* transcripts did not detect grossly abnormally sized products. RT-PCR analysis was performed on total mRNA extracted from control fibroblasts (C) and fibroblasts taken from an affected family member (P, II.2). All twelve exons of *LMNA* were interrogated in a stepwise fashion with over-lapping amplicons spanning exons 1-4, 2-6, 4-7, 5-9, 8-11, and 10-12. Full length *LMNA* cDNA was also amplified (exons 1-12) as well as the cDNA that produces the lamin C protein isoform (2-10). PCR amplicons were all of the appropriate size and no differentially expressed products were detected between the control and affected fibroblasts.

Table S2. Primer sequences used for RT-PCR amplification of the *LMNA* gene mRNA transcript and anticipated amplicon sizes.

Exons Amplified	Forward Primer	Reverse Primer	Anticipated Product
Exons 1-4	GCTGGAGCTGAG-CAAAGTGCGTGAGG[1]	GTCCAGCTTGGCAGAATAAGTC	508bp
Exons 2-3	AAGCGCGCAATACCAAGAAG	CTTGGTCTCACGCAGCTCT[2]	308bp
Exons 2-6	AAGCGCGCAATACCAAGAAG	GACAGGCGTAGCCTCTCCTC	820bp
Exons 4-7	AGTGAGGAGCTGCGTGAGAC	ACTGGTCCTCATTGGACTTGTT	754bp
Exons 5-9	AGAAGACTTATTCTGCCAAGCTG	ACTTCTTCCCCAGTGGAGTTGAT	823bp
Exons 8-11	CAACAAGTCCAATGAGGACCAG	AGGTCCCAGATTACATGATGCT	641bp
Exons 10-12	ATCAACTCCACTGGGGAAGAAG	GGCATGAGGTGAGGAGGAC	466bp
Exons 1-12	GCTGGAGCTGAG-CAAAGTGCGTGAGG[1]	ACATGATGCTGCAGTTCTGG[1]	1685bp
Exons 2-10 (Lamin C)	AAGCGCGCAATACCAAGAAG	CGGCTACCACTCACGTGGTG[3]	1360bp
GAPDH[4]	TGAAGGTCGGAGTCAACGGATTTGGTCATGTGGGCCATGACCTCCACCAC		983bp

[1]: Primer does not cross an exon:exon junction. All remaining primers do span an exon:exon junction (for example, the forward primer for amplification of exons 2-6 spans the exon 1:exon 2 junction).

[2]: Underlined nucleotide is a mismatch to the wildtype spliced mRNA transcript at the exon 3:exon 4 junction, however, is a match to a proposed mutation induced abnormally spliced mRNA product predicted by *in silico* analysis to utilize an upstream 5′ cryptic donor splice site.

[3]: Reverse primer is specific for a portion of exon 10 differentially spliced and present only in the mRNA transcript that gives rise to the lamin C protein isoform.

