ARTICLE

Mutations in Contactin-1, a Neural Adhesion and Neuromuscular Junction Protein, Cause a Familial Form of Lethal Congenital Myopathy

Alison G. Compton,[1,9] Douglas E. Albrecht,[2,10] Jane T. Seto,[1,3] Sandra T. Cooper,[1,3] Biljana Ilkovski,[1] Kristi J. Jones,[1] Daniel Challis,[4,5] David Mowat,[4,6] Barbara Ranscht,[7] Melanie Bahlo,[8] Stanley C. Froehner,[2] and Kathryn N. North[1,3,*]

We have previously reported a group of patients with congenital onset weakness associated with a deficiency of members of the syntrophin-α-dystrobrevin subcomplex and have demonstrated that loss of syntrophin and dystrobrevin from the sarcolemma of skeletal muscle can also be associated with denervation. Here, we have further studied four individuals from a consanguineous Egyptian family with a lethal congenital myopathy inherited in an autosomal-recessive fashion and characterized by a secondary loss of β2-syntrophin and α-dystrobrevin from the muscle sarcolemma, central nervous system involvement, and fetal akinesia. We performed homozygosity mapping and candidate gene analysis and identified a mutation that segregates with disease within *CNTN1*, the gene encoding for the neural immunoglobulin family adhesion molecule, contactin-1. Contactin-1 transcripts were markedly decreased on gene-expression arrays of muscle from affected family members compared to controls. We demonstrate that contactin-1 is expressed at the neuromuscular junction (NMJ) in mice and man in addition to the previously documented expression in the central and peripheral nervous system. In patients with secondary dystroglycanopathies, we show that contactin-1 is abnormally localized to the sarcolemma instead of exclusively at the NMJ. The *cntn1* null mouse presents with ataxia, progressive muscle weakness, and postnatal lethality, similar to the affected members in this family. We propose that loss of contactin-1 from the NMJ impairs communication or adhesion between nerve and muscle resulting in the severe myopathic phenotype. This disorder is part of the continuum in the clinical spectrum of congenital myopathies and congenital myasthenic syndromes.

Introduction

The dystrophin-associated protein complex (DAPC) is a large, tightly associated oligomeric complex of proteins located within the skeletal muscle membrane.[1] The DAPC is thought to connect the extracellular matrix to the intracellular cytoskeleton and stabilize the plasma membrane against stresses imposed during muscle contraction or stretch (reviewed in Ervasti and Sonnemann[2]). Mutations in several members of the DAPC result in human muscle disease.[3] Many members of the DAPC, e.g., utrophin (MIM 128240), α-dystroglycan (MIM 128239), biglycan (MIM 301870), the syntrophins, and dystrobrevins, are also highly expressed at the neuromuscular junction (NMJ). The syntrophins and dystrobrevins form a cytoplasmic subcomplex within the DAPC, binding directly to dystrophin (MIM 300377) and utrophin (MIM 128240). This subcomplex serves as a membrane scaffold, targeting signaling proteins such as ion channels, kinases, and nNOS (MIM 163731) to the sarcolemma. In normal human muscle, syntrophins and α-dystrobrevin (MIM 601239) also localize to the NMJ. In addition, α1-syntrophin (MIM 601017) and α-dystrobrevin localize to the sarcolemma of both fast and slow fibers; β1-syntrophin (MIM 600026) is located predominantly at the sarcolemma of type 2 fibers, whereas β2-syntrophin (MIM 600027) is largely expressed at the sarcolemma of type 1 fibers.[4] In mice, however, β2-syntrophin and α-dystrobrevin-1 expression are largely restricted to the NMJ.[5,6]

Previously, we have identified 16 patients with congenital onset muscle weakness and myopathic features on muscle biopsy (out of 162 patients with congenital onset myopathy or dystrophy of unknown cause) with isolated secondary deficiency of the syntrophin-α-dystrobrevin subcomplex by immunohistochemistry.[4] Primary mutations in members of the syntrophin-dystrobrevin complex were excluded in all patients. Although the functional consequence of syntrophin and α-dystrobrevin loss is not known in humans, several knockout mouse models that lack syntrophins and dystrobrevins have been generated to elucidate their roles in skeletal muscle and nerve. However, the loss of α1- and β2-syntrophin in mice does not affect muscle structure or function, and they do not exhibit signs of a muscular dystrophy.[7–9] Mice lacking

[1]Institute for Neuromuscular Research, The Children's Hospital at Westmead, Sydney, NSW 2145, Australia; [2]Department of Physiology and Biophysics, University of Washington, Seattle, WA 98195, USA; [3]Discipline of Pediatrics and Child Health, Faculty of Medicine, University of Sydney, NSW 2006, Australia; [4]Department of Pediatrics, School of Women's and Children's Health, University of New South Wales, Sydney, NSW 2052, Australia; [5]Division of Obstetrics and Maternal Fetal Medicine, Royal Hospital for Women, Randwick, NSW 2031, Australia; [6]Department of Medical Genetics, Sydney Children's Hospital, Sydney, NSW 2031, Australia; [7]Burnham Institute for Medical Research, La Jolla, CA 92037, USA; [8]Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria 3050, Australia
[9]Present address: Mitochondrial and Metabolic Research Group, Murdoch Childrens Research Institute, Royal Children's Hospital, Melbourne, VIC 3052, Australia
[10]Present address: The Jain Foundation, Inc., Redmond, WA 98052, USA
*Correspondence: kathryn@chw.edu.au
DOI 10.1016/j.ajhg.2008.10.022.

α-dystrobrevin exhibit a mild muscular dystrophy[10,11] and cardiomyopathy.[12] Interestingly, loss of either α-dystrobrevin or α1-syntrophin also result in abnormalities in NMJ structure including reduced number of junctional fold openings to the synaptic cleft and reduced junctional AChR levels,[8–13] suggesting that the syntrophin-dystrobrevin subcomplex may have a role in synaptic maturation and remodeling. Adding additional weight to this hypothesis is the finding that the syntrophin-dystrobrevin subcomplex is differentially regulated during fetal development not only in mice but also in humans.[14,15] Unlike other members of the DAPC, the expression of members of the syntrophin-dystrobrevin subcomplex is also altered in response to innervation and denervation, implicating a participation in nerve-muscle communication[15].

On the basis of these observations, we hypothesized that the underlying cause of muscle weakness in our patient cohort may be neuropathic rather than myopathic. In this study, we used homozygosity mapping, gene-expression array, and a candidate gene approach to identify the disease-causing gene, *CNTN1* (MIM 600016), in the affected family members from the large consanguineous Egyptian family first described in Jones et al.[4] The *CNTN1* gene encodes for contactin-1, a neural adhesion molecule of the immunoglobulin (Ig) superfamily. We show contactin-1 expression at the NMJ in normal human and mouse skeletal muscle, and its mislocalization to the sarcolemma in patients with secondary dystroglycanopathy. To our knowledge, this is the first report implicating mutations in contactin-1 as a cause of human disease. We suggest that a spectrum exists including both severe lethal myopathy associated with fetal akinesia and congenital myasthenic syndromes much like those seen in the synaptopathies caused by mutation in proteins expressed at the NMJ, such as in AChR subunits and rapsyn.[16,17]

Material and Methods

Patient Material

Patient material was obtained from four affected infants and nine unaffected family members from a large consanguineous family of Egyptian background that have been briefly reported previously.[4] Informed consent for tissue and DNA samples was obtained from parents and other contributing family members. Skeletal-muscle biopsy samples were obtained from the four affected infants for immunohistochemical and immunoblot analysis as well as genomic DNA isolation and cDNA synthesis. Blood lymphocytes (n = 8) and buccal swab (n = 1) was obtained for total DNA isolation from unaffected family members. The study protocol was approved by the ethics committees of The Children's Hospital at Westmead, Sydney, Australia.

