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Supplemental information

ABHD16A deficiency causes a complicated form of hereditary spastic paraplegia associated with intellectual disability and cerebral anomalies

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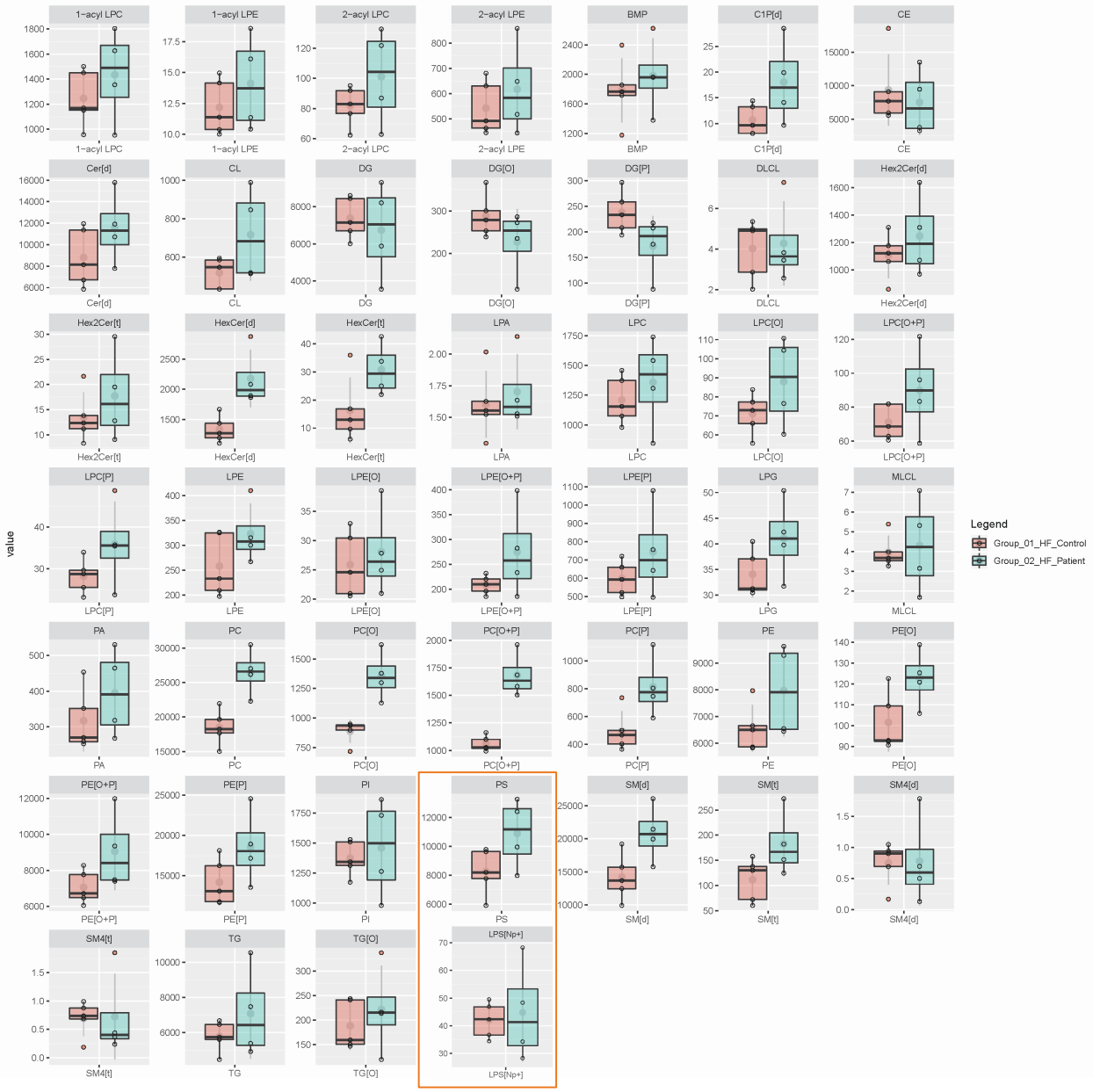
Figure S1: Lipidomics analysis of patient and control fibroblasts.



