Supporting Information

Supp. Methods

Linkage and homozygosity mapping

Linkage analysis was carried out as part of a previous PhD thesis (Lo, 2009) on five families including family A, C, D, G and H by using whole genome microsatellite markers. The analysis was done by parametric linkage analysis with MLINK. Mode of inheritance was set as autosomal recessive with full penetrance. Liability class was set as 1, and allele frequencies were provided by deCODE Genetics.

To confirm the result from linkage analysis, SNP array analysis was carried out in two affected siblings from family A and one affected child from family C. Autozygosity mapping was performed using the plug-in software Homozygosity Detector within the BeadStudio suite. Regions of shared homozygosity that segregated with disease were visually inspected using the Illumina Genome Viewer within the BeadStudio suite.

Fragment analysis

We performed fragment analysis of *ROGDI* intron 1-2 using FAM labelled forward primer (sequence available upon request). Fragments were analysed with Gene Mapper software.

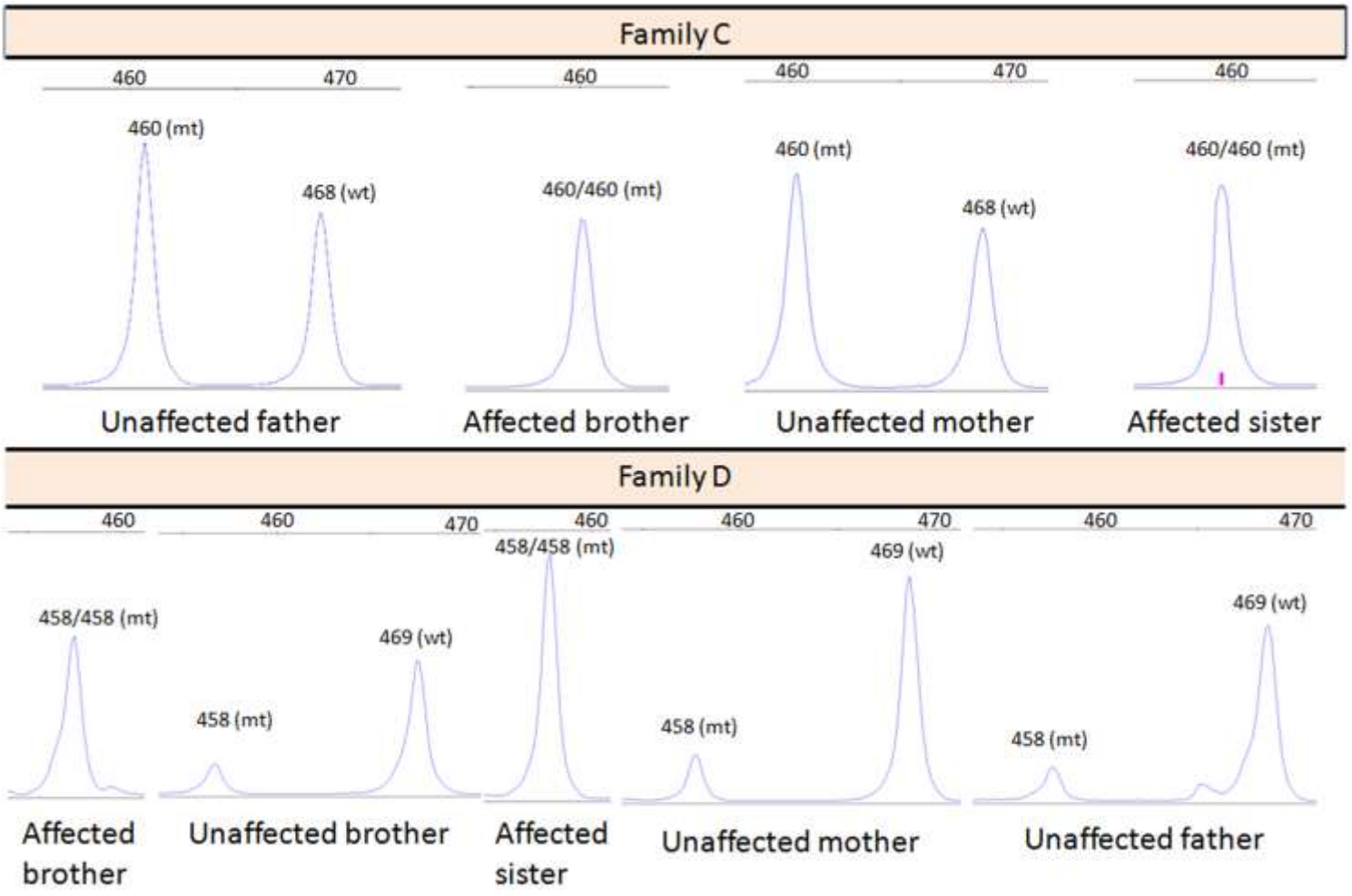
Exome Sequencing

All sequencing reads were aligned to the hg19 build of the human reference genome using the software novoalign (www.novocraft.com). SNP and indel calling were performed using Samtools version 0.18 and were annotated using the software ANNOVAR (Wang, et al., 2010). Candidate variants were filtered on the basis of function (as predicted by ANNOVAR), and the 1,000 genomes (www.1000genomes.org) and NHLBI exome sequencing project (http://evs.gs.washington.edu/EVS/) frequencies.

