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High Rate of Recurrent *De Novo* Mutations in Developmental and Epileptic Encephalopathies

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Developmental and epileptic encephalopathy (DEE) is a group of conditions characterized by the co-occurrence of epilepsy and intellectual disability (ID), typically with developmental plateauing or regression associated with frequent epileptiform activity. The cause of DEE remains unknown in the majority of cases. We performed whole-genome sequencing (WGS) in 197 individuals with unexplained DEE and pharmaco-resistant seizures and in their unaffected parents. We focused our attention on *de novo* mutations (DNMs) and identified candidate genes containing such variants. We sought to identify additional subjects with DNMs in these genes by performing targeted sequencing in another series of individuals with DEE and by mining various sequencing datasets. We also performed meta-analyses to document enrichment of DNMs in candidate genes by leveraging our WGS dataset with those of several DEE and ID series. By combining these strategies, we were able to provide a causal link between DEE and the following genes: *NTRK2*, *GABRB2*, *CLTC*, *DHDDS*, *NUS1*, *RAB11A*, *GABBR2*, and *SNAP25*. Overall, we established a molecular diagnosis in 63/197 (32%) individuals in our WGS series. The main cause of DEE in these individuals was *de novo* point mutations (53/63 solved cases), followed by inherited mutations (6/63 solved cases) and *de novo* CNVs (4/63 solved cases). *De novo* missense variants explained a larger proportion of individuals in our series than in other series that were primarily ascertained because of ID. Moreover, these DNMs were more frequently recurrent than those identified in ID series. These observations indicate that the genetic landscape of DEE might be different from that of ID without epilepsy.

Introduction

Epilepsy is often associated with major comorbidities, most frequently intellectual disability (ID), which affects 25% of children with epilepsy.[1,2] Conversely, the frequency of lifetime history of epilepsy ranges from 7%–15% for individuals with mild to moderate ID to 45%–82% for those with severe ID.[3] The co-occurrence of epilepsy and ID can involve at least two non-exclusive mechanisms. In some cases, uncontrolled seizures can be detrimental to developing cortical networks and can lead to regression and poor cognitive outcomes in children.[4] The term epileptic encephalopathy (EE) has been used to designate disorders where the epileptic activity itself contributes to cognitive

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slowing or regression, and EE can occur in a child with or without preexisting developmental delay.[5] In other cases, a single genetic or environmental process is sufficient to induce both seizures and cognitive impairment.[6] For instance, mutations that induce specific synaptic defects might result in aberrant connectivity and seizures, as well as alter synaptic plasticity and cause learning disabilities. The term developmental encephalopathy (DE) has been proposed to designate disorders where developmental delay emerges before the presence of epileptic activity or in the presence of infrequent epileptic activity.[5] Because it is not always easy to dissect the contribution of each of these mechanisms and because some genetic disorders can involve both mechanisms in the same or in different individuals, the term developmental and epileptic encephalopathy (DEE) has been coined to refer to conditions

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characterized by ID and epilepsy where both mechanisms might play a role.[5]

Recently, parent-child exome sequencing studies in cases of sporadic DEE have shown that *de novo* mutations (DNMs) are an important cause of DEE. However, only a minority of the studied cases were solved by these approaches, thus underlining the genetic heterogeneity of DEE and the need to sequence large cohorts to increase the power to identify genes associated with DEE.[7,8] With an average of ~1 DNM affecting the coding sequence of an individual, one of the challenges has been to determine whether the candidate DNMs are pathogenic or coincidental. To address this, Samocha et al. published a statistical framework that determines the rate of *de novo* variants per gene per class of variant (e.g., missense, nonsense, frameshift, or canonical splice site [CSS]) in order to determine whether there is gene enrichment for a particular variant class in the studied cohort and thus provide evidence that the observed DNMs are most likely implicated in the disease.[9] This strategy was recently successfully employed in meta-analyses of DNMs identified from various sequenced trios affected by ID and/or developmental disorders for the identification of genes enriched in DNMs in these cohorts.[10,11]

In this study, we performed whole-genome sequencing (WGS) on 197 DEE individuals and their unaffected parents. We focused our analyses on DNMs (single-nucleotide variations [SNVs], small insertions or deletions [indels]), and copy-number variations (CNVs) affecting coding or splice-site regions. To identify genes implicated in DEE, we performed meta-analyses of the DNMs identified in our series and those found in other studies of DEE or ID trios and looked for genes statistically enriched in DNMs. We also performed targeted sequencing and leveraged our network of collaborators and gene-matching tools to find additional similarly affected individuals with DNMs in some of our prioritized genes and thus provide additional support for their implication in the disease. On the basis of these collective approaches, we provide herein evidence implicating DNMs in eight genes in DEE.

Subjects and Methods

Subjects

The DEE series screened by WGS (n = 197 trios) was recruited at three centers in Canada—the Sainte-Justine University Hospital Center in Montreal (HSJ; 99 trios), the Toronto Western Hospital (TWH; 35 trios), and the Hospital for Sick Children in Toronto (HSC; 63 trios)—after the study was approved by the research ethics boards and informed consent was obtained from each participant or legal guardian. This series, referred to as the Canadian Epilepsy Network (CENet) DEE cohort, included subjects with diverse DEE phenotypes. The criteria used for the selection of these individuals were as follows: (1) intractable epilepsy defined as an absence of response to two appropriate and well-tolerated anti-epileptic therapies (AEDs) over a 6-month period and an average of at least one focal, generalized tonic-clonic, myoclonic, tonic, atonic, or absence seizure or epileptic spasm per month during the period of poor control; (2) ID or global developmental delay (GDD); (3) absence of malformations or focal and multifocal structural abnormalities on brain MRI; and (4) absence of parental consanguinity and family history of epilepsy, ID, or autism in first-degree relatives. Each individual was classified into a specific epilepsy syndrome when possible (Table S1). The majority (~90%) had had array comparative genome hybridization performed on a clinical basis, and only those with no pathogenic or possibly pathogenic CNVs were included. Many of the individuals were previously screened and found to be negative for mutations in various DEE gene-panel tests. A subset of candidate genes identified in the course of this study were sequenced in a cohort composed of 595 individuals with DEE of unknown cause (Table S2), most of whom had been tested for mutations in genes previously associated with DEE, as well as for pathogenic CNVs, as previously described.[12] We were also able to recruit, through various collaborations, additional subjects with DNMs in candidate genes; these DNMs were identified by clinical or research exomes. Informed consent was similarly obtained from these individuals or their legal guardians.

WGS

WGS was performed at the McGill University and Genome Quebec Innovation Center as part of the Illumina Genome Network (IGN) according to the IGN standard procedure. In brief, genomic DNA extracted from blood samples was subjected to an additional cleaning step with the ZR-96 DNA Clean & Concentrator-5 Kit (Zymo) and then used for generating sequencing libraries with the TruSeq DNA PCR-Free Library Preparation Kit according to the manufacturer's procedure. Sequencing was done either on the HiSeq 2000 (100 bp paired-end reads; one genome per three lanes) or on the HiSeq 2500 (125 bp paired-end reads; one genome per two lanes) such that a minimum final coverage of 30× was attained after data processing.

WGS Data Processing, Variant Calling, and Analyses

The Illumina sequencing reads were generated with bcl2fastq v.1.8.4. Trimmomatic v.0.32 was used to remove bad-quality reads and to trim the read edges with a lower quality. The filtered reads were aligned to reference *Homo sapiens* assembly b37 (GRCh37) with BWA-mem v.0.7.10 for the creation of a binary alignment map (BAM) file. Read-set BAM files from different sequencing lanes for the same sample were merged into a single global BAM file with Picard v.1.123. Regions containing multiple base mismatches were realigned locally with Picard. Once local regions were realigned, Picard was also used to recalculate the coordinates of read mates and to mark duplicates for removal. Individual base-quality values were recalibrated with the Genome Analysis Toolkit (GATK) v.3.3-0. Genotypes were called with the GATK HaplotypeCaller, and all variant calls were merged and recalibrated in three different sensitivity tranches with GATK according to its recommended best practices. All variant sites were annotated with a custom version of ANNOVAR.[13] Only variants with positions covered at ≥10× and supported by at least four variant reads constituting ≥25% of the total reads for each called position were considered. Rare variants included those present with a minor allele frequency (MAF) of ≤0.005 in 1000 Genomes, GoNL, ExAC Browser v.0.3, or the NHLBI Exome Sequencing Project Exome Variant Server (EVS) or ≤2% in the unaffected parents from the entire trio dataset. Variant segregation (in child-parent

trios) was analyzed with an in-house script. We identified putative DNMs by excluding those present in the genomes of the parents and those with a MAF $\geq$ 0.001 in the ExAC Browser. Potential *de novo* variants outside the exonic and splice consensus regions were further excluded if they were present in small-repeat regions (for SNVs and indels), present in Alu regions (for indels), or had an SNV variant quality-score recalibration (VQSRT) from $\geq$99.90 to 100.00 or an indel VQSRT different than PASS. We visually inspected the sequencing reads carrying putative DNMs in each trio by using the Integrative Genomics Viewer (IGV)[14] to exclude obvious false positives or inherited variants. Putative DNMs affecting the coding and consensus splice regions were validated by Sanger sequencing in the corresponding trio.

CNV Analyses

CNVs were identified by two algorithms: Lumpy, whose calls integrate multiple breakpoint signals, and PopSV, whose calls rely on deviation from normalized read depths across samples.[15,16] Default parameters were used unless otherwise specified. For PopSV, 5 kb bin scans of the genome were used. We filtered CNV calls to exclude those with a size < 1 kb and with a quality-value (PopSV) or evidence-set (Lumpy) score $\leq$ 0.1%. CNVs falling in regions of segmental duplications were also excluded. To identify *de novo* CNVs, we excluded those present in any of the parents' samples from the entire dataset, those in population controls from 1000 Genomes, and those from the CNV map of high-quality datasets of common variants.[17] *De novo* CNVs called by both Lumpy and PopSV were prioritized for validation. Potential *de novo* CNVs detected by only one algorithm, and thus likely to be enriched with false positives, were considered for validation only if they affected exonic regions and if they could not be ruled out as inherited or false positives upon visual inspection by IGV of the reads near the breakpoints. CNVs were validated in the trio by standard qPCR (TaqMan assay) and/or by Sanger sequencing.

Targeted Sequencing Using the Molecular Inversion Probe (MIP) Technique

Seven of our initially prioritized genes (*DHDDS* [OMIM: 608172], *RYR2* [OMIM: 180902], *HECW2* [OMIM: 617245], *GABRB2* [OMIM: 600232], *NUS1* [OMIM: 610463], *NTRK2* [OMIM: 600456], and *CLTC* [OMIM: 118955]) were selected for MIP sequencing in a cohort of 595 individuals with DEE. We used a multiplex targeted-capture strategy to target the coding exons and intron-exon boundaries (a minimum of 5 bp of flanking sequence) in each of the seven genes. Single-molecule MIPs (smMIPs) were used as previously described[18] with minor modifications detailed below. The molecular tag within the probe consisted of five random nucleotides that allowed for distinction of genomic molecules and a high-confidence consensus call. Library preparation remained the same as described by O'Roak et al.,[19] except the ratio of probe to genomic DNA was adjusted to 2,000:1, a 10-fold increase from the previously reported ratio. We performed sequencing on an Illumina HiSeq 2500 to generate 100 bp paired-end reads. Raw read mapping and processing were performed as previously described.[12] Private variants (absent from SNP public databases: ExAC Browser v.0.3, EVS, and 1000 Genomes) predicted to affect the protein sequence (missense variants, nonsense variants, indels, and CSSs) were validated by Sanger sequencing in the proband and the parents.

Gene-Specific DNM Enrichment

We used the DenovolyzeR open-access program to assess whether a specific gene is enriched in DNMs in subjects with DEE, GDD, and/or ID.[20] This *R* package program is based on gene-specific mutation rates.[9] DNM gene-specific p values calculated by DenovolyzeR for loss-of-function (LoF) variants (nonsense variants, CSSs, and frameshift indels) and functional variants (missense and LoF variants) were further corrected for multiple testing (Bonferroni correction) on the basis of the 19,618 genes with available mutation rates on which DenovolyzeR based its calculation and the number of tests (two; for LoF and functional categories; i.e., 2 × 19,618 = 39,236). A corrected p value (c.p value) < 0.05 was considered statistically significant. To increase statistical power, we performed a meta-analysis to combine DNMs identified herein with those previously reported from trio whole-exome sequencing (WES) done on other DEE cohorts.[8,21,22] We also performed another meta-analysis to combine DNMs from the DEE cohorts with those from exome or genome sequencing from published ID cohorts.[10,11,23–28] Only studies consisting of more than ten trios were included in these meta-analyses (Table S3). To further increase the power to detect gene-specific DNM enrichment in genes whose mutations are not yet an established cause of DEE, we applied a strategy similar to that of Lelieveld et al.,[11] who excluded from their meta-analysis trios with DNMs found in their curated list of genes previously associated with ID. Therefore, we performed a meta-analysis after excluding trios with DNMs affecting the autosomal-dominant or X-linked genes mentioned in this list (n = 572), which also includes genes associated with DEE, or trios with such mutations in 21 genes not reported in this list but subsequently found to be enriched with DNMs by Lelieveld et al. and/or by the recent Deciphering Developmental Disorders (DDD) trio sequencing study.[10,11]

Clustering of *De Novo* Missense Variants

We used the open-source program Denovonear, which had been used in the DDD study,[10,29] to calculate the probability of the proximity of *de novo* missense variants in genes of interest on the basis of one million simulations weighted by the context trinucleotide rates. We considered a p value < 0.01 to be statistically significant.

Results

We performed WGS on 197 individuals with DEE and their unaffected parents. The average coverage of the genomes was 37.9×, and 99% of the genome (GRCh37) bases were covered at $\geq$ 10× (Figure S1). The average number of SNVs and indels per genome was ~4,182,490 and ~23,532, respectively (Table S4). The average number of CNVs per subject, excluding those in segmentally duplicated regions, varied between 275 (PopSV) and 400 (Lumpy). In total, we detected an average of 66 high-quality DNMs (61 SNVs and 5 indels) that passed IGV inspection (~75% of total DNMs calls) per individual, translating into a mutation rate of $\sim 1.2 \times 10^{-8}$ DNMs per diploid genome per generation, which is in the range reported from other WGS trio studies.[24,30–32]

We next focused our attention on the putative DNMs that affect coding and CSS regions and that passed IGV

Table 1. Genes Affected by Pathogenic or Likely Pathogenic Variants in the CENet Cohort

Variant Type	Genes Whose Mutations Are an Established Cause of DEE and/or ID	Candidate Genes
DNMs (n = 53)	**(n = 44)**	**(n = 9)**
Missense	*SCN1A* (3), *SCN2A* (3), *SCN8A* (4), *KCNT1* (3), *CACNA1A* (2), *GNAO1* (2), *ATP1A3* (1), *CDKL5* (1), *COL4A1* (1), *DDX3X* (1), *DNM1* (1), *FGF12* (1), *GABRG2* (1), *HECW2* (1), *KCNA2* (1), *KCNQ2* (1), *MED13L* (1), *MEF2C* (1), *NAA10* (1), *PPP2R1A* (1)	*NTRK2* (2), *DHDDS* (1), *GABBR2* (1), *GABRB2* (1), *RAB11A* (1), *SNAP25* (1)
Nonsense	*SCN1A* (2), *ANKRD11* (1), *HIVEP2* (1), *IQSEC2* (2), *NF1* (1), *SYNGAP1* (1)	–
Frameshift	*ARID1B* (1), *CDKL5* (1), *IQSEC2* (1), *KIAA2022* (1)	*CLTC* (1), *NUS1* (1)
Canonical splice site	*SCN1A* (1), *SCN8A* (1)	–
***De Novo* CNVs (n = 4)**	**(n = 3)**	**(n = 1)**
Deletions	deletion (exons 21–23) of *DNMT3A* (1), deletion encompassing *PCDH19* (1)	deletion (exon 2) of *NUS1* (1)
Duplications	duplication encompassing *UBE3A* (1)	–
Inherited Recessive SNVs or Indels (n = 6)	**(n = 6)**	**(n = 0)**
Bi-allelic	*WWOX* (1), *SZT2* (1), *NAGA* (1), *TBC1D24* (1)	–
Hemizygous	*SLC9A6* (1), *IQSEC2* (1)	–

The number of individuals affected by pathogenic or likely pathogenic variants in the specified genes is indicated in parentheses.

inspection. We were able to validate by Sanger sequencing 95% of these calls. In total, 288 DNMs were validated (1.46 DNMs/trio), representing an average of ~1.37 *de novo* SNVs and 0.09 indels per individual, which is in the range of what was observed in a previous WES study of DEE trios (Table S5).[8] We did not detect any DNMs in the coding or CSS regions of 39 probands (20%) (Figure S2A). Of only *de novo* SNVs, 7.8% (nonsense and CSS variants) are predicted to cause a loss of function, whereas 72% are predicted to cause a missense change (Figure S2B). We compared the *de novo* SNV rates observed in our DEE individuals with those observed in unaffected siblings of individuals with an autism spectrum disorder (ASD) (66.5% missense and 4.8% LoF)[33] or in Icelandic control individuals (82% missense and 2.7% LoF).[32] We found more LoF SNVs in our EE subjects than in the exomes of control siblings ($p = 0.03$, binomial exact test) or Icelandic genomes ($p = 0.00002$, binomial exact test), suggesting that a subset of these variants contributes to the disease.

We also searched for *de novo* CNVs. In total, 12 CNVs were called as *de novo* by both Lumpy and PopSV, and all were successfully validated by qPCR and/or Sanger sequencing. In addition, 35 putative *de novo* CNVs encompassing exonic regions were identified by only one of the algorithms; six of these putative CNVs were confirmed to be *de novo* by qPCR, 17 were inherited, and 12 were false positives. In total, 10/18 validated *de novo* CNVs, including five deletions and five duplications, affected exonic regions (Table S6).

Likely Pathogenic Variants Identified in the CENet Series

For all DNMs and rare recessive (bi-allelic and X-linked hemizygous) variants affecting the coding regions or CSSs, we assessed the involvement of the corresponding genes in epilepsy or related neurodevelopmental disorders by searching PubMed (gene name and "epileptic encephalopathy," "epilepsy," "seizure," "mental retardation," or "intellectual disability") and verifying the gene's OMIM description. Using the American College of Medical Genetics and Genomics 2015 guidelines for interpreting sequence variants,[34] we initially identified pathogenic or likely pathogenic variants in 50/197 (25%) subjects in genes that, when mutated, have been shown to cause DEE and/or ID. Of these, 88% were explained by DNMs, and 12% were caused by inherited recessive mutations (Tables 1, S5, and S7).

We also identified pathogenic *de novo* CNVs in three individuals, including an 8 Mb deletion encompassing *PCDH19* (OMIM: 300460) in a female individual, a 5.2 Mb duplication corresponding to the 15q11–q13 region located between the recurrent breakpoints BP2 and BP3, and a 3.4 kb exonic deletion of *DNMT3A* (OMIM: 615879), all of which have been previously associated with ID and/or epilepsy.

