**Next generation sequencing (NGS)**

Genomic DNA was extracted from full blood using standard methods. For whole exome sequencing (WES) of patient 1, 2, 12, 13 and their respective parents, exome targets were enriched using Nextera Rapid Capture Exome Kit and products were sequenced on Illumina HiSeq 2000 instrument using version 4 chemistry with a paired end 2 x125 base-paired protocol.

In patient 3 the homozygous *PIGT* mutation was detected by using an epilepsy gene panel that tested 85 genes. Genomic DNA was extracted from full blood using standard methods. A targeted NGS library was prepared from 15 ng of template DNA using the Ion AmpliSeq library 2.0 kit and custom primers following the manufacturer's instructions (Life Technologies). The library DNA was clonally amplified onto the Ion Spheres Particles (ISPs) by emulsion PCR using an Ion OneTouch 2 system and the Ion PGM Template OT2 200 kit (Life Technologies). ISPs were sequenced on an Ion PGM sequencer as per the manufacturer's instructions (Life Technologies). Sequences were mapped to hg19 in the Torrent suite software (Life Technologies) and variant calling was achieved in the Strand NGS software (Avadis) with a minimum of 20-fold read depth.

WES sequencing identified the homozygous mutation in patient 4. Coding regions were enriched with the Human All Exons Kit SureSelect XT (Agilent) and the library was sequenced with 150bp paired-end reads on a HiSeq 2000 (Illuimina). Reads were mapped to the reference genome GRCh37/hg19 using BWA (0.7.12-r1039). FreeBayes-Software (Version 0.9.20) was used for variant calling.

WES was performed on DNA from patient 5 using Agilent Sure SelectQXT Human All Exon v6 enrichment kit (Agilent). Enriched library was sequenced on Illumina HiSeq 1500 platform (Illumina). Variant calling and alignment was performed using SureCall software (Agilent) and annotations were performed using Variant Studio software (Illumina). For the analysis of quality and read coverage IGV tool was applied[29].

WES was performed on DNA from patient 7, 9 and 11 as part of the Deciphering Developmental Disorders project (reference).

**Commented [B1]:** Firth HV, Wright CF (2011). The Deciphering Developmental Disorders (DDD) study. *Dev Med Child Neurol* **53:**702-703.

In patient 12 a trio WGS was performed as part of NIHR GOSH/UCL BRC funded RaPS project ormbrc-2012-1. DNA was extracted using Chemagic-STAR (Hamilton USA). Whole genome gDNA libraries were prepared using TruSeq DNA PCR-Free Library Prep (Illumina, USA) following manufacturer's advice starting with 1ug of sheared gDNA. Parental samples were pooled at equimolar concentrations and sequenced on Illumina NextSeq 550 High-Output Mode (29 hours). Patient samples were sequenced on Illumina HighSeq 2500 Dual Flow Cell, Rapid Run Mode (27 hours) Mapping and variant calling were performed using a GENALICE appliance running GENALICE Map 2.5.5 including Mapping, Variant Calling and the Population Calling module for trio analysis (GENALICE BV, Netherlands). GENALICE default configuration files were used for WGS mapping, and trio variant detection.

**Variant identification, prediction and verification**

Exome data from patient 1, 2, 13 and 14 was analyzed and variants prioritized using the Cologne Center for Genomics' VARBANK database analysis tool (https://varbank.ccg.uni-koeln.de). wAnnovar[30] annotated variants were filtered for recessive mode of inheritance according to the family history. In patients 1 and patient 2 we identified two compound heterozygous variants in *PIGT* (NM_015937.5: c.1472T>A, p.Leu491His, and c.1484+2T>A) that segregated in biparental mode. The paternally inherited missense mutation of a highly conserved amino acid was predicted to be probably damaging by PolyPhen-2[31] and MutationTaster[32] and had a CADD score[33] of 24.2. The maternally inherited (c.1484+2T>A) was a splice variant with a CAAD score of 22.9. Both variants were yet not found in healthy individuals (gnomAD). In patients 13 and patient 14 one homozygous missense mutation (NM_015937.5: c.709G>C, p.Glu237Gln) that leads to an exchange of a highly conserved amino acid was predicted by SIFT, MutationTaster, and PolyPhen-2 to be pathogenic. 16 heterozygos carriers were identified in the gnomAD database of this variant and Sanger sequencing confirmed biparental inheritance.

Variants from patient 3 resulting from epilepsy gene panel sequencing were reduced by allele frequency ≥2% and SNPs observed in more than 2 samples for each analyzed sample batch were filtered out. Genetic non-synonymous/splice site variants were evaluated through database

searches such as dbSNP, Exome Variant Server, the Exome Aggregation Consortium database (ExAC), and HGMD Professional. One homozygous missense mutation (NM_015937.5: c.709G>C, p.Glu237Gln) that leads to an exchange of a highly conserved amino acid was predicted by SIFT, MutationTaster, and PolyPhen-2 to be pathogenic. 16 heterozygos carriers were identified in the gnomAD database of this variant and Sanger sequencing confirmed biparental inheritance.

Rare variants (allele frequency < 1%) in patient 4 were filtered out based on healthy individuals from ExAC and 1KGP. Prioritization of the remaining variants was done with PhenIX (http://compbio.charite.de/ PhenIX/)[34]. MutationTaster was used to predict the pathogenic effect of the compound heterozygous mutatations (NM_015937.5: c.494-2A and c.1582G>A; p.Val528Met). The maternally inherited c494-2A>G variant affects a consecutive splice site and was predicted to be pathogenic by MutationTaster. This variant was observed in 17 healthy in heterozygous carriers (gnomAD). The paternally transmitted c.1582G>A transition was shown to be pathogenic by Pagnamenta, et al.[35]

Variants with a MAF below 0.01 % in genes associated with ID[36] remained after filtering and were assessed with bioinformatic tools (Polyphen2, Mutation Taster and Sift). The biparental transmission of the homozygous pathogenic variant (NM_015937.5: c.1582G>A; p.Val528Met) was confirmed in the affected sister by Sanger sequencing.

In case 7 a homozygous sequence variant c.1079G>T was identified using trio WES performed by the Deciphering Developmental Disorders project. The c.1079G>T p.(Gly360Val) variant is predicted to result in the substitution of a highly conserved glycine at position 360 for valine. It was absent or at extremely low frequency in controls in Exome Sequencing Project, 1000 Genomes or ExAC and there are multiple lines of computational evidence support a deleterious effect. This has been supported by functional data with reduced levels of GPI-anchored proteins reported in leukocytes in two clinically affected brothers homozygous for this variant. Sanger sequence analysis of exon 9 of the PIGT gene confirmed homozygosity for sequence variant c.1079G>T p.(Gly360Val). Segregation analysis showed that the variant was biparentally inherited from parents who are both heterozygous for this variant. The same sequence variant was also detected in the homozygous state in a clinically affected sibling. In accordance with ACMG guidelines the

homozygous sequence variant c.1079G>T was classified as likely to be pathogenic, and explained the clinical features in the proband and his affected siblings.

In case 12 the Ingenuity Variant Analysis™ software (QIAGEN, USA) was used to identify rare variants predicted to result in loss of function, or to have a functional effect on the protein. Variants with a frequency of ≤ 0·5% in 1000 Genomes, ExAC , and Exome Variant Server were investigated. A homozygous PIGT variant c.550G>A was identified. This is a highly conserved residue present in low freqency in Exac with pathogenicity supported by the majority of in silico tools.