Supplementary Online Content


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eFigure 6. COS7 cells were co-transfected with 1.5 μg GFP-tagged sec61, HA-tagged CPT1C wild-type or mutant, HA-empty vector and GFP-empty vector as specified in the picture.

This supplementary material has been provided by the authors to give readers additional information about their work.
**Figure 1.** Diagram of the filtering strategy for the exome sequencing data.

- **Filter 1:** Non-Autosomal Variants removed
  - 44,258 Single Nucleotide Variants
    - 1,804 Indels

- **Filter 2:** Homozygous Variants removed
  - 43,600 Single Nucleotide Variants
    - 1,772 Indels

- **Filter 3:** Variants in dbSNP Build 134 and 1000 Genomes removed
  - 31,089 Single Nucleotide Variants
    - 1,069 Indels

- **Filter 4:** Intronic and/or Synonymous Variants removed
  - 3,646 Single Nucleotide Variants
    - 4 Indels

- **Filter 5:** Variants in ESP Database removed
  - 46 Single Nucleotide Variants
    - 0 Indels

- **Filter 6:** Non-segregating Variants removed
  - 13 Single Nucleotide Variants
    - 0 Indels

- **Final:** 1 Single Nucleotide Variant
  - 0 Indels
**eFigure 2.** Comparison of spectral and structural parameter of Nβ and Nβ (Arg37Cys) of human CPT1C.

(A) Superposition of $^1$H-$^15$N correlation NMR spectra of wild-type Nβ and Nβ(Arg37Cys). The spectra were recorded in the presence of dodecyltrimethylammonium chloride (DDAC) at pH 5.6, 35 °C and a $^1$H frequency of 700 MHz. (B) $^1$H$^N$ chemical shift differences, Dd($^1$H$^N$), between Nα and Nα (Arg37Cys), and Nβ and Nβ(Arg37Cys). The borders of the secondary structure elements of Nα, helices α1, α2, and α2′, are indicated. (C) Comparison of Nβ and Nβ(Arg37Cys) secondary $^{13}$Cα chemical shifts, d($^{13}$Cα), defined as the difference between observed and tabulated, random coil $^{13}$Cα shifts. Positive and negative shifts indicate helical and extended backbone conformations, respectively (Wishart & Case, 2001). The borders of all possible Nα and Nβ secondary structure elements, helices α1, α2, α2′, and sheets β1, β2 are indicated (Rao et al, 2011).
eFigure 3. iPSC-derived motor neurons from an unaffected subject were stained for SMI-32 (Covance, SMI-32R, 1:1,000) and CPT1C (Proteintech, 12969-1-AP; 1:1,000).

The nuclear DAPI stain is blue. Scale bar=20 μm.
**eFigure 4.** Western blot analysis of CPT1C.

(antibody developed following the indications in the previous study by Carrasco et al., 2012, by Sigma-Genosys; cropped image) expression in different samples. 1) Ganglion (the majority are sensory neurons); 2) ventral horn of the spinal cord (the majority are motor neurons); and 3) cortex from WT mice.
**eFigure 5.** COS7 cells were transfected with 1.5 μg HA-tagged CPT1C and signal was detected using anti-HA (Covance, clone 16B12, 1:5,000), anti-Calreticulin antibody (Calbiochem, 208910, 1:1,000), and anti-AIF antibody (Millipore, AB16501, 1:1,000).

The nuclear DAPI stain is blue. Scale bar=20 μm.
**eFigure 6.** COS7 cells were co-transfected with 1.5 μg GFP-tagged sec61, HA-tagged CPT1C wild-type or mutant, HA-empty vector and GFP-empty vector as specified in the picture.

Co-Immunoprecipitation was detected using an anti-HA antibody (Covance, clone 16B12, 1:5,000) and anti-GFP antibody (IP: molecular probe, A-11122; IB: abcam, ab290, 1:5,000).