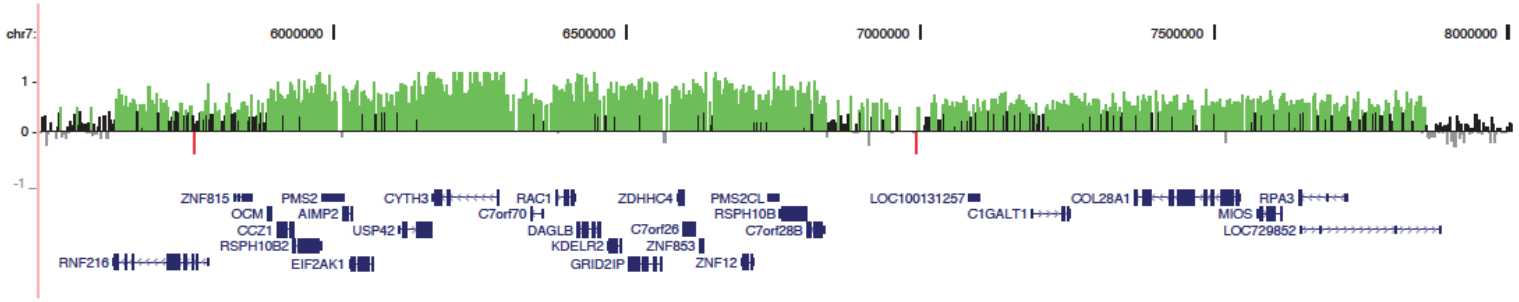
Transcript analysis

cDNA was obtained by standard procedures from short-term cultivated peripheral blood mononuclear cells (PBMC) of the affected child and both parents from families B, E and J.

Relative expression of *ROGDI* was quantified by real time PCR with specific primers spanning *ROGDI* exons 3–5 and the comparative DDCt method using *HPRT1* (RefSeq accession number NM_000194.2) as a reference gene, as previously described (Schossig et al., 2012a). Direct cDNA sequencing was performed in family E and family J by RT-PCR amplification of the entire *ROGDI* coding region with a forward primer in exon 1 (ccatggccaccgtgatg), a reverse primer in exon 11 (ttcctggagacaagctcctg) and subsequent sequence analysis using internal primers (available upon request). In family E, the forward primer in exon 1 was used in combination with a wildtype-specific reverse primer (ggtaagcaggtaaatggcttc) at the position of mutation c.366dupA in exon 6 (the affected child of family E had inherited this mutation from his father) to preferentially amplify transcripts from the maternal allele in the affected child. Subsequent sequence analysis was performed with the wildtype-specific reverse primer.



Supp. Figure S1. Fragment analysis in families C and D. Numbers indicate fragment length.



Supp. Figure S2. Oligo array CGH results for the affected child from Family I shown in UCSC genome browser (chr7:5,500,000-8,000,000, NCBI Build 36, hg17). Deviations of probe $\log_2$ ratios from zero are depicted by vertical grey/black lines, with those exceeding a threshold of 1.5 standard deviations from the mean probe ratio colored green and red to represent relative gains (duplications) and losses (deletions), respectively. Genes are depicted in blue below the CGH data.

Supp. Table S1. LOD scores on chromosome 16

θ / Marker	0.0	0.01	0.05	0.1	0.2	0.3	0.4
D16S521	-infini	-5.59	-2.35	-1.17	-0.31	-0.04	0.02
D16S3065	-infini	-0.57	0.56	0.82	0.75	0.47	0.18
D16S423	3.05	2.97	2.64	2.23	1.44	0.74	0.22
D16S418	-infini	-0.58	0.57	0.85	0.81	0.53	0.23
D16S3062	-infini	-3.92	-1.40	-0.54	0.00	0.09	0.06

Supp. Table S2. Whole exome sequencing summary metrics per sample

Sample	Family	Target enrichment	Target size (bp)	Total number of unique reads	Mean target coverage	Total number of variants
sample I	A	Illumina TruSeq	62,286,318	7,870,652	7.6	15,959
sample II	C	Nimblegen SeqCap EZ	34,459,145	171,506,673	130.7	16,085
sample III	C	Illumina TruSeq	62,286,318	35,822,484	22.4	16,231
sample IV	D	Illumina TruSeq	62,286,318	10,002,954	9.9	15,875
sample V	I	Illumina TruSeq	62,286,318	28,014,742	18.1	15,359

Target enrichment = method used to selectively capture the coding regions of the genome; target size = unique number of target bases in the experiment; total number of unique reads = number of reads that are not marked as duplicates after alignment; mean target coverage = mean coverage of targets that received at least coverage depth = 2 at one base

Supp. References

Lo C-N. 2009. Genetics in Epilepsy [PhD thesis]. London: University College London. 319 p.

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