Linkage Analysis

Genotyping was performed at the Australian Genome Research Facility (AGRF), Melbourne, with the 5 cM ABI PRISM Linkage Mapping Set version 2.5 marker set. An additional 15 microsatellite markers were genotyped for fine mapping in all individuals and an additional three individuals. The genotyping was cleaned of Mendelian and double-recombinant errors with MERLIN.[18] Error rates were low, indicating that the pedigree was correct. We carried out multipoint homozygosity mapping assuming a fully penetrant recessive model with a rare allele frequency of 0.0001. The initial genome-wide scan LOD score based on the eight family members yielded a peak LOD of 2.9 at 12q. Analysis with the additional fine-mapping marker data and refinement of genetic maps increased evidence for linkage to highly significant (LOD = 3.99) and narrowed the linkage region to 14cM at 12p12.1-q12. Because the pedigree was too large (31 bits), the final multipoint mapping was carried out by dropping individuals from the analysis until the pedigree was analyzable with exact multipoint methods. Haplotype results were combined to delineate the critical region.

DNA Isolation and Mutation Analysis

Total DNA extraction from blood lymphocytes (n = 8) or cheek swab (n = 1) was performed with proteinase K or sodium dodecyl sulfate (SDS) digestion. Genomic DNA was isolated from 1 × 8 μm cryosection of frozen skeletal muscle (n = 4) with the GenomiPhi DNA amplification kit (Amersham Biosciences). We synthesized cDNA by RT-PCR from mRNA extracted from frozen muscle (patient V:4) and used PCR primers designed to amplify the entire cDNA in overlapping segments (primer sequences for additional genes screened available upon request). We analyzed PCR fragments by direct sequencing using ABI Prism (ABI100 Version 3.2). Sequences were compared with the published NCBI sequences. To confirm sequence changes found in cDNA, we amplified the corresponding exons from genomic DNA using genome-specific primers. We based codon numbering of *CNTN1* on the reported amino acid sequence, using the first methionine as an initiation codon (NCBI accession numbers NM_001843.2 and NM_175038). Control genomic DNA was screened by a PCR-RFLP test utilizing a MnlI restriction site abolished by the *CNTN1* 871 dupT mutation (primers REgCNTN-1: 5′-GGGCCAAAATGT GACC-3′, REgCNTN-2: 5′-aagccctccatgtgattt-3′). For all primers used for *CNTN1* amplification, see Table S1 available online. Alignments of contactin sequences from a variety of vertebrate species were performed with the alignment program MAFFT.

In Silico Analysis

We used the University of California Santa Cruz (UCSC) Genome Browser (July 2003 freeze), the National Center for Biotechnology Information (NCBI) Entrez Genome Map Viewer, and the Ensembl Human Genome Server to search for known genes, expressed sequence tags, and putative genes in the 12p12.1-q12 region. This region contained 174 known gene sequences; however, 91 sequences were multiple entries of the same gene because of alternative splicing and seven were annotation errors within the Browser (i.e., had no *Unigene* identifier) leaving 76 genes within our region. We performed bioinformatic analysis of public gene-expression databases to prioritize our candidate genes in this region to those expressed in muscle and nerve tissue. We used expression data from brain as a substitute for nerve because data is incomplete for gene expression in nerve and the spinal cord. Because public databases may contain high levels of inaccuracy, we used these two independent data sets to allow crossvalidation of results, GeneCards' electronic Northern (eNorthern), and NCBI SAGEmap (Serial Analysis of Gene Expression). Of the 76 known genes in our candidate region, 34 are expressed in muscle, and 44 are

expressed in the brain. Except for one gene (CFTR/MRP), all muscle genes are also expressed in the brain.

Expression-Array Analysis of Patients and Control Skeletal Muscle

Total RNA was isolated from postmortem muscle biopsies taken from three patients (V:2, V:3, and V:4) and six normal controls of similar age-matched controls. RNA was extracted with Trizol reagent (Invitrogen) in accordance with the manufacturer's instructions and then further purified with a QIAGEN RNeasy kit. The concentration of total RNA was measured by absorbance at 260 nm and RNA quality was assessed on an Agilent bioanalyzer 2100. RNA target labeling, hybridization to Affymetrix Human Genome U133 plus 2.0 arrays, and chip scanning were performed by the Center for Array Technologies at the University of Washington with standard Affymetrix protocols and an Affymetrix 3000 scanner. Data from the raw chip scans were uploaded to the Genesifter (Vizix Labs) server and normalized by mean intensity, and comparisons between the control and patient population were performed with two sample t tests with a p value of 0.05. Genes mapping to the region of chromosome 12 that contained the mutation were analyzed for significant changes.

Immunohistochemistry and Immunoblotting

Muscle biopsy samples were available from all four infants and showed the muscle to be structurally normal with no signs of dystrophy. Biopsies were snap frozen in liquid-nitrogen-cooled isopentane and stored in liquid nitrogen until required. Indirect immunofluorescence was performed on 8-μm-thick cryosections with antibodies against all members of the DAPC especially those associated with congenital muscular dystrophy (dystrophin, [α-, β-, δ-, and γ-] sarcoglycans, laminin α2, β-spectrin, and α-dystroglycan) according to standard procedures.[4] All antibodies were obtained from Novacastra Laboratories (Newcastle upon Tyne, UK) unless otherwise stated. Antibodies raised against α1-syntrophin (SYN17), β1-syntrophin (SYN35), β2-syntrophin (SYN28)[5,19] and α-dystrobrevin-1 (DB670) and −2 (DB2)[6] were provided by S.C Froehner. A rabbit polyclonal antibody raised against sarcospan was generously provided by Dr. Louis Kunkel (Boston, MA). Monoclonal anti-laminin α2 (MAB1922) was obtained from Chemicon, and clone 4H8-2 was obtained from Alexis. Antibodies to the cytoplasmic domain of the α7-integrin chain [A2(346) and B2(347)] were kindly provided by S.J. Kaufman and used as previously described.[20,21] α-Bungarotoxin, Alexa Fluor 488 conjugate was obtained from Molecular Probes, and polyclonal contactin-1 antibody (AF904) was obtained from R&D Systems. For α-dystroglycan (using clone VIA4-1 from Upstate Biotechnology), we treated the muscle with ice-cold acetic acid:ethanol (1:1) for 1 min. For immunodetection of α-dystrobrevin (DB670 and DB2[6]), cryosections were fixed with 3% paraformaldehyde at room temperature for 10 min and then rinsed in PBS. Sections were blocked in 2% BSA (bovine serum albumin) in PBS (phosphate-buffered saline) at room temperature for 15 min. Sections were incubated in primary antibody (diluted in 2% BSA) at room temperature for 2 hr or 4°C overnight and then were washed three times in PBS. Anti-mouse CY3-conjugated, anti-mouse Alexa-488-conjugated, anti-goat CY3-conjugated, and anti-rabbit CY3-conjugated secondary antibodies (Jackson Immunoresearch Laboratories) were applied, and sections were incubated at room temperature for 1 hr. After an additional three washes in PBS, coverslips were applied over Immumount. Images were captured with a Leica SP2 scanning confocal microscope. Immunoblot analysis was performed as described previously.[22] We performed all immunohistochemical staining and immunoblot studies where possible using age-matched muscle biopsies with normal histology as controls.

Results

As part of our initial cohort,[4] we ascertained four patients who belonged to a large consanguineous family of Egyptian descent (Figure 1A). Individuals V:2, V:3, V:4, and V:7 had a similar clinical presentation. Reduced fetal movements were detected on ultrasound during the second and third trimester. All infants were born between 28 and 35 weeks' gestation and pregnancy was complicated by multiple problems including polyhydramnios and growth retardation. Three of the children showed no spontaneous movements and died shortly after birth. One infant (V:7) survived to 1 month of age but required intubation and ventilation from birth. She remained ventilator dependent because of poor respiratory effort. However, there was no evidence of primary lung pathology requiring increased ventilatory settings. Infant V:7 had low birth weight and was noted to be hypotonic at birth with absent deep tendon reflexes, no suck or swallow, an absent Moro reflex, and paucity or absence of spontaneous movement. She had dysmorphic features including scaphocephaly, an oval face, hypertelorism, and a high arched palate. She was unresponsive to external stimuli. Her hands were small with simian creases, arachnodactyly with overlapping fingers, and marked camptodactyly. She had flexion contractures in most joints but most notably the elbows and hips. Echocardiogram and head ultrasound were normal. Muscle mass was generally reduced and all serum levels were normal, including creatine kinase levels (70 U/l; normal 0–230 U/l). She died 1 month after birth (equivalent of 36 weeks' gestation developmentally) from respiratory failure precipitated by aspiration. An autopsy confirmed that the gross morphology of all organs was normal including the central nervous system. Light microscopy of her skeletal muscle showed a minor variation in fiber size (7–15 microns) and a mild increase in collagen fibrils between muscle fibers. Electron microscopy revealed a moderate number of foci in which small numbers of contiguous sarcomeres were disrupted with disorganization of the Z-band (Figures 1B and 1B′) and a reduced number of mitochondria. The myofilaments were also somewhat disordered within these foci, reminiscent of minicores. The nerve biopsy was histologically normal. Based on the clinical presentation and histological findings in muscle, the diagnosis in this family was concluded to be a severe congenital myopathy.

