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Supplemental Data

De Novo Mutations in *NALCN* Cause a Syndrome Characterized by Congenital Contractures of the Limbs and Face, Hypotonia, and Developmental Delay

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Figure S1

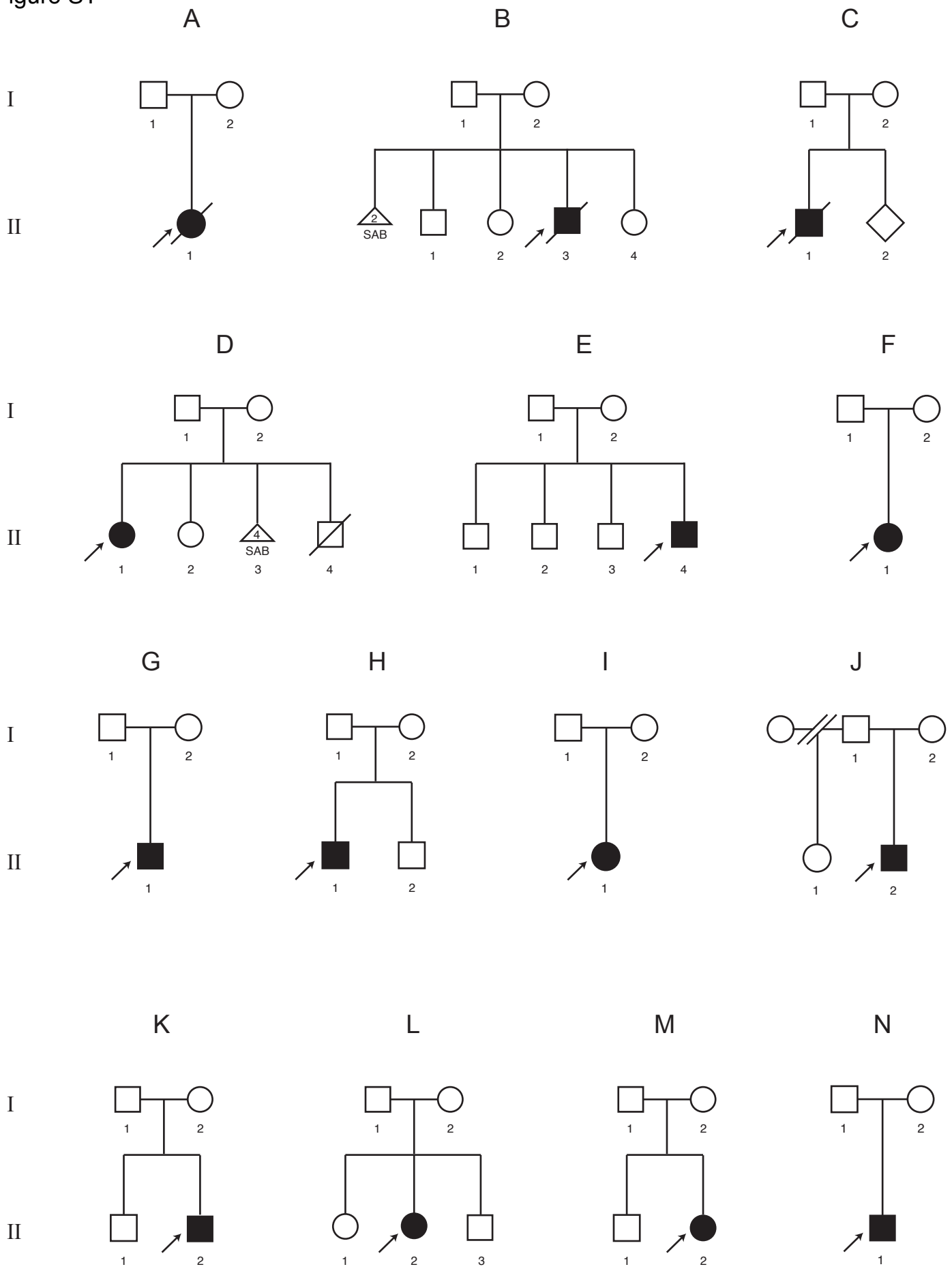
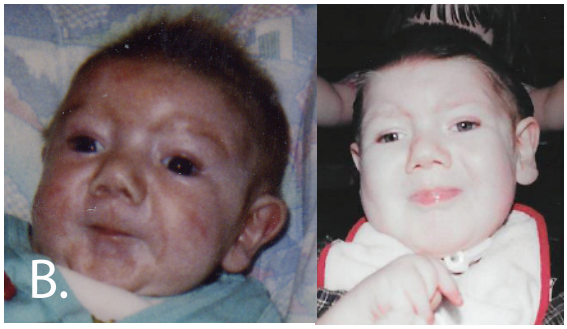
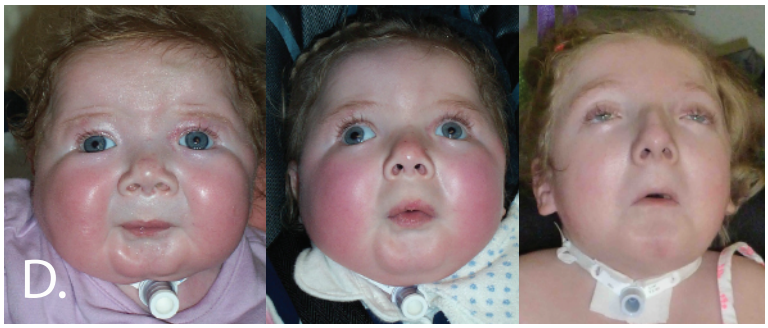