Lipidomics analysis of patient and control fibroblasts. The lipidome of four different patient fibroblasts (Individuals P3, P4, P7, P9) were compared with four independent control fibroblast lines. Boxplots of summed total levels of major lipid classes are shown for control (red) and patients (blue). Value on the y-axis are semi-quantitative lipid abundances (response of analyte divided by that of the internal standard multiplied by the concentration of the internal standard) PS and LPS are indicated in an orange box; PS is elevated in patients whereas LPS levels are not changed. In addition, changes in other lipid major classes were observed; PC, PE and different members of the ether lipid family (PC[O], PC[P], PE[O], PE[P]) are elevated in patients.

METHODS

Sequencing

The study was approved by the Children's Hospital of Eastern Ontario Research Ethics Board and institutional review boards of the University of California, San Diego. Informed consent was obtained from families. Exome sequencing of the six affected individuals from Family 1, 2 and 3 was performed in 2015 as part of the Care4Rare Canada research project (see Beaulieu *et al* [1] for technical details). Segregation of the *ABHD16A* variants in individuals from Family 1, 2 and 3 was performed by Sanger sequencing (primers available on request) at McGill University Health Center Research Institute (Montreal, QC, Canada). Trio exome sequencing of the proband and parents from Family 4 was performed by next-generation sequencing in a clinical laboratory. Family 4 was enrolled into the Care4Rare research program in 2019 after the clinical trio exome did not identify any variants to explain the proband's phenotype. The raw genomic data of these three family members were repatriated and processed through the Care4Rare Canada research bioinformatic pipeline. The four other affected individuals with homozygous variants in *ABHD16A* from Family 4 and 5 were identified via interrogation of the Geno2MP platform (Genotype to Mendelian Phenotype Browser, (URL:http://geno2mp.gs.washington.edu [(April, 2020) accessed])). Exome sequencing and data processing for these four individuals were performed by the Genomics Platform at the Broad Institute of MIT and Harvard (Cambridge, MA, USA) with an Illumina Nextera exome capture (~38 Mb target) and sequenced (150 bp paired reads) to cover >80% of targets at 20x and a mean target coverage of >100x. Exome sequencing data was processed through a pipeline based on Picard and mapping done using the BWA aligner to the human genome build 38. Variants were called using Genome Analysis Toolkit (GATK) HaplotypeCaller package version 3.5. Segregation of the *ABHD16A* variants in

individuals from Family 5 and 6 was performed by Sanger sequencing (primers available on request) at the Children's Hospital of Eastern Ontario (Ottawa, ON, Canada).

Patient fibroblast cell lines

Primary fibroblast cells from affected individuals P3 and P4 (Family 2) and P7 (Family 4) were derived and established from a skin biopsy by The Centre for Applied Genomics (Toronto, Canada). Primary fibroblast cells from affected individual P9 (Family 5) were derived and established from a skin biopsy at the University of California in San Diego (UCSD IRB protocol 171094, San Diego, USA) (see Chai *et al* [2] for further details).

RNA analysis

Total RNA was obtained from affected and control fibroblast cell lines with the RNeasy Mini Kit (QIAGEN) and reverse transcribed into complementary DNA (cDNA) with iScript kit (BioRad Laboratories) according to manufacturers' instructions. Control reactions without reverse transcriptase were prepared in parallel. cDNA was amplified with gene-specific primers and iQ SYBR Green Supermix (BioRad Laboratories) and read on a CFX96 Touch Real-time PCR Detection System (BioRad Laboratories). Gene expression was quantified using the standard Ct method with CFX software (BioRad Laboratories), and all data corrected against GAPDH as an internal control. The primer sequences used to amplify cDNA from exons 5-6 of NM_021160.2:

ABHD16A_ex4-5_F1 AAGTGGTGCCGTTTTCTCAC

ABHD16A_ex4-5_R1 ATGGTGATGAACTGCCGGTA

Fibroblast cell lines from five healthy, age and gender matched individuals were used as controls for the RT-PCR analysis (GM05381 (5-year-old male), GM07522 (19-year-old female),

GM07532 (16-year-old female), GM00038 (9-year-old female), GM00969 (2-year-old female) Coriell Institute, Hamden, USA).