The muscle biopsies from all four infants (V:2, V:3, V:4, and V:7) were stained with antibodies to dystrophin, α-, β-, γ-, and δ-sarcoglycan, sarcospan, α-dystroglycan, laminin α2, β-dystroglycan, and collagen VI. All showed normal expression and localization at the muscle membrane

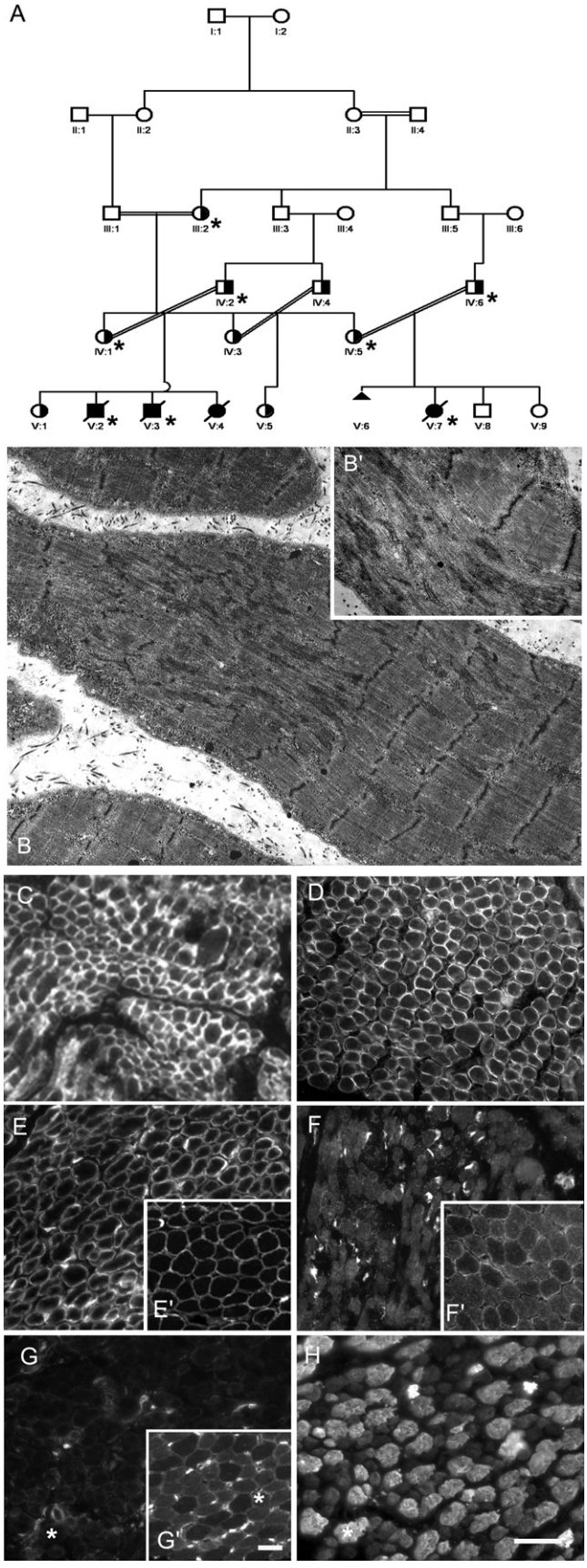

Figure 1. Family Pedigree and Skeletal Muscle Pathology

(A) Pedigree indicating family members studied with linkage analysis (initial mapping performed on individuals marked with an asterisk) and genotype. Filled symbols indicate affected individuals and half-filled symbols indicate carrier status (assessed by genotyping). All empty symbols indicate family members for which samples were unavailable for testing.

(B and B′) Electron micrograph of skeletal muscle from affected individual V:7. Fibers contained foci of sarcomeric disruption and z-line streaming. (B) shows a micrograph magnification at 4500×, and (B′) shows a micrograph magnification at 10,000×.

(C–H) Immunohistochemistry of muscle sections from patient V:7 (C, D, E, F, G, and H) and age-matched control (E′, F′, and G′) for members of the dystrophin-associated protein complex. Shown are (C) α-dystroglycan, (D) α1-syntrophin, (E and E′) β1-syntrophin, (F and F′) α-dystrobrevin-2, (G and G′) β2-syntrophin, and (H) slow myosin. All members of the dystrophin-associated protein complex were expressed normally except select members of the syntrophin-α-dystrobrevin subcomplex. All affected family members showed an absence of sarcolemmal membrane expression for β2-syntrophin (which is normally expressed at the sarcolemma of slow fibers (G′ marked with an asterisk in the age-matched control) and α-dystrobrevin. Slow myosin shows the presence of slow fibers in the patient biopsy. Scale bars represent 40 μm. Biopsies were taken from quadriceps at developmental age 35 weeks' gestation.

(Figure 1C and data not shown). Integrin α7 staining was absent; however, sequencing of the *ITGA7* (MIM 600536) gene identified no mutations within its coding region.[23] Integrin α7 deficiency is now commonly recognized as a secondary phenomenon in inherited myopathies.[23,24] We stained the muscle with antibodies to members of the syntrophin-α-dystrobrevin subcomplex and found consistent abnormalities in all four patients. In patients and age-matched controls, both α1-syntrophin and β1-syntrophin localized to the muscle membrane (Figures 1D and 1E). Developmentally, β1-syntrophin is expressed at the sarcolemmal membrane of all fiber types[15] as is observed in Figures 1E and 1E′ (age-matched control). However, in all four patients, both β2-syntrophin and α-dystrobrevin were absent from the muscle membrane (Figures 1F and 1G). Immunohistochemistry with an antibody raised against slow myosin heavy chain (Figure 1H) shows the presence of slow fibers (which would normally express β2-syntrophin) in the patient biopsy. Interestingly, α-dystrobrevin-1 and -2 were retained at the NMJ even though these molecules were lost from the sarcolemmal membrane (Figure 1F). We performed immunoblot analysis of β2-syntrophin on the skeletal muscle biopsy from patient V:4 compared to age-matched control. A diffuse band of ~59 kDa (the expected size for β2-syntrophin) was detected in normal control muscle but not in the patients' muscle (data not shown). Antibodies raised against α-dystrobrevin do not work reliably on immunoblotting to allow study. We previously sequenced the coding regions of β2-syntrophin and α-dystrobrevin as well as α1-syntrophin in these patients and did not identify any mutations.[4]

