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Supplemental Data

A Peroxisomal Disorder of Severe Intellectual Disability, Epilepsy, and Cataracts Due to Fatty Acyl-CoA Reductase 1 Deficiency

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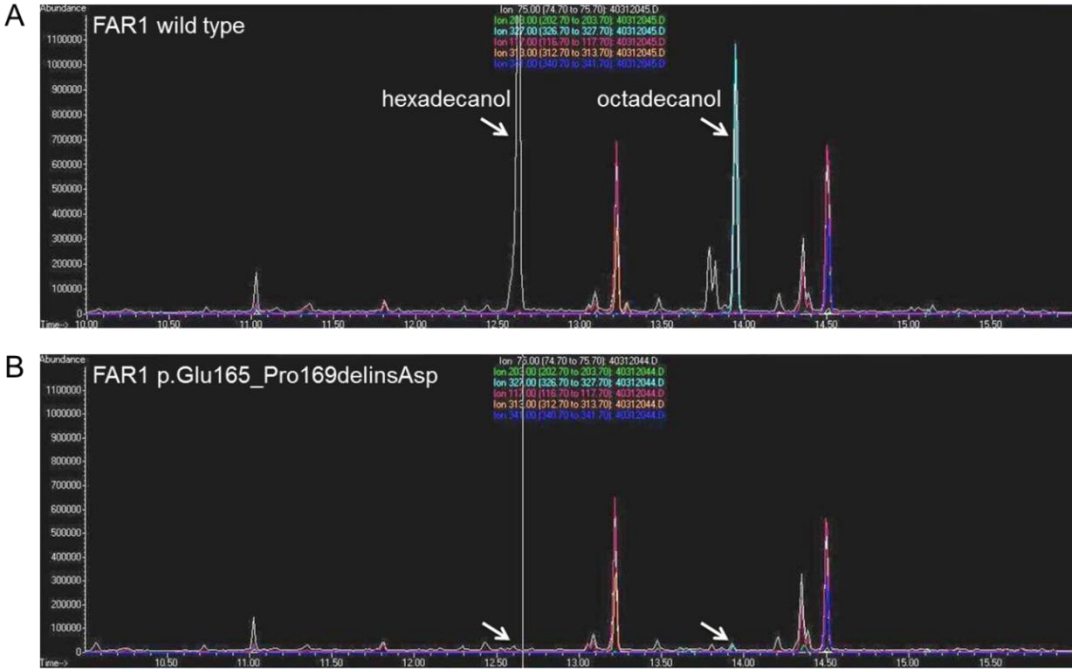
Figure S1

Results of the gas-chromatography and mass spectrometry on lipid extracts



These are the primary data produced by the gas-chromatography mass-spectrometry. **A:** For wild type FAR1, high peaks for hexadecanol and octadecanol are detected. **B:** For altered FAR1 (p.Glu165_Pro169delinsAsp as an example) there is almost no detectable hexadecanol and octadecanol. This strongly suggests a loss of function mutation. The other two mutations produce similar results.

FAR1 enzyme activity assay was performed as follows: Site-directed mutagenesis (QuickChange site-directed mutagenesis kit, Stratagene, La Jolla, USA), was performed as per manufacturer's instructions to introduce all three mutations c.495_507delinsT, c.787C>T, and c.1094A>G (that lead to the alterations p.Glu165_Pro169delinsAsp, p.Arg263*, and p.Asp365Gly, respectively) into a human fatty acyl-CoA reductase 1 cDNA (hFAR1, GenBank™/EBI data bank accession number AY600449) in the pCMV6-XL6 vector (pCMV6-XL6-hFAR1) (Origene Technologies, Rockville, MD). On day 1, 1.8×10^6 HEK293 cells (American Type Culture Collection) were plated into a 10-cm dish in low glucose Dulbecco's modified Eagle's medium (Life Technologies, Carlsbad, USA) supplemented with 10% (v/v) fetal calf serum, 100 units/ml penicillin, and 100 µg/ml streptomycin sulfate. The next day, cells were transfected with 10 µg of pCMV6-XL6-hFAR1 containing wild type or mutation-containing sequences using Lipofectamine 2000 (Life Technologies, Carlsbad, USA) according to standard protocol. Forty-eight hours later the cells were washed once with phosphate-buffered saline (PBS) and harvested into PBS. Lipids were extracted into chloroform using the Bligh and Lyer method. Aliquots of the chloroform extracts were taken and the solvent was removed under mild conditions in a nitrogen flow. In order to mask polar groups, the derivating agent MSTFA (N-methyltrimethylsilyltrifluoracetamide) was added to the residues forming the corresponding trimethylsilyl ether from the hydroxyl groups or the corresponding trimethylsilyl ester from the carboxy groups, respectively. These silylated extracts were separated by gas chromatography and detection was carried out by electron impact (EI) mass spectrometry in scan mode with reference spectra from NIST Standard Reference Database Number 69. Hexadecanol and octadecanol were also measured. The quantification of the absolute amounts in 1 ml of each extract was carried out after calibration with hexadecanol and octadecanol. We performed six biological replicates.