[4]: GAPDH was amplified as an internal amplification control

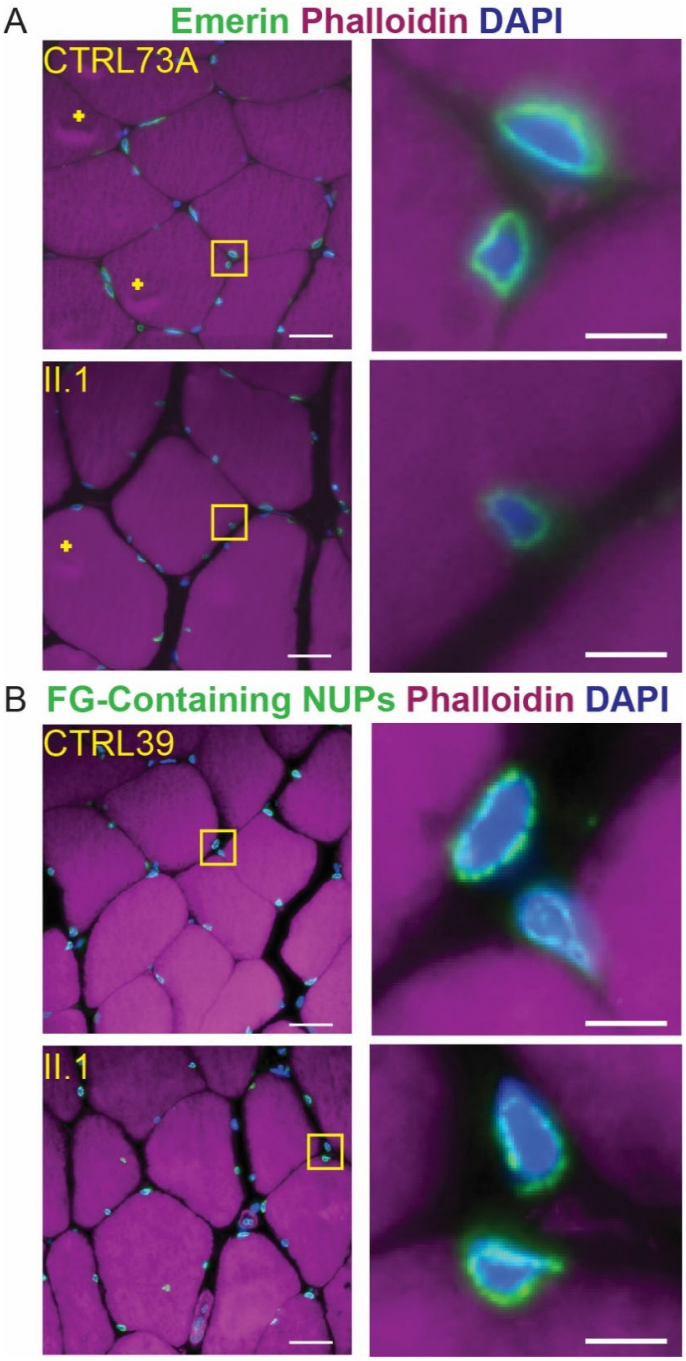

Figure S2: Emerin and nuclear pore proteins are properly localized in individuals with the *LMNA* mutation. A) Skeletal muscle biopsy from individual II.1 stained with an antibody against emerin, an inner nuclear envelope protein, reveals nuclear envelope localization, like that of the control (CTRL73A). Scale Bar = 30 μm. B) Skeletal muscle biopsy from individual II.1 stained with an antibody against FG repeat-containing nuclear pore proteins reveals nuclear envelope localization, like that of the control (CTRL39). Scale Bar = 30 μm.