Figure S1. Pedigrees of families of persons diagnosed with CLIFAHDD Syndrome. Case identifiers for each individual shown correspond to those in Table 1 and Figures 1, S2, and S3. Each pedigree depicts a simplex family (i.e., one person affected with CLIFAHDD syndrome) and a *de novo* mutation in *NALCN* was identified in each affected individual.



6 months 5 years

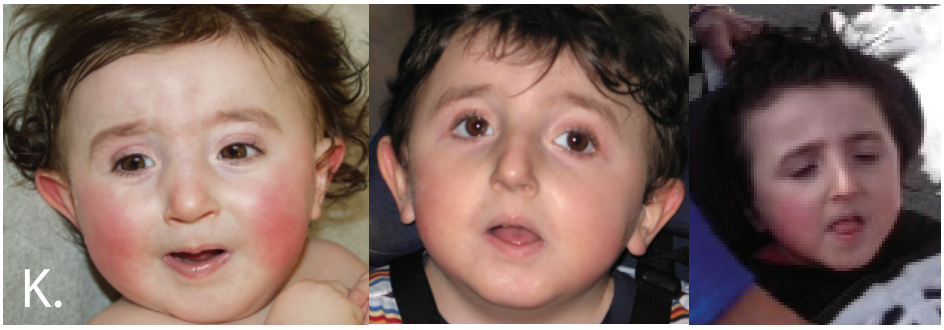


10 months 18 months 9 years

1 month 6 months 6 years 23 years

3 months 3 years 8 years

Figure S2



10 months 3.5 years 7 years

3 months 18 months 5 years 7 years

Figure S2. Change in facial characteristics over time of six persons with CLIFAHDD Syndrome. Case identifiers for the individuals shown correspond to those in Table 1 and Figures 1, S1, and S3. Note that the similarity of features is maintained over time, in particular the midface (e.g., nasal root and bride, columella, nares).

Figure S3. Facial characteristics of individuals with CLIFAHDD syndrome who were originally diagnosed with DA2A, DA2B, or DA1. Case identifiers for the individuals shown in this figure correspond to those in Table 1 and Figures 1, S1, and S2.

