**Supplementary material**

**Variants in *CPLX1* in two families with autosomal-recessive severe infantile myoclonic epilepsy and ID**

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**Running title**: *CPLX1* in ID and myoclonus epilepsy

**Conflicts of interest**: none declared.

**Materials and methods**

***Whole exome sequencing (WES)***

We performed trio-based WES in a cohort of 311 patients with unsolved ID. DNA was extracted from peripheral blood leukocytes of affected individuals and their parents according to standard methods. Exomes were enriched using the SureSelect XT Human All Exon 50 Mb kit, version 5 (Agilent Technologies) according to manufacturer's instructions in an automated fashion using the Bravo Liquid Handling Platform (Agilent) and 3µg of input material. Sequencing was performed as 101 bp paired-end reads on HiSeq 2500 systems (Illumina). Reads were aligned against the human assembly hg19 (GRCh37) using Burrows–Wheeler Aligner (BWA v 0.7.5). We performed variant calling using SAM-tools (v 0.1.18), PINDEL (v 0.2.4t) and ExomeDepth (v1.0.0). Subsequently, variants were filtered using the SAMtools varFilter script with default parameters except for the maximum read depth (-D) and the minimum P-value for base quality bias (-2), which were set to 9999 and 1e-400, respectively, and custom scripts. The variants were then inserted into an in-house database.

To discover putative de novo variants, variants present in the parents of an affected individual, in the 1000 Genomes Project or in less than four of 5,165 in-house controls, which had a variant quality of <30, or which did not pass the filter scripts were filtered out. Raw read data of the remaining variants were then checked using the Integrative Genomics Viewer (IGV). To discover putative homozygous and compound heterozygous variants or X-linked variants which may be disease-causing, we filtered out variants that were already present in frequencies of at least 1% in our 5,165 in-house control exomes, the 1000 Genomes Project or in the ExAc database. We also filtered variants with a variant quality less than 30, or a read depth below 7 and variants that did not pass the filter scripts. For the compound heterozygous variants, the frequency filters were applied to both variants and the variants were only filtered out if both compound heterozygous variants had frequencies >1%. Raw read data of the remaining variants were then checked using the Integrative Genomics Viewer (IGV). For the remaining variants, the affected genes were checked to see if they were listed as disease-associated in the OMIM database or in an in-house curated list of autosomal recessive and X-linked recessive genes including, but not limited to, the DDG2P gene list or the gene list published by Kochinke et al. Prediction algorithms such as SIFT, Polyphen2, MutationTaster or CADD were used to determine potential pathogenicity of variants.

***Sanger sequencing***

Sanger sequencing of *CPLX1* was performed to verify thevariants. PCR was performed on a DNA sample of the affected children and the unaffected siblings and parents using standard conditions with primer pairs covering exon 3 and exon 4 of the *CLPX1* gene. In a second step, sequencing analysis of exon 3 and 4 of the *CLPX1* gene was performed in all family members. The PCR products were purified (PCR Clean-up using ExoSAP-IT, Affymetrix, Santa Clara, CA, USA) and directly sequenced (BigDye Terminator v1.1 Cycle Sequencing Kit; Applied Biosystems, Foster City, CA, USA) on an automated analyser (ABI 3130XL Genetic Analyzer; Applied Biosystems, Foster City, CA, USA). Primer pairs can be provided on request.

***Clinical information of the independent screening cohorts***

The cohort of 25 patients comprised individuals with unexplained ID and spasms, which could not be attributed to a clinically recognizable syndrome by experienced clinical geneticists. In addition, clinically relevant chromosomal aberrations had to be excluded previously by chromosomal microarray analysis, and fragile-X testing had to be normal. Inclusion criteria to this cohort were developmental delay (DD)/ID with an IQ <70, spasms without further specification and consanguineous parents, suggesting an autosomal-recessive inheritance.

The screening cohort comprised 96 index cases with epileptic encephalopathy and ID and pedigrees compatible with autosomal recessive inheritance, e.g. due to parental consanguinity and/or ≥1 similarly affected sibling. Disease onset was in childhood. Among the 96 cases with epileptic encephalopathy and ID, not any pathogenic/likely pathogenic variant or variant of unknown significance was detected.

***Sequencing analysis in the additional sample of 25, respectively 96 samples***

Exons 2-4 of *CPLX1* (OMIM\*605032, NM\_006651.3, ENST00000304062.10, 4p16.2) of all samples were amplified by PCR (primer sequences and PCR conditions on request). Samples were sequenced by Next Generation Sequencing using NexteraXT technology and MiSeq instrument and results were evaluated using SeqNext software (JSI medical systems, Kippenheim, Germany) and HGMD Professional (www.biobase-international.com). Variants are reported according to HGVS nomenclature.