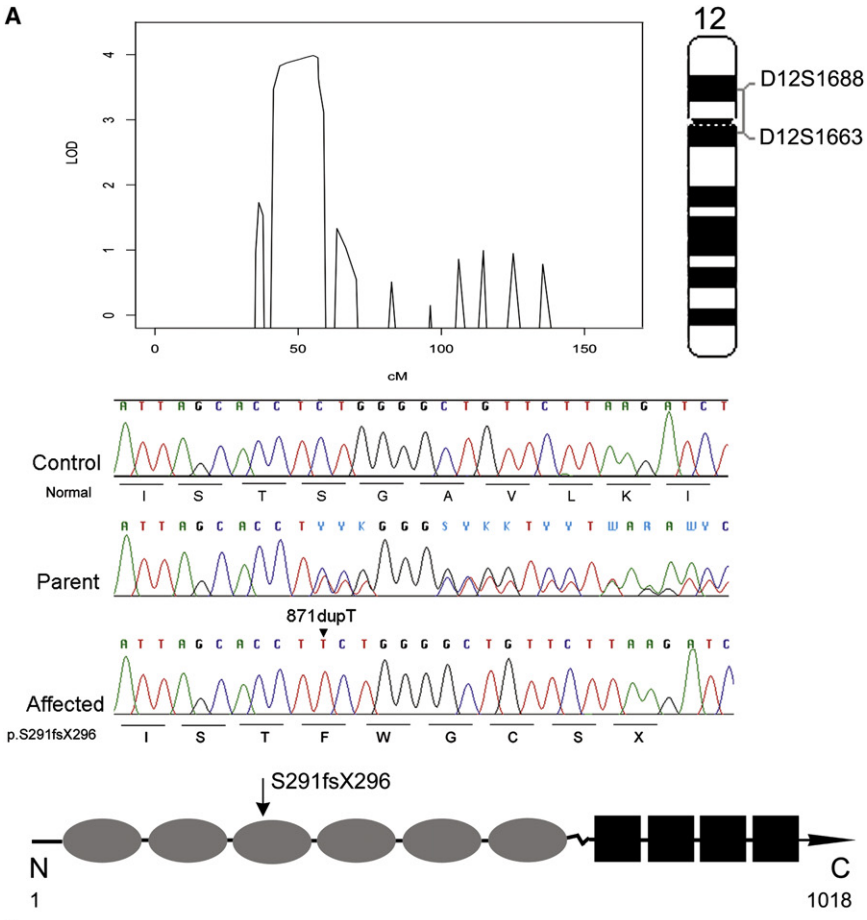

Figure 2. Linkage and Mutation Analysis

(A) Parametric multipoint linkage analysis results of the chromosome 12 disease locus in this Egyptian family. An idiotype of chromosome 12 reports the position of the locus within the flanking recombination markers D12S1688 and D12S1663. Electropherograms show the mutation containing region in control, unaffected parent and affected infant. The duplication of a thymine between position 871 and 872 is indicated by an arrow. Both the control and the affected normal protein sequences are shown below the respective electropherogram. A schematic of the contactin-1 protein shows the position of the amino acid mutation, S291fsX296, in relation to the full-length 1018 amino acid protein (gray ovals represent Ig-like domains, black boxes indicate FNIII domains, and the C-terminal arrow indicates the GPI-anchor). (B) The nucleotides and amino acids flanking the insertion (gray shaded) are highly conserved in different species (human contactin-1 nucleotides 848–894, amino acids 262–314). Orthologs in *Homo sapiens*, *Pongo troglodytes*, *Pongo pygmaeus*, *Mus musculus*, *Rattus norvegicus*, *Bos taurus*, *Gallus gallus*, *Xenopus laevis*, and *Danio rerio* are shown. Stars indicate conserved residues.

We thus carried out multipoint homozygosity mapping on eight family members (including three of the four affected individuals, Figure 1A) and identified a single, fully informative region at 12p12.3-q13.11 of 25cM in which only affected children were homozygous. The nonaffected family members (unaffected carriers) were heterozygous for this region. Further analysis with additional microsatellite markers confirmed coinheritance of the haplotype with disease status in all family members (n = 13) and further narrowed the region of homozygosity to 14cM (flanked by markers D12S1688 and D12S1663), with a parametric LOD score of 4 (Figure 2A). There were no other obvious candidate loci. This region (12p12.1-q12) contains 76 known genes (retrieved from the UCSC Genome Browser, NCBI Entrez Genome Map Viewer, and Ensembl Human Genome Server); only some genes had identified functions, whereas others encode "hypothetical" proteins with yet-to-be-determined function. We constructed an in silico genomic map of the region, using public databases (GeneCards' eNorthern and NCBI SAGEmap) and prioritized genes for mutation screening on the basis of expression levels (i.e., present in skeletal muscle and brain) and putative function or known interactions. We excluded a number of candidate genes by sequence analysis (n = 24; *PEPP2*, *IAPP*, *LDHB*, *KCNJ8*, *ABCC9*, *KRAS2*, *BHLHB3*, *SSPN*, *ITPR2*, *STK38L*, *OVCH1*, *IPO8*, *DDX11*, *MGC50559*, *LOC196394*, *FLJ10652*, *DNM1L*, *YARS2*, *SYT10*, *KIF21A*, *FGD4*, *BICD1*, *MGC24039*, and *C12orf14*).

Concurrently, we performed gene-expression-array assays on skeletal muscle RNA (matched for age and fiber type proportion) from three patients (V:2, V:3, and V:4) and six control samples to compare the expression levels of the 76 genes within the linkage region. Fourteen genes within this region were identified as being differentially expressed in patients versus controls. We had previously excluded mutations in half of the genes identified, as being differentially expression in patients versus controls

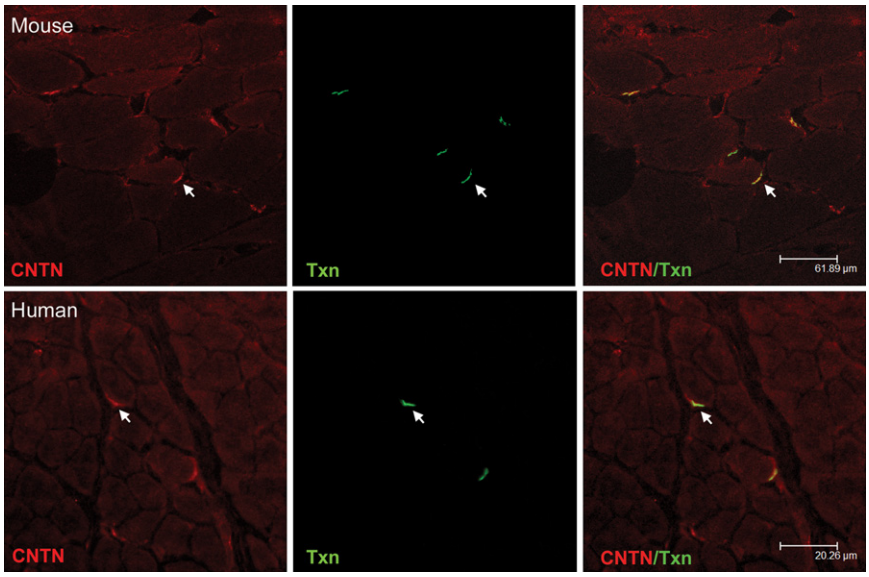

Figure 3. Contactin-1 Localization at the NMJ in Both Human and Mouse Skeletal Muscle

Confocal immunofluorescence in normal human and mouse quadriceps muscle showing overlap between contactin-1 (CNTN) and α-bungarotoxin (Txn) used to highlight the acetylcholine receptors of the NMJ. Arrows are used to indicate an example of this staining.

(*ABCC9*, *STK38L*, *DNM1L*, *DDX11*, *ITPR2*, *IAPP*, and *MGC24039*), by sequence analysis. Of the remainder, the most striking alteration in expression was seen for *CNTN1* with a 7.9- and 16.7-fold decrease in expression in patients compared to controls depending on the probe set used (227202_at and 227209_at, respectively). In addition, analysis using Genesifter annotated *contactin-1* as "present" for all six control samples but "absent" in all three patient samples.

We sequenced the coding region of the contactin 1 gene (*CNTN1)* and identified a homozygous thymidine duplication, c.871dupT, within exon 8 in all four affected infants (Figure 2A). The thymidine duplication (c.871dupT) in exon 8 is predicted to result in a reading frameshift introducing a premature stop codon (S291fsX296). The truncating mutation occurs within the third Ig domain, present within both human contactin-1 transcripts[25] and is likely to be rapidly degraded by nonsense-mediated decay. The mutation was heterozygous in the parents of affected individuals and cosegregated with disease in each family member. The mutation was not present on 121 healthy controls of mixed ethnicity (242 chromosomes) or 27 unrelated myopathy patients (54 chromosomes). Multiple alignments of contactin-1 orthologs from vertebrates and invertebrates demonstrated that both the nucleotide and amino acid sequences surrounding this duplication mutation (nucleotides 871 and 872 in human *CNTN1* sequence NM_001843) are highly conserved during evolution (Figure 2B). We have also sequenced contactin-1 in the other members of the patient cohort with secondary syntrophin-dystrobrevin deficiencies described in Jones et al.[4] but did not identify any disease-causing mutations. This suggests that this clinical and immunohistochemical phenotype is genetically heterogeneous, and we consider other proteins that interact with the syntrophins, dystrobrevins and now contactin-1 as potential disease candidates for these patients.

