nature research

Corresponding author(s): Carsten G Bonnemann, Teresa M. Dunn

Last updated by author(s): Apr 6, 2021

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	ImageLab Studio (BioRad) was used for western blot image acquisition. Leica application suite Advanced Fluorescence software was used for confocal microscopy image acquisition. Quantstudio real-time software v1.1 was used for real-time PCR data acquisition.
Data analysis	Quantstudio real-time software v1.1 was used for real-time PCR data analysis. Graphpad/Prism v9 software was used for statistical analysis and generation of the graphs. FIJI (ImageJ) was used for analysis of microscopy images. Adobe Illustrator was used for compilation of the figures.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All data and material generated and analysed within the main figures and supplementary material of this study are available upon request from the authors. All requests for raw and analysed data and materials related to this article will be reviewed by the respective institution to verify if the request is subject to any intellectual property or confidentiality obligations. Some patient-related data, including genetic sequencing data, not included in the manuscript or its supplements, were generated as part of clinical care and may be subject to patient confidentiality. Any data and materials that can be shared will be released via a Material Transfer Agreement.

Databases: The following databases were searched for presence of variants reported in this manuscript: the Genome aggregation database (https://gnomad.broadinstitute.org), the Decipher database (https://www.deciphergenomics.org), Leiden database (https://www.lovd.nl) and the TopMed database (https://bravo.sph.umich.edu/freeze8/hg38/).

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No sample size calculation was performed. We obtained and processed as many human serum/plasma samples from the individuals in the patient cohort who consented to participate in research studies. The proof of concept mechanistic studies in cells throughout this study were exploratory. At least 3 (and in most cases more) replicates for each in vitro experiment was performed.
Data exclusions	No data were excluded from analysis.
Replication	The MS data on HEK293 cells, fibroblasts with siRNA intervention, and serum samples were generated by different labs. The same primary fibroblast cell lines were tested with siRNA intervention at each lab with at least 3 replicates in each lab (3 independent platings) and MS data was generated for each plating. The serum/plasma samples were aliquoted and data were generated by multiple MS runs of the same sample, not from multiple blood draws. iPSC-hMN MS data in each relevant figure is from multiple MS measurements of the one plating and differentiation experiment. The HEK293 knockout cell experiments were obtained from 3 different platings and each plating was measured by MS. When performed, replication attempts were successful.
Randomization	Randomization is not relevant to this study as there are no interventional studies performed in the human subjects or animal models.
Blinding	The MS lipidomic analysis of serum samples, iPSC-derived hMN, and fibroblasts was performed blinded.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☐	☒ MRI-based neuroimaging

Antibodies

Antibodies used	Antibodies for immunocytochemistry: Anti-HB9 antibody, Developmental Studies Hybridoma Bank, Catalog#: 81.5C10 Anti-Choline Acetyltransferase, Aves Labs, Catalog#: 6727986 Beta-III-Tubulin (Tuj1), Covance, Catalog #: MMS-435P SPTLC1, BD Biosciences, Catalog # 611304 TDP-43, proteintech, Catalog# 12892-1-AP Antibodies for western blotting: GAPDH, Millipore, Catalog# MAB374 SPTLC1, BD Biosciences, Catalog # 611304
Validation	All antibodies were validated per manufacturer or previous publications. The staining patterns observed were consistent with expected cellular localization of the target antigens. Western blot bands were near the expected molecular weight for the target protein.

1. Anti-HB9 antibody, Developmental Studies Hybridoma Bank, Catalog#: 81.5C10. has been validated for IF (See PMID: 32571478)
2. Anti-Choline Acetyltransferase, Aves Labs, Catalog#: 6727986. Validated per manufacturer.
3. Beta-III-Tubulin (Tuj1), Covance, Catalog #: MMS-435P. Validated per manufacturer.
4. SPTLC1, BD Biosciences, Catalog # 611304. Validated per manufacturer.
5. TDP-43, proteintech, Catalog# 12892-1-AP. Validated per manufactuer.

Antibodies for western blotting:
GAPDH, Millipore, Catalog# MAB374. Validated per manufacturer
SPTLC1, BD Biosciences, Catalog # 611304. Validated per manufacturer

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Fibroblasts (primary cell lines from patients), iPSC (Coriell), HEK293 (ATCC)
Authentication	iPSCs were authenticated by g-banded karyotype analysis, sequence verification of targeted CRISPR-Cas9 genomic edits, and antibody staining of motor neuron markers following differentiation. HEK293 cells were not authenticated.
Mycoplasma contamination	All fibroblast and iPSC cell lines routinely tested negative for mycoplasma. HEK293 cells were not tested.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

Human research participants

Policy information about studies involving human research participants

Population characteristics	The patients were evaluated at different centres by neuromuscular specialists and medical geneticists per standard of clinical diagnosis and clinical care. A total of 11 patients (6F, 5M) ages 8-62 years of age were evaluated. The patients were referred by their treating physicians to observational and biorepository research studies and after informed consent, additional research tests that were not part of routine clinical care were performed (e.g. skin biopsy for fibroblast cultures, research based genetic testing, serum sample collection, muscle MRI images).
Recruitment	The subjects were self-referred or referred to research teams by their treating physicians. Subjects were referred to research teams for further evaluation of their disease and genetic variants identified. This is therefore a self-selected subject group to include those who have had access to extensive genetic testing and/or access to clinical care.
Ethics oversight	Intramural NIH IRB NINDS protocol 12-N-0095, Washington University, protocol #201308083, McMaster University protocol REB 14-595-T, and University of Massachusetts protocol 13788_10.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Magnetic resonance imaging

Experimental design

Design type	Standard clinical neuromuscular MRI was performed. No experimental MRI protocols were used.
Design specifications	Patient 1 underwent one neuromuscular MRI at age 16 years.
Behavioral performance measures	Standard neuromuscular MRI was performed, no behavioral performance was measured.

Acquisition

Imaging type(s)	Structural
Field strength	1.5T
Sequence & imaging parameters	T1-weighted spin echo and short tau inversion recovery (STIR) of the lower extremities on a 1.5-T Aera Siemens system. Slices were 5 mm thick. The gap between slices was 10 mm.
Area of acquisition	Non-contrast images were obtained from pelvis, thighs and lower legs in the axial plane.

Diffusion MRI ☐ Used ☒ Not used

Preprocessing

Preprocessing software	No preprocessing was used
Normalization	No normalization was used

Normalization template	No template was used
Noise and artifact removal	No noise removal or artifact removal were used
Volume censoring	No volume censoring was used

Statistical modeling & inference

Model type and settings	No statistical modeling was used
Effect(s) tested	No statistical effects were tested

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference (See Eklund et al. 2016)	None used
Correction	None used

Models & analysis

n/a	Involved in the study
☒	☐ Functional and/or effective connectivity
☒	☐ Graph analysis
☒	☐ Multivariate modeling or predictive analysis