Supporting Information to

Intellectual-disability-associated mutations in the ceramide transport protein gene CERT lead to aberrant function and subcellular distribution

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Figure S1, dupAA mutation does not affect the function of CERT in the KI cells

(A) Schematic representation of the location of genome-edited sequences of human CERT1. The coding regions and 3′-UTR are shown in black and white rectangles, respectively. The red highlighted sequence (upper panel) and red line (lower panel) represent an extended sequence caused by a patient-derived mutation (blue). In the KI cells, edited gene sequences with desired gene insertions are shown (lower panel).

Neo, neomycin-resistant gene.

(B) WT, CERT1 KO, CERT KI, and CERTdupAA KI HCT116 cells were analyzed by western blotting. Heterozygous and homozygous KI-CERT mutants are shown as Neo/+ or Neo/Neo, respectively. hyper-p-CERT, hyperphosphorylated CERT; d/hypo-CERT, de/hypophosphorylated CERT.

(C) WT, CERT1 KO, CERT KI, and CERTdupAA KI HCT116 cells were cultured with 250 ng/ml lysenin for 1 h. Cellular viability was measured by the lactate dehydrogenase cytotoxicity assay. Data show mean ± SEM; n = 3 (**p < 0.01; n.s., not significant).

(D) WT, CERT1 KO, CERT KI, and CERTdupAA KI HCT116 cells were cultured with L-[U-14C]serine for 16 h. Metabolically labeled lipids separated on a TLC plate were visualized (representative image, left) and labeled SM was quantified (right). PE, phosphatidylethanolamine; PS, phosphatidylserine. Data show mean ± SEM; n = 4 (**p < 0.01; n.s., not significant).
Figure S2, Sequence alignment of CERT proteins
(A) Comparison of the amino acid sequence of CERT in various organisms. Homo sapiens (H.s.) CERT (ProUnitKB accession number: Q9YSP4-2), Mus musculus (M.m.) CERT (Q9EQG9-2), Xenopus laevis (X.l.) CERT (Q6NRZ4), Danio rerio (D.r.) CERT (Q5M7Y0-2), Drosophila melanogaster (D.m.) CERT (Q9Y128), and Caenorhabditis elegans (C.e.) CERT (Q93569) are shown. Alignment was generated using CLUSTALW (http://www.genome.jp) and Jalview (http://www.jalview.org) software. Residues were colored using the clustalx color scheme implemented in Jalview. The black lines above the sequences represent the PH domain, coiled-coil domain, and START domain. The red lines above the sequences represent the disorder regions predicted by PSIPRED (http://bioinf.cs.ucl.ac.uk/psipred/). The black rectangles represent the SRM and FFAT motif. ID-associated mutations are indicated by black arrowheads.
(B) The Phylogenetic tree of CERT in various organisms was constructed by the neighbor joining tree method using Jalview software. Sequence similarities between each pair based on BLOSUM62 are shown.
Table S1

| WISC-V index               | Composite score | Percentile rank | 95% confidence interval | Qualitative Intellectual Classification |
|----------------------------|-----------------|-----------------|--------------------------|---------------------------------------|
| Verbal comprehension (VCI) | 45              | <0.1            | 42-60                    | Extremely low                          |
| Visual Spatial (VSI)       | 67              | 1               | 63-77                    | Very low                              |
| Fluid Reasoning (FRI)      | 72              | 3               | 67-81                    | Very low                              |
| Working Memory (WMI)       | 51              | 0.1             | 47-63                    | Extremely low                          |
| Processing Speed (PSI)     | 60              | 0.4             | 56-74                    | Extremely low                          |
| Full Scale (FSIQ)          | 55              | 0.1             | 51-63                    | Extremely low                          |

Table S1. Wechsler Intelligence Scale for Children - Fifth Edition (WISC-V)

WISC-V of the proband at 8 years and 2 months of age showed a mild intellectual disability (FISQ composite score of 55). The Visual Spatial Index (VSI) and Fluid Reasoning Index (FRI) were in the very low part of the Qualitative Intellectual Classification, while the Verbal Comprehension Index (VCI), Working Memory Index (WMI), and Processing Speed Index (PSI) were in the extremely low part.
Table S2

| Gene   | Location genomics | Protein/DNA     | Genotype/Matches the clinical phenotype | ACMG Classification | OMIM phenotype (#OMIM)/inheritance |
|--------|-------------------|-----------------|----------------------------------------|---------------------|------------------------------------|
| ABCA4  | (NM_000350.2)     | p.Gly1961Glu/c.5882G>A | Heterozygosity/No                       | Pathogenic; rs1800553 | Stargardt disease 1 (#248200), autosomal recessive |
| ANO5   | (NM_001130105.1)  | p.Gly231Val/c.692G>T | Heterozygosity/No                       | Pathogenic; rs137854523 | Muscular dystrophy, limb-girdle, autosomal recessive 12 (#611307), autosomal recessive |
| LTBP2  | (NM_000428.2)     | p.Pro432Leu/c.1295C>T | Heterozygosity/No                       | Pathogenic; rs201818754 | Microspherophakia and/or megalocornea, with ectopia lentis and with or without secondary glaucoma (#251750), autosomal recessive |
| ABCC6  | (NM_001171.5)     | p.Arg1141*/c.3421C>T | Heterozygosity/No                       | Pathogenic; rs72653706 | Pseudoxanthoma elasticum, forme fruste (#177850), autosomal dominant |
| XYLTI  | (NM_022166.3)     | c.1588-3C>T       | Heterozygosity/No                       | Benign; rs201818754  | Desbuquois dysplasia 2 (#615777), autosomal recessive |
| KAT6A  | (NM_006766.4)     | p.Ser448Gly/c.1342A>G | Heterozygosity/No                       | Benign; rs149459548  | Arboleda-Tham syndrome (#616268), autosomal dominant |
| KCNT1  | (NM_020822.2)     | p.Leu386Phe/c.1156C>T | Heterozygosity/No                       | Uncertain significance; rs780875110 | Developmental and epileptic encephalopathy 14 (#614959), autosomal dominant |
| WDR11  | (NM_018117.11)    | p.Tyr994Cys/c.2981A>G | Heterozygosity/No                       | Uncertain significance | Hypogonadotrophic hypogonadism 14 with or without anosmia (#614858), autosomal dominant |
| NALCN  | (NM_052867.2)     | p.Ser515Arg/c.1545C>G | Heterozygosity/No                       | Likely pathogenic    | Congenital contractures of the limbs and face, hypotonia, and developmental delay (#616266), autosomal dominant |
| SETBP1 | (NM_015559.2)     | p.Asp1292Glu/c.3876C>G | Heterozygosity/No                       | Likely benign; rs139106261 | Schinzel-Giedion midface retraction syndrome (#269150), autosomal dominant |
| COL4A3BP| (NM_001130105.1)  | p.Pro749fs/ c.2242_2243dupAA | Heterozygosity/Yes                      | Pathogenic          | Mental retardation, autosomal dominant 34, (#616351), autosomal dominant |

**Proband variants**

Other gene variants found in the proband exome are listed with information from the American College of Medical Genetics and Genomics (ACMG) Classification and the Online Mendelian Inheritance in Man (OMIM). **COL4A3BP** is an alternative name of **CERTI**.