Contactin-1 expression has not been extensively studied in tissues other than the peripheral and central nervous systems. However, very low levels of contactin-1 mRNA have previously been reported in human skeletal muscle.[26] The low levels of contactin-1 expression in skeletal muscle is supported by our gene-expression-array control data, which in turn demonstrated a significant reduction in the contactin-1 RNA transcript in muscle from affected patients. Contactin-1 protein expression levels in normal human and mouse skeletal muscle were below detection levels by immunoblot analysis, although a correctly sized band could be detected in mouse cerebellum in which contactin-1 is abundantly expressed (see Berglund et al.[27] and data not shown). Immunohistochemical studies revealed that contactin-1 expression is restricted to the NMJ in both mouse and human skeletal muscle (Figure 3, n = 3 each). Because of sampling-site variability and lack of sufficient patient muscle, additional examination of NMJ morphology could not be performed. However, immunohistochemistry was performed on the quadriceps muscles from the *cntn1* null[27] and wild-type littermate mice for the syntrophins-dystrobrevin subcomplex. In contrast to loss of β2-syntrophin and α-dystrobrevin from the sarcolemma in human muscle lacking contactin-1, no difference in staining was detected at the sarcolemmal membrane for any members of this subcomplex in wild-type or *cntn1* null mice (data not shown). Junctional staining was also normal for these proteins. Specifically, β2-syntrophin staining was restricted to the NMJ in both wild-type and *cntn1* null mice, and no difference was detected in either the junctional or sarcolemmal staining of the different α-dystrobrevin isoforms (data not shown).

The role of contactin-1 at the neuromuscular junction is unknown. In *Drosophila* follicle cells, contactin forms a complex with neurexin IV and neuroglian, which are necessary for septate-junction formation and localization.[28] In the absence of α-dystroglycan, both neurexin and contactin are promiscuously mislocalized, whereas neuroglian expression is unaltered by loss of α-dystroglycan[29]. On this basis, we used immunochemistry to determine whether this relationship between contactin and α-dystroglycan is maintained in human skeletal muscle.

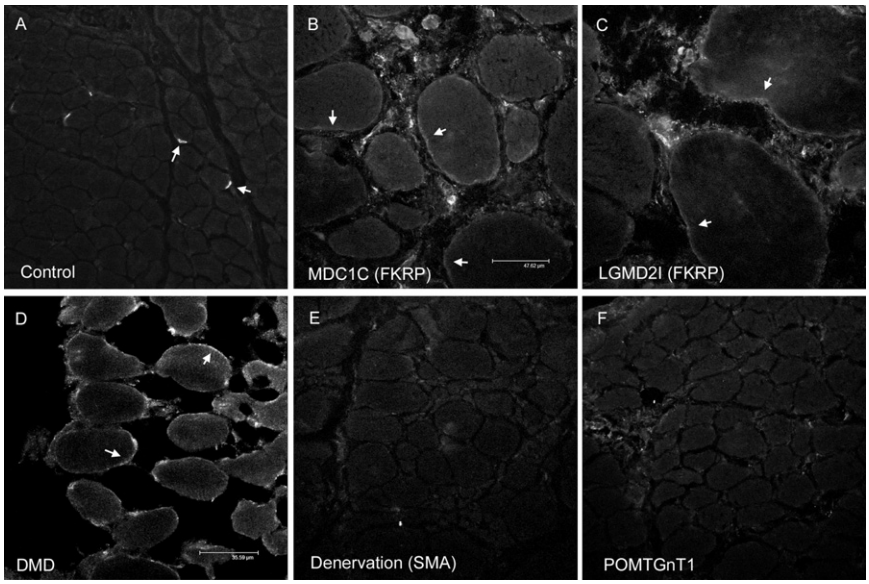

Figure 4. Contactin-1 Is Mislocalized to the Sarcolemmal Membrane in the Absence of Glycosylated α-Dystroglycan
Confocal immunofluorescence of skeletal muscle biopsies from secondary α-dystroglycanopathy patients (mutations in FKRP [B and C] and POMTGnT1 [D]), (E) denervation disorder (SMA [MIM 253300]), and (F) Duchenne muscular dystrophy (DMD) as compared to (A) control muscle with polyclonal anti-contactin-1 antibodies. Contactin-1 expression is mislocalized to the sarcolemmal membrane in response to loss of glycosylated α-dystroglycan (indicated with arrows in [B], [C], and [D]) but not in response to generalized dystrophic changes or denervation. In the control (A), contactin-1 is retained at the NMJ (indicated with arrow). The control shown is not age matched for all biopsies because NMJs are not present in all human biopsies as a result of sampling.

In control skeletal muscle, contactin-1 expression was restricted to the NMJ (Figures 3 and 4A). However, in patients with secondary dystroglycanopathies (due to mutations in the putative glycosyl transferases FKRP [MIM 606612], Fukutin [MIM 253800], and POMTGnT [MIM 606822]; n = 6), contactin-1 was not restricted to the NMJ but was also found on the sarcolemmal membrane (Figures 4B–4D). Sarcolemmal expression of contactin-1 was not observed in either denervated (n = 4) or dystrophinopathy (MIM 310200, n = 4) muscle (Figures 4E and 4F), suggesting that the loss of glycosylated membrane-bound α-dystroglycan is associated with the contactin-1 mistargeting and is not secondary to the neuropathic or dystrophic process. These data suggest that in humans, as in *Drosophila*, the glycosylation state of α-dystroglycan influences the localization of contactin-1.

Discussion

The congenital myopathies are a clinically and genetically heterogeneous group of neuromuscular disorders that present with hypotonia and areflexia, poor muscle bulk, and generalized weakness and are usually defined by a pathological hallmark on muscle biopsy, e.g., nemaline bodies, cores, and central nuclei. To date, the genes responsible for inherited myopathies encode proteins with a variety of structural and metabolic functions (reviewed in North[30]) including components of the muscle contractile machinery, proteins that mediate calcium release from the sarcoplasmic reticulum, and proteins involved in cytoskeleton reorganization and endocytosis. There is clinical overlap between severe congenital myopathies and congenital myasthenic syndromes due to mutations in components of the NMJ such as acetylcholine receptor subunits and rapsyn.[16,17] Both disorders can present with fetal akinesia sequence, characterized by paucity of movement in utero, and severe weakness and joint contractures at birth. In this study, we report on a disease-causing mutation in *CNTN1*—the gene encoding the glycosyl phosphatidylinositol (GPI)-anchored neural adhesion molecule contactin-1—that results in a severe lethal congenital myopathy with fetal akinesia and nonspecific myopathic features on muscle biopsy.

In skeletal muscle, contactin-1 is primarily localized to the NMJ. We propose that reduced expression of contactin-1 at the NMJ causes a defect in neuromuscular transmission, resulting in the severe myopathic phenotype. We have previously demonstrated that denervation results in secondary deficiency of members of the syntrophin-dystrobrevin complex,[15] which is normally localized to the NMJ. The loss of β2-syntrophin and α-dystrobrevin in affected family members is probably secondary to the defect in neuromuscular transmission or signaling. These data further emphasize the overlap in the clinical spectrum of congenital myopathies with fetal akinesia and congenital myasthenic syndromes and suggest that it is not only the proteins necessary for neuromuscular signal transduction but also those related to NMJ adhesion that are important for normal muscle development and growth.

