**Molecular genetic studies:**

Trio exome sequencing was performed on a Genome Analyzer HiSeq 2000 system (Illumina) after in-solution enrichment of coding (exonic) and adjacent intronic sequences (SureSelect Human all Exon 50 Mb kit v4; Agilent, Santa Clara, CA) and indexing of samples for multiplex-sequencing (Multiplexing Sample Preparation Oligonucleotide Kit; Illumina). A read alignment was performed with BWA (version 0.5.8) to the human genome assembly hg19 and single nucleotide variants and small insertions and deletions were called with SAMtools (version 0.1.7). Filtering for variants warranted the exclusion of HapMap SNPs present in dbSNP 135 with an average heterozygosity greater than 0.02. Next, we filtered for variants present in more than 15 of > 7,000 in-house exomes from individuals with unrelated diseases. Variant annotation was performed with custom scripts and only non-synonymous variants were considered further. No convincing point mutations or small deletions or insertions were observed in known genes for neurodegenerative or neuromuscular diseases. Further analysis of the WES data was then performed using algorithms (Pindel) for the detection of deletions, insertions, inversions, tandem duplications and other structural variants.

**Proteome profiling:**

Materials

Following materials were purchased from Sigma-Aldrich, Germany: ammonium bicarbonate (NH4HCO3), guanidine hydrochloride (GuHCl) and iodoacetamide (IAA). Dithiothreitol (DTT) was bought from Roche Diagnostics, Mannheim, Germany. Trypsin Gold, Mass Spectrometry Grade was purchased from Promega, Madison, USA. All chemicals for ultra-pure HPLC solvents such as formic acid (FA), trifluoroacetic acid (TFA) and acetonitrile (ACN) were purchased from Biosolve, Valkenswaard, the Netherlands.

Tissue lysis

The skeletal muscle biopsy (Musculus quadriceps femoris) derived from an index patient (female, Caucasian) and the respective two controls (matched for age, sex and muscle group) were lysed in a buffer comprising 125 mM Tris buffer (pH 6.4), 4% SDS, 10% beta-mercaptoethanol [1.3 M], 10% glycerin, 0.001% bromphenol blue, 4 M urea and protease inhibitor mix (Roche cOmplete EDTA-free Protease Inhibitor Cocktail). The approximate protein content of all three samples was estimated to be 0.625 µg/µL (based on the 1D-PAGE gel band intensity). Normally, these lysates are used for the routine diagnostic work-up in terms of immunoblot studies focusing on proteins for which variants in the corresponding genes are causative for the clinical manifestation of a neuromuscular phenotype.

Ice cold ethanol precipitation, carbamidomethylation and in solution protein digestion

Muscle lysates (protein extract routinely used for immunoblot-analyses of diagnostically relevant proteins) corresponding to ~1 µg of protein were diluted with 10-fold excess of ice-cold ethanol and stored at -40°C for 1 h followed by centrifugation at 4°C at 25,000 *g* for 30 min. The supernatant was discarded and 100 µL of ice-cold acetone was added to the precipitated proteins and centrifuged under same conditions as mentioned above for 10 min. Next, the supernatant was discarded, and the samples were left to dry under the laminar flow hood. Next, each protein pellet was resolubilized in 8 µL of 1 M GuHCl and samples were diluted with 50 mM NH4HCO3 (pH 7.8) to lower the GuHCl concentration to 0.2 M. Reduction of disulfide bonds was carried out with 10 mM DTT and incubation at 56°C for 30 min followed by alkylation of free thiols with 30 mM IAA at room temperature for 30 min in dark. Next, trypsin was added in a 1:40 w/w ratio of enzyme to substrate and the samples were incubated at 37°C overnight. After incubation, each sample digest was acidified with 10% TFA to pH < 3.0 and desalting was performed using C18 10 µL tips (Proxeon Biosystems) according to the manufacturer’s instructions. Finally, the eluates were completely dried in a SpeedVac and reconstituted in 15 µL of 0.1% TFA for subsequent LC-MS/MS analysis.

LC-MS/MS analysis

Each sample was analyzed using an Ultimate 3000 RSLC HPLC system coupled to a Q Exactive (both Thermo Scientific). Peptides were preconcentrated on a 100 µm x 2 cm C18 trapping column for 10 min using 0.1% TFA at a flow rate of 12 µL/min followed by separation on a 75 µm x 50 cm C18 main column (both Acclaim Pepmap nanoviper, Thermo Scientific) with a 187 min LC gradient ranging from 3-35% of B (84% ACN in 0.1% FA) at a flow rate of 250 nL/min. The Q Exactive was operated in data-dependent acquisition mode and MS survey scans were acquired from m/z 300 to 1,500 at a resolution of 70,000 using the polysiloxane ion at m/z 371.1012 as lock mass.[2] The fifteen most intense ions were isolated with a 2.0 m/z window and fragmented by higher energy collisional dissociation (HCD) with a normalized collision energy of 27%, taking into account a dynamic exclusion of 12 s. MS/MS were acquired at a resolution of 17,500. Automated gain control (AGC) target values and fill times were set to 2 × 105 and 120 ms for MS and 5 × 104 and 250 ms for MS/MS, respectively, using an under-fill ratio of 5%.

MS data analysis

MS data was analyzed using Proteome Discoverer 3.01. Database searches were performed using Sequest HT using a human Swissprot database (downloaded October 16, 2022; 20,328 target entries). Trypsin was selected as protease allowing a maximum of two missed cleavages. Carbamidomethylation of Cys and oxidation of Met were set as fixed and variable modifications, respectively. Precursor and product ion mass tolerances were set to 10 ppm and 0.02 Da, respectively. Only peptide-spectrum-matches (PSM) to sequences between 7-30 amino acids were considered and rescored using INFERYS.[4] Poster-error-probabilities (PEP) were determined using Percolator[3] and all MS/MS spectra that did not yield PSMs passing a 1% false discovery rate were researched using additional variable modifications (deamidation of N, Q; N-terminal pyro-Q; protein N-terminal acetylation and loss of protein N-terminal M). Only PSM to sequences between 7-35 amino acids were evaluated by Percolator. Label free quantitation (LFQ) was performed using the Minor Feature Detector, Feature Mapper, and Precursor Ion Quantified nodes, considering only high-confidence protein-unique peptides, and normalizing the samples based on total peptide amount to compensate for differences in sample loading. Only proteins that were quantified either in the index patient or in both controls were considered and low abundance resampling was used for imputation of missing values. All data was filtered for 1% false discovery rate (PSM, peptide, and protein level). Regulated proteins were defined as follows: the relative standard deviation (RSD) of the normalized abundances of the two control samples had to be <30%[1] ,and the protein had to be at least 2-fold up/down-regulated in the index patient compared to the average of the controls. All corresponding proteins were used to generate a high-confidence STRING-based protein/protein interaction network map, after removing proteins that were absent in the patient and quantified with less than 3 protein-unique peptides in the controls.

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