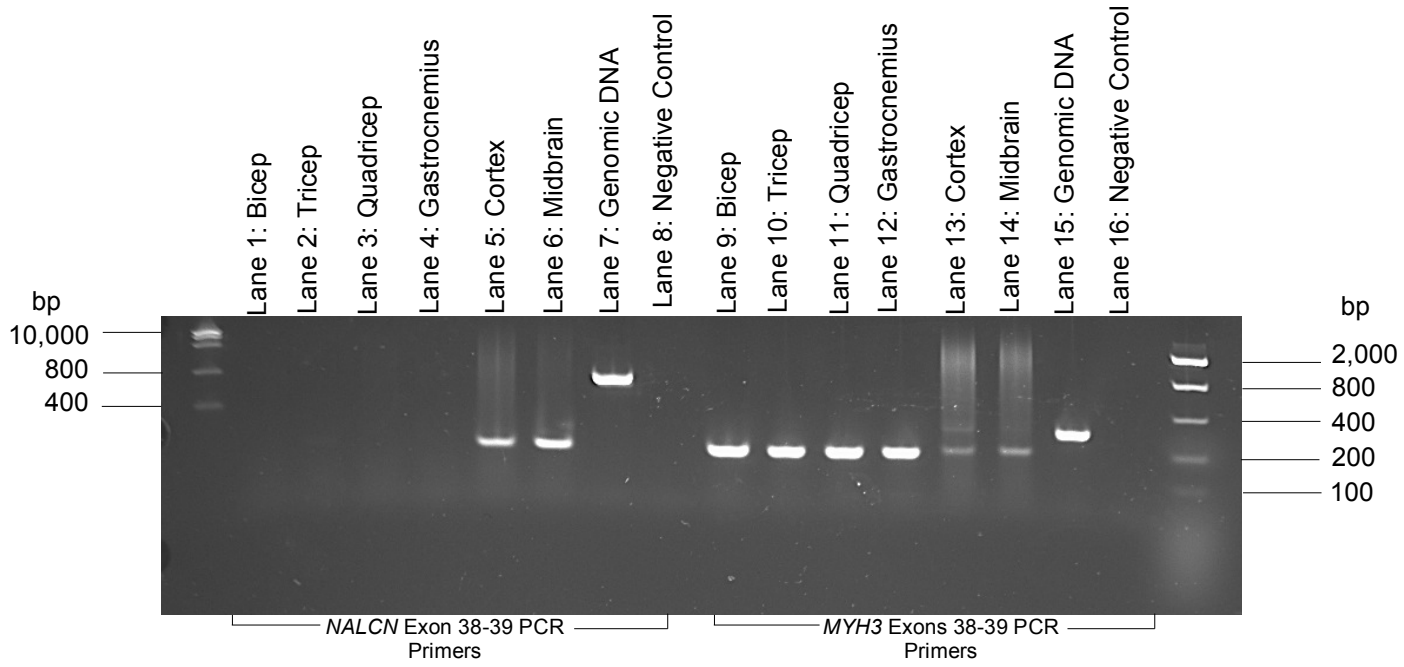


Figure S4. Expression of *NALCN* and *MYH3* as detected in cDNA derived from fetal tissues.

RNA was extracted from the cortex and midbrain of a 113-day-old male fetus using the Qiagen RNEasy fibrous tissue mini kit. The fetal tissues were obtained 2-3 days prior to RNA extraction from the Laboratory for Developmental Biology (LDB) and stored in RNAlater at 4^{o}C for preservation. A total of 35.4 mg of fetal cortex and 23.0 mg of fetal midbrain were collected in 300 µL buffer RLT and BME solution. RNA concentrations isolated from the samples were then quantified using a Nanodrop spectrophotometer. Additionally, RNA was isolated from muscle samples representing the bicep, tricep, quadricep vastus lateralis, and gastrocnemius medial head from a 127-day-old male fetus acquired July 31, 2009 from the LDB. After total RNA was extracted from all acquired fetal samples, cDNA was produced following the Life Technologies Superscript III First-Strand Synthesis SuperMix protocol. The cDNA samples were stored at -20^{o}C for preservation. Finally, a PCR was conducted to amplify cDNA and genomic DNA control samples using primers for exons 38-39 of *NALCN* and *MYH3* at 58^{o}C and products were visualized with a 2% agarose gel.

MYH3 primers were expected to yield amplicons of 313 bp on genomic DNA (Lane 15) and 206 bp on cDNA (Lanes 9-14) while *NALCN* primers had predicted amplicon sizes of 868 bp on genomic DNA (Lane 7) and 212 bp on cDNA (Lanes 1-6). Neural cDNA samples amplified with *NALCN* primers produced visible bands (Lanes 5-6). However, *NALCN* expression was not detected in fetal skeletal muscle tissue (Lanes 1-4).

Domain	Transmembrane Segment	Uniprot Prediction	Hydropathy Profile Prediction	Corresponding Exons	CLIFAHDD Mutations
1	IS5	183-203	177-203	Exons 5-9	p.Q177P
	IS6	302-322	296-325		p.L312I, p.V313G, p.E327L
2	IIS5	510-530	505-531	Exons 13-15	p.L509S, p.F512V, p.T513N
	IIS6	579-599	573-602		p.Y578S, p.L590F
3	IIIS5	1016-1036	1016-1044	Exons 26-31	p.V1006A, p.I1017T
	IIIS6	1136-1156	1130-1160		p.T1165P, p.R1181Q
4	IVS5	1336-1356	1334-1361	Exons 36-39	
	IVS6	1427-1447	1421-1450		p.I1446M

Table S1. Predicted coordinates of the S5 and S6 transmembrane segments of NALCN.

Coordinates of amino acid residues of the pore-forming S5 and S6 segments of NALCN as predicted by the Uniprot database algorithms and by a hydropathy profile analysis of NALCN. Mutations identified in individuals with CLIFAHDD syndrome are listed in the last column, in the same row as the nearest transmembrane segment (S5 or S6) as depicted in Figure 2. Uniprot transmembrane predictions for Q8IZF0 were obtained from http://www.uniprot.org/uniprot/Q8IZF0. Hydropathy profile prediction was obtained from Lee, Cribbs, Perez-Reyes FEBS Letters 1999. Exons are numbered relative to transcript ENST00000251127.