Contactin-1 is a member of the immunoglobulin superfamily with a common structure of six C2 Ig-like domains, four fibronectin type III (FNIII) repeats, and a GPI anchor.[25] Ig-like and FNIII domains are common building blocks of many extracellular proteins involved in ligand recognition and cell adhesion. They are also the main motifs of several intracellular proteins associated with the contractile apparatus of muscles such as titin, obscurin, and myotilin. Contactin-1 expression has been extensively studied in the mouse central nervous system and is thought to play

a role in the establishment and stabilization of synaptic connections.[31,32] In the mouse central and peripheral nervous systems, contactin-1 is associated with several signaling molecules present in the extracellular matrix of neurons and glia. Several lines of evidence suggest a critical role for contactin-1 in the regulation of neurite growth, synapse formation, fasciculation, and myelin organization (reviewed in Falk et al.[33]).

Several different contactin-1 knockdown models have been generated so that the in vivo functions of contactin-1 could be determined. Knockdown of *cntn1* expression in *Xenopus* impairs neurogenesis and outgrowth of sensory axons.[34] Antibody perturbation induces misguidance of central axons of proprioceptive dorsal root ganglion neurons of chicks.[35] Contactin-1 null mice are severely ataxic, exhibit muscle weakness and growth retardation, and die within the first 18 days of life. The mice show defects in the micro-organization of the cerebellar circuitry,[27] disruption of paranodal junctions,[36] and impaired synaptic plasticity.[37] In the affected infants in our study family, central and peripheral nervous system involvement could not be well documented because of their early death. The one infant who survived for 1 month responded poorly to external stimuli. Although thought to be a possible consequence of birth asphyxia, this clinical phenotype may be associated with contactin-1 mutations. Skeletal muscle function and NMJ morphology and function have not been studied in detail in our patient group or in the contactin-1 null mouse; however, leg splaying was evident in contactin-1 null mice, suggestive of muscle weakness and reminiscent of the "frog-leg" posture adopted by patients with congenital myopathy.

The mutation in *CNTN1* described in this study introduces a premature stop codon at position 296 within the third Ig-like domain. Little is known about the contactin-1 binding partners in skeletal muscle; however, this domain is crucial for tenascin binding in the nervous system[38,39] and would be abolished with this mutation. Tenascin-C (MIM 187380) has been predominantly studied within the central nervous system; however, one study showed expression also at the neuromuscular and myotendinous junctions.[40] Tenascin-C-deficient mice exhibit long-term impairment of skeletal muscle reinnervation, suggesting that tenascin-C plays an important role in the formation and stabilization of the NMJ.[41] Contactin-1 also interacts with specific sodium channel isoforms in the central and peripheral nervous system,[42–46] but whether this interaction occurs with the skeletal-muscle-specific isoforms has yet to be investigated. The syntrophins also directly associate with voltage-gated sodium channels in brain and skeletal muscle.[47] In mice, mutations in the neuronal voltage-gated sodium channel, $Na_v1.6$ (MIM 600702) cause an ataxic phenotype (i.e., *med* mouse; neurological mutant with "motor endplate disease"[48]) or muscular dystrophy (i.e., a spontaneous mutation resulting in muscle degeneration; *dmu* mouse[49]). Both mouse models are characterized by early-onset progressive paralysis of the hind limbs due to muscle atrophy during the second week of life and subsequent lethality in the first month of life. The *med* mouse also presented with Purkinje cell degeneration, much like the *cntn1*-null-mouse model.

Unlike the majority of DAPC members, the syntrophins and dystrobrevins in skeletal muscle are regulated by neural signaling; their expression levels and localization are altered by loss of innervation (as seen in human denervation disorders).[15] Glycosylation of α-dystroglycan is regulated in a similar manner.[50] The potential interaction between the syntrophin-dystrobrevin subcomplex and α-dystroglycan within the DAPC and contactin-1 may contribute to the central nervous system defects seen in both patients with muscle disease and the knockout mouse models (*cntn1*, *med*, and *dmu*). In several secondary α-dystroglycanopathies, loss or abnormal glycosylation of brain dystroglycan can also result in CNS defects as well as delayed NMJ formation and a delay in muscle formation.[51] Both the syntrophin (α1- and β2-) and dystrobrevin (α- and β-) knockout mouse models are also reported to exhibit subtle functional defects, such as performance in sensorimotor tests, that cannot be explained by NMJ abnormalities[9,52].

The abnormalities in syntrophins and α-dystrobrevin in patients with a mutation in *CNTN1* suggests an association between contactin-1 and the syntrophin-dystrobrevin subcomplex. Another member of the DAPC, biglycan regulates the membrane expression of the syntrophin-dystrobrevin complex in skeletal muscle. Biglycan null mice show abnormal syntrophin-dystrobrevin expression, similar to our patient cohort;[53] we have excluded primary biglycan mutations in all of these patients.[54] Biglycan interacts directly with α-dystroglycan and, in *Drosophila*, expression of α-dystroglycan at the sarcolemmal membrane precludes incorrect membrane localization of contactin.[29] In this study, we show that contactin-1 is also mislocalized to the sarcolemmal membrane in humans with secondary dystroglycanopathies. Dystroglycan is normally expressed in the brain, and loss of dystroglycan in the brain-specific dystroglycan knockout mouse results in structural and functional brain abnormalities.[55] Similarly, in several secondary α-dystroglycanopathies, loss or abnormal glycosylation of brain dystroglycan also results in CNS defects, as well as abnormal NMJ formation and delayed terminal muscle formation.[51] In combination, these data suggest that contactin-1 may also contribute to disease pathogenesis in a wider spectrum of disease phenotypes including the dystroglycanopathies. Although the precise function of contactin-1 in skeletal muscle is unknown, it is possible that its absence results in disruption of signaling pathways through the DAPC and has a deleterious effect on muscle membrane function or integrity, accounting for the muscle weakness observed in patients with *CNTN1* mutations. This hypothesis warrants further investigation.

In summary, we have identified a mutation within the neural adhesion molecule, contactin-1 in patients with

lethal congenital myopathy, characterized by the secondary loss of β2-syntrophin and α-dystrobrevin from the sarcolemma and central nervous system involvement. To our knowledge, this is the first time contactin-1 has been linked to human disease. In patient muscle biopsies, we show that contactin-1 transcript levels are significantly decreased compared to controls and demonstrate that contactin-1 is localized to the NMJ. The NMJs in patients with *CNTN1* mutations may thus be compromised, because of the absence, mislocalization, or disruption in signaling capabilities of various NMJ-associated proteins, leading to muscle weakness. To our knowledge, our work is the first to link disruptions of contactin-1 to human disease and further emphasizes the overlap in the clinical spectrum of congenital myopathies and congenital myasthenic syndromes.

Supplemental Data

Supplemental Data include one table and can be found with this article online at http://www.ajhg.org/.

Acknowledgments

This work was supported by grants from the Muscular Dystrophy Association of USA (S.C.F. and K.N.N.) and the NHMRC (K.N.N.) and the NIH (PO1 NS046788 to S.C.F. and NS038297 to B.R.). M.B. is supported by an NHMRC Career Development Award. We thank Dr. Alex Kan, Department of Anatomical Pathology, The Children's Hospital at Westmead, for interpretation of muscle electron microscopy. We acknowledge and thank Joanna Raftery, Dr. Nan Yang, and Dr. Daniel MacArthur for input into prioritization and sequence analysis of candidate disease genes and Vivienne Tobias, Department of Anatomical Pathology, South Eastern Area Laboratory Services, Sydney for initial muscle histology and sample handling collection (V:2, V:3, and V:4).