Targeted MIP Sequencing

From the WGS results of our first 120 DEE trios, we prioritized seven of our best candidate genes (*CLTC*, *DHDDS*, *GABRB2*, *HECW2*, *NTRK2*, *RYR2*, and *NUS1*) for targeted resequencing in 595 unsolved cases of DEE. These genes were selected on the basis of the documentation of predicted-damaging DNMs in at least two individuals with unsolved DEE from the CENet series or in one individual with unsolved DEE from the CENet series and in at least one previously reported individual with DEE and/or ID. Exon 1 of *NUS1* was excluded from the analysis because it was poorly

covered across the samples (18% of the target bases at ≥10×), possibly because of its high GC content. On average, 90% of the target bases were covered at ≥10× in 476 samples. Reduced coverage was obtained in the remaining 119 cases such that only 70% of the target bases reached ≥10×, probably as a result of poor DNA quality. Four predicted-damaging missense variants absent from the ExAC Browser were identified, each in a single DEE subject, in *NTRK2* (c.1301A>G [p.Tyr434Cys] [GenBank: NM_006180.4]), *GABRB2* (c.730T>C [p.Tyr244His] [GenBank: NM_021911.2] and c.911C>T [p.Ala304Val]), and *HECW2* (c.4484G>A [p.Arg1495Lys] [GenBank: NM_020760.1]). These variants were validated to be *de novo* by Sanger sequencing. Interestingly, two of the DNMs affecting GABRB2 (p.Tyr244His) and NTRK2 (p.Tyr434Cys) were also recurrent in the CENet series. The missense variant (p.Arg1495Lys) in HECW2 was also recurrent given that it was previously reported as a *de novo* variant in a DDD case.[10] Recently, DNMs in *HECW2* have been shown to cause DEE.[25,35]

Involvement of *NTRK2*, *GABRB2*, *CLTC*, *DHDDS*, and *NUS1* in DEE

We next sought to identify additional DEE or ID individuals who carry DNMs in the candidate genes that were prioritized for MIP sequencing by mining GeneMatcher[36] and DDD research variants in DECIPHER[37] and by contacting our network of collaborators. Through this approach, we were able to obtain additional supporting evidence for the involvement of the following genes in DEE.

NTRK2

Our strategy involving trio WGS and targeted sequencing led to the identification of three DEE individuals carrying *de novo* predicted-damaging missense variants in *NTRK2* (GenBank: NM_006180.4); these included an individual with the c.2159C>T (p.Thr720Ile) variant and two unrelated individuals with the same c.1301A>G (p.Tyr434Cys) variant. In addition, we identified two other individuals with the *de novo* p.Tyr434Cys missense variant through clinical WES.

In total, we identified four individuals with the p.Tyr434Cys missense variant. All subjects with this missense variant had severe GDD or ID and optic nerve hypoplasia with visual impairment, and three had significant feeding impairment (Table 2 and Supplemental Note). Three of them presented with epileptic spasms in the first few months of life and subsequently developed intractable seizures of various types in association with multifocal epileptic activity on electroencephalography (EEG), whereas the remaining individual had startle-like myoclonic events at 12 hr of life and developed, at 5 years of age, focal seizures that caused impaired awareness and occasionally evolved into bilateral tonic-clonic seizures. Clustering analysis using the Denovonear algorithm indicated that the presence of the p.Tyr434Cys variant in four individuals with similar phenotypes is statistically significant ($p = 0.0001$).

The subject with the p.Thr720Ile missense variant had moderate to severe ID, ASD, and intractable generalized tonic-clonic and focal seizures with impaired awareness starting at the age of 2.5 years. Unlike the individuals with the p.Tyr434Cys missense variant, she had hyperphagia and early-onset obesity from the age of 3 years. Interestingly, Yeo et al. reported that an individual carrying the *de novo* c.2165A>G (p.Tyr722Cys) variant, affecting an amino acid residue adjacent to Thr720, presented with a phenotype similar to that of our subject; characteristics of this phenotype included excessive weight gain, moderate ID, language delay, autistic features, hypotonia, and seizures.[38]

NTRK2 encodes the TRKB receptor, a member of the neurotrophin receptor tyrosine kinase family.[39] TRKB has high affinity for brain-derived neurotrophic factor (BDNF) and for neurotrophin-4. BDNF-TRKB signaling is a critical regulator of neuronal development and function.[40] The p.Tyr434Cys variant is located at the beginning of the transmembrane domain of NTRK2 (Figure 1A). The fact that this *de novo* variant has been identified in four individuals with a similar phenotype suggests that it confers a specific property to the protein, possibly via a gain-of-function or a dominant-negative mechanism. The p.Thr720Ile and p.Tyr722Cys variants cluster in the catalytic domain of NTRK2 (Figure 1A). *In vitro* studies indicate that p.Tyr722Cys impairs BDNF-induced TRKB receptor autophosphorylation and downstream signaling.[38] It is currently unknown whether p.Thr720Ile affects NTRK2's function in a similar way, but its proximity to p.Tyr722Cys and the similar phenotypes of both individuals carrying these variants suggest that this could be the case. Interestingly, mice expressing 25% of normal TRKB levels are hyperphagic and overweight.[41] Altogether, our findings unequivocally show that DNMs in *NTRK2* cause DEE.

GABRB2

Our WGS and MIP screens identified three DEE individuals carrying DNMs in *GABRB2* (GenBank: NM_021911.2), including the c.911C>T (p.Ala304Val) variant in one subject and the recurrent c.730T>C (p.Tyr244His) variant in two subjects. Two other individuals with DNMs in *GABRB2*, one with the c.830T>C (p.Leu277Ser) variant and another with the c.373G>A (p.Asp125Asn) variant, were identified by the DDD study.[10] We also identified from WES and targeted gene-panel sequencing six individuals with *de novo* missense variants in *GABRB2*, including one with the same c.830T>C (p.Leu277Ser) variant found in the DDD subject, one with c.851C>A (p.Thr284Lys), one with c.878G>C (p.Arg293Pro), one with a missense c.908A>G (p.Lys303Arg) variant adjacent to c.911C>T (p.Ala304Val) (which was identified in our MIP screen), one with c.946G>A (p.Val316Ile), and one with a *de novo* c.236T>C (p.Met79Thr) variant. This latter individual was previously reported to have a *de novo* frameshift in *CHAMP1* (c.1876_1877delAG [p.Ser626Leufs] [GenBank: NM_032436.2]), which also most likely contributes to the cognitive impairment of the subject (F3-II.1 in Isidor et al.[42]). All of these *de novo* missense variants are predicted

Table 2. Summary of the Clinical Features in Cases with DNMs in *NTRK2* (GenBank: NM_006180.4)

Individual	Gender	Age at Last Examination	DNM (Detection)	Cognitive and Behavioral Features	Epilepsy Diagnosis	Age at Seizure Onset	Seizure Types	AEDs	EEG	Brain MRI	Associated Neurological Features and Seizure Outcome
HSC0103	male	2 years, 9 months	c.1301A>G (p.Tyr434Cys) (WGS[a])	severe GDD	IS	3 days	ES, Fo	VGB, ACTH, LEV, CLB, TPM, VPA	modified hyps.	optic nerve hypoplasia	limb hypertonia and hyperreflexia, acquired microcephaly, visual impairment, swallowing difficulties, intractable seizures
indvSLIJ	male	6 years, 3 months	c.1301A>G (p.Tyr434Cys) (cWES[b])	severe ID, ASD	DEE	12 hr with recurrence at 5 years	M, FIA	OXBZ, DZP	DS, TIRDA	optic nerve hypoplasia	hypotonia, lower-limb spasticity, visual impairment, seizures controlled on OXBZ for 1 month
T25821	female	4 years, 7 months	c.1301A>G (p.Tyr434Cys) (MIPS)	severe GDD, severe ID	IS	4 months	ES, To	prednisolone, VGB, B6, LEV, CLB, TPM, LCM, KD, VPA, RFN, ZNS, CBD, DZP, PHT	MF, hyps.	optic nerve hypoplasia, myelination delay	acquired microcephaly, hypotonia, subtle choreoathetosis, visual impairment, feeding difficulties, intractable seizures, high tolerance to painful stimuli (parents report)
HF303	male	4 years, 3 months	c.1301A>G (p.Tyr434Cys) (cWES[b], WGS[c])	severe GDD, suspected severe ID, ASD	IS	4 months	ES, FIA	PB, LEV, ACTH, VGB, CLB, ZNS, DZP, CBD	DS, MF	optic nerve hypoplasia	limb hypotonia, visual impairment, swallowing difficulties, intractable seizures, high tolerance to painful stimuli (parents report)
HSJ0335	female	9 years	c.2159C>T (p.Thr720Ile) (WGS[a])	GDD, moderate to severe ID, ASD	DEE	2.5 years (febrile size at 23 months)	febrile, FIA, GTC, SE	CLB, *LEV*, TPM, VPA, CBZ	normal, DS after SE	delayed myelination, reduced WM, ventriculomegaly, thin CC	swallowing difficulties, hyperphagia after 3 years of age, no seizures for 2 years under CBZ

Underlining indicates treatment with clinical response (decreased seizure frequency or severity), and italics indicates a negative response (aggravation of seizure frequency and/or severity). Abbreviations are as follows: WGS, whole-genome sequencing; cWES, clinical whole-exome sequencing; MIPS, molecular inversion probe sequencing; GDD, global developmental delay; ID, intellectual disability; ASD, autism spectrum disorder; IS, infantile spasms; DEE, developmental and epileptic encephalopathy; Fo, focal; FIA, focal impaired awareness; ES, epileptic spasm; M, myoclonic; To, tonic; GTC, generalized tonic-clonic; SE, status epilepticus; AED, anti-epileptic therapy; ACTH, adrenocorticotropin; B6, vitamin B6; CBD, cannabidiol; CLB, clobazam; CBZ, carbamazepine; DZP, diazepam; KD, ketogenic diet; LCM, lacosamide; LEV, levetiracetam; OXBZ, oxcarbazepine; PB, phenobarbital; PHT, phenytoin; RFN, rufinamide; TPM, topiramate; VGB, vigabatrin; VPA, valproic acid; ZNS, zonisamide; EEG, electroencephalography; hyps., hypsarrhythmia; DS, diffuse slowing; MF, multifocal; TIRDA, temporal intermittent rhythmic delta frequency activity; MRI, magnetic resonance imaging; WM, white-matter tracts; and CC, corpus callosum.

[a]CENet.

[b]GeneDx.

[c]HudsonAlpha study.

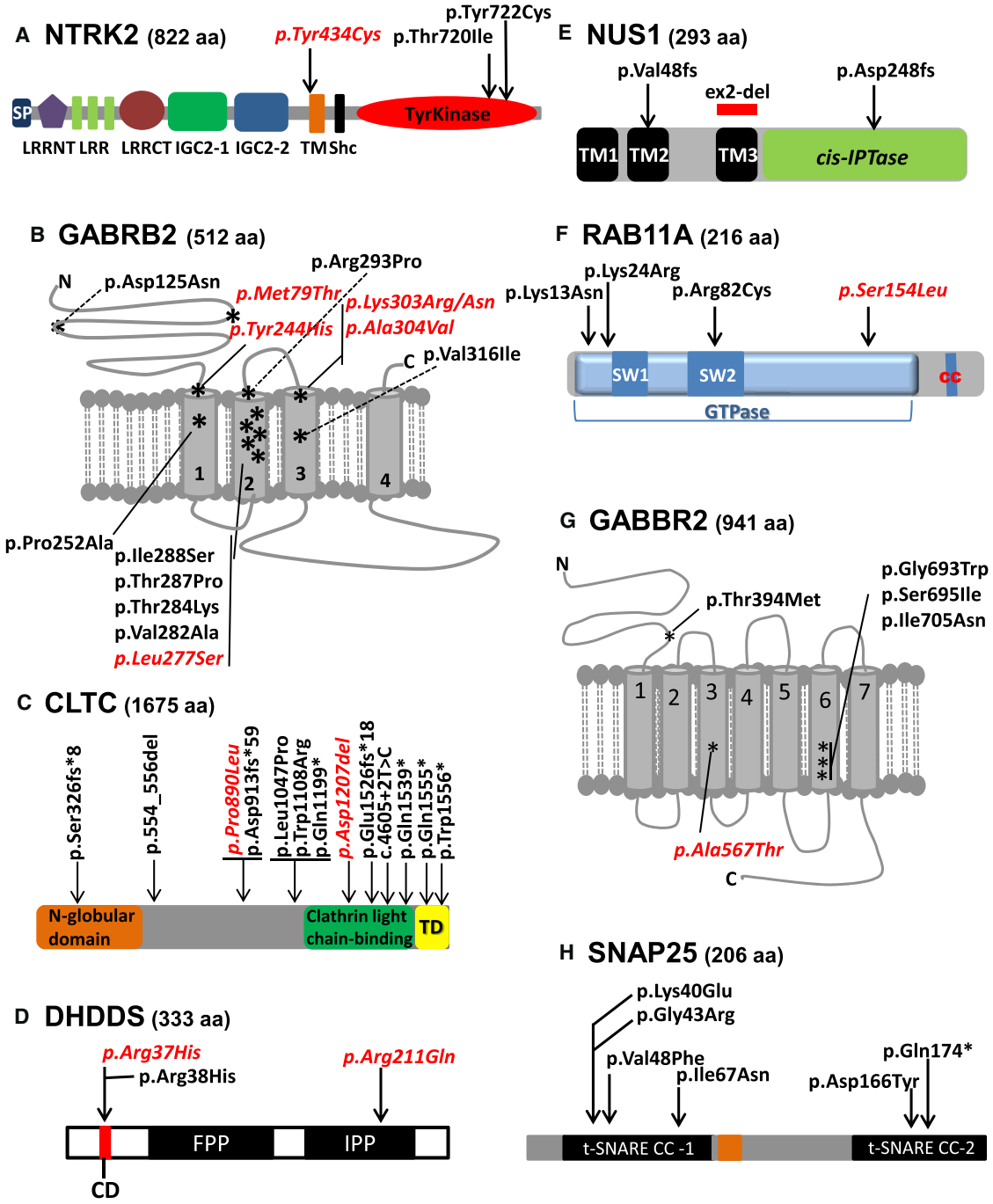

Figure 1. Localization of *De Novo* Variants in Protein Domains Encoded by Genes of Interest
GABRB2 (A), CLTC (B), NUS1 (C), NTRK2 (D), and DHDDS, SNAP25, GABBR2, and RAB11A (E). Recurrent *de novo* variants are in italics and red font. The transmembrane domains of GABRB2 and GABBR2 are labeled 1–4 and 1–7, respectively. Abbreviations are as follows: TM, transmembrane domain; TD, trimerization domain; SP, signal peptide; LRRNT, leucine-rich repeat N-terminal domain; LRR, leucine-rich repeat; LRRCT, leucine-rich repeat C-terminal domain; IGC2, immunoglobulin C-2 type 1 domain; IGC2-2, immunoglobulin C-2 type 2 domain; Shc, SHC1 interaction domain; IPP, isopentenyl diphosphate binding site; CD, catalytic domain; FPP, farnesyl diphosphate binding site; SW, switch domain; and CC, prenylation residue.

to be damaging (PolyPhen-2, SIFT, and CADD). Their localization in GABRB2 is shown in Figure 1B.

We were able to obtain detailed clinical information for all of these 11 individuals (Table 3 and Supplemental Note). They all displayed moderate to severe ID (or severe GDD), with the exception of the individual with the p.Val316Ile variant, who achieved normal milestones at 21 months of age. Most individuals had microcephaly (n = 7/11), which was acquired in six individuals and congenital in the seventh. Within the first year of life, most individuals developed refractory seizures (predominantly myoclonic seizures and absences), which sometimes

evolved into myoclonic status epilepticus or non-convulsive status epilepticus. Some individuals developed focal seizures with impaired awareness or autonomic seizures, tonic seizures, atonic seizures, and/or rarely generalized tonic-clonic seizures. In five of the individuals, the epilepsy remained refractory despite multiple drug trials. Two individuals were given a trial of vigabatrin and showed marked deterioration. Responses to lamotrigine, valproate, levetiracetam, or high-dose steroids were observed in five individuals. Axial hypotonia, spasticity, dystonia, and choreoathetosis appeared to be common features. Cortical visual impairment was present in 3/11 individuals. Brain MRI was usually normal, although delayed myelination or diffuse T2 hypersignal in the subcortical white matter was noted in three individuals.

GABRB2 encodes the β2 subunit of the $GABA_A$ receptor, a neuronal pentameric ionotropic ligand-gated chloride channel that induces synaptic inhibition when activated by its agonist GABA.[43] Variants in other $GABA_A$ receptor subunits encoded by *GABRA1* (OMIM: 137160), *GABRB1* (OMIM: 137190), *GABRB3* (OMIM: 137192), and *GABRG2* (OMIM: 137164) are established causes of DEE. Three individuals with DNMs in *GABRB2* and detailed phenotypic information have been previously published: one of these subjects, who carries the DNM c.236T>C (p.Met79Thr), also found in one of our subjects, showed generalized seizures and moderate ID; another one displayed ID, seizures (of unspecified type), and cortical visual impairment (c.754C>G [p.Pro252Ala]); and the last one was found to have early-onset myoclonic encephalopathy (c.859A>C [p.Thr287Pro]).[44–46] Four additional subjects with *de novo* missense mutations in *GABRB2*, including c.845T>C (p.Val282Ala), c.863T>G (p.Ile288Ser), c.909G>T (p.Lys303Asn), and c.911C>T (p.Ala304Val), have been reported.[47–49] The amino acid residues affected by the latter two of these DNMs (p.Lys303Asn and p.Ala304Val) were also found to be mutated in our series. These four individuals appear to show ID or GDD and epilepsy, but no detailed clinical information was available.

Out of the 13 *GABRB2* DNMs previously reported or described herein, ten encode variants clustered within a stretch of 60 amino acids (positions 244–304) encompassing three transmembrane domains and/or their boundaries (p value = 0.000002, Denovonear) (Figure 1B). These clustering variants appear to be mostly associated with severe GDD or ID and, with the exception of p.Arg293Pro, intractable generalized seizures and DEE. So far, only one of these *de novo* missense variants, p.Thr287Pro, has been functionally tested in transfected HEK293 cells and found to reduce cell-surface expression and peak current amplitudes of $GABA_A$ channels.[46] It is currently unknown whether the other *de novo* missense variants in GABRB2 behave similarly to p.Thr287Pro, especially the closely clustering or recurrent ones (p.Tyr244His, p.Leu277Ser, p.Lys303Leu, and p.Ala304Val), which might confer specific properties to the protein such as gain-of-function or dominant-negative effects. Collectively, the previously and presently reported individuals with DNMs in *GABRB2* confirm that *de novo* missense mutations in *GABRB2* can cause a DEE phenotype.