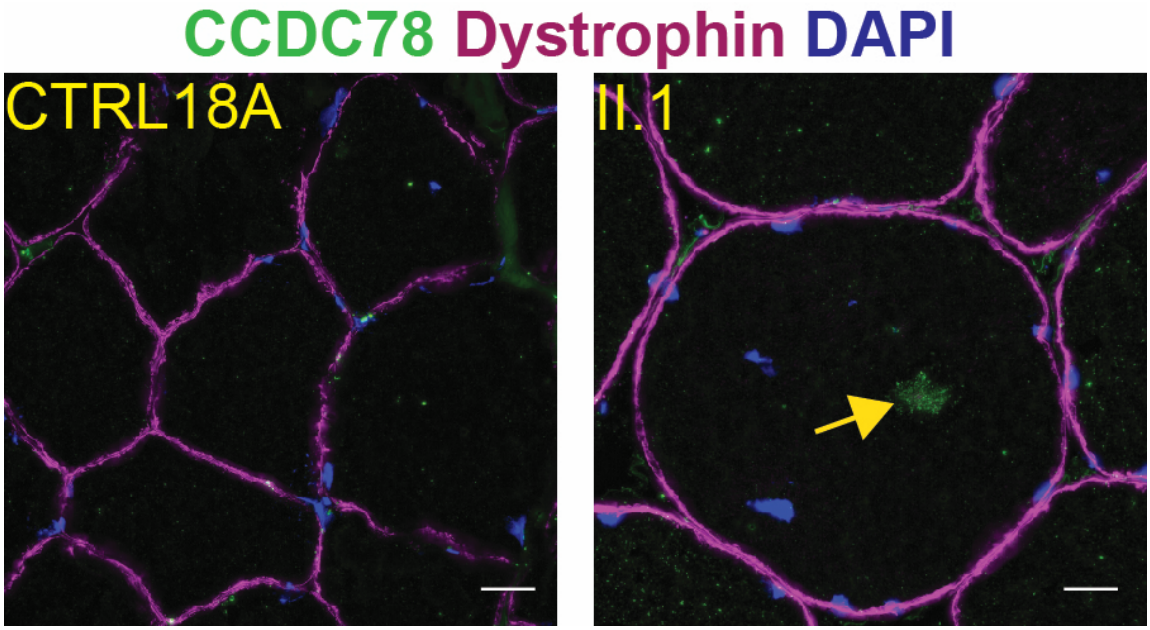

Figure S3. CCDC78 accumulation in the cytoplasm is obvious when the muscle cell periphery is marked with antibodies to dystrophin. Skeletal muscle biopsy tissue from individual II.1 stained with antibodies against CCDC78 and dystrophin shows obvious CCDC78 accumulation within the cytoplasm that is outlined by the dystrophin staining just under the sarcolemmal membrane. This demonstrates that the cytoplasmic CCDC78 staining in Figures 7 and 8 is not due to bleed through from fluorescent signal from other channels. Cytoplasmic CCDC78 accumulation is absent in the muscle of an unrelated control (CTRL18A). Scale bar = 30 μm.

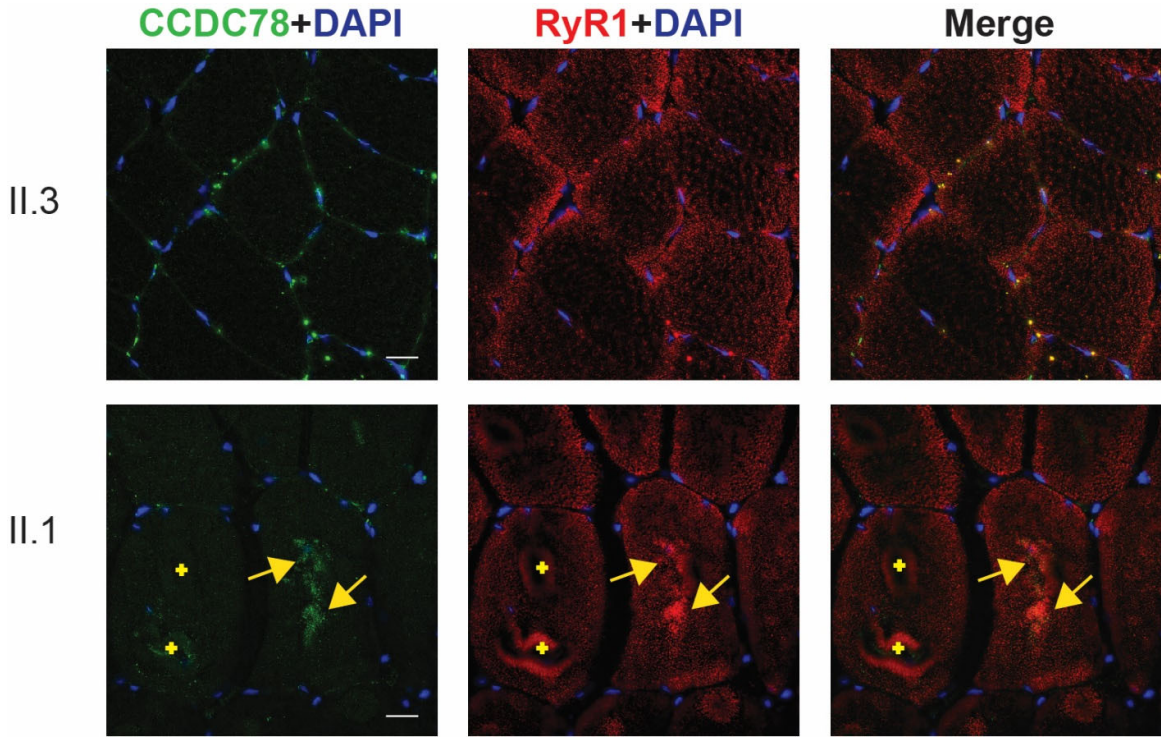

Figure S4. RyR1 accumulation is absent in individual II.3. Skeletal muscle biopsy from individual II.3, who possesses the *LMNA* mutation but lacks the *CCDC78* variant, stained with antibodies against CCDC78 and RyR1 show normal CCDC78 and RyR1 localization. In contrast, skeletal muscle biopsy from II.1, a sibling of II.3 who possesses both the *LMNA* and *CCDC78* variants, shows abnormal accumulation of both CCDC78 and RyR1 that colocalize within the cytoplasm. + = ice crystal artifacts from tissue preservation.