Received: August 27, 2008
Revised: October 16, 2008
Accepted: October 29, 2008
Published online: November 20, 2008

Web Resources

The URLs for data presented herein are as follows:

Ensembl Human Genome Server, http://www.ensembl.org/
Entrez Genome Map Viewer, http://www.ncbi.nlm.nih.gov/mapview/
MAFFT, http://align.bmr.kyushu-u.ac.jp/mafft/online/server/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/OMIM
University of California, Santa Cruz (UCSC) Genome Browser, http://genome.ucsc.edu/

Accession Numbers

The protein sequences reported in this paper are as follows: *Pan troglodytes* contactin 1 isoform 1 XM_001167985.1, *Mus musculus* NP_031753, *Rattus norvegicus* NP_476459, *Bos Taurus* NP_776705, *Gallus gallus* NP_001004381, *Danio rerio* NP_851300, *Xenopus laevis* BAA13100, *Homo sapiens* NP_001834, and NP_778203. The cDNA sequences reported in this paper are as follows: *Homo sapiens* NM_001843 and NM_175038, *Pan troglodytes* XM_001167985, *Pongo pygmaeus* CR857808, *Mus musculus* NM_007727, *Rattus norvegicus* NM_057118, *Bos Taurus* NM_174280, *Gallus gallus* NM_001004381, *Xenopus laevis* D86505, and *Danio rerio* (1a) NM_180969.

References

1. Ervasti, J.M., Kahl, S.D., and Campbell, K.P. (1991). Purification of dystrophin from skeletal muscle. J. Biol. Chem. *266*, 9161–9165.
2. Ervasti, J.M., and Sonnemann, K.J. (2008). Biology of the striated muscle dystrophin-glycoprotein complex. Int. Rev. Cytol. *265*, 191–225.
3. Zatz, M., de Paula, F., Starling, A., and Vainzof, M. (2003). The 10 autosomal recessive limb-girdle muscular dystrophies. Neuromuscul. Disord. *13*, 532–544.
4. Jones, K.J., Compton, A.G., Mills, M.A., Yang, N., Peters, M.F., Kunkel, L.M., Froehner, S.C., and North, K.N. (2003). Deficiency of the syntrophins and α-dystrobrevin in patients with inherited myopathy. Neuromuscul. Disord. *13*, 456–467.
5. Peters, M.F., Kramarcy, N.R., Sealock, R., and Froehner, S.C. (1994). β2-Syntrophin: Localization at the neuromuscular junction in skeletal muscle. Neuroreport *5*, 1577–1580.
6. Peters, M.F., Sadoulet-Puccio, H.M., Grady, R.M., Kramarcy, N.R., Kunkel, L.M., Sanes, J.R., Sealock, R., and Froehner, S.C. (1998). Differential membrane localization and intermolecular associations of α-dystrobrevin isoforms in skeletal muscle. J. Cell Biol. *142*, 1269–1278.
7. Kameya, S., Miyagoe, Y., Nonaka, I., Ikemoto, T., Endo, M., Hanaoka, K., Nabeshima, Y., and Takeda, S. (1999). α1-Syntrophin gene disruption results in the absence of neuronal-type nitric-oxide synthase at the sarcolemma but does not induce muscle degeneration. J. Biol. Chem. *274*, 2193–2200.
8. Adams, M.E., Kramarcy, N., Krall, S.P., Rossi, S.G., Rotundo, R.L., Sealock, R., and Froehner, S.C. (2000). Absence of α-syntrophin leads to structurally aberrant neuromuscular synapses deficient in utrophin. J. Cell Biol. *150*, 1385–1397.
9. Adams, M.E., Kramarcy, N., Fukuda, T., Engel, A.G., Sealock, R., and Froehner, S.C. (2004). Structural abnormalities at neuromuscular synapses lacking multiple syntrophin isoforms. J. Neurosci. *24*, 10302–10309.
10. Grady, R.M., Grange, R.W., Lau, K.S., Maimone, M.M., Nichol, M.C., Stull, J.T., and Sanes, J.R. (1999). Role for α-dystrobrevin in the pathogenesis of dystrophin-dependent muscular dystrophies. Nat. Cell Biol. *1*, 215–220.
11. Grady, R.M., Zhou, H., Cunningham, J.M., Henry, M.D., Campbell, K.P., and Sanes, J.R. (2000). Maturation and maintenance of the neuromuscular synapse: Genetic evidence for roles of the dystrophin-glycoprotein complex. Neuron *25*, 279–293.
12. Grady, R.M., Akaaboune, M., Cohen, A.L., Maimone, M.M., Lichtman, J.W., and Sanes, J.R. (2003). Tyrosine-phosphorylated and nonphosphorylated isoforms of α-dystrobrevin: Roles in skeletal muscle and its neuromuscular and myotendinous junctions. J. Cell Biol. *160*, 741–752.
13. Akaaboune, M., Grady, R.M., Turney, S., Sanes, J.R., and Lichtman, J.W. (2002). Neurotransmitter receptor dynamics

