

## ***De novo* stoploss variants in *CLDN11* cause hypomyelinating leukodystrophy**

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## Supplementary material and methods

### Next-generation sequencing

#### Individual 1

DNA was extracted from peripheral blood using the Gentra Puregene Blood Kit (Qiagen, Germany) according to the manufacturer's instructions. Trio exome sequencing (ES) was performed using a Sure Select Human All Exon 50 Mb V5 Kit (Agilent, USA) and a HiSeq2500 (Illumina, USA) as previously described (Kremer *et al.*, 2017). Mitochondrial DNA was derived from off-target exome reads as previously described (Griffin *et al.*, 2014). ExomeDepth was used for the detection of copy number variations (CNVs) (Plagnol *et al.*, 2012). For the analysis of *de novo*, autosomal dominant and mitochondrial variants, only variants with a minor allele frequency (MAF) of less than 0.1% in an in-house database containing over 20,000 exomes (Munich Exome Server) were considered. For the analysis of autosomal recessive and X-linked variants (homozygous, hemizygous or compound heterozygous) variants with a MAF of less than 1.0% were considered.

#### Individual 2

DNA was extracted from peripheral blood using salting out method. Enrichment was done using a Sure Select Human All Exon V7 Kit (Agilent, USA). Proband-only ES was performed on a HiSeq1500 (Illumina, USA). Data analysis was performed as described previously except that hg38 (GRCh38) was used as the human reference genome version (Ploski *et al.*, 2014). For autosomal recessive inheritance (homozygous or putative compound heterozygous variants), all variants with a frequency of less than 1.0% in the Genome Aggregation Database (gnomAD v2.1.1) and an in-house database (more than 1000 Polish exomes) were considered. For dominant inheritance, variants with a frequency of less than 0.01% in both databases were analyzed. The prioritized *CLDN11* variant was subsequently confirmed in the proband and tested in parents using next-generation sequencing of a PCR amplicon encompassing the variant. Library was prepared using Nextera XT Library Prep Kit (Illumina, USA), sequencing was performed on a HiSeq1500 (Illumina, USA).

### Individual 3

Trio ES was performed using a SureSelect Kit (Agilent, USA) and a HiSeq 2000 (Illumina, USA). The sequencing reads were aligned to the human reference genome version hg19 (GRCh37) using Bowtie 2 (Langmead and Salzberg, 2012). The data was further analyzed using a semi-automated bioinformatics pipeline previously described (Tarailo-Graovac *et al.*, 2016).

### **Sanger sequencing**

Sanger sequencing of index individuals, their parents and the unaffected brother of individual 3 was done to confirm the identified variant and its *de novo* status. Oligonucleotide primer sequences are available upon request.

### **RNA sequencing**

RNA of individual 3 was extracted from cultured human dermal fibroblasts (passage 3) using the AllPrep RNA Kit (Qiagen, Germany) and quantified using Qbit RNA Broad Range assay kit (Life Technologies, USA). RNA integrity number (RIN) was determined with the Agilent 2100 Bioanalyzer (RNA 6000 Nano Kit, Agilent, USA). Library preparation was performed automated on a Bravo NGS Option B (Agilent, USA). 1µg of RNA was poly(A)- selected, fragmented and reverse transcribed with the Elute, Prime and Fragment Mix (Illumina, USA). A-tailing, adaptor ligation and library enrichment were performed as described in the TruSeq Stranded mRNA Sample Prep Guide (Illumina, USA). cDNA libraries were assessed for quality on a LabChip GX (Perkin Elmer, USA) and quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Life Technologies, USA). RNA libraries were sequenced on an Illumina HiSeq4000 platform. The STAR aligner (v 2.4.2a) with modified parameter settings (--twopassMode=Basic) was used for split-read alignment against the human genome assembly hg19 (GRCh37) and UCSC known Gene annotation (Dobin *et al.*, 2013). To quantify the number of reads mapping to annotated genes we used HTseq-count (v0.6.0) (Anders *et al.*, 2015). If, for a gene, the 95th percentile of the coverage across all samples was below 1 FPKM the gene was considered “not expressed” and discarded from later analysis. Next, RNA-seq data was analyzed in order to detect differentially expressed genes compared to 156 control fibroblasts by applying the specialized method OUTRIDER (Kremer *et al.*, 2017; Brechtmann *et al.*, 2018).

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