CLTC

Our WGS trio screen identified a *de novo* frameshift variant (c.4575dupA [p.Glu1526fs*18]) in *CLTC* (GenBank: NM_004859.3) in an individual with moderate ID associated with severe refractory seizures (absence, myoclonic, tonic, generalized tonic-clonic, and focal seizures). We obtained detailed clinical information on 11 additional individuals with DNMs in *CLTC*, four of whom were identified by the DDD study[10] and seven of whom we identified through clinical WES (Table 4 and Supplemental Note). We were able to obtain detailed clinical information for all 12 of these individuals. Most of these individuals presented with early-onset hypotonia and GDD, which evolved over time into mild to severe ID (or borderline intelligence). Four individuals also developed ataxia. When performed, neuromuscular investigations (electromyography and biopsy) were negative. Two individuals had pharmaco-resistant epilepsy with a preponderance of myoclonic and generalized tonic-clonic seizures. One individual had one isolated seizure and is now seizure free after being weaned from medication. Two other individuals had severe GDD or ID with seizures, starting between the ages of 1 and 2 years, that were well controlled with valproate or levetiracetam. Interestingly, three of the ID subjects (one sequenced by the CAUSES [Clinical Assessment of the Utility of Sequencing and Evaluation as a Service] study and another sequenced in the context of the Undiagnosed Patient Program at the Ospedale Pediatrico Bambino Gesù in Rome) had a recurrent *de novo* missense mutation (c.2669C>T [p.Pro890Leu]), which was also reported in a DDD trio for which we were not able to obtain phenotypic information. The presence of the same DNM in *CLTC* in four independent subjects was statistically significant for missense clustering (p = 0.0000001, Denovonear). The positions of these various DNMs in *CLTC* are shown in Figure 1C.

CLTC encodes the widely expressed clathrin heavy chain 1, which is involved in endocytosis, intracellular trafficking, and synaptic recycling.[50,51] Recently, a *de novo* frameshift in *CLTC* (c.2737_2738dupGA [p.Asp913Glufs*59]) was reported in a subject with GDD, unclassified epilepsy, and dysmorphic features.[52,53] In their study of 800 probands with ID, Leliveld et al.[11] reported two additional DNMs, c.4615C>T (p.Glu1539*) and c.3621_3623del (p.Asp1207del), the latter of which was also identified in one of our DEE subjects. *CLTC* is predicted to be intolerant of LoF mutations and has a pLi score of 1.00 according to the ExAC Browser.[54] The phenotypic spectrum associated with these individuals is heterogeneous and ranges from mild ID or learning disability to severe ID or DEE. Interestingly, individuals with refractory epilepsy were found to carry variants in the first section of the clathrin light-chain binding domain, whereas truncating DNMs affecting the C terminus of CLTC tended to be associated with hypotonia, GDD, and ID (Figure 1C).

Table 3. Summary of the Clinical Features of Individuals with DNMs in *GABRB2* (GenBank: NM_021911.2)

Individual	Gender	Age at Last Examination	DNM (Detection)	Cognitive and Behavioral Features	Epilepsy Diagnosis	Age at Seizure Onset	Seizure Types	AEDs	EEG	Brain MRI	Associated Neurological Features and Seizure Outcome
1242500	female	9.3 years	c.236T>C (p.Met79Thr) (cWES[a])	GDD, severe ID	DEE	11 months	A or FIA	LEV	normal	arachnoid cyst	acquired microcephaly, axial hypotonia, spasticity, ataxia, minor dysmorphic traits (short perineum, tapered fingers, short broad great toes), seizures controlled with LEV
K.02591	female	10 years	c.373G>A (p.Asp125Asn) (WES[b])	GDD, moderate ID	DEE	6 years	febrile, GTC	VPA	ND	normal	acquired microcephaly, no seizures (responded to VPA; off medication)
indvLB	female	1.5 years	c.878G>C (p.Arg293Pro) (WES)	GDD	no seizures	NA	NA	NA	normal	normal	severe psychomotor delay, generalized dyskinesia, dystonia, cortical visual impairment
CNSA01M	male	4 years	c.908A>G (p.Lys303Arg) (targeted gene panel)	GDD, severe ID	EOEE	1 day	Fo, MF, To	VPA, LEV, TPM, LTG	MF, slow background	diffuse T2 hypersignal in white matter at birth and 18 months	acquired microcephaly, neonatal feeding difficulties, nonambulation, hypotonia, spasticity, dystonia, rare seizures under TPM
T21213B	female	14 years, 6 months	c.911C>T (p.Ala304Val) (MIPS)	GDD, severe ID	DEE	4 years	M, A, At, non-convulsive SE	CLB, *VGB*, pred., TPM, HCT, VPA, LTG, LEV, CZP, SULTH	biF SW or sharp SW	normal	acquired microcephaly, nonambulation, hypotonia, intractable seizures
HSJ0753	female	4 years	c.730T>C (p.Tyr244His) (WGS[c])	severe GDD	DEE	4 months	M, GTC, MSE	LEV, VPA, TPM, B6, DZP, CLB, PB, PHT, *CBD*, KD	biF SW, hyps., continuous diffuse SW	normal (at 9 days and at 1 year)	acquired microcephaly, nonambulation, axial hypotonia, spasticity, nystagmus, cortical visual impairment, intractable seizures
T23211	female	5 years, 1 month	c.730T>C (p.Tyr244His) (MIPS)	GDD, severe ID	DEE	<5 months	To, Fo, autonomic, M, SE	PB, LEV, CZP, P5P, B6, FOL, *VGB*, TPM, CBZ, NZP, OXBZ, VPA	MF (biF predominant), slow background	delay in myelination, reduction of white matter	congenital microcephaly, axial hypotonia, peripheral hypertonia, cortical visual impairment, choreoathetosis, dystonia, failure to thrive, intractable seizures

(*Continued on next page*)

Table 3. ***Continued***

Individual	Gender	Age at Last Examination	DNM (Detection)	Cognitive and Behavioral Features	Epilepsy Diagnosis	Age at Seizure Onset	Seizure Types	AEDs	EEG	Brain MRI	Associated Neurological Features and Seizure Outcome
HA076	male	15 years, 8 months	c.830T>C (p.Leu277Ser) (WES)	GDD, severe ID	DEE	4 years, 8 months	M, At, A, GTC	VPA, TPM, CZP, CLB, LEV, LTG	slow, rhythmic notched slow waves	MF T2 hypersignal in white matter at 2 years, normal at 4 and 9 years	spasticity, poor coordination, broad-base gait, seizure control with LVT and LTG
G64518	female	10 years	c.830T>C (p.Leu277Ser) (WES[b])	GDD, severe ID	DEE	2 years	GTC, A, febrile	VPA, LTG	high-amplitude rhythmic slow waves	mild increase in LVs at 2 years; normal at 3 years	acquired microcephaly, brisk reflexes, seizure control with LTG
31841	male	17 days	c.851C>A (p.Thr284Lys) (WES)	severe GDD	EME	7 days	M, To	PB, LEV, MDZ, biotin, FOL, B6	BS	normal	hypotonia, jitteriness, back arching, apneas, intractable seizures, deceased at age 17 days
3001866	female	21 months	c.946G>A (p.Val316Ile) (cWES[a])	language delay	DEE	12 months	A or Fo, GTC	LEV, OXBZ, CNZ, ZNS	normal	normal	apneas, neuroendocrine cell hyperplasia of infancy, intractable seizures

Individual 1242500 was also previously identified with a pathogenic *de novo* mutation in *CHAMP1* (Isidor et al.[42]). Underlining indicates treatment with clinical response (decreased seizure frequency or severity), and italics indicates a negative response (aggravation of seizure frequency and/or severity). Abbreviations are as follows: NA, not applicable; ND, not done; cWES, clinical whole-exome sequencing; WGS, whole-genome sequencing; MIPS, molecular inversion probe sequencing; GDD, global developmental delay; ID, intellectual disability; DEE, developmental and epileptic encephalopathy; EOEE, early-onset epileptic encephalopathy; EME, early myoclonic encephalopathy; A, absence; At, atonic; GTC, generalized tonic-clonic; SE, status epilepticus; MSE, myoclonic status epilepticus; To, tonic; M, myoclonic; FIA, focal impaired awareness; Fo, focal; MF, multifocal; CZP, clonazepam; FOL, folinic acid; HCT, hydrocortisone; LTG, lamotrigine; MDZ, midazolam; NZP, nitrazepam; P5P, pyridoxal 5-phosphate; pred., prednisone; SULTH, sulthiam; AED, anti-epileptic therapy; LEV, levetiracetam; VPA, valproic acid; TPM, topiramate; CLB, clobazam; VGB, vigabatrin; B6, vitamin B6; DZP, diazepam; PB, phenobarbital; PHT, phenytoin; CBD, cannabidiol; KD, ketogenic diet; OXBZ, oxcarbazepine; ZNS, zonisamide; EEG, electroencephalography; biF, bi-frontal predominance; BS, burst suppression; SW, spike-wave; hyps., hypsarrhythmia; MRI, magnetic resonance imaging; WM, white-matter tracts; and LV, lateral ventricle.
[a]Baylor College of Medicine and Miraca.
[b]DDD study.
[c]CENet.

Table 4. Summary of the Clinical Features of Individuals with DNMs in *CLTC* (GenBank: NM_004859.3)

Individual	Gender	Age at Last Examination	DNM (Detection)	Cognitive and Behavioral Features	Epilepsy Diagnosis	Age at Seizure Onset	Seizure Types	AEDs	EEG	Brain MRI	Associated Neurological Features and Seizure Outcome
PBSD	female	11 years, 2 months	c.977_980delCAGT (p.Ser326Cysfs*8) (cWES[a])	GDD, borderline IQ at 5 years	no seizures	NA	NA	NA	NA	T2 hypersignal in white matter (hypomyelination)	ADHD, impulsivity, poor socialization skills, mild hypotonia, wide-based gait
5289183	male	20 years, 5 months	c.1660_1668del (p.Met554_Tyr556del) (cWES[a])	borderline IQ, learning disabilities	NA	14 years	one seizure	LEV	normal	normal	progressive paraparesis with lower-limb spasticity, ataxia, myoclonus, one seizure without recurrence under LEV, no seizures for 4 years off meds
indvAA	male	3 years, 2 months	c.2669C>T (p.Pro890Leu) (cWES[b])	GDD	no seizures	NA	NA	NA	normal	normal	mild ataxia, possible myoclonus
CAUSES1	male	4 years, 7 months	c.2669C>T (p.Pro890Leu) (WES)	GDD, suspected ID	no seizures	NA	NA	NA	NA	normal	mild hypotonia, oral and motor apraxia, suspected ADHD
18052017	female	30 years	c.2669C>T (p.Pro890Leu) (WES[c])	moderate ID	no seizures	NA	NA	NA	normal	normal	bradykinesia, bradypsychism, hypomimia, hypokinesia, clumsiness, attention instability
indvPAR	male	16 years	c.3140T>C (p.Leu1047Pro) (trio cWES)	severe ID	DEE	1 year	suspected FIA	VPA	non-specific irritative pattern, no foci	thin and short CC with hypoplasia of its posterior part, wide Virchow-Robin spaces	neonatal-onset hypotonia, no speech, acquired microcephaly, severe gastroinstestinal reflux, no seizures under VPA
273692	male	4 years	c.3322T>C (p.Trp1108Arg) (WES[d])	severe GDD, suspected severe ID	DEE	2 years	M, GTC, possible gelastic seizures	LEV	abnormal	pontocerebellar atrophy, delayed myelination	nonambulation, spasticity, dystonia, myoclonus, neonatal feeding difficulties, visual impairment, seizure control with LEV
DDD261801	male	10 years, 7 months	c.3595C>T (p.Gln1199*) (WES[d])	mild GDD, mild ID	no seizures	NA	NA	NA	NA	normal	neonatal-onset hypotonia, congenital ptosis, poor social skills
indvMB	female	7.5 years	c.3621_3623del (p.Asp1207del) (WES)	GDD, severe ID	DEE	3 years	febrile GTC, M, To	VPA, LTG, CLB, CZP, LEV, TPM, LCM	MSW, biF	thin CC, T2 hypersignal in white matter, enlarged LVs	acquired microcephaly, severe hypotonia, ataxia, oral and motor apraxia, intractable seizures
HSC0054	female	23 years	c.4575dupA (p.Glu1526Argfs*18) (WGS[e])	GDD, moderate ID	DEE	5 months	A, M, To, GTC, Fo	CLB, VPA,HCTZ, LEV, LTG, KD	gen. SW and PSW	delayed myelination, normal at 20 years	neonatal hypotonia, scoliosis, intractable seizures until puberty, no seizures under LEV and LTG

(*Continued on next page*)

Table 4. *Continued*

Individual	Gender	Age at Last Examination	DNM (Detection)	Cognitive and Behavioral Features	Epilepsy Diagnosis	Age at Seizure Onset	Seizure Types	AEDs	EEG	Brain MRI	Associated Neurological Features and Seizure Outcome
LDKQS	male	12 years, 10 months	c.4605+2T>C (cWES[a])	GDD, moderate ID	no seizures	NA	NA	NA	NA	normal	hypotonia, neonatal feeding difficulties, sensorineural hearing loss
DDD0280	female	6 years	c.4663C>T (p.Gln1555*) (WES[d])	GDD, moderate to severe ID	no seizures	NA	NA	NA	NA	ND	hypotonia
281177	male	11 years	c.4667G>A (p.Trp1556*) (WES[d])	moderate ID	no seizures	NA	NA	NA	NA	ND	neonatal hypotonia

Underlining indicates treatment with clinical response (decreased seizure frequency or severity). Abbreviations are as follows: NA, not applicable; ND, not done; cWES, clinical whole-exome sequencing; WGS, whole-genome sequencing; GDD, global developmental delay; IQ, intelligence quotient; ID, intellectual disability; DEE, developmental and epileptic encephalopathy; FIA, focal impaired awareness; M, myoclonic; GTC, generalized tonic-clonic; To, tonic; A, absence; Fo, focal; AED, anti-epileptic therapy; LEV, levetiracetam; VPA, valproic acid; LTG, lamotrigine; CLB, clobazam; CZP, clonazepam; TPM, topiramate; LCM, lacosamide; KD, ketogenic diet; HCTZ, hydrochlorothiazide; EEG, electroencephalography; MSW, multifocal spike-wave; biF, bi-frontal predominance; gen. SW, generalized spike-wave; PSW, poly-spike and wave; MRI, magnetic resonance imaging; WM, white-matter tracts; CC, corpus callosum; LV, lateral ventricle; and ADHD, attention-deficit hyperactivity disorder.

[a]GeneDx.
[b]Radboud University Medical Center.
[c]Ospedale Pediatrico Bambino Gesù.
[d]DDD study.
[e]CFNet.

DHDDS

WGS identified a *de novo* missense variant (c.110G>A [p.Arg37His]) in *DHDDS* (GenBank: NM_024887.3) in one of our DEE individuals (HSJ0762). We identified, by clinical WES, another DEE individual who carries the same *de novo* p.Arg37His. Interestingly, this missense variant lies adjacent to p.Arg38His, which was reported in a DEE subject from the Epi4K study.[7] Clustering analysis indicated that the presence of these two *de novo* variants in three individuals is statistically significant ($p = 0.0005$, Denovonear). In addition, we identified by clinical or research WES two other individuals with DEE and the *de novo* missense mutation c.632G>A (p.Arg211Gln) in *DHDDS* and obtained detailed clinical information on a third subject, also with the same *de novo* p.Arg211Gln (indvNCJ herein), who was previously reported in a recent WES study of ID trios.[11] The positions of these various identified *de novo* variants in DHDDS are shown in Figure 1D, and their associated phenotypes are summarized in Table 5 and detailed in the Supplemental Note.

These five individuals with DNMs in *DHDDS* presented with a generalized epilepsy disorder with myoclonic seizures, either as myoclonic absences or as isolated cortical myoclonus, and sometimes with light sensitivity or fever susceptibility. Two of these individuals also presented with other generalized seizure types, including atonic seizures or generalized tonic-clonic seizures. In three individuals, EEG revealed clear generalized spike-wave discharges (and additional photosensitivity in one individual). The seizures were aggravated by levetiracetam in two individuals, but favorable responses to valproic acid were observed. Interestingly, all individuals presented with marked hypotonia, and four had mixed movement disorders including ataxia, tremors, and dystonia.

DHDDS encodes dehydrodolichyl diphosphate synthase (also known as hCIT), which is essential for dolichol monophosphate (Dol-P) synthesis and global N-linked glycosylation.[55] The Arg37 and Arg38 residues fall into an evolutionarily conserved stretch of five amino acids (positions 34–38) corresponding to the catalytic domain of the enzyme (Figure 1D). The crystal structure and mutagenesis studies done on the bacterial Dhdds enzyme (*M. luteus* undecaprenyl diphosphate synthase [UDPS]) show that the Arg203 residue, which is equivalent to Arg211 in the human DHDDS, is critical for the homoallylic binding to the substrate isopentenyl diphosphate.[56,57] The identification of recurrent or clustering DNMs in individuals with a similar phenotype in *DHDDS* is highly suggestive of pathogenicity.

A homozygous missense variant (c.124A>G [p.Lys42Glu]) was previously found in *DHDDS* in consanguineous families affected by retinitis pigmentosa.[58,59] In addition, bi-allelic truncating or splicing variants in *DHDDS* were reported in a case of type 1 congenital disorder of glycosylation with severe GDD and refractory seizures.[60] We hypothesize that null alleles of *DHDDS* disrupt brain development only in the context of a recessive genotype, whereas the DNMs

Table 5. Summary of the Clinical Features of Individuals with DNMs in *DHDDS* (GenBank: NM_024887.3) and *NUS1* (GenBank: NM_138459.4)

ID	Gender	Age at Last Examination	Gene	DNM (Detection)	Cognitive and Behavioral Features	Epilepsy Diagnosis	Age at Seizure Onset	Seizure Types	AEDs	EEG	Brain MRI	Associated Neurological Features and Seizure Outcome
indvSG	female	5 years, 1 month	*DHDDS*	c.110G>A (p.Arg37His) (cWES[a])	GDD, severe ID	DEE	18 months	MA photo+, GTC, febrile Fo	VPA, LTG, *LEV*, ETH, VPA	gen. SW, photo+	normal	hypotonia, short stature, intractable seizures
HSJ0762	male	5 years, 6 months	*DHDDS*	c.110G>A (p.Arg37His) (WGS[b])	GDD	DEE	1 years	M, A, At, fever sensitive	*LEV*, VPA	gen. SW, diffuse slowing	normal	hypotonia, tremor, wide-based gate, ataxia, no seizures for 1 year on VPA
indvEF	female	5 years, 6 months	*DHDDS*	c.632G>A (p.Arg211Gln) (cWES[c])	GDD, borderline IQ	DEE	4 years	MA	*LEV*, LTG, OXBZ	epileptiform	normal, Chiari I malformation	hypotonia, tremor, ataxia, inattention, obesity, seizures controlled with OXBZ
MDB31882	male	35 years	*DHDDS*	c.632G>A (p.Arg211Gln) (WES[d])	GDD, severe ID	DEE	6–9 years	M	VPA, benzodiazepines	gen. PSW	normal	gen. tremor, facial myokimia, bradykinesia, hypomimia, rigidity, freezing and impaired postural reactions, frontal lobe impairment features, no seizures since the age of 9 years, normal glycosylation assay, current therapy: VPA, clonazepam, tetrabenazine
indvNCJ	female	7 years, 1 month	*DHDDS*	c.632G>A (p.Arg211Gln) (cWES[e])	GDD, moderate to severe ID	NA	7 years	M	none	normal	normal	ataxia, myoclonus, tremor, dystonia, short stature, no treatment initiated yet for cortical myoclonus, normal glycosylation assay
indvKW	male	7 years, 11 months	*NUS1*	c.743delA (p.Asp248Alafs) (cWES[c])	GDD, severe ID	DEE	12 months	M, GTC	LEV	biF epileptiform	normal	ataxia with LEV, lack of coordination, seizures controlled with LEV
HSJ0623	male	15 years	*NUS1*	c.128_141dup (p.Val48Profs)*7 (WGS[b])	GDD, moderate ID, ASD	DEE	10 months	MA, At, febrile GTC	VPA, LTG, LEV, ETH, CZP, CBZ, Stiri., CLB	diffuse slowing, biF or gen spikes	normal	ADHD, tremor, seizures controlled under VPA and CLB
HSJ0627	female	20 years	*NUS1*	exon 2 deletion (WGS[b])	motor delay, mild ID	DEE	2.5 years	M status, MA, At	VPA, LEV, CLB, FEL, LTG, CZP	gen. SW and PSW	normal	tremor, dysarthria, seizures controlled on VPA, LTG, and CZP

Underlining indicates treatment with clinical response (decreased seizure frequency or severity), and italics indicates a negative response (aggravation of seizure frequency and/or severity). Abbreviations are as follows: NA, not applicable; cWES, clinical whole-exome sequencing; WGS, whole-genome sequencing; GDD, global developmental delay; ID, intellectual disability; IQ, intelligence quotient; ASD, autism spectrum disorder; DEE, developmental and epileptic encephalopathy; MA, myoclonic absence; MA photo+, myoclonic absences with photosensitivity; GTC, generalized tonic-clonic; Fo, focal; M, myoclonic; A, absence; At, atonic; AED, anti-epileptic therapy; FEL, felbamate; VPA, valproic acid; LTG, lamotrigine; LEV, levetiracetam; ETH, ethosuximide; CZP, clonazepam; CBD, cannabidiol; CLB, clobazam; LTG, lamotrigine; OXBZ, oxcarbazepine; Stiri, stiripentol; EEG, electroencephalography; gen., generalized; SW, spike-wave; photo+, photosensitive; PSW, poly-spike and wave; biF, bi-frontal predominance; MRI, magnetic resonance imaging; and ADHD, attention-deficit hyperactivity disorder.