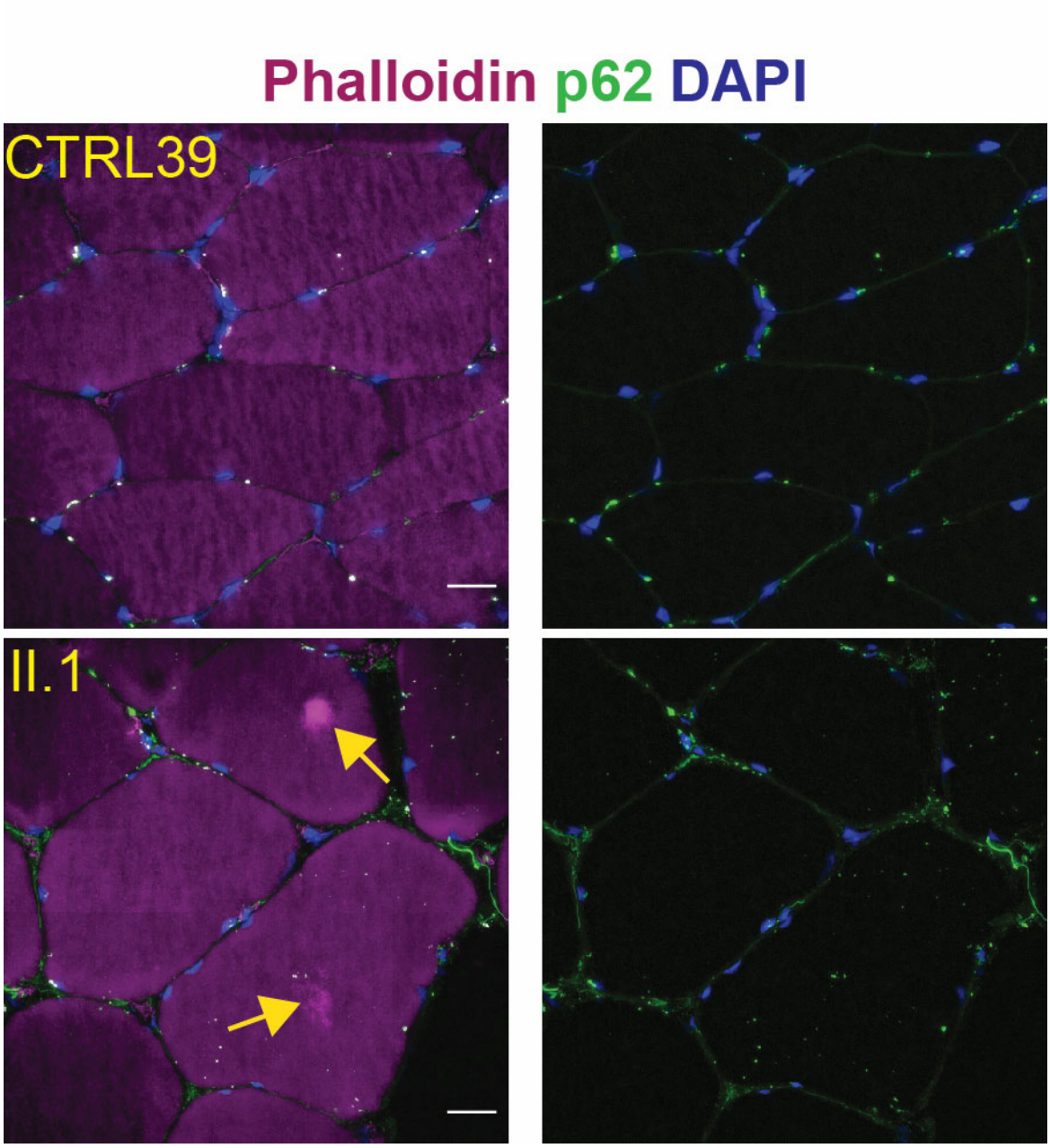

Figure S5. The muscle cores in profoundly affected family members are not marked by p62. Skeletal muscle biopsy from individual II.1 stained with an antibody against the p62 autophagy protein and phalloidin show muscle cores that lack staining with an antibody to p62. No cores and no p62 staining are observed in the control muscle (CTRL39). Scale bar = 30 μm.