studied in vivo by reversible photo-unbinding of fluorescent ligands. Neuron *34*, 865–876.
14. Kramarcy, N.R., and Sealock, R. (2000). Syntrophin isoforms at the neuromuscular junction: Developmental time course and differential localization. Mol. Cell. Neurosci. *15*, 262–274.
15. Compton, A.G., Cooper, S.T., Hill, P.M., Yang, N., Froehner, S.C., and North, K.N. (2005). The syntrophin-dystrobrevin subcomplex in human neuromuscular disorders. J. Neuropathol. Exp. Neurol. *64*, 350–361.
16. Michalk, A., Stricker, S., Becker, J., Rupps, R., Pantzar, T., Miertus, J., Botta, G., Naretto, V.G., Janetzki, C., Yaqoob, N., et al. (2008). Acetylcholine receptor pathway mutations explain various fetal akinesia deformation sequence disorders. Am. J. Hum. Genet. *82*, 464–476.
17. Vogt, J., Harrison, B.J., Spearman, H., Cossins, J., Vermeer, S., ten Cate, L.N., Morgan, N.V., Beeson, D., and Maher, E.R. (2008). Mutation analysis of CHRNA1, CHRNB1, CHRND, and RAPSN genes in multiple pterygium syndrome/fetal akinesia patients. Am. J. Hum. Genet. *82*, 222–227.
18. Abecasis, G.R., Cherny, S.S., Cookson, W.O., and Cardon, L.R. (2002). MERLIN–rapid analysis of dense genetic maps using sparse gene flow trees. Nat. Genet. *30*, 97–101.
19. Peters, M.F., Adams, M.E., and Froehner, S.C. (1997). Differential association of syntrophin pairs with the dystrophin complex. J. Cell Biol. *138*, 81–93.
20. Song, W.K., Wang, W., Sato, H., Bielser, D.A., and Kaufman, S.J. (1993). Expression of α7 integrin cytoplasmic domains during skeletal muscle development: Alternative forms, conformational change, and homologies with serine/threonine kinases and tyrosine phosphatases. J. Cell Sci. *106*, 1139–1152.
21. Burkin, D.J., and Kaufman, S.J. (1999). The α7β1 integrin in muscle development and disease. Cell Tissue Res. *296*, 183–190.
22. Cooper, S.T., Lo, H.P., and North, K.N. (2003). Single section western blot: Improving the molecular diagnosis of the muscular dystrophies. Neurology *61*, 93–97.
23. Peat, R.A., Smith, J.M., Compton, A.G., Baker, N.L., Pace, R.A., Burkin, D.J., Kaufman, S.J., Lamandé, S.R., and North, K.N. (2008). The diagnosis and etiology of congenital muscular dystrophy. Neurology *71*, 312–321.
24. Pegoraro, E., Cepollaro, F., Prandini, P., Marin, A., Fanin, M., Trevisan, C.P., El-Messlemani, A.H., Tarone, G., Engvall, E., Hoffman, E.P., et al. (2002). Integrin alpha 7 beta 1 in muscular dystrophy/myopathy of unknown etiology. Am. J. Pathol. *160*, 2135–2143.
25. Berglund, E.O., and Ranscht, B. (1994). Molecular cloning and in situ localization of the human contactin gene (*CNTN1*) on chromosome 12q11-q12. Genomics *21*, 571–582.
26. Reid, R.A., Bronson, D.D., Young, K.M., and Hemperly, J.J. (1994). Identification and characterization of the human cell adhesion molecule contactin. Brain Res. Mol. Brain Res. *21*, 1–8.
27. Berglund, E.O., Murai, K.K., Fredette, B., Sekerkova, G., Marturano, B., Weber, L., Mugnaini, E., and Ranscht, B. (1999). Ataxia and abnormal cerebellar microorganization in mice with ablated contactin gene expression. Neuron *24*, 739–750.
28. Faivre-Sarrailh, C., Banerjee, S., Li, J., Hortsch, M., Laval, M., and Bhat, M.A. (2004). Drosophila contactin, a homolog of vertebrate contactin, is required for septate junction organization and paracellular barrier function. Development *131*, 4931–4942.
29. Schneider, M., Khalil, A.A., Poulton, J., Castillejo-Lopez, C., Egger-Adam, D., Wodarz, A., Deng, W.M., and Baumgartner, S. (2006). Perlecan and dystroglycan act at the basal side of the drosophila follicular epithelium to maintain epithelial organization. Development *133*, 3805–3815.
30. North, K. (2008). What's new in congenital myopathies? Neuromuscul. Disord. *18*, 433–442.
31. Ranscht, B. (1988). Sequence of contactin, a 130-kd glycoprotein concentrated in areas of interneuronal contact, defines a new member of the immunoglobulin supergene family in the nervous system. J. Cell Biol. *107*, 1561–1573.
32. Faivre-Sarrailh, C., Gennarini, G., Goridis, C., and Rougon, G. (1992). F3/F11 cell surface molecule expression in the developing mouse cerebellum is polarized at synaptic sites and within granule cells. J. Neurosci. *12*, 257–267.
33. Falk, J., Bonnon, C., Girault, J.A., and Faivre-Sarrailh, C. (2002). F3/contactin, a neuronal cell adhesion molecule implicated in axogenesis and myelination. Biol. Cell *94*, 327–334.
34. Fujita, N., Saito, R., Watanabe, K., and Nagata, S. (2000). An essential role of the neuronal cell adhesion molecule contactin in development of the xenopus primary sensory system. Dev. Biol. *221*, 308–320.
35. Perrin, F.E., Rathjen, F.G., and Stoeckli, E.T. (2001). Distinct subpopulations of sensory afferents require F11 or axonin-1 for growth to their target layers within the spinal cord of the chick. Neuron *30*, 707–723.
36. Boyle, M.E., Berglund, E.O., Murai, K.K., Weber, L., Peles, E., and Ranscht, B. (2001). Contactin orchestrates assembly of the septate-like junctions at the paranode in myelinated peripheral nerve. Neuron *30*, 385–397.
37. Murai, K.K., Misner, D., and Ranscht, B. (2002). Contactin supports synaptic plasticity associated with hippocampal long-term depression but not potentiation. Curr. Biol. *12*, 181–190.
38. Zisch, A.H., D'Alessandri, L., Ranscht, B., Falchetto, R., Winterhalter, K.H., and Vaughan, L. (1992). Neuronal cell adhesion molecule contactin/F11 binds to tenascin via its immunoglobulin-like domains. J. Cell Biol. *119*, 203–213.
39. Brummendorf, T., Hubert, M., Treubert, U., Leuschner, R., Tarnok, A., and Rathjen, F.G. (1993). The axonal recognition molecule F11 is a multifunctional protein: Specific domains mediate interactions with Ng-CAM and restrictin. Neuron *10*, 711–727.
40. Daniloff, J.K., Levi, G., Grumet, M., Rieger, F., and Edelman, G.M. (1986). Altered expression of neuronal cell adhesion molecules induced by nerve injury and repair. J. Cell Biol. *103*, 929–945.
41. Cifuentes-Diaz, C., Faille, L., Goudou, D., Schachner, M., Rieger, F., and Angaut-Petit, D. (2002). Abnormal reinnervation of skeletal muscle in a tenascin-C-deficient mouse. J. Neurosci. Res. *67*, 93–99.
42. Kazarinova-Noyes, K., Malhotra, J.D., McEwen, D.P., Mattei, L.N., Berglund, E.O., Ranscht, B., Levinson, S.R., Schachner, M., Shrager, P., Isom, L.L., et al. (2001). Contactin associates with Na+ channels and increases their functional expression. J. Neurosci. *21*, 7517–7525.
43. Liu, C.J., Dib-Hajj, S.D., Black, J.A., Greenwood, J., Lian, Z., and Waxman, S.G. (2001). Direct interaction with contactin targets voltage-gated sodium channel Na(v)1.9/NaN to the cell membrane. J. Biol. Chem. *276*, 46553–46561.

44. McEwen, D.P., Meadows, L.S., Chen, C., Thyagarajan, V., and Isom, L.L. (2004). Sodium channel beta1 subunit-mediated modulation of Nav1.2 currents and cell surface density is dependent on interactions with contactin and ankyrin. J. Biol. Chem. *279*, 16044–16049.
45. Shah, B.S., Rush, A.M., Liu, S., Tyrrell, L., Black, J.A., Dib-Hajj, S.D., and Waxman, S.G. (2004). Contactin associates with sodium channel Nav1.3 in native tissues and increases channel density at the cell surface. J. Neurosci. *24*, 7387–7399.
46. Rush, A.M., Craner, M.J., Kageyama, T., Dib-Hajj, S.D., Waxman, S.G., and Ranscht, B. (2005). Contactin regulates the current density and axonal expression of tetrodotoxin-resistant but not tetrodotoxin-sensitive sodium channels in DRG neurons. Eur. J. Neurosci. *22*, 39–49.
47. Gee, S.H., Madhavan, R., Levinson, S.R., Caldwell, J.H., Sealock, R., and Froehner, S.C. (1998). Interaction of muscle and brain sodium channels with multiple members of the syntrophin family of dystrophin-associated proteins. J. Neurosci. *18*, 128–137.
48. Burgess, D.L., Kohrman, D.C., Galt, J., Plummer, N.W., Jones, J.M., Spear, B., and Meisler, M.H. (1995). Mutation of a new sodium channel gene, Scn8a, in the mouse mutant 'motor endplate disease'. Nat. Genet. *10*, 461–465.
49. De Repentigny, Y., Cote, P.D., Pool, M., Bernier, G., Girard, S., Vidal, S.M., and Kothary, R. (2001). Pathological and genetic analysis of the degenerating muscle (*dmu*) mouse: A new allele of Scn8a. Hum. Mol. Genet. *10*, 1819–1827.
50. Leschziner, A., Moukhles, H., Lindenbaum, M., Gee, S.H., Butterworth, J., Campbell, K.P., and Carbonetto, S. (2000). Neural regulation of α-dystroglycan biosynthesis and glycosylation in skeletal muscle. J. Neurochem. *74*, 70–80.
51. Taniguchi, M., Kurahashi, H., Noguchi, S., Fukudome, T., Okinaga, T., Tsukahara, T., Tajima, Y., Ozono, K., Nishino, I., Nonaka, I., et al. (2006). Aberrant neuromuscular junctions and delayed terminal muscle fiber maturation in alpha-dystroglycanopathies. Hum. Mol. Genet. *15*, 1279–1289.
52. Grady, R.M., Wozniak, D.F., Ohlemiller, K.K., and Sanes, J.R. (2006). Cerebellar synaptic defects and abnormal motor behavior in mice lacking alpha- and beta-dystrobrevin. J. Neurosci. *26*, 2841–2851.
53. Mercado, M.L., Amenta, A.R., Hagiwara, H., Rafii, M.S., Lechner, B.E., Owens, R.T., McQuillan, D.J., Froehner, S.C., and Fallon, J.R. (2006). Biglycan regulates the expression and sarcolemmal localization of dystrobrevin, syntrophin, and nNOS. FASEB J. *20*, 1724–1726.
54. Peat, R.A., Gecz, J., Fallon, J.R., Tarpey, P.S., Smith, R., Futreal, A., Stratton, M.R., Lamande, S.R., Yang, N., and North, K.N. (2008). Exclusion of biglycan mutations in a cohort of patients with neuromuscular disorders. Neuromuscul. Disord. *18*, 606–609.
55. Moore, S.A., Saito, F., Chen, J., Michele, D.E., Henry, M.D., Messing, A., Cohn, R.D., Ross-Barta, S.E., Westra, S., Williamson, R.A., et al. (2002). Deletion of brain dystroglycan recapitulates aspects of congenital muscular dystrophy. Nature *418*, 422–425.