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Supplemental Data

Mutations in *GLDN*, Encoding Gliomedin, a Critical Component of the Nodes of Ranvier, Are Responsible for Lethal Arthrogryposis

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SUPPLEMENTAL DATA

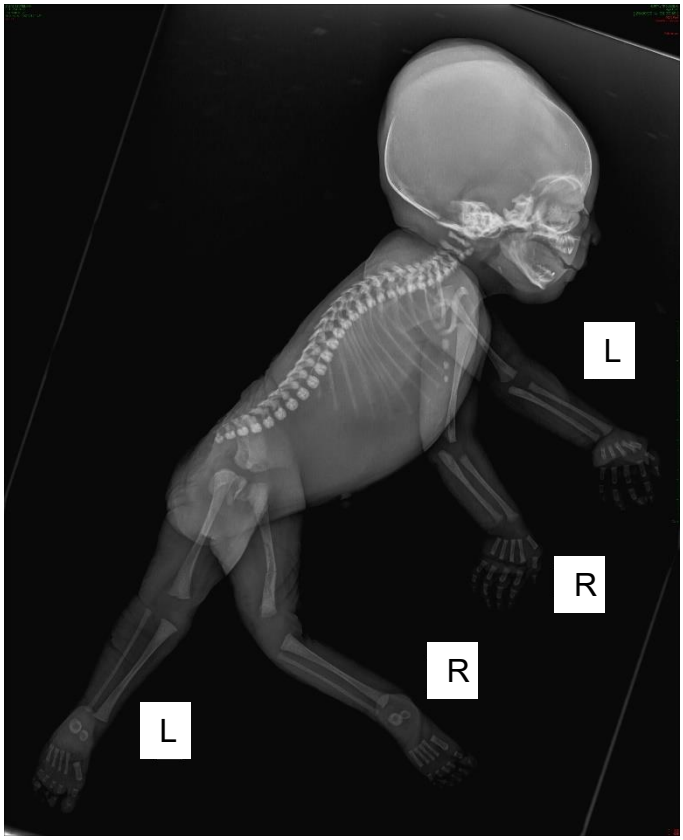

Figure S1: X-ray of affected individual II:1 of family 1 revealed marked extension of lower limbs associated with extension of handles and anomalies of the thoracic spine. L: left; R: right

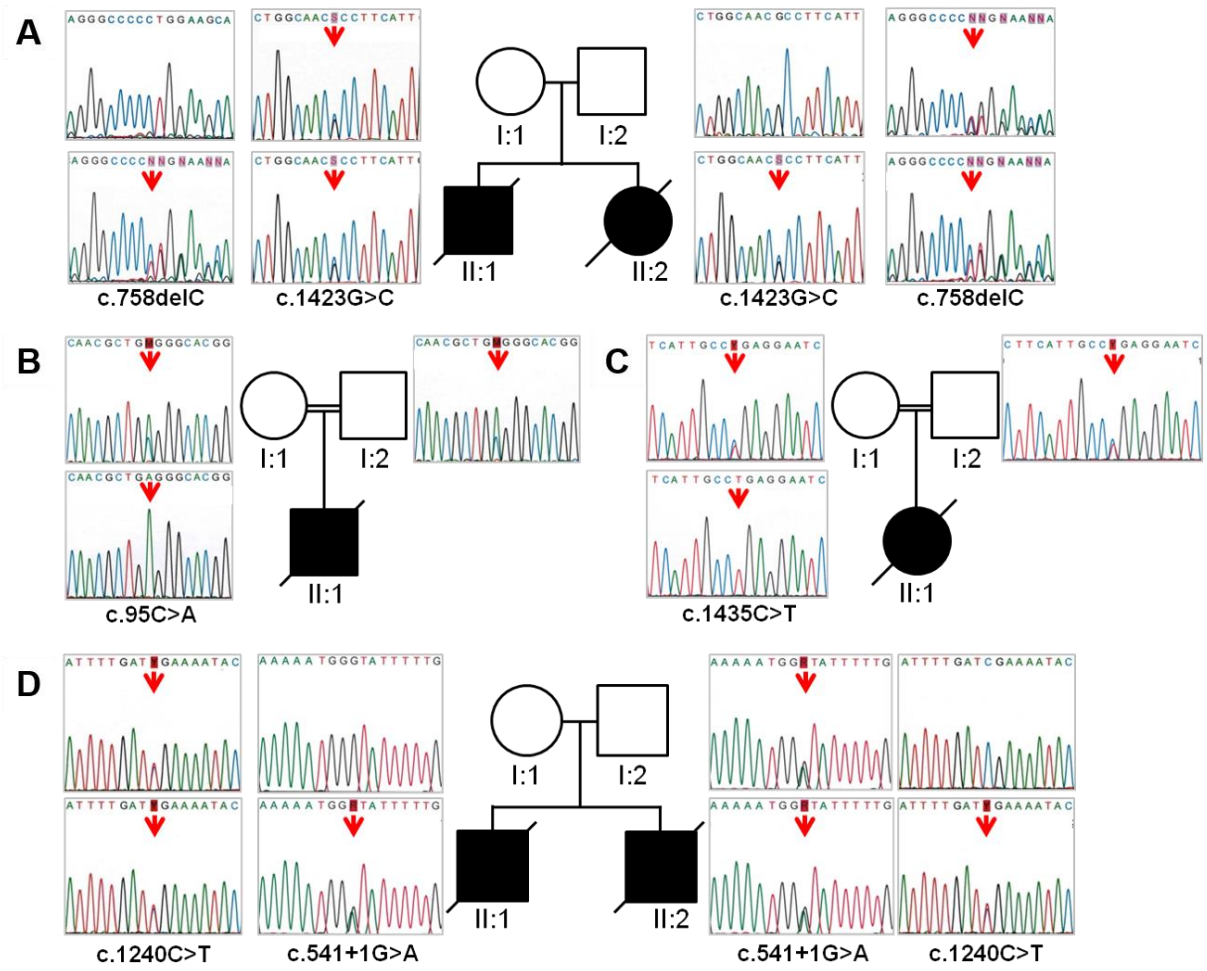

Figure S2: Sanger sequencing of mutations identified in *GLDN* in AMC families. Pedigrees for families 1 (A), 2 (B) and 3 (D) and 4 (C) are shown. Arrows indicate mutant nucleotide positions. The nucleotide changes based on NM_181789.2 reference sequence are indicated. Open symbols: unaffected; filled symbols: affected.