Western blot analysis

Western blot analysis was conducted to assess protein levels in fibroblast cells derived from affected individuals and controls. Cells were lysed in radioimmunoprecipitation assay buffer containing 10 mg/mL each of aprotinin, phenylmethanesulfonyl fluoride, and leupeptin (all from Sigma) for 20 min at 4°C, followed by centrifugation at 13,000xg for 15 min and retrieval of supernatants. Total protein concentrations were determined by Bradford protein assay (BioRad Laboratories). Protein samples were resolved by SDS-PAGE, transferred onto nitrocellulose membrane and incubated in blocking solution (Tris-buffered saline (TBS), 5% non-fat milk, 0.1% Tween-20) for 1 h at room temperature followed by overnight incubation with primary antibody at 4°C (BAT5/ABHD16A, Abcam ab185549). Membranes were washed with TBS and 0.1% Tween-20 three times followed by incubation with secondary antibody (HRP conjugated anti-rabbit; BioRad Laboratories) for 1 h at room temperature. Blots were visualized by autoradiography using the Clarity Western ECL substrate (BioRad Laboratories). Fibroblast cell lines from five healthy, age and gender matched individuals were used as controls for the Western Blot analysis (GM01652 (11-year-old female), GM05381(5-year-old male), GM07522 (19-year-old female), GM07532 (16-year-old female), GM08399 (19-year-old female), Coriell Institute, Hamden, USA).

Proliferation assay

Fibroblast cells were plated in triplicate at a density of 3000 cells/well in 96 well dishes. 24 h later, wells were imaged for 72 h using the IncuCyte ZOOM and confluency was assessed using IncuCyte ZOOM confluence processing analysis tool.

Plasma sample collection

Whole blood drawn into EDTA containing tubes were collected from individuals P1 and P2, the unaffected sibling and both parents from Family 1. Whole blood samples were also collected from individuals P3 and P4 and both parents from Family 2. Medication and fasting details for each participant were collected at the time of blood draw. Blood tubes were kept on ice until centrifugation. They were centrifuged at 1500xg for 10 min at 4°C. The plasma was collected from the top layer, leaving behind the buffy coat and red blood cells. The samples were kept on ice during plasma collection. Four-hundred µL of plasma was then aliquoted into 2 pre-chilled vials and frozen immediately at -80°C.

Fibroblast cell culture for lipidomics assay

Cell lines for 4 patients (P3 and P4 (Family 2), P7 (Family 4), P9 (Family 5)) and five control cell lines (GM01652 (11-year-old female), GM05381(5-year-old male), GM07522 (19-year-old female), GM07532 (16-year-old female), GM08399 (19-year-old female), Coriell Institute, Hamden, USA) were grown in Ham's F10 Medium (Wisent Inc., St-Jean-Baptiste, Canada) supplemented with 10% fetal bovine serum (FBS; Sigma, St. Louis, USA), penicillin/streptomycin cocktail (Hyclone, Logan, USA) to a final concentration of 100 U/ml and L-glutamine (Hyclone, Logan, USA) to a final concentration of 2 mM. All cells were

treated in the same way to reduce variability including using the same lot number for media and its additives, the use of the same incubator, and same technician tending to the cells. When confluent, cells were maintained for another 8 days before collection. Stationary cells were harvested by trypsinization, washed twice with phosphate buffered saline and then washed with 0.9% saline before the cell pellet was frozen and stored at -80°C. An aliquot of each cell suspension was taken before the final pelleting and cell number obtained for normalization purposes. All cell pellets were collected on the same day.

Lipidomics assays

Frozen plasma and fibroblast cell pellets were shipped on dry ice to the Laboratory Genetic Metabolic Diseases, Amsterdam UMC, in Amsterdam for lipidomic analysis as previously described.[3]

REFERENCES

1. Beaulieu, C.L., Majewski, J., Schwartzentruber, J., Samuels, M.E., Fernandez, B.A., Bernier, F.P., Brudno, M., Knoppers, B., Marcadier, J., Dyment, D., et al. (2014). FORGE Canada Consortium: outcomes of a 2-year national rare-disease gene-discovery project. Am J Hum Genet. *94*, 809-817.
2. Chai, G., Webb, A., Li, C., Antaki, D., Lee, S., Breuss, M.W., Lang, N., Stanley, V., Anzenberg, P., Yang, X., et al. (2021). Mutations in Spliceosomal Genes PPIL1 and PRP17 Cause Neurodegenerative Pontocerebellar Hypoplasia with Microcephaly. Neuron. *109*, 241-256 e249.
3. Vaz, F.M., McDermott, J.H., Alders, M., Wortmann, S.B., Kolker, S., Pras-Raves, M.L., Vervaart, M.A.T., van Lenthe, H., Luyf, A.C.M., Elfrink, H.L., et al. (2019). Mutations in PCYT2 disrupt etherlipid biosynthesis and cause a complex hereditary spastic paraplegia. Brain. *142*, 3382-3397.