Supplementary methods

Gene-panel sequencing

Gene-panel sequencing was performed with DNA from peripheral blood. Enrichment was done with an Illumina Enrichment Kit (Nextera Rapid Capture Custom Enrichment Kit) and the respective libraries were sequenced on a NextSeq500 sequencer (Illumina, San Diego, CA, USA). Alignment and variant calling was performed with SeqMule (v1.2),[1] (FastQC (version: 0.11.2), BWA-MEM (version: 0.7.8-r455), SAMtools (rmdup; version: 0.1.19-44428cd), SAMtools (filter; version: 0.1.19-44428cd), SAMtools (index; version: 0.1.19-44428cd, and GATKLite (realign; version: 2.3-9-gdcdccbb). Genome version hg19 was used for the alignment. Three variant caller were applied for variant detection (GATKLite UnifiedGenotyper (variant; version: 2.3-9-gdcdccbb), SAMtools (mpileup; version: 0.1.19-44428cd), FreeBayes (version: 0.9.14-14-gb00b735)). Variants called by at least two programs were considered for further analysis. The resulting variant files were combined (GATK, v3.6, CombineVariants) and processed with KGGSeq (v1.0, 14/Apr./2017).[2] Core genes were: *AAAS, ARL6IP1, ATL1, ATL3, CLTCL1, DNMT1, DST, FAM134B, FLVCR1, GLA, GMPPA, IKBKAP, KIF1A, NAGLU, NGF, NTRK1, PRDM12, RAB7A, SCN9A, SCN10A, SCN11A, SPTLC1, SPTLC2, TRPA1, TTR, WNK1*.

Deletions/duplications of single exons are not routinely analyzed using next generation sequencing. In rare individual cases, random homologies, repeat expansions, homopolymers, paralogues, pseudogenes or misalignments can lead to false positive or false negative results. The examination cannot rule out the existence of a cell mosaic and balanced aberrations. Sequence changes in the promoter and in the majority of the intron areas as well as rearrangements are not detected with the analysis. Variants of the mitochondrial genome are not analyzed.

Variants with a frequency of the rarer allele (MAF) of >0.75% in relevant databases (ExAC, dbSNP, 1000G, ESP, GnomAD) and variants that localize outside the coding region

and the canonical splice sites, synonymous variants, and variants that are not recorded bi-directionally, are usually not considered in the evaluation. When evaluating the bioinformatic pathogenicity of variants (e.g. PolyPhen-2, MutationTaster, SIFT, CADD), it must be taken into account that the programs used only provide information on possible pathogenicity. The prioritization of the potentially pathogenic or clinically relevant changes takes place based on the clinical information available to us and with regard to the clinical question. Potentially pathogenic changes that are not currently related to the clinical picture or are otherwise assessed as non-pathogenic (e.g. through segregation analyzes) are not reported. Variants of unclear clinical significance[3] are only reported if, based on the available clinical information and the available literature, a connection with the clinical phenotype is conceivable.

References

1. Guo Y, Ding X, Shen Y, Lyon GJ, Wang K. SeqMule: automated pipeline for analysis of human exome/genome sequencing data. Sci Rep 2015;5:14283.

2. Li MX, Kwan JS, Bao SY, Yang W, Ho SL, Song YQ, et al. Predicting mendelian disease-causing non-synonymous single nucleotide variants in exome sequencing studies. PLoS Genet 2013;9:e1003143.

3. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405-424.