[a]Baylor College of Medicine.
[b]CENet.
[c]GeneDx.
[d]Ospedale Pediatrico Bambino Gesù.
[e]Radboud University Medical Center.

documented in our study cause DEE via a dominant-negative or gain-of-function mechanism.

NUS1

In *NUS1* (GenBank: NM_138459.3), we identified two DNMs in individuals from our WGS trio study; these included a frameshift variant in exon 1 (c.128_141dup [p.Val48Profs*7]) in one individual and a ~1.3 kb deletion encompassing the entire exon 2 in the other. In addition, we identified a *de novo* truncating variant in *NUS1* (c.743delA [p.Asp248Alafs*4]) by clinical WES in an individual with DEE (Table 5, Supplemental Note, and Figure 1E). These individuals with DNMs in *NUS1* all presented with GDD (or isolated motor delay) which eventually evolved into mild to severe ID. Furthermore, they all presented with generalized myoclonic seizures (with myoclonic status epilepticus in one individual and with myoclonic absences in two individuals). All individuals showed other generalized seizure types, including atonic seizures (drop attacks) or generalized tonic-clonic seizures. EEG revealed either generalized epileptic activity or bi-frontal epileptic discharges. Movement disorders, including tremor (in two individuals) and ataxia (in one individual), were also common. Altogether, this clinical phenotype is highly reminiscent of the one we observed in individuals with DNMs in *DHDDS*.

NUS1 encodes Nogo-B receptor (NgBR), which physically interacts with DHDDS to stabilize the dehydrodolichyl diphosphate synthase complex and potentiate its enzymatic activity.[55,61] Both indel mutations identified in this study affect upstream exons and thus have the potential to induce nonsense-mediated decay of the transcript. In addition, both variants are predicted to abolish the conserved C-terminal domain, which is required for the interaction with DHDDS.[61] The deletion of exon 2 causes an in-frame deletion of amino acids 139–180, leading to the loss of transmembrane domain 3 (TM3), which is critical for the proper topology of NUS1. Previously, a homozygous missense mutation affecting its C terminus (c.869G>A [p.Arg290His]) was identified in two siblings with type 1a congenital disorder of glycosylation and a severe phenotype of early-onset refractory epilepsy, congenital scoliosis, developmental delay with hypotonia, microcephaly, hearing and visual impairment, and severe cortical atrophy.[62] This mutation was found to decrease cis-PTase activity when expressed with hCIT (DHDDS) in yeast. In addition, Szafrans et al. reported individuals with early-onset seizures and 6q22.1 microdeletions centered on a 250 kb critical region that includes only *NUS1* and the promoter of *SLC35F1*.[63]

Collectively, our finding of DEE individuals with two truncating DNMs and one *de novo* whole-exon deletion in *NUS1*, the reported DEE individuals with *NUS1* microdeletions, and the fact that NUS1 is a functional direct interactor of DHDDS suggest that heterozygous mutations in *NUS1* can cause DEE, possibly via a mechanism of haploinsufficiency. This is in agreement with the fact that *NUS1* does not tolerate LoF variants, as suggested by the ExAC Browser, in which no such LoF mutations have been reported (pLi = 0.87).[54] The more severe phenotype previously observed in the siblings with the homozygous p.Arg290His variant could be due to a more dramatic reduction in NUS1 activity as a result of the recessive nature of a potentially hypomorphic mutation. Failure to identify other individuals with *NUS1* truncating mutations from the MIP screen or other published EE trios could be due in part to reduced capture efficiency of exon 1, which encodes 137/293 (~47%) amino acids of NUS1. Indeed, in the ExAC Browser, exon 1 of *NUS1* is, on average, poorly covered by WES in comparison with the rest of the exons of the gene.

Meta-analyses of DNMs from DEE and DEE-ID Cohorts

In order to further assess the involvement of various candidate genes in DEE, we sought to determine whether DNMs were enriched in certain genes in a series of affected individuals by taking advantage of a statistical framework that is based on the use of gene-specific mutation rates.[9] To increase power, we meta-analyzed DNMs from our DEE cohort along with DNMs from published WES studies of DEE trios (combined DEE trios = 624; Table S3). In total, 12 genes were found to be statistically enriched with LoF and/or functional DNMs (Table 6); mutations in all of these genes are now considered causative of DEE.

Given that epilepsy is a frequent comorbidity of ID, we performed a second meta-analysis combining the DNMs from published ID trios with those from the DEE cohorts used above (DEE-ID cohorts: 5,948 trios and 7,778 DNMs). In total, 111 genes were found to be enriched with functional and/or LoF DNMs, and 37 of these were found to be mutated in at least one DEE individual (Table S8). Interestingly, DNM enrichment has not been previously documented for 22/111 genes, including nine genes that have either not been directly associated with ID or DEE (*BTF3* [OMIM: 602542], *CHD3* [OMIM: 602120], *FBXO11* [OMIM: 607871], *PLK5, SETD1B* [OMIM: 611055], and *SF1* [OMIM: 601516]) or been described only in single or few individuals and therefore represent candidates pending additional evidence (*CLTC*,[52] *GABBR2* [OMIM: 607340],[12,27] and *PHIP* [OMIM: 612870]).[64] Among these, only *GABBR2*, *PHIP*, and *CLTC* had some DNMs in DEE individuals, whereas the rest had DNMs only in the ID cohorts (Table S8).

Lelieveld et al. recently showed increased power to detect ID-associated genes in a meta-analysis after excluding individuals with DNMs in genes previously found to be causally linked to ID.[11] We applied a similar strategy here to both the DEE-ID cohorts and excluded individuals with DNMs in any of the genes mentioned in the list established by these authors.[11] We also removed the individuals with DNMs in genes that showed DNM enrichment from the recent meta-analyses done on trios with ID or developmental disorders.[10,11] This retained 4,424 trios from the combined DEE-ID cohorts. As a result, three additional genes from the DEE-ID cohort showed

Table 6. Genes Enriched with DNMs in the DEE Cohorts

	De Novo LoF Variants				*De Novo* Functional Variants			
Gene	**Observed**	**Expected**	**p Value**	**c.p Value**	**Observed**	**Expected**	**p Value**	**c.p Value**
CDKL5	3	0	8.57E−9	0.0003*	5	0	8.90E−10	3.49E−5*
DNM1	0	0	1	1	6	0	1.03E−11	4.04E−7*
GABRB3	0	0	1	1	4	0	7.58E−9	0.0003*
GNAO1	0	0	1	1	4	0	4.47E−9	0.0002*
IQSEC2	3	0	6.76E−9	0.00026*	3	0	1.14E−5	0.45
KCNQ2	0	0	1	1	4	0	1.69E−7	0.007*
KCNT1	0	0	1	1	4	0.1	9.12E−7	0.036*
SCN1A	7	0	1.84E−17	7.2E−13*	14	0.1	6.37E−27	2.5E−22*
SCN2A	0	0	1	1	7	0.1	3.81E−12	1.49E−7*
SCN8A	1	0	0.007	1	7	0.1	3.08E−12	1.21E−7*
SLC35A2	2	0	4.74E−7	0.018*	3	0	4.89E−7	0.019*
STXBP1	1	0	0.006	1	6	0	1.27E−12	4.98E−8*

c.p value is the corrected p value = p value × 2 × 19,618 (significant < 0.05, indicated by an asterisk). LoF variants are nonsense, frameshift, and CSS *de novo* variants. Functional variants are LoF and missense variants.

modest but significant enrichment of functional DNMs; these included *GABRB2* (c.p value = 0.036), *RAB11A* (OMIM: 605570; c.p value = 0.036), and *SNAP25* (OMIM: 600322; c.p value = 0.042), all of which were found with predicted-damaging DNMs in individuals from our CENet DEE cohort, as well as in individuals from the ID cohorts.

Additional Supporting Evidence for the Involvement of *RAB11A*, *GABBR2*, and *SNAP25* in DEE

Our meta-analyses of the DEE-ID trios showed significant enrichment of DNMs in *GABBR2*, *PHIP*, *CLTC*, *RAB11A*, *SNAP25*, and *GABRB2*, whose mutations have not yet been confirmed as causes of DEE. With the exception of *PHIP*, we found predicted-damaging DNMs in all of these genes in individuals from the CENet series. We further validated the involvement of *GABRB2* and *CLTC* in DEE by identifying additional individuals in the context of our MIP screen or other WGS or WES studies (see above). As shown below, we also provide additional evidence for the involvement of *RAB11A*, *GABBR2*, and *SNAP25* in DEE.

RAB11A

We found a *de novo* predicted-damaging missense variant in *RAB11A* (c.244C>T [p.Arg82Cys] [(GenBank: NM_004663.3]) in a CENet individual with refractory epileptic spasms and erratic myoclonus with developmental regression. She subsequently developed focal seizures and severe ID. Using WES, we also identified another predicted-damaging *de novo* missense mutation in *RAB11A* (c.71A>G [p.Lys24Arg]) in an individual with moderate GDD and abnormal EEG but with no seizures reported so far. Three additional individuals with DNMs in *RAB11A*, including two individuals with the same variant (c.461C>T [p.Ser154Leu]) and another individual with a different variant (c.39A>C [p.Lys13Asn]), were identified in the context of the DDD study.[10] We were able to obtain detailed clinical information on the individuals who had the p.Ser154Leu variant and showed moderate GDD without epilepsy. The other individual from the DDD study had abnormalities of the nervous system according to DECIPHER, but we could not get additional clinical information. Brain atrophy and/or abnormalities of the corpus callosum were noted for three of the individuals with available MRI information (Table 7 and Supplemental Note).

RAB11A encodes a GTPase that regulates the recycling of a wide range of receptors at the cell surface.[65] Interestingly, RAB11A regulates synaptic plasticity by modulating the endocytic recycling of NTRK2 and AMPA receptors at the post-synaptic membrane of neurons.[66–68] The highly conserved Arg82 residue is located in the nucleotide-sensitive switch domain II of RAB11A and is involved in binding to the RAB11A effector FIP3.[69,70] The p.Lys24Arg, p.Lys13Asn, and p.Ser154Leu variants do not affect any of the nucleotide-sensitive switch domains of RAB11A (Figure 1F). The fact that *RAB11A* is enriched with DNMs in the DEE-ID cohorts and was found to have a recurrent *de novo* missense in two individuals of the DDD cohort suggests that DNMs in this gene can cause a DEE or ID phenotype.

GABBR2

We identified from our WGS a *de novo* missense mutation in *GABBR2* (c.2077G>T [p.Gly693Trp] [GenBank: NM_005458.7]) in one CENet subject who presented with focal seizures with impaired awareness and later developed epileptic spasms while on carbamazepine. He remains with refractory focal and generalized tonic-clonic seizures, severe ID, severe limb and axial hypotonia, and hyporeflexia (Table 7 and Supplemental Note). The Epi4K Consortium

Table 7. Summary of the Clinical Features of Individuals with DNMs in *RAB11A* (GenBank: NM_004663.4), *GABBR2* (GenBank: NM_005458.7), and *SNAP25* (GenBank: NM_003081.3)

Individual	Gender	Age at Last Examination	Gene	DNM (Detection)	Cognitive and Behavioral Features	Epilepsy Diagnosis	Age at Seizure Onset	Seizure Types	AEDs	EEG	Brain MRI	Associated Neurological Features and Seizure Outcome
HK055	male	5.5 years	*RAB11A*	c.71A>G (p.Lys24Arg) (WES)	GDD, moderate ID	no seizures	NA	NA	NA	abnormal background activity, no epileptic charges	central brain atrophy, bilateral periventricular white-matter damage, thin CC	acquired microcephaly, axial hypotonia, obesity, aggressive behavior
HSJ0637	female	9.5 years	*RAB11A*	c.244C>T (p.Arg82Cys) (WGS[a])	GDD, severe ID	IS	4 months	M, ES, Fo	NZP, CLB, VGB, TPM, VPA, LEV	modified hyps., M, diffuse slowing with M spikes	atrophy, partial agenesis of CC, delayed myelination, decreased NAA	acquired microcephaly, axial hypotonia
24631	male	4 years	*RAB11A*	c.461C>T (p.Ser154Leu) (WES[b])	moderate GDD	no seizures	NA	NA	NA	NA	partial agenesis of the CC	distractible, possible ADHD
84049	female	9 years, 11 months	*RAB11A*	c.461C>T (p.Ser154Leu) (WES[b])	moderate ID	no seizures	NA	NA	NA	NA	ND	possible hyperactivity, obesity
HSJ0048	male	14 years	*GABBR2*	c.2077G>T (p.Gly693Trp) (WGS[a])	severe GDD, severe ID	DEE, IS	11 months	FIA, ES, GTC	CBZ, VGB, VPA, TPM, CLB, PHT, LEV, LCM, LTG	modified hyps.	increased sub-arachnoid spaces	axial and limb hypotonia, hyporeflexia, scoliosis, hypersalivation
HSJ0745	male	23 years	*SNAP25*	c.496G>T (p.Asp166Tyr) (WGS[a])	GDD, moderate ID	DEE	18 months	GTC, FIA	VPA, CLB	gen. SW, CSWS	mild diffuse cortical atrophy	apneas, bradycardia, severe constipation, minor dysmorphic traits, no seizures for 2 years on VPA

Underlining indicates treatment with clinical response (decreased seizure frequency or severity). Abbreviations are as follows: NA, not applicable; ND, not done; WES, whole-exome sequencing; WGS, whole-genome sequencing; GDD, global developmental delay; ID, intellectual disability; IS, infantile spasms; DEE, developmental and epileptic encephalopathy; M, myoclonic; ES, epileptic spasm; Fo, focal; FIA, focal impaired awareness; GTC, generalized tonic-clonic; AED, anti-epileptic therapy; NZP, nitrazepam; CLB, clobazam; VGB, vigabatrin; TPM, topiramate; VPA, valproic acid; LEV, levetiracetam; CBZ, carbamazepine; PHT, phenytoin; LCM, lacosamide; LTG, lamotrigine; EEG, electroencephalography; hyps., hypsarrhythmia; gen. SW, generalized spike-wave; MRI, magnetic resonance imaging; CC, corpus callosum; ADHD, attention-deficit hyperactivity disorder; CSWS, continuous spike and wave during sleep; and NAA, N-acetylaspartate.

[a]CENet.

[b]DDD study.

reported two *de novo* predicted-damaging missense variants (c.2114T>A [p.Ile705Asn] and c.2084G>T [p.Ser695Ile]) in *GABBR2* in two individuals with unsolved infantile spasms.[8] Lopes et al. recently reported a *de novo* missense mutation in *GABBR2* (c.1699G>A [p.Ala567Thr]) in an individual with severe ID and Rett-syndrome-like features but no seizures.[27]

GABBR2 encodes a γ-aminobutyric acid type B receptor that inhibits neuronal activity through G-protein-coupled second-messenger signaling at both the presynaptic and post-synaptic membranes, where it regulates neurotransmitter release and the activity of ion channels.[71] This receptor is the target of baclofen, a medication often used to treat spasticity. The hypotonia and hyporeflexia observed in our subject might therefore reflect underactivation at the neuromuscular junction or in spinal motor control centers. Interestingly, unlike the missense mutation identified by Lopes et al., which affects TM3 of GABBR2, the three DNMs in the individuals with infantile spasms affect TM6 of the protein ($p = 0.001$, Denovonear), suggesting that these TM6 variants are specific to DEE. Meta-analysis of DNMs from DEE-ID cohorts showed an enrichment of functional DNMs in *GABBR2*. In addition to the subject with the c.1699G>A (p.Ala567Thr) variant from Lopes et al., the DDD cohort contained two other individuals with the p.Ala567Thr variant and one individual with a c.1181C>T (p.Thr394Met) variant that affects the N-terminal extracellular region of the receptor.[10] We conclude that *de novo* missense mutations in *GABBR2* have the potential to cause DEE or ID with no seizures, depending perhaps on where they affect the protein.

SNAP25

We identified from our WGS a *de novo* missense mutation in *SNAP25* (c.496G>T [p.Asp166Tyr] [GenBank: NM_003081.3 and NM_130811.2]) in a male with DEE. He presented with apneas, GDD, nocturnal generalized tonic-clonic seizures, and focal seizures with impaired awareness and progressively developed moderate ID (Table 7 and Supplemental Note). SNAP25 is a member of the SNARE complex and is required for the exocytosis of neurotransmitters during synaptic transmission by mediating synaptic vesicle fusion.[72,73] Developmentally regulated alternative splicing of two similar exon 5 sequences of *SNAP25* generates two isoforms (a and b), which differ only by nine residues in this exon 5. Various mutant *Snap25* mouse lines displayed cognitive deficit and seizures or susceptibility to seizures.[74,75] SNAP25 interacts with STXBP1, another SNARE synaptic protein in which variants are known to cause DEE.[26,76] So far, only two *de novo* mutations have been reported in *SNAP25*: a missense mutation affecting both isoforms (c.142G>T [Val48p.Phe] [GenBank: NM_003081.3 and NM_130811.2]) in an individual with DEE[77] and a missense mutation affecting a conserved residue in exon 5 of only *SNAP25b* (c.200T>A [p.Ile67Asn] [GenBank: NM_130811.2]) in a girl showing congenital myasthenia, cerebellar ataxia, and ID.[78] Both mutations affect the N-terminal t-SNARE coiled-coil homology domain of SNAP25. The *de novo* missense variant identified in our cohort (p.Asp166Tyr) is predicted to be damaging (by SIFT, PolyPhen-2, and CADD) and alters a conserved residue in the second t-SNARE coiled-coil homology domain common to both isoforms (Figure 1H). In addition, three DNMs in *SNAP25* (c.118A>G [p.Lys40Glu] [GenBank: NM_130811.2], c.127G>C [p.Gly43Arg], and c.520C>T [p.Gln174*]) were recently reported in the DDD study, but no detailed clinical information was available on these.[10] Collectively, these findings support the involvement of *SNAP25* mutations in DEE.