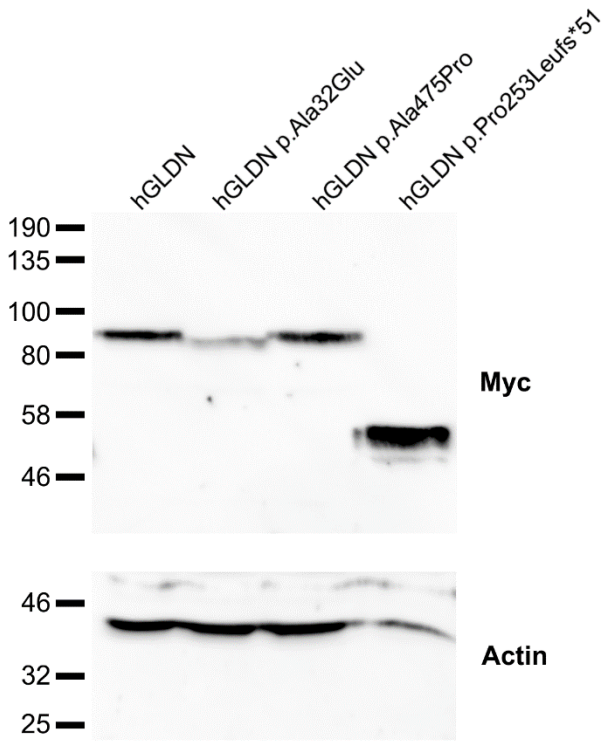

Fig. S3: Protein levels of *GLDN* mutants. Cell lysates were prepared from CHO cells transiently transfected with myc-tagged human WT and mutant gliomedin (hGLDN). Cells were solubilized in 1% Triton X-100, 140 mM NaCl, 20 mM Tris-HCl, pH 7.4 containing protease inhibitors (2 mM EDTA, 1 µg/ml Leupeptin, 1 µg/ml Aprotinin, and 0.5 mM Phenylmethylsulfonyl Fluoride, Sigma-Aldrich), agitated on ice for 15 min, then centrifuged at 18,900 X *g* for 30 min. The supernatants were saved and the protein concentrations were determined using the BCA kit (Sigma-Aldrich). 100 µg (WT, p.Ala32Glu, and p.Ala475Pro) and 25 µg (p.Pro253Leufs*51) of proteins were denatured in SDS sample buffer for 2 min at 90°C, then separated on 7.5% SDS-PAGE gels and transferred onto nitrocellulose membranes. The membranes were blocked for 1 hour using 5% powdered skim milk in PBS with 0.5% Tween-20 and incubated with a mouse monoclonal antibody Myc (1/1,000; Roche), or a rabbit antiserum against actin as a loading control (1/2,000; Sigma-Aldrich). After several washes, blots were incubated with the appropriate peroxidase-coupled secondary antibodies (1/5,000; Jackson ImmunoResearch) for 1 hour and washed several times. Immunoreactivity was

revealed using the BM chemiluminescence kit (Roche) and visualized on a G:BOX gel imaging and analysis system (Syngene). Protein levels were normal for p.Ala475Pro and decreased for p.Ala32Glu variants. By contrast, the protein level of hGldn bearing the p.Pro253Leufs*51 variant were 10 fold higher than those of WT, and appeared at a lower molecular weight as expected. Molecular weight markers are shown on the left (in kDa).

Table S1. Sequences of Primers

Designation	**Primer sequences (5'-3')**
1- Detection of *GLDN* mutation	
Family 1	
GLDN718-F	5’-TGGCCAGGAAACATCCCAAA-3’
GLDN718-R	5’-AGCATTAAATGGCCATCTTCCC-3’.
GLDN736-F	5’-TCCCTCCCCTTTCCTTCCC-3’
GLDN736-R	5’-GGACAAAACCCTCCTCCCTC-3’
Family 2	
GldnEx1A-F	5’-GCCACCACTACTGTCCCC-3’
GldnEx1A-R	5’-GCTCAACTCGGCCAGGAA-3’
Family 3	
GldnEx4-F	5’-CTCTGCCATCACCATCCCC-3’
GldnEx4-R	5’-GTGGGACCAAGAAGTATACCCT-3’.
GldnEx10A-F	5’-CTCACAGCATTGCCCAAGG-3’
GldnEx10A-R	5’-ACCCTCATATCTTTGGTGTCTGT-3’.
Family 4	
GldnEx10A-F	5’-CTCACAGCATTGCCCAAGG-3’
GldnEx10A-R	5’-ACCCTCATATCTTTGGTGTCTGT-3’.
2- Long Range RT-PCR amplification of *GLDN* transcripts	
GLDN-Ex1Fq	5’-CCGCACCACCCCAAGA-3’
GLDN-Ex10Rq	5’-CGTGTTGCACCACTGAGAATGT-3’