SUPPLEMENTARY METHODS

Whole-exome sequencing

Paired-end whole-exome-enriched libraries were prepared from genomic DNA isolated from the peripheral blood of the proband and parents using an in-house-developed capture reagent from Roche NimbleGen (Madison, WI, USA) that targets coding genes from the Consensus Coding Sequence Project (CCDS)[1], RefSeq[2], and Vega[3] gene models as well as microRNAs. Libraries were sequenced on the HiSeq 2000 platform (Illumina, San Diego, CA) using 100-bp reads generating on average 24 Gb of data per library. Sequencing reads were then aligned to the March 2006 human reference assembly (NCBI36/hg18) using the Burrows-Wheeler Aligner (BWA)[4] resulting in 95% of targeted regions at 20x coverage or more.

Variants were called with SAMtools Pileup[5] and filtered using the following criteria: Phred-like variant quality score of at least 40 (or 30 if observed on both strands) and a minimum 15% reads containing the variant. Mutations were annotated for frameshift insertions/deletions, splice-site, missense, and nonsense variants as well as functional effect using the ANNOVAR package[6] and the dbNSFP database[7]. Common benign variants were excluded by filtering high-quality variants against 1000 Genomes Pilot[8] and an internal database comprised of approximately 600 control exomes.

Variants of interest were confirmed by di-deoxy sequencing: flanking PCR primers (sequence available on request) were designed to amplify the exon of interest in both forward and reverse directions. The resulting fragment was capillary sequenced in the study trio. Analysis of the sequence traces was carried out using the SNP-detector software[9] and by visual assessment.

References

1. Pruitt KD, Harrow J, Harte RA, Wallin C, Diekhans M, Maglott DR, et al. The consensus coding sequence (CCDS) project: Identifying a common protein-coding gene set for the human and mouse genomes. *Genome Res*. 2009;19(7):1316-1323.

2. Pruitt KD, Tatusova T, Klimke W, Maglott DR. NCBI Reference Sequences: current status, policy and new initiatives. *Nucleic Acids Res*. 2009;37(Database issue):D32-36.

3. Wilming LG, Gilbert JG, Howe K, Trevanion S, Hubbard T, Harrow JL. The vertebrate genome annotation (Vega) database. *Nucleic Acids Res*. 2008;36(Database issue):D753-760.

4. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*. 2009;25(14):1754-1760.

5. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The Sequence Alignment/Map format and SAMtools. *Bioinformatics*. 2009;25(16):2078-2079.

6. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res*. 2010;38(16):e164.

7. Liu X, Jian X, Boerwinkle E. dbNSFP: A lightweight database of human nonsynonymous SNPs and their functional predictions. *Hum Mutat*. 2011;32(8):894-899.

8. Altshuler DM, Gibbs RA, Peltonen L, Dermitzakis E, Schaffner SF, Yu F, et al. Integrating common and rare genetic variation in diverse human populations. *Nature*. 2010;467(7311):52-58.

9. Zhang J, Wheeler DA, Yakub I, Wei S, Sood R, Rowe W, et al. SNPdetector: a software tool for sensitive and accurate SNP detection. *PLoS Comput Biol*. 2005;1(5):e53.

SUPPLEMENTAL MATERIALS - ADDITIONAL VARIANTS FOUND IN PROBAND

I. Other *de novo* mutations found on whole-exome sequencing

- *EPX*: associated with autosomal recessive eosinophil peroxidase deficiency (MIM 131399). Single mutation (c.1106C>A, p.P369H) observed, and the phenotype of eosinophil peroxidase deficiency was unrelated to the proband's symptoms.
- *CYP26A1* (MIM 602239): no known phenotype
- *RUNDC3A* (MIM 605448): no known phenotype
- *TMEM8B* (HGNC:21427: transmembrane protein gene with no known phenotype

II. Variants found on clinical sequencing

- *CACNA1S*
  - rs16847674 – MAF 0.137
  - rs9427714 – MAF 0.173
  - rs4915477 – MAF 0.376

Minor allele frequency (MAF) from 1000Genomes phase 1

- *CLCN1*
  - c.461A>G - recorded in dbSNP (rs111482384). Minor allele frequency of 0.01 in 1000 Genomes phase 1. Predicted to be benign (PolyPhen) and tolerated (Sift).
  - IVS2-4C>T - unknown

Supplementary Table 1 – Comparison of estimated costs for trio-based whole-exome sequencing (WES) versus clinical diagnostic costs (base cost before institutional mark-up) in affected family.

Test	Lab charges
CACNA1S – Exons 11 & 30	$420
SCN4A – Exon 12	$463
SCN4A – Exon 13	$315
CLCN1 sequencing	$1050
Chromosome microarray	$1,580
Chromosomes	$940
MECP2 sequencing	$1,280
EEG (no interpretation)	~$700
Video EEG	$4,233
MRI brain/spine	$2,359
CSF Neurotransmitters	$190
CSF amino acids	$240
EMG & nerve conduction	$250-$2,000
Plasma amino acids	$240
Urine organic acids	$230
Acylcarnitine profile	$280
TOTAL Estimated Clinical cost	**$14,770- $16,520**
TOTAL Estimated trio WES cost	**$12,000**