Pattern of DNMs Associated with DEE

Out of the 53 pathogenic or likely pathogenic *de novo* point variants identified in our CENet series, 35 are missense and 15 are LoF, resulting in a missense/LoF ratio of 2.5 (Table S5). We examined the list of DNMs identified in the Epi4K series of individuals with DEE and found a similar ratio of pathogenic or likely pathogenic missense variants to LoF variants (n = 56 DNMs; missense/LoF ratio = 43/13 = 3.3).[8] Interestingly, these observed missense-to-LoF ratios of *de novo* pathogenic or likely pathogenic variants in both the CENet ($p = 0.004$, two-tailed Fischer's exact test) and Epi4K ($p = 0.0004$, two-tailed Fischer's Exact test) series were significantly higher than those similarly observed in 192 published trios with moderate to severe ID (WES and WGS) and detailed phenotypic and pathogenic variant information (missense/LoF ratio = 36/42 = 0.85).[23,24,26,28] Remarkably, out of all the pathogenic or likely pathogenic DNMs identified in the CENet series (n = 53), ~45% were also independently reported in ClinVar (n = 24 [19 missense and 5 LoF]) (Table S5). This rate of recurrent pathogenic and likely pathogenic DNMs was significantly higher in the CENet DEE series than in the exomes or genomes of the 192 previously published trios with moderate to severe ID (out of 80 pathogenic or likely pathogenic variants, only 19 [13 missense and 6 LoF] were also reported independently in ClinVar) ($p = 0.0012$, two-tailed Fischer's exact test).[23,24,26,28]

Discussion

In this study, we performed WGS on 197 individuals with DEE and their unaffected parents. We initially identified pathogenic variants in 53/197 (27%) individuals, including 50 with point mutations in genes previously found to be causally linked to DEE or ID, one with a recurrent pathogenic CNV (15q11–q13 duplication), and two with CNVs encompassing genes previously associated with ID or DEE (*PCDH19* and *DNMT3A*). Moreover, we were able to explain DEE in ten additional individuals from the series by identifying DNMs in candidate genes for which we provide additional evidence for their involvement in DEE (*NTRK2*, *GABRB2*, *CLTC*, *DHDDS*, *NUS1*, *RAB11A*, *GABBR2*, and *SNAP25*). Overall, our approach allowed us to obtain a molecular diagnosis in 63/197 (32%)

individuals. It is important to note that the diagnostic yield of WGS would have most likely been higher in an unbiased series given that many of our subjects had previously been screened by targeted sequencing and/or array genomic hybridization. Interestingly, two of the four pathogenic *de novo* CNVs identified in our series would have been missed by clinical array genomic hybridization because of their size (<5 kb), providing some support for the added value of WGS.

The main cause of DEE in our series was *de novo* point mutations (53/63 solved cases), whereas the remaining cases could be explained by inherited mutations (6/63 solved cases) or *de novo* CNVs (4/63 solved cases). *De novo* missense variants explained a larger proportion of individuals with DEE in our series than of individuals ascertained because of ID in other series. Interestingly, more than half of these pathogenic missense mutations were recurrent, suggesting that at least a subset of them confer a specific property to the protein, such as dominant-negative or gain-of-function effects. Shohat et al. recently showed that, compared with genes with missense mutations, genes with LoF mutations were associated with different pathways across neuro-developmental disorders such as ID, ASD, and schizophrenia.[79] For instance, genes with missense variants involved in neuro-developmental disorders code for proteins that show a higher number of protein interactions than those encoded by genes with LoF variants. Together, these data raise the possibility that the genetic landscape of DEE is enriched with gene products that act as protein hubs. It would be important to understand why these hubs are specifically associated with DEE.

Of the eight genes highlighted herein for their involvement in DEE, we were not able to show *de novo* gene enrichment for three of them: *NTRK2*, *DHDDS*, and *NUS1*. However, the multiple occurrences of DNMs affecting the same conserved amino acid residues in NTRK2 and DHDDS in individuals with a similar phenotype nonetheless represent strong evidence implicating the encoding genes in DEE. Indeed, other DEE-related genes with site-specific recurrent DNMs, such as *GRIN2D* (OMIM: 300776) and *FGF12* (OMIM: 601513), did not also show DNM enrichment in our meta-analyses. Genetic forms of neuro-developmental disorders that are caused by recurrent DNMs associated with gain-of-function or dominant-negative effects tend to be rare because there are typically a smaller number of variants that can confer such effects than of variants that can induce haploinsufficiency. It is thus likely that meta-analyses involving larger numbers of subjects will be necessary for identifying these rare forms of DEE. No DNM enrichment was observed for *NUS1* in our meta-analysis, possibly because of the poor capture of its exon 1, which represents almost half of the entire coding sequence of this gene. However, the identification of three DNMs in *NUS1* (including two truncating variants and a microdeletion) in DEE individuals with similar phenotypes and the fact that NUS1 is a functional direct interactor of DHDDS strongly support the involvement of this gene in DEE.

Several of the DEE-related genes highlighted in this study code for proteins that interact directly or indirectly with other proteins encoded by genes associated with epilepsy. Such proteins include (1) DHDDS and NUS1, which form a complex for the synthesis of dolichol monophosphate;[55,61] (2) SNAP25, which interacts with the DEE-associated STXBP1 for the docking of neurotransmitter vesicles; and (3) RAB11A, which is involved in the endocytosis of NTRK2.[68] In addition, both GABRB2 and GABBR2 belong to the family of GABAergic receptors, which include other members involved in epilepsy (encoded by *GABRA1* [OMIM: 137160], *GABRB1* [OMIM: 137190], *GABRB3* [OMIM: 137192], and *GABRG2* [OMIM: 137164]). The identification of multiple genes acting along pathways or playing biological functions that have already been linked to epilepsy raises the possibility that many of the major pathways involved in DEE have been identified. Stratifying genetic forms of DEE on the basis of the involvement of these pathways could facilitate the development of tailored therapies.

Supplemental Data

Supplemental Data include Supplemental Acknowledgments, a Supplemental Note, two figures, and eight tables and can be found with this article online at https://doi.org/10.1016/j.ajhg.2017.09.008.

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Web Resources

1000 Genomes Project, http://browser.1000genomes.org/index.html
CADD, http://cadd.gs.washington.edu/
ClinVar, https://www.ncbi.nlm.nih.gov/clinvar/
Denovonear, https://github.com/jeremymcrae/denovonear
DenovolyzeR, http://denovolyzer.org/

DDD Research Variants, https://decipher.sanger.ac.uk/ddd#research-variants
ExAC Browser, http://exac.broadinstitute.org/
GenBank, https://www.ncbi.nlm.nih.gov/genbank/
GoNL, https://molgenis95.gcc.rug.nl/
GATK Best Practices, https://software.broadinstitute.org/gatk/best-practices
NHLBI Exome Sequencing Project (ESP) Exome Variant Server, http://evs.gs.washington.edu/EVS/
OMIM, http://www.omim.org
PubMed, https://www.ncbi.nlm.nih.gov/pubmed/
PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/
PopSV, http://jmonlong.github.io/PopSV/
SIFT, http://sift.jcvi.org/

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Supplemental Data

High Rate of Recurrent *De Novo* Mutations in Developmental and Epileptic Encephalopathies

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Supplemental Note: Case Reports

1) Individuals with *de novo* variants in *NTRK2* (NM_006180.4):

HSC0103; *NTRK2* (c.1301A>G: p.Tyr434Cys): This is a 3.5 years old male. He was born full term, 41 weeks by vaginal delivery. At 3 days of life, he developed episodes of extension epileptic spasms involving both his arms and legs with his eyes rolling up, lasting for less than 1 sec. At one month of age, the episodes became more frequent, with 3-4 clusters per day, each of them lasting 2-3 mins. They were now described as extension of both arms and legs together with flexion of the trunk and upward rolling of his eyes. He was diagnosed with infantile spasms, which subsequently progressed to multiple seizure types. At his baseline, he experiences 4-5 spasms per day as well as approximately ten episodes of upward tonic eye deviation with fluttering of the eyelids. He failed multiple AEDs: vigabatrin, ACTH, levetiracetam, clobazam, topiramate, valproic acid. He subsequently developed other types of seizures. He has problem swallowing and is fed through an NG tube. On examination his weight is 13.4, below the 3%. He is microcephalic with a head circumference at 46.5 cm, far below the 3%. He has severe global developmental delay. He is non-ambulatory and nonverbal. There is no dysmorphism. He has truncal hypertonia with increased tone of the extremites and exaggerated deep tendon reflexes at all levels. His pupils are symmetrical and reactive but he is not following, not fixating and there is no nystagmus. His multiple EEGs showed modified hypsarrythmia and abnormal visual evoked potential.

CGH microarray was normal. Ecocardiogram was normal. Recent MRI showed normal brain signal intensity with no evidence of focal lesion or diffusion restriction, but with hypoplasia of the optic chiasm and both optic nerves. Trio WGS sequencing identified a *de novo* missense variant in *NTRK2* (NM_006180.4:c.1301A>G: p.Tyr434Cys).

indvSLIJ; *NTRK2* (c.1301A>G: p.Tyr434Cys): This male individual was born by caesarean section due to transverse lie. His birthweight was 3.8 lbs 5oz, and birth length was 21.1 inches. Head ultrasound was done as part of his workup, which was normal. He had his first seizure within 12 hours of birth which resolved by day 10 of life. Details are unclear, however by parental report these presented as startle like events. He had some difficulties with breastfeeding after birth. However, no problems with bottle feeding are reported. The parents noted that he was not able to sit unassisted at 6 months of age. By 1 year, he was seen by Neurology service for global developmental delay. He was also referred to an ophthalmologist then and was diagnosed with optic nerve hypoplasia bilaterally. He has significant visual impairment. ECI intervention was started and he began receiving OT, PT, and speech therapy. At the age of 4 years, he was diagnosed with autism. Regarding specific developmental milestones, the parents reported that he sat up at 12 months, walked at 14 months, and said his first word at 16 months of age. He has on examination hyper-reflexia in legs, Babinski signs, and hypertonia in ankles, findings consistent with spastic diplegic cerebral palsy. Brain MRI at the age of 5 years showed moderate loss of the bilateral optic nerves and optic chiasm. At the age of 5 years he was admitted to the hospital with prolonged status epilepticus. He developed several months later focal seizures with impaired awareness, sometimes progressing to bilateral tonic clonic seizures. The events were described as zoning out, drooling, tremor-like shaking of the body, and fall if not held. They lasted 20-30 seconds each. He is on oxcarbazepine therapy 30 mg/kg/day, with no further seizures for 1 month. The wake and sleep EEGs reveal diffuse background slowing for age without discernable posterior rhythms and right temporal intermittent rhythmic delta frequency activity (TIRDA) that might indicate an underlying epileptic focus. His current problems include: GDD, hypotonia, ASD, severe ID, speech delay (says 15 words at the age of 5y7mo), visual impairment and seizures. Previous investigations were normal and included plasma amino acids, array CGH, Fragile X, and lactate of 1.8. Clinical trio WES (GeneDx) revealed a *de novo* mutation in *NTRK2* (NM_006180.4:c.1301A>G: p.Tyr434Cys).

T25821; *NTRK2* (c.1301A>G: p.Tyr434Cys): Concerns about individual T25821, a 4.5 year old girl and the fourth child of unrelated Ashkenazi Jewish parents, arose at 6 weeks when her head control remained poor. She has made little developmental progress subsequently. She can sometimes smile with a spasm but does not smile responsively and does not laugh. She only started fixing transiently at 2 years and has cortical visual impairment. Currently, she has no voluntary movement other than putting her hand in her mouth, she smiles but not always responsively, she has no reliable communication and is dependent for all activities of daily living. This individual developed epileptic spasms, both flexor and extensor, at 4 months with series lasting a few minutes. At 4 months, prednisolone controlled spasms within 2 days but they subsequently relapsed and she remained refractory to anti-epileptic therapy including: vigabatrin, levetiracetam, topiramate, lacosamide, valproate, rufinamide, zonisamide, diazepam, phenytoin, cannabis oil and the ketogenic diet. At 2

years, spasms continued to occur frequently with 3-5 spasms every 15 minutes. They comprised multiple types with eye rolling up and to either side or to the midline lasting 1-2 seconds but sometimes longer and could involve extension of the upper limbs. By 2 years, isolated spasms had reduced to 3 times per day. She also has tonic seizures every few days. At 6 months, she lost the ability to feed and required nasogastric feeding until 16mths when she had a percutaneous endoscopic gastrostomy. Episodes of uncontrollable crying occurred nightly at age 2 years, often followed by vomiting. On examination, she was hypotonic with poor head control, had noisy respiration and a persistent cough and had subtle choreoathetosis, also involving her tongue. MIP-targeted sequencing of *NTRK2* identified a *de novo* missense in this gene (NM_006180.4:c.1301A>G: p.Tyr434Cys).

HF303; *NTRK2* (c.1301A>G: p.Tyr434Cys): The case was the first child of a 28 year old woman who suffered from migraines, heartburn and iron deficiency during the pregnancy. The case was born at term by spontaneous vaginal delivery at 2.8 Kg without significant complications. At approximately 2 weeks of age, abnormal eye movements developed and a dilated eye exam demonstrated a small optic nerve. At 4 months of age, he developed epileptic spasms that responded to ACTH, but other seizures developed. Other AEDs were started with vigabatrin helping the most. He had storms of dystonia and hyperventilation that responded to diazepam. A lumbar puncture was only significant for low 5-HIAA 173 (nl=179-711) and HVA 358 (nl=450-1132). Genetic testing showed a negative array CGH, *CDKL5* sequencing epilepsy gene panel, Williams panel, DYT1 and 15q13 methylation profile. Seizures continue with the development of focal seizures with impaired awareness and atonic seizures. Other AEDs were tried, including zonisamide, clonazepam and diazepam. He was also started on taurine and hemp oil has alternative therapies. He remains non-verbal with global developmental delay despite aggressive treatment of his seizures. Feeding problems started as an infant with severe gastroeosophageal reflux, requiring NG-tube placement. MRI at 6 months old demonstrated hypoplasia of the optic nerves, tracts and chiasm. He was diagnosed with ASD at 3 years of age by a developmental pediatrician using the DSM criteria. He was later seen in an autism clinic where his diagnosis was verified by a pediatric neurologist examination and scores on the Social Responsiveness Scale (Total Raw Score 109; T-Score 80) and Autism Symptoms Questionnaire (Total Score 11) that were well within the range for autistic disorder. His cardiac evaluation revealed a normal echocardiogram with normal cardiac anatomy and biventricular systolic function. Family history was significant for mother having restless leg syndrome and chronic constipation, and father having chronic diarrhea. On the parental side of the family, there was a history of learning disabilities, ADHD and hypertension. His examination demonstrated a thin boy with BMI on the 2%ile. He was non-verbal with poor social interactions. Cranial nerves were unremarkable but eye movements were roving with nystagmus. Motor examination demonstrated appendicular hypotonia, reduced bulk but normal strength. His deep tendon reflexes were reduced but symmetric. Sensory, gait and coordination examinations were unremarkable. Trio exome sequencing done independently at 2 sites (GeneDx; Hudson-alpha) identified a *de novo* missense variant in *NTRK2* (NM_006180.4:c.1301A>G: p.Tyr434Cys). No other likely pathogenic variants were reported in the exome of this individual.

HSJ0335; *NTRK2* (c.2159C>T:p.Thr720Ile): Individual HSJ0335 is a 11 years old girl and the fourth child of non-consanguineous parents from Guatemala. She was born at term after an uneventful

pregnancy, by iterative C-section. The perinatal evolution was unremarkable (BW 2690g, APGAR 5-9-9) apart from physiological jaundice treated with phototherapy. She presented with global developmental delay and poor weight gain at 4 months of age. She has remained with a moderate global delay and evolved towards moderate-severe intellectual deficiency. She started holding her head at 6 months, could sit with support at 9 months but without support only at 18 months. She started rolling front-back at 15 months, never crawled but could walk with support at 27 months. She started walking independently at 3 years of age. She remains clumsy with a tendency for anteropulsion when standing. She said her first words at 2 years of age but with a slow progression (3 words at 3 years, 15 words at 6.5 years). She was oblivious of others, has little interests for toys, presents stereotypic rocking of the trunk and wriggling of the hands. She was diagnosed with autism spectrum disorder at 3 years of age, and with moderate-severe intellectual deficiency at 6 years of age without formal neuropsychological evaluation. When last seen at 9.5 years, she could walk short distances but used a wheelchair for longer distances, she could climb stairs while holding the railing and could drive a tricycle. She used approximately 50 words, could juxtapose 2 words, understood simple commands and could count up to 5. She could grasp objects and throw them but could not draw or dress herself. She presented 2 febrile generalized tonic-clonic seizures at 23 and 26 months. She developed rare afebrile focal seizures around 2.5 years old: she would scream, look terrified with behavioral arrest for a few seconds and 1-2 hours post-ictal fatigue. The parents did not seek medical attention for these episodes. At 5.5 years of age, she developed tonic-clonic seizures proceeded by a scream, lasting 1-3 minutes and occurring 2-4 times per week. The seizures were refractory to clobazam, were aggravated by levetiracetam but were partially controlled by topiramate (from daily seizures to monthly seizures). She still presented occasional prolonged generalized tonic-clonic seizures (status epilepticus), with or without fever, occurring every 2 months. Valproic acid was added to topiramate at 6.5 years of age and she was seizure-free for 10 months. Unfortunately, the seizures recurred despite increased doses and the valproic acid was replaced by carbamazepine at 7 years of age. She had only one short recurrence 2 years ago and has remained seizure-free since then, on carbamazepine monotherapy. 4 EEGs were done between ages 15 months and 6 years and were normal. Another EEG at 6.5 yo post-status epilepticus showed diffuse slowing, more marked at the left temporal area. She had been followed initially by the genetics and dietary services for failure to thrive and swallowing difficulties. She was fed through NG tube for a few months. Her weight generally followed the 10^{th} percentile from the age of 1 to 2.5 years of age (with a height around the 3^{rd} centile). However, she suddenly started gaining weight around 3 years of age, eventually reaching the 95^{th} percentile at 9.5 years of age (with a height at the 50^{th} percentile). She is now described as hyperphagic and must be supervised to prevent overeating. On last examination, she was smiling but could not maintain eye contact. She had stereotypic movements of the hands with flapping when excited. She had no dysmorphic traits apart from obesity. The motor exam was unremarkable. She could walk with help and had a wide-based stance. She has had an extensive investigation which was negative, including an array cGH and Fragile X screen, serum sialic acid and amino acid screen and urinary organic acid screen, urinary purines/pyrimidines, GUAC/creatine, *MECP2* sequencing. Her lactate was initially mildly increased (3.4) but normalized on four other tests. Pyruvate was normal. Her brain MRI at 20 months revealed delayed myelination (corresponding to that of a 13 months old child), mild reduction of global white matter tracts with mildly enlarged lateral ventricles, thin corpus callosum (posteriorly), normal spectroscopy. A brain

MRI at 6 years of age revealed bilateral atrophy of the hippocampi, decreased volume of the anterior commissure and mild increased subarachnoid spaces. The myelin was felt to be normal. An ophtalmological examination at 20 months of age was unremarkable (no optic nerve hypoplasia). Her genome sequencing revealed a *de novo* missense variant in *NTRK2* : NM_006180.4: c.2159C>T: p.Thr720Ile.

2) Individuals with *de novo* variants in *GABRB2* (NM_021911.2):

1242500; *GABRB2* (c.236T>C: p.Met79Thr): This 9 year female was the only child of non-consanguineous parents. The family history was unremarkable. The pregnancy, screening ultrasounds, delivery and birth parameters were normal. Neonatal examination was notable for hypotonia and dysmorphic features with round face and hypertelorism, everted lower lip, fifth finger clinodactyly and short broad great toenails. Her subsequent development was globally delayed. She walked at 4 years. Her language acquisition was also very late, slow and limited. She has severe ID. Ophthalmologic examination showed hypermetropia and astigmatism. Brain MRI and EEG were normal. This individual presented clinical seizures with altered consciousness (mostly absences), which started at birth (diagnosed at 11 months) and responded to levetiracetam. Clinical WES identified a *de novo* pathogenic truncating variant in *CHAMP1* (NM_021911.2: c.1876 1877delAG: p.Ser626Leufs (case published as case F3-II.1 in Isidor et al. 2016 PMID: 26751395). A recent revision of the exome data flagged a missense variant in *GABRB2* as likely pathogenic (NM_021911.2: c.236T>C: p.Met79Thr) and was subsequently confirmed by Sanger sequencing to be *de novo*.

K.02591; *GABRB2* (c.373G>A: p.Asp125Asn): This 10 year old female has moderate intellectual disability. She sat at 12 months and walked at 23 months. First words were at about 23 months. She babbled at 2.3 years and progressed to single words over next 2-3 years. She is hypermobile in her joints. She experienced febrile seizures and intermittent tonic clonic seizures from 6 years of age. She was treated with valproate and seizures stopped after several years. She is now seizure free on no AEDs. Brain MRI is normal. EEG was not done. Trio exome sequencing done as part of the DDD Study revealed a de novo missense in GABRB2 (NM_021911.2:c.373G>A: p.Asp125Asn).

CNSA01; *GABRB2* (c.908A>G: p.Lys303Arg): This is a 4 years old boy who presented with neonatal seizures on the first day of life. He was born after a normal pregnancy at 39 weeks' gestation. He had unremarkable familial and prenatal history. Birth weight, length, and head circumference were within normal ranges. We initially recorded focal seizures and then multifocal seizures (seizures recorded from both sides at 14 days of age). EEG background consisted of bilateral abnormalities. Neonatal MRI revealed diffuse white matter abnormalities on T2 weighted sequences. Feeding difficulties were observed during the neonatal period. Dystonic movements were observed at 3 months of age. Head growth slowed at 3 month-old and is now below the first percentile. Several antiepileptic drugs were tried. A combination of valproate, lamotrigine and topiramate was associated with a decrease in seizure frequency and intensity. Rare seizures were observed after 18 months of age. Dystonic movements became the prominent motor symptom. Gabapentin seemed to have a good efficacy on this symptom. It was difficult to distinguish clinically whether the motor symptoms were

seizures or dystonic movements. Repeat EEGs were conducted to elucidate this question that would impact the choice in treatment. This individual has axial hypotonia from early life. Feeding difficulties remained present. Perendoscopic gastrostomy was performed to provide adequate nutritional requirements. He has severe intellectual disability. At age of 4, he is seizure free but dystonic movements are observed. He is not ambulatory and non-verbal. Targeted gene panel sequencing revealed a de novo variant in GABRB2 (NM_021911.2:c.908A>G: p.Lys303Arg).

T21213B; *GABRB2* (c.911C>T: p.Ala304Val): T21213B is a 14 year old girl with profound ID who is nonverbal and cannot walk independently. Her parents are unrelated and her paternal grandfather had focal epilepsy. She was born vaginally at term following a normal pregnancy with normal growth parameters. She had mild initial feeding difficulties as she did not suck well. She was otherwise developmentally normal until 6 months of age when she was not able to sit or hold her head appropriately. Due to continued developmental concerns an EEG was performed at 15 months of age, which was severely encephalopathic approaching hysparrythmia in sleep. There were no seizures at this time. Treatment of the EEG with clobazam resulted in some improvement in both EEG and development. Vigabatrin caused a deterioration both electrographically and clinically. Hydrocortisone resulted in improvement on EEG and development. Multiple attempts to wean the hydrocortisone over the next 2 years resulted in regression of development. She had no seizures until 3.5 years of age when she presented with regression associated with multiple seizure types. Recurrent periods of deterioration were associated with weaning of steroids and would begin with her becoming unsteady and not feeding for a day followed by the onset of absence, myoclonic and atonic seizures with an encephalopathic EEG. If her hydrocortisone dose was not increased at that stage then over several days the seizures became frequent and she developed non-convulsive status epilepticus. These periods of regression and seizures lasted 3 to 14 days. During these she would have two types of absence seizures: typical brief absence seizures with no motor semiology as well as absence seizures with a significant myoclonic component. These seizures, captured on video-EEG, began with brief eyelid flickering and eyes rolling upward followed by loss of tone with low amplitude rhythmic myoclonic movements of arms. The EEG during these showed 3 Hz GSW. The episodes of non-convulsive status did not respond to AED therapy and only resolved after several days of increased hydrocortisone. Multiple trials of AEDs to prevent these periods of deterioration and allow weaning of the hydrocortisone failed resulting in her receiving hydrocortisone at varying doses continuously for 12 years. She has had no seizures occurring outside the episodes of deterioration which occur several times per year correlating with the hydrocortisone being weaned to a low level. On examination between periods of regression she is a happy child. She has no dysmorphic features but her height, weight and head circumference all below the 3rd percentile. She has generalised hypotonia with normal strength and reflexes. Metabolic investigations and MRI imaging were unremarkable.

HSJ0753; *GABRB2* (c.730T>C:p.Tyr244His): Individual HSJ0753 is a 4 years old girl and the 2nd child of non-consanguinous French-Canadian parents. She was born at term after an uneventful pregnancy (BW 4035g, HC 35cm, APGAR 9-9-9). She was hospitalized for one week at 3 days of life for irritability and feeding difficulties (chocking episodes). On examination, she had no visual contact,

major axial hypotonia and spasticity. She has profound ID. Cortical blindness was confirmed following normal ophtalmological examination and normal visual evoked potentials and electroretinogram. She developed an early-onset myoclonic epilepsy with initial seizures at 4 months of age. Her seizures consist of erratic myoclonic jerks involving the eyelids and limbs, sometimes with nystagmoïd eye movements, lasting seconds to minutes but with recurrent episodes of prolonged myoclonic status epilepticus. Her seizures have been refractory to levetiracetam, valproic acid, topiramate and a bolus of pyridoxine (100 mg). They responded partially to benzodiazepine, phenobarbital and phenytoin. They were aggravated by cannabidiol. They did not respond to the ketogenic diet, which she attempted twice with incomplete compliance. At 14 months of age, she has developed continuous erratic myoclonus when she is awake that disappears during sleep. She occasionally present with episodes of tonic limb extension. In the last year, she has had a few brief generalized tonic-clonic seizures. She is currently treated with phenobarbital, clonazepam and topiramate. Her initial EEG at 4 months of age revealed generalized 2 Hz spike-wave discharges, predominant in both frontal areas. At 6 months of age, the EEG showed rhythmic high amplitude slow waves (2.5Hz) and high-amplitude poly-spike and slow wave discharges at 2-3Hz for 3-15 seconds. The EEG evolved towards hypsarrhythmia with electrodecremental responses at 7 months of age but she never presented clinical epileptic spasms (and no clinical correlates were observed on video-EEG monitoring). Since the age of 26 months, the EEGs have revealed diffuse continuous spike-waves at 2Hz. On last examination at 4 years of age, she had no eye contact, had severe axial hypotonia, limb hypertonia with diffuse hyperreflexia. A progressive microcephaly was noted at 17 months of age (42.5 cm, $<2^{nd}$ percentile)**.** She had 2 brain MRIs (9 days and 1 year) that were normal. Her blood lactates were found to be elevated at two occasions (2.9 and 4.4 mmol/L) but have repeatedly been normal since then. An extensive metabolic screen was normal including ammonia, serum amino acid screen, urinary organic acid screens, glycosylation, long chain fatty acids, acylcarnitines, free and esterified carnitine, urinary purines/pyrimidines, GUAC/creatine, CSF lactate, amino acids, neurotransmitters). Sequencing of a panel of 126 epileptic encephalopathy genes (MNG) and of the mitochondrial DNA were non-diagnostic. Genome sequencing revealed *a de novo* missense in *GABRB2* (NM_021911.2: c.730T>C:p.Tyr244His).

T23211; *GABRB2* (c.730T>C:p.Tyr244His): Individual T23211 is a 6 year old girl and the fifth pregnancy of consanguineous Iraqi parents. Four previous pregnancies include a first trimester miscarriage, a boy who died at 20 days of age due to complications of maternal gestational diabetes and a boy who died at 18 months attributed to meningitis in Iraq. She presented at 5 months with global developmental delay, failure to thrive and microcephaly. On admission she was observed to have previously unrecognised tonic seizures. She was admitted and treated with phenobarbitone and levetiracetam, reducing the tonic seizures from 7 per day to 2 per day. She had choreoathetoid movements which gradually resolved after ceasing phenobarbitone. She became encephalopathic within 24 hours of starting vigabatrin at 8 months for persistent seizures. She had increased drooling and tonic seizures with eye flickering associated with stertorous breathing, desaturation and tachycardia. Seizures escalated over time and culminated in admission due to status. At 10 months she developed focal autonomic seizures and focal tonic seizures requiring multiple hospital admissions. She was admitted to hospital for 6 weeks at 15 months due to repeated vomiting and escalation of seizure frequency leading to an episode of status epilepticus. With treatment she settled back to having

5-10 brief seizures per day comprising tonic extension followed by eyelid flickering. She required nasogastric tube feeding from 6 months until she had a percutaneous endoscopic gastrostomy at 2.5 years. She has profound global intellectual impairment and cannot roll over or sit, is non-verbal and has cortical visual impairment. Investigations done and found negative include aCGH, Mito and POLG panel of mitochondrial mutations (Victorian Clinical Genetics Service). Targeted MIPs sequencing of *GABRB2* identified a missense in this gene that was confirmed by Sanger sequencing to be *de novo* (NM_021911.2:c.730T>C:p.Tyr244His).

G64518; *GABRB2* (c.830T>C: p.Leu277Ser): This ten year old girl is the second child of unrelated parents; 3 siblings are well and there is no significant family history. She was born after an uncomplicated pregnancy, with a birth weight of 2.5 kg. There were no neonatal problems, and she breast fed well for 4 months. There were no early concerns about development, she sat at 7 months. Although she was noisy, her vocalisations did not contain vowel sounds. She pulled to stand at thirteen months, but has never walked alone, although she does ambulate with hand held or a walking frame. She has only two words, used occasionally and communicates her wants by tapping. She developed a generalised epilepsy at age two; this was worsened with treatment with valproate. A small dose of lamotrigine was effective in controlling her seizures. Off treatment, she has mild absence seizures and febrile tonic-clonic seizures. Her general health is good; she has episodic panting respiration. She is generally happy and content. She has developed a mild microcephaly, her growth is otherwise normal. Testing (normal results) has included; microarray, MECP2 and TCF4 mutation testing, very long chain fatty acids, transferrin iso-electric focussing, white cell enzymes, routine haematology, biochemistry and thyroid function testing. Cerebral MRI showed mild ventricular dilatation at age 2, but was normal at age 3. Whole exome sequencing as part of the DDD study identified a de novo missense variant in *GABRB2* (NM_021911.2; c.830T>C: p.Leu277Ser).

HA076; *GABRB2* (c.830T>C: p.Leu277Ser): This 15-year-old male is the only child of non-consanguineous white Welsh parents. He was conceived by IVF. There was no family history of learning disability or epilepsy. Hewas born by emergency caesarean section for breech position at 38 weeks gestation. Birth weight was 4.12Kg (75-91st centile), length 56cm (98-99.6th centile) and OFC 39cm (slightly above 99.6 centile). He breast fed well. However, at 8-9 months there were concerns about poor feeding, lack of weight gain, and slow development. A Paediatric Neurologist diagnosed global developmental delay. He sat at 11-12 months and walked just before 2 years. At 4 years and 8 months He presented with clusters of myoclonic seizures. He was diagnosed with epileptic encephalopathy. The onset of seizures was associated with loss of language skills. He went from using several words and animal noises to no speech. The seizures responded rapidly to Valproate but within 2 weeks he began having head nods and clusters of myoclonic seizures with loss of posture. He was noted to have some mild left leg weakness and increased leg tone bilaterally. He had brisk but symmetrical reflexes. Topiramate and Clonazepam were started. The Valproate and Clonazepam were stopped at 6 years 4 months and Levetiracetam was started. Reviewed at 9 years his antiepileptic medications were still Levetiracetam and Topiramate. He was having occasional absences. The drop attacks had stopped. His speech development was limited (2-3 words). His fine motor skills were poor

and he had a mildly broad-based gait. He could run, jump and climb, but could not ride a bike. He could finger feed and just about use a spoon. This individual's height was 124.5cm (9th centile), weight 26Kg (25th centile) and OFC 54.5cm (75th centile). He was subtly dysmorphic with an alternating convergent squint, full and everted lower lip and small epicanthic folds. He had two symmetrical hair whorls on his scalp. The rest of his neurological examination was normal. At age 9 years he had a 10 minute episode of dystonia following an intramuscular dose of prochlorperazine (given to treat an episode of vomiting). His Topiramate was stopped, but Levetiracetam alone was unable to control seizures. Clobazam was restarted. At 11 years the Clobazam was replaced with Lamotrigine. At 13 years of age he had 3 generalised tonic-clonic seizures following an attempt to stop his Levetiracetam. Reviewed recently (at 15 years and 8 months of age) this individual has severe intellectual disability. He attends a specialist school for children with severe learning difficulties. He has minimal speech with only 2-3 recognisable words and 3-4 hand signs. He communicates what he wants by pointing or taking a person to the object. He makes some sounds (e.g. car noises). He is not toilet trained. He has poor concentration and is very active. He has no sense of danger. He is sociable and has good eye contact. He has occasional brief emotional outbursts, usually due to frustration. He is on Levetiracetam and Lamotrigine which controls his seizures well (one observed absence in the past year). Investigations: At 2 years his EEG was normal. Repeated at 4y8m (2 weeks after seizure onset and on treatment) his EEG showed marked slow background (2-3Hz delta along with rhythmical 3-4Hz slow components diffusely but more marked in anterior quadrants) frequent runs of higher amplitude rhythmical 2-3Hz delta (maximal in temporal to occipital regions, more marked on right) on four occasions becoming generalised and prolonged (8-10s) showing 'notched morphology' and more marked on right. During these four electroclinical events he leant forward, raised his arms to side and shuffled in chair. Sleep EEG at 6y0m showed abnormal sleep - mostly slow wave sleep. On waking he removed the electrodes but the background appeared low amplitude and slow. MRI at 2 years of age showed multiple small focal areas of abnormal increased signal in the periventricular and deep white matter mainly within the frontal lobes. MRI scan was repeated at 4y10m and 9y3m with no significant change. Extensive biochemical investigation was normal. Genetics testing included basic karyotype, subtelomeric screening with FISH, array CGH, Fragile X syndrome, ARX, Angelman methylation and Severe Infantile Epilepsy 35 gene Panel - were all normal. Trio-based whole-exome sequencing identified a *de novo GABRB2* missense variant (NM_021911.2; c.830T>C: p.Leu277Ser) in this individual.

31841; *GABRB2* (c.851C>A: p.Thr284Lys): Individual 31841 was the third pregnancy of unrelated parents. He was born quickly at 38+5/7 weeks by normal vaginal delivery with no concerns at birth. He has two older siblings, both in good health. There is unconfirmed family history of a paternal uncle having a childhood seizure disorder. On day 5, individual 31841 presented to hospital at the suggestion of the community nurse as he was not opening his left eye and was having difficulty feeding. His mother had been manually pulling his tongue down to get a bottle in and he had been feeding quite well while she did this. He examined normally on day 5 and was sent home with referral for ophthalmological review. On day 7 the community nurse raised further concerns regarding abnormal hand movements and "jerkiness". His parents had noticed decreased activity, poor feeding and jitteriness that day. The individual was admitted to hospital and on examination he was noted to be hypotonic with back arching and jitteriness. He was having tonic seizures captured on amplitude-

integrated EEG. He was loaded with phenobarbitone leading to a burst-suppression pattern on EEG. Individual 31841 required intubation and ventilation due to increasing apnoeic episodes. He was started on levetiracetam as well as phenobarbitone. Formal EEG showed epileptiform bursts associated with myoclonic jerks, along with suppression, consistent with early myoclonic encephalopathy. He was started on biotin, folinic acid and pyroxidine, all to no effect and all metabolic screens were normal. He was started on IV midazolam due to increasing seizure activity but continued to have seizures with multifocal myoclonus (diaphragm, feet and hands), right sided tonic episodes and lip smacking. These episodes lasted up to 10min with upwards of 20 episodes per day. On day 9 of this admission the decision was made to palliate the individual and he passed away at 17 days old. Clinical aCGH was found normal. WES identified a de novo missense in *GABRB2* (NM_021911.2: c.851C>A:p.Thr284Lys)

3001866; *GABRB2* (c.946G>A: p.Val316Ile): This 21 month old female presented with focal seizures progressing to become bilateral tonic clonic at 12months of age. She also has apneic/cyanotic episodes which have become more frequent. The seizure semiology consists of staring with eyes fluttering followed by left arm twitching and subsequent progression to all extremities. She stopped breathing and turned blue. The seizure lasted 2-3 minutes and then she was apneic for 4 minutes. Parents performed CPR (mouth breathing) for 4 minutes and then she became limp and subsequently slept for hours. There was no triggering factors. She was evaluated in the emergency department and blood work was normal. She has had no myoclonic or atonic seizures. Her motor development is normal but she has probable language delay (uses only 2 words at 21 months of age). Echo, EKG and Holter monitoring were all normal. Chest CT which showed NEHI (Neuroendocrine hyperplasia of infancy) which can cause hypoxemia and she is using oxygen during sleep. MRI brain at 1 yr of age show few punctate foci of low signal on the hemosiderin sensitive sequence within the cerebellum, which may reflect foci of tiny prior hemorrhage. EEGs and video-EEGs were normal. A Sleep study at14 months of age showed mild obstructive sleep apnea and periodic Legs movements. Sleep study (21 months of age): There were no apneas for more than 15 sec and no bradycardias. There was one oxygen desaturation (5 sec). The average oxygen saturation was 98% on RA; normal study on room air. Clinical microarray testing was negative. Clinical WES (BCM-Miraca) identified a private missense in *GABRB2* (NM_021911.2: c.946G>A: p.Val316Ile) that was shown by Sanger sequencing to be *de novo*.

3) Individuals with *de novo* variants in *CLTC* (NM_004859.3):

PBSD; *CLTC* (c.977_980delCATG: p.Ser326Cysfs*8): This girl was first seen in Genetic and Neurology clinics for delayed development, hyperactivity and impulsivity at age 5 years. Birth weight was 2.9 kg. at full term. The family history was negative for delays or genetic problems. A younger sister was normal. Parents noted that she said a few words early, but her language did not progress and she did not begin to talk until age 2. She did not put words together until age 3. She crawled late, at 14 months of age, and walked by 18 months. Further motor progress was slow, and she was late in running and feeding herself. She struggled with poor fine motor skills and was always clumsy. Intensive

interventions with speech, occupational and physical therapies were helpful, but she fell further behind her peers. As she got older, poor social skills became more of an issue. Although generally kind and affectionate, she would grab objects from other children, played poorly with her peers, and demonstrated poor understanding of social rules and cues. This improved with behavioral therapies, but continued to be a problem. IQ testing at age 5 showed Full Scale IQ of 79 and Verbal Comprehension Index 80, Perceptual Reasoning Index 82, Processing Speed 78. She had learning gaps and it was difficult for her to remember and learn new things. At age 5 she knew letters of the alphabet, but could not read or identify single words. She was poor at math, and had superficial concept formation. She had a nice personality, followed directions, and did not have behavioral problems, other than hyperactivity and social difficulties. Issues with gross motor delays continued. A physical examination at age 5 showed mildly large size (95 pc) attributed to tall parents, minimal epicanthic folds, mild hypotonia, trace deep tendon reflexes and poor fine motor skills. Her gait was mildly wide based. An MRI, at age 5 demonstrated "gray-white matter junction signal abnormality seen in the bilateral temporal, frontal and periatrial white matter regions. These findings most likely represent hypomyelination." A metabolic disorder was suspected, but an acylcarnitine profile showed only mild increase in glutaryl, hexanoyl, and decanoyl acylcarnitines, and was normal on repeat testing. Urine organic acids were normal. A microarray was not done. Stimulant therapy for ADHD was begun, and she had good response with decreased hyperactivity and better focus, but learning continued to lag. At age 11 her academic skills in math and language arts were assessed at the 3rd grade level. Trio exome sequencing (GeneDx) showed a c.989_992delCATG (p.Ser330Cysfs*8) in *CLTC* (NM_004859.3), a frameshift, not found in either parent. No other likely pathogenic variants were identified.

5289183; *CLTC* (c.1660_1668del: p.Met554_Tyr556del): This is a 20 year old male. He was born full term with a birth weight of 3.3 kg following an uncomplicated pregnancy. His neonatal course was unremarkable. He had mild gross motor delays and began sitting at 8 months and walking at 15 months. As a toddler, he fell frequently and was clumsy. Around age 6 years old, he began showing signs of a gait abnormality characterized by lower limb spasticity, mild truncal instability, and a slight hand tremor bilaterally. For the next 6 years, he experienced progressive spasticity with weakness in the lower extremities, brisk reflexes, intermittent myoclonic jerks, ataxia and a mild resting hand tremor. He underwent bilateral tendon lengthening at age 11 years old with no improvement in gait. By the age of 13, his gait stabilized and has remained unchanged since with no further progression. He is able to jump up on two feet, but cannot jump or balance on one foot. At age 14 years old, he had a single seizure event and video EEG showed some discharges, but no seizure activity. He was treated with anti-epileptic drugs for 2 years, after which the medication was discontinued since there was no further seizure activity. He has a history of cognitive delays. Delays were initially noticed when he entered school and presented with difficulties with reading and counting. A neuropsychological evaluation in 2003 and was revealing with a full scale IQ score of 69 and a second one in 2007 was revealing for a full scale IQ score of 72, consistent with low-average cognitive function. He began receiving special education at age 7 and graduated from high school. He has difficulty with abstract thoughts and complex reasoning and his cognitive functioning remains in the low-average range. He is one of 15 siblings and is of Ashkenazi Jewish descent with an unremarkable family history. He had a medical evaluation with normal results for his brain MRI, c-spine MRI, temporal bone CT, EEG,

electromyelogram, nerve conduction studies, echocardiogram and EKG. He had normal genetic and metabolic testing including chromosomes, Fragile X, mtDNA, lactate, pyruvate, urine mucopolysaccharides and oligosaccharides, creatine kinase, very long chain fatty acids and plasma amino acids. Trio whole exome sequencing done at GeneDx was revealing for a heterozygous *de novo* nonframeshift mutation in *CLTC* (NM_004859.3:c.1660_1668del: p.(Met554_Tyr556del).

indvAA; *CLTC* (c.2669C>T:p.Pro890Leu): This 4-year-old boy was born at term following a pregnancy with oligohydramnion in the last weeks and an uneventful delivery. After birth he shortly needed oxygen, but recovered quickly and was send home at day two. At three weeks of age he was admitted to the hospital for 5 days because of an RSV-infection. He underwent an adenoidectomy at two years of age and grommets were inserted because of multiple ear infections and delayed speech development. He has a global developmental delay. Speech development is more delayed than motor development. He started walking at 19 months of age and he falls easily. At three years of age he spoke several single words. At four years of age he sporadically uses two word sentences and starts to imitate words and behavior. He understands simple assignments. There are no overt behavioral problems, but he is easily distracted and under-aroused regularly. He loves water. His developmental age was tested at 18,5 months at an actual age of 35,5 months. On exam at the age of three years and two months his weight was 18.8 kg (+1.4 SD), height 97.6 cm (-0.91 SD). His head circumference is just below the 50th percentile. He has a rather long philtrum, a full lower lip with open mouth behavior, and a high palate. He drools continuously. He has mildly lax ligaments. At three years of age the child neurologist observed mild ataxic movements and a myoclonic jerk. EEG and MRI brain were normal. Chromosomal SNP array showed a likely benign paternal deletion 8p23.3p23.2, without OMIM-genes in the deletion. There were no homozygous regions. FMR1 analysis was normal. Trio whole exome sequencing (Radboud UMC; Nijmegen) showed only a *de novo* missense variant in *CLTC* (NM_004859.3:c.2669C>T: p.Pro890Leu).

CAUSES-1; *CLTC* (c.2669C>T: p.Pro890Leu): This 5-year-old boy was born at 39 weeks to non-consanguineous parents and has one brother with spina bifida and another brother with iris heterochromia. During the pregnancy maternal serum PAPP-A level was low at 15 weeks. There was a maternal short cervix, and contractions were present at 33 weeks gestation. Maternal steroids were administered and bedrest from 33-37 weeks was advised. Delivery was unremarkable and APGARS were 8 (1 min), 9 (5 min), and 9 (10 min). He rolled at 6 months, sat unsupported at 8 months and took his first steps were at 2 years 11 months. He had his first words at 2.5 years. At 4.5 years he had 6 words. He has never had seizures. Formal IQ testing at 4.5 years noted intellectual disability/GDD but the severity was unspecified as the assessments differed between home, school and clinic. He has oral and motor apraxia, poor attention and suspected ADHD. MRI brain and spine was normal. Formal ophthalmological and cardiological assessments were normal. On exam at the age of 4.5 years and two months his weight was 16.2 kg, height 96 cm. His head circumference was 52 cm. Blood pressure was 107/78 mmHg. He was alert and friendly and made excellent eye contact. He had an immature gait. He had an immature pincer grasp but could scribble. He knew his body parts and some colors. Cranial nerve functions were normal. He had mildly reduced tone with normal strength. He had normal reflexes with down-going plantar reflexes. Chromosomal SNP array and 15q13 methylation was normal. Whole

exome sequencing in trio with his non-affected parents as part of the CAUSES study demonstrated a *de novo* missense variant in *CLTC* (NM_004859.3: c.2669C>T:p.(Pro890Leu).

18052017; *CLTC* (c.2669C>T: p.Pro890Leu): This 30 year-old female was born from nonconsanguineous parents after an uneventful pregnancy and delivery. Two younger brothers (24 and 20 year-old) were healthy. Psychomotor delay and behavioral features (aggressiveness and impaired social interaction) were noticed during the first years of life. From the age of 4 years, oculo-manual and gross motor incoordination, and proximal limb rigidity became evident; persistent impairment of social skills was reported. Nevertheless, at that age, her cognitive development was reported as normal. During the following years, a cognitive decline was noticed, and at the age of 11, when she was fist investigated for diagnostic purposes, mild intellectual disability, drooling and gait incoordination were reported. Brain MRI, and neurophysiological studies were normal. Extensive neurometabolic work-up provided normal results except for a mild persistent hyperphenylalaninemia (180-240 microM; normal values: 60-120) associated with marginal reduction of urinary neopterin (0.15 mmol/mol creat; n.v. 0.2-1.7). PAH mutation analysis disclosed compound heterozygosity for the c.453T>A (p.Asp151Glu) and c.1139C>T (p.Thr380Met) variants. A similar biochemical alteration and the same PAH genotype was documented in one of the two unaffected brothers. In the following years, hypo- and bradykinesia emerged in association with dysphagia, hyporexia and weight loss with an intermitting course. At the age of 13, tetrahydrobiopterin (BH4) loading test proved to normalize blood Phe/Tyr ratio, and the reduced pteridine derivative was added to the therapy with a stabilization of the clinical condition. Looking for an alternative molecular cause explaining the individual's condition, BH4 synthetic pathway was further explored by molecular analysis of PTS, GCH1, and SPR genes, but no functionally relevant variant was identified. Similarly, possible involvement of FMR1 was excluded. On the last examination at the age of 30, she exhibited bradykinesia and bradypsychism, hypomimia and clumsiness, moderate intellectual disability (WAIS IQ 45), attention instability and verbal reiteration with relatively good adaptive skills. Brain MRI and DaTSCAN were normal. Trio-based WES identified a de novo missense change, (NM_004859.3: c.2669C>T: p.Pro890Leu), in *CLTC* as the only excellent candidate underlying the trait.

indvPAR; *CLTC* (c.3140T>C: p.Leu1047Pro)

This 16 years old boy is the third and only affected child of unrelated Caucasian parents. Family history was unremarkable. Pregnancy was uncomplicated. BW: 2860 g (-1.24 SD), BL: 48 cm (-0.81 SD), BHC: 34 cm (-0.93 SD). Hypotonia was recorded at birth and remained prominent till now. Feeding was difficult because of poor sucking, was and complicated by a severe gastroesophageal reflux (GER) that persisted through infancy, causing reported inhalation pneumonias. At the age of 10 years, he had Nissen fundoplication and gastrostomy. Development milestones were severely delayed: he controlled head position at age 9 months, could sit unsupported at age 13 years old. He had severe to profound intellectual disability, with limited interaction, no speech, no purposeful use of the hands. Seizures probably begin during the first months, but were initially considered to be vagal malaise related to his GER. Treatment with VPA was initiated at 2 years of age and resulted in the disappearance of clinical seizure. EEG showed nonspecific irritative pattern, without foci. He was noted to have spasticity of the lower limbs in infancy, but this stiffness evolved to choreo-athetotic movements clearly noted at age of

6 years, with some myoclonic jerks. Eye tracking was abnormal, with saccades but no clear oculomotor apraxia. Hypotonia lead to progressive kyphoscoliosis that required surgical arthrodesis T2 to sacrum at 14 years of age. MRI in infancy showed thin, short corpus callosum, with hypoplasia of its posterior part, wide Virchow-Robin spaces, and normal gyration, cerebellum and brainstem. Diffuse hypersignals in the frontal, temporal and parietal white matter was noted at age 3 years, that did not evolved between the age of 3-6 years. Syringomyelia T4-T7 was observed at the time of spine surgery. ERG and evoked visual potentials were normal in infancy. When examined at 13 years of age, he was 135 cm tall(-2.72SD), weighted 33 kg (-1.84SD) and had a HC: 49 cm (-3.64SD). Oval-shaped face, upslanted palpebral fissures, long nose with bulbous tip, tented upper lip, wide mouth, big central incisors, microretrognathia, and pointed chin. In infancy: ridged metopic suture was noted, but this anomaly vanished with time. Trio clinical exome sequencing identified a de novo missense in CLTC

273692; *CLTC* (c.3322T>C: p.Trp1108Arg): At 12 weeks of pregnancy, screening risk indicated a high risk of Down's and CVS was performed which gave a normal chromosome result (karyotype). Delivery was at term complicated by cord around the neck. At day one, he was noted not to cry for feeds and was sleepy. A few hours later he became jittery. He went home at three days of age. He was reviewed in the Genetics Clinic aged seven months. For the past two weeks, he had required NG tube feeding. He was on treatment with Baclofen to prevent stiffness and on antireflux medication. Developmentally he could smile at his mother when her face was close to his. He had a head circumference of 44cm between 2nd and 9th centile, length 65cm and weight 7.25 kilos. He did not have any dysmorphic features helpful for making a diagnosis. He was fairly hairy at birth but no longer hirsute. He had relatively small jaw. Posture was abnormal with arching of his back and generalised stiffness. He had right convergent squint but no nystagmus. Brain MRI scan had been reported as showing a small cerebellum. Nerve conduction studies were normal, EMG had some features of a myopathic process. Ophthalmology assessment showed normal structural eye features. Seizures have become more of a problem. He was suspected at two years of age to have myoclonic jerks and was referred for a further neurological opinion regarding seizures. Seizures have been confirmed and now settled on levetiracetam. On review at age four years, his phenotype was that of the quadriplegic cerebral palsy, significant intellectual disability, bilateral convergent squint and cortical visual immaturity. Head circumference aged was at 48.5 cm (9th centile). He was continuing with gastro-oesophageal reflux and was gastrostomy fed.

261801; *CLTC* (c.3595C>T: p.Gln1199*): This 10 years/7month old male is the middle of three children to a non-consanguineous Caucasian couple. He was born at term after an uncomplicated pregnancy. His father has joint hypermobility but there is no other relevant family history. Hypotonia and poor head control were noted in the neonatal period. Although ptosis was not recognised at that stage, it is believed to be congenital. Early investigations concentrated on congenital myasthenic syndrome in view of the hypotonia, ptosis and easy fatigability. Repetitive stimulation testing and Tensilon testing both gave normal results, as did Ach receptors antibodies and a metabolic screen including lactate and CK levels. DYT1, RAPSN, DOK7 and array CGH analysis were all normal. He first presented to Genetics at 5 years and 4 months, at which point, he was repeating his reception year

in school because of developmental delay and intellectual disability. He was able to make an attempt at writing his first name but only recognised the letters within it and could only count to 5. There were also concerns about speech delay and limited vocabulary. When reviewed again at 10 years and 7 months, he was estimated to be working at 6 – 7 year-old level and Special Needs input was being planned for secondary education. His hypotonia and hypermobility are still present but less pronounced and neurological examination is otherwise normal. He has frequent injuries, when unable to protect his face in a fall. He becomes easily fatigued and his parents often discover he has put himself to bed. When tired, both the ptosis and slurring of speech worsen. He has some obsessions, for example requiring smart, matching clothes, with exactly the correct leg length and wanting to wear a tie at all times. He also has some ritualistic behaviour such as needing a new book and pencil after a single use. His social skills are poor and he is a sensitive child, who is prone to stress and anxiety. He has had a normal MRI scan of his head and has never had any seizures. He has zygomatic hypoplasia and often has peri-orbital puffiness and discoloration. Trio exome sequencing as part of the DDD study identified a *de novo* nonsense variant in *CLTC* (NM_004859.3:c.3595C>T: p.Gln1199*).

indvMB; *CLTC* (c.3621_3623del: p.Asp1207del): indvMB is the second child of healthy, non-consanguineous parents with unremarkable family history. She has two healthy sisters. She was born at term, following uneventful pregnancy and delivery, with a weight of 3140 g, length 49.5 cm and head circumference 34.5 cm. She had severe global developmental delay since her first months of life: she sat at 3.5 years, stood up with support at 4, walked a few steps at 6, and did not develop any understandable language. The first years of life were also complicated by feeding difficulties, because of a severe gastroesophageal reflux, significantly improved after antireflux surgery at 5 years. The epilepsy of indvMB started at 3 years. She first had febrile tonico-clonic seizures, then febrile and afebrile seizures.When she was 4 years old, daily myoclonic jerks of her four limbs appeared, as well as tonic seizures with upgaze. The epilepsy was pharmacoresistant, partly because of a poor tolerance to many treatments: lamotrigin induced vomiting and worsened myoclonias, the introduction of clonazepam or clobazam was associated with behavioral troubles, valproate induced asthenia and levetiracetam acute pancreatitis. Finally, the epilepsy was partially controlled by a combination of topiramate and lacosamide, but her EEG remained pathologic. First EEG showed interictal multifocal spikes and spike – waves with bifrontal predominance, generalised spike-waves during myoclonic jerks. EEG at 7 years old was characterized by bifrontal slow-waves throughout the recording, without spikes. At the age of 8 years, she was unable to speak, she could understand very simple commands, had some autistic features with fluctuating eye contact. She could grab objects but had poor fine motor skills. Her weight was 27 kg (+0,5 DS), her height was 120 cm (-1 DS), her head circumference was 49 cm (-2 DS) with acquired microcephaly. She had no malformation but mild facial dysmorphic features: upslanting palpebral fissures, long filtrum with thin upper lip, prominent ears, scattered and slow growing hair. Neurological examination revealed severe hypotonia, ataxia and weak osteotendinous reflexes. Brain MRI at 2 years, 3 years and 6 years showed thin corpus callosum, hypersignal of the subcortical white matter (T2), and widened lateral ventricles. Metabolic studies (plasma lactate, pyruvate, ammonia, plasma amino acids and urin organic acids chromatographies, plasma acylcarnitine profile, isoelectric focusing of serum transferrine, urinary guanidinoacetate and creatine, lumbar punction, urine purines/ pyrimidins measurements) were normal. The following studies were

also normal: study of the mitochondrial respiratory chain on skin sample, karyotype, array-CGH, study of the methylation of the Prader-Willi/Angelman locus on chromosome 15, study of MECP2 and TBC1D24 genes, and an optical microscope examination of the hair. Finally, a trio based WES was performed and revealed a heterozygous de novo 1 amino acid inframe deletion in *CLTC* (NM_004859.3: c.3621_3623del: p.Asp1207del).

HSC0054; *CLTC* (c.4575dupA: p.Glu1526fs*18): This is a 23 years old female. She is the product of an uncomplicated pregnancy and delivery. Her parents are non-consanguineous. Family history is unremarkable. She had global developmental delay. She walked at the age of 4 years. She currently has moderate intellectual disability and can recognize letters but cannot read. She has perseverative behavior, characterized by some obsessive compulsive symptoms. She also has a somewhat dysmorphic face with abnormally long teeth. She has flat feet, scoliosis and had low muscle tone as a child (normal tone now). Limited neurological examination was normal. She has a history of 4 seizure types. Her epilepsy began at 5 months of age with seizures characterized by abnormal eye movements. Before 1 year, she developed absence seizures and another type of seizure that could be interpreted as myoclonic or tonic seizures. These occurred 50-60 times per day until puberty. She had her first GTCS at 11 years and then continued to have one GTCS every 3 months. She has had focal seizures from the age of 12 or 13 years characterized by panic, a terrified look on her face and she would pace back and forth and scream for 1-2 minutes. Past treatments include Clobazam, Valproic acid, Acetazolamide and the Ketogenic diet. She is currently on Levetiracetam and Lamotrigine. Her last known seizure was at the age of 20 yrs and has been seizure free since. EEGs have shown generalized spike and wave and polyspike and wave discharges and independent inter-ictal epileptiform discharges at F4 and over both mid temporal regions. MRI showed immature myelination in both temporal lobes and thinning of the corpus callosum as a child; a later scan as an adult was reported as normal and a neuroglial cyst noted in the right posterior centrum semiovale. Trio WGS identified a *de novo* frameshift variant in *CLTC* (NM_004859.3: c.4575dupA: p.Glu1526fs*18)

LDKQS; *CLTC* (c.4605+2T): This individual is currently a 12-year-10-month-old male whose neonatal history was remarkable for difficult feeding. Breastfeeding was originally attempted, however he was unable to latch and was eventually bottle fed. Originally the difficulty feeding was attributed to a short frenulum although concerns for hypotonia were also noted. Developmental milestones were delayed throughout infancy. He started to walk around age 2.5 years of age and began to talk around 3.5 years of age. In early childhood he was diagnosed with sensory motor difficulties including auditory hypersensitivity, tactile hypersensitivity, and visual hypersensitivity to light. At approximately seven years of age, he was diagnosed with a "cookie bite" sensorineural hearing loss, and was subsequently fitted for hearing aids. The hearing aids improved his hearing, but did not noticeably improve his speech ability. Also at age seven, it was discovered that he has left ventricular noncompaction (LVNC) of the left ventricular apex. This is felt to be a mild asymptomatic form of LVNC. Throughout his life, has had hypotonia, particularly noticeable in his core. He has also struggled with chronic constipation. At age 11, the child was seen by a neuropsychologist for intellectual disability. Full scale IQ was 44 on the WISC IV. Testing showed him functioning significantly below average in the areas of verbal comprehension, perceptual reasoning, working

memory, and processing speed. He has a speech and language disorder. He struggles with anxiety, which is controlled through medications. At age 12, he is able to read some books and his able to ride a bike. His gait and run are normal. Overall, he is in good general health. Trio clinical exome sequencing (GeneDx) revealed a de novo variants in CLTC (NM_004859.3: c.4605+2T

DDD00280; *CLTC* (c.4663C>T:p.Gln1555*): This female individual now aged 6 years/8month was referred to Clinical Genetics for assessment aged 3.5 years. She was the 2nd child of nonconsanguinous parents and her elder brother has a diagnosis of autism. Her mother has 3 healthy children from a previous relationship. There is no other family history of note. She was born at 36 weeks gestation after an uneventful pregnancy. She was in good condition at birth and weighed 7lb 14oz. She was noted to be hypotonic in the neonatal period and had gastro oesophageal reflux. At the Age 3.5 years, there were concerns regarding developmental delay and speech delay. She walked at 19 months and by 3 ½ was described as clumsy with joint hypermobility. She was provided with ankle supports and required a buggy for distances. She was beginning to put 2 or 3 words together. She was drooling, had glue ear and was described as a snorer with a poor sleep pattern. She had a sleep study suggestive of obstructive sleep apnoea and had adenotonsillectomy but symptoms recurred. On examination her OFC was 50.4 cm (+1 SD) with height and weight on the 75th centile. She had rough wiry hair out of keeping with the rest of the family. She had a tall forehead, a broad nasal tip, a high arched palate, a shallow philtrum and a wide mouth. She had bilateral 5th finger clinodactyly. When reviewed at 6 years her mother raised concerns that her appearance had coarsened with time and that her voice sounded hoarse. She still was not toilet trained. She was putting 3 or 4 words together and had obtained a place at a specialised additional needs school. At this time her height and weight were on the 98th centile. Bone age was normal. Abdominal ultrasound detected mild dilatation of the right renal pelvis but no masses or other abnormalities were detected. Thyroid function was normal and urinary metabolic screen was negative. Trios WES as part of the DDD Study revealed a *de novo* variant in *CLTC* (NM_004859.3: c.4663C>T: p.Gln1555*).

281177; *CLTC* (c.4667G>A: p.Trp1556*): Individual 281177 is an 11 year old male who was born after an uneventful pregnancy by planned caesarean section at 40 weeks gestation weighing 3.5Kg [-.02 SD]. There were no perinatal problems, there was some delay in attaining head control but no other concerns were noted in the first year. He sat unaided at 6 months and walked unaided at 12 months. He was generally healthy throughout infancy. The first significant cognitive concern was related to his delay in acquisition of both receptive and expressive language. He did not have clear words until he was over three years old. He was first seen by clinical genetics services aged 7 years 8 months for investigation of learning disability. At that age his height was 127.3 cm [.26 SD], weight 25.7 kg [.26 SD] and his head circumference was 54.3 cm [.34 SD]. He had no major dysmorphisms or malformations. There were no focal neurological signs. He was generally a pleasant and cooperative boy but his parents reported significant behavioral problems at home, in particular he would become in appropriately angry over minor issues. He has been formally assessed for autistic spectrum disorder but he did not fulfill the diagnostic criteria. Following the clinical genetics appointment DNA was taken for array CGH which was normal. He was recruited to the DDD Study in 2013. On trio-based

exome sequencing a *de novo* nonsense mutation was identified in *CLTC* (NM_004859.3:c.4667G>A: p.Trp1556*).

4) Individuals with *de novo* variants in *DHDDS* (NM_024887.3):

indvSG; *DHDDS* (c.110G>A: p.Arg37His): This is a globally delayed, nonverbal girl who recently started walking independently at 4 years of age. She has dozens of seizures per day that started at 18 months of age which are exquisitely photosensitive- even going outside in the sunlight sets her off. Her seizures consist of eyelid fluttering lasting a few seconds often with throwing her head back, suggesting absence seizures with eyelid myoclonia. There is no post-ictal state. No medication has been successful at treating her seizures (valproic acid, lamotrigine, levetiracetam, ethosuxamide); however, parents feel valproic acid (divalproex) had made the greatest difference but she still has dozens of seizures daily. Brain MRI was normal at the age of 12 months. Other investigations found negative include clinical array CGH and comprehensive epilepsy panel sequencing from Transgenomics. Clinical WES at BCM-Miraca diagnostic laboratory did not reveal any likely pathogenic variant, however, it identified a missense in *DHDDS* (NM_024887.3; c.110G>A:p.Arg37His) which was subsequently confirmed to be *de novo* by Sanger sequencing in the parents and the child.

HSJ0762; *DHDDS* (c.110G>A: p.Arg37His): This is a 6 years old male with normal antenatal and postnatal history born to non-consanguineous parents. Low tone was evident since birth and always showed some tremor when performing an action. He sat at 9 months and walked at 21 months. He said his first words at the age of 2 and at 5 years of age he was able to communicate with sentences. At 12 months of age he started having myoclonic seizures requiring hospitalization; brain MRI and lumbar puncture (LP) including amino acids were normal. EEG showed generalized epileptiform activity. He started on high dose levetiracetam and became somnolent therefore dose reduced. At 22 months of age he experienced "staring spells" 2-3 seconds during times of illness and a few episodes of sudden falling to the ground. At the age of 25 months the EEG showed abnormal background with no gradient and generalized discharges. He experienced ongoing myoclonic seizures only at time of fevers and atypical absence seizures also increased with fever. Levetiracetam was increased to 70mg/kg/day and valproate was added resulting in a better seizure control. At the age of 36 months he showed a wide based gait, difficulty running, tremor, 4 point crawl up stairs. At age 5 years he was still having atypical absences with atonic semiology a couple of times a week (was seizure free for 1 year 2015-2016). No further myoclonic events. VPA increased in 2016 and no seizures since. Other Investigations done and found negative: aCGH, epilepsy panel (GeneDx), MELAS/MERFF, Carnitine, acylcarnitine, amino acids, organic acids, ammonia, 2nd LP normal CSF/serum glucose, neurotransmitters. Trio WGS sequencing identified a *de novo* missense in *DHDDS* (NM_024887.3: c.110G>A:p.Arg37His).

indvEF; *DHDDS* (c.632G>A: p.Arg211Gln): This individual is a six-year-old female with a history of global developmental delay, hypotonia, tremor, ataxia, and seizures. Hypotonia (axial and appendicular) was first noted around seven months of age. Around the same time, head tremors, eventually progressing to involving her arms and legs, were also noted. In retrospect, her parents feel she had tremors in early infancy. The tremor is mild, present at rest but worsens with activity. It is absent during sleep. Her early development was normal, with rolling over and sitting up at six months,

pulling to stand at ten months, and walking by 14.5 months. As she began to walk, she was noted to be ataxic and has continued to have an unsteady gait. Otherwise, her development was relatively normal up until age two years. Evidence of global developmental delay manifested after age two years. She has global developmental delay, although she has not had regression and is making progress. She has difficulty processing information and her level of understanding is unclear. Her seizures began around four years of age and were initially described as staring blankly and eye fluttering, followed by a postictal period of weakness and confusion. An EEG in the past was abnormal with epileptiform activity. Levetiracetam was started but discontinued because she would not tolerate it. Seizures occurred every two to three months. Seizures are now well controlled with lamotrigine. Brain imaging was essentially normal and did not reveal a cause for her neurologic symptoms (a Chiari I malformation was identified). She was born full term at 8 lbs 12 ounces and 21.5 inches long. Past medical history is significant for laryngomalacia and torticollis in infancy. She has had a substantial weight gain in the setting of insatiable appetite and is undergoing endocrinological workup for Cushing's syndrome and thyroid disorders. Family history is unremarkable. Her father had seizures in childhood, but not now. Her mother is in good health. Neither parent has intellectual disability, tremors, or ataxia. She has a sister and a brother who are both growing and developing normally. No one else in the family has health problems like indvEF. Physical exam at age 5 years revealed thick, dark hair, hypotonia and unsteady gait, but she was able to walk on her own. Speech was relatively easy to understand but she displayed some articulation errors. Genetic work up (chromosome microarray and deletion testing for spinal muscular atrophy) was initially normal. Trio WES (GeneDx) revealed a *de novo* missense in DHDDS (NM_024887.3; c.632G>A: p.Arg211Gln).

MDB31882; *DHDDS* (c.632G>A: p.Arg211Gln): This 35 year-old male was born after an uneventful pregnancy and normal delivery from nonconsanguineous healthy parents. After an unremarkable neonatal period, global developmental delay became evident. He acquired trunk control at 18 months, and walked unsupported at 30 months. Impaired social skills, repetitive behaviors, sensory-perceptual abnormalities, and language development delay suggested the diagnosis of autistic spectrum disorder, at the age of 4 years. From early infancy, he showed fluctuations in mood and activity levels with periods of marked anxiety and restlessness alternated with periods of apathy and hypokinesia. At the age of 2 years, distal upper limb tremor was noticed; which became associated with postural, action and stimulus-sensitive multifocal non-epileptic cortical myoclonus (involving trunk and upper limbs), from the age of 6, which turned out to be generalized during the following two years. From the age of 9, paroxysmal eyelid myoclonias and staring were associated with generalized polyspike-waves on the EEG recording. Epileptic seizures were successfully treated with sodium valproate, which did not affect movement disorders and clinical fluctuation. From late adolescence forward, this individual experienced a progressive switch to an hypokinetic rigid syndrome associated with generalized tremor. Clinical status continued to fluctuate between status of increased tremulousness and multifocal myoclonus (lasting 1 week/month) and akinesia and catatonia (lasting 1 week/month). Several pharmacological attempts (piracetam, benzodiazepines, dopaminergic and anti-dopaminergic drugs) resulted ineffective in improving neuromotor disorders and/or preventing clinical fluctuations. On the last examination at the age of 35 years, he showed generalized tremor, facial myokimia, poly-mini-myoclonus of the fingers, bradykinesia, hypomimia, rigidity, freezing and impaired postural reactions. Neuropsychological and behavioral phenotype was characterized by severe intellectual disability and

frontal lobe impairment features with verbal perseveration, disinhibition and unsuitable joviality. Structural and functional evaluation of brain (MRI, 1H-MRS, EMG, Flash and pattern visual-, motor- and somatosensorial-evoked potentials) were normal. An extensive neurogenetic and neurometabolic work-up, including IEF profiling of transferrins, failed in identifying any diagnostic cue. WES identified a heterozygous predicted-damaging missense in DHDDS (NM_024887.3: c.632G>A:p.Arg211Gln) that was subsequently confirmed by Sanger sequencing to be *de novo*.

indvNCJ; *DHDDS* (c.632G>A: p.Arg211Gln): This individual is a 7 year old female with a moderate-severe intellectual disability and a movement disorder. She is the second child of healthy parents and was born after an uncomplicated pregnancy. She had a secondary caesarian section because of abnormal CTG. Her APGAR scores were 6 after 1 minute and 8 after 5 minutes. pH umbilical artery 7.21 (Base excess -2.0), pH umbilical vein 7.25 (Base excess -1.8). Her birth weight was 4035 gram. At the age of 3 months she had an airway infection due to RS virus for which ventilation and tube feeding was needed for several days. Around the age of 1 year parents noticed she developed different than their older child. At the age of 1 year and 10 month she was formally tested and global developmental delay was identified. She functioned at the level of a 1 year old (Bayley Scales of Infant Development (BSID)-II-NL non-verbal, rough score 61, development index 55, Dutch non-speech test receptive and perceptive language below first percentile). When she started walking at the age of 2,5 years, parents noticed a movement disorder. She said her first words at the age of 3,5 year, and speaks full sentences at the age of 7 years. The family history revealed neither movement disorders nor intellectual disability nor seizures. At the age of 1 year and 4 months parents thought she might have Rett syndrome. Clinical and molecular investigation (MECP2) showed no evidence for this diagnosis. Also array CGH (Agilent 180 K custom HD-DGH microarray; (AMADID-nr 27730)) was done around that period and showed a normal female pattern. At the age of 4.5 years, physical exam revealed no dysmorphisms except for a high palate, missing tooth element and a hyperpigmentation at the right side of the thorax. An intention tremor was seen and ataxia was suspected. Genetic analysis of the FMR1-gene showed no CGG-expansion and whole exome sequencing filtered for genes causing developmental delay no potential pathogenic variants were identified (Radboud MC, Netherlands). At the age of 5.5 years eye examination including examination of the fundi showed no abnormalities. The pediatric neurologist noted that the child displayed abnormal movement with especially dystonia, but also random movements of the face, eyebrows and mouth. No intention tremor was seen. Metabolic pediatrician clinically saw no evidence for a disorder of metabolism. Analysis panel movement disorders and open exome analysis initially revealed no potential pathogenic variants (Radboud MC, Netherlands). Lumbar puncture, MRI and MR spectrometry revealed no abnormalities. At the age of 7 years reanalysis of exome data revealed a *de novo* missense in *DHDDS* (c.632G>A: p.Arg211Gln). Standard metabolic investigation in blood and urine has been performed including sialo-transferines, but no abnormalities were identified. She recently had her first seizures. They started with jerky movements from her right arm and in lesser extent her right leg. She remained conscious during this period. On video the movement are classified as cortical myoclonus. After half an hour her whole body cramped for one minute. She had several periods of these insults afterwards. Her movement disorder is described as jerky movements especially in action, partly related to ataxia, partly related to myoclonus. A recent EEG was of limited assessability, but no clear epileptiform activity is seen.

5) Individuals with *de novo* variants in *NUS1* (NM_138459.4):

indvKW; *NUS1* (c.743delA: p.Asp248Alafs*4): This is a male (8years, 9 months) whose had global developmental delay and currently shows moderate ID. He has language delay and is difficult to understand. Seizures started at the age of 12 months as generalized myoclonic epilepsy versus convulsive epilepsy, nocturnal jerks. EEG testing showed bifrontal epileptiform activity. The seizures are controlled with Levetiracetam. History of ataxia post doses of Levetiracetam was initially noted and was later resolved but incoordination persists. Following periods of increased seizure activity he has regression of language. Brain MRI (2y3mo) and array CGH were normal. Clinical exome sequencing (GeneDx) revealed a de novo frameshift mutation in NUS1 (NM_138459.4: c.743delA: p.Asp248Alafs*4).

HSJ0623; *NUS1* (c.128_141dup: p.Val48Profs*7): Individual HSJ0623 is a 15 years old boy and is the first child of a non-consanguineous French-Canadian couple. The pregnancy was unremarkable except for preterm contractions for which the mother was placed on bed at 7 months of gestation. The individual was born at term after an unremarkable delivery. He presented with low birth weight (2 489g, 3rd percentile) but without signs of perinatal distress (APGAR 9-10-10). He presented at 10 months of age with seizures and mild motor delay. His initial seizures were described as myoclonic absences with behavioural arrest, facial and palpebral myoclonus, lasting 5-10 seconds and occurring 5 times per day. The seizures responded to valproic acid. He had one febrile tonic-clonic seizure at 18 months of age. He presented with atonic seizures (drops) at 2 years of age, sometimes with a vague sensory aura, occurring up to 100 times per day. These seizures were quite refractory to treatment (various combinations of valproic acid, lamotrigine, levetiracetam, ethosuximide, clonazepam, carbamazepine and stiripentol), but responded to a combination of valproic acid and clobazam at age 7.5 years old. He has had only 2 seizures since 10 years of age on this combination. EEGs revealed diffuse background slowing with rhythmic bifrontal high amplitude rhythmic theta discharges. A video-EEG monitoring at 7.5 years revealed diffuse background slowing and epileptic discharges with bi 2082 frontal small amplitude spikes with secondary generalization manifesting as absence seizures or head drops. The walked at 16 months of age. However, he can now run, climb stairs, play sports. He is described as clumsy on fine motor tasks (he can eat and dress independently but needs help with buttons and zip and uses adapted pencils to write). He has moderate intellectual deficiency and was diagnosed with autism spectrum disorder (ADI and ADOS) at 7 years of age. He spoke his first words at one year of age and now communicates with short sentences. He can engage in brief conversations. He is known for inattention but has not tolerated the side effects of psychostimulants (Adderal and Ritalin). He receives special education in a TEACH classroom. On examination at 10 years of age, his head circumference was 56 cm (98th percentile), his height was 154 cm (>97th percentile). He has no dysmorphic traits and his neurological exam is entirely normal apart from mild postural and kinetic tremor without other signs of cerebellar impairment (no ataxia, dyskinesia, dysarthria, etc). On investigation, a brain CT scan at 18 months and a brain MRI at 8 years of age were unremarkable. The karyotype, CGH and Fragile X screens were normal. Serum lactate, ammonia, amino acid screen were normal, as was a urinary organic acid, purines/pyrimidines, creatine and GUAC profiles. *SCN1A* sequencing was negative. Whole genome sequencing revealed a *de novo* variant in *NUS1* (NM_138459.4:c.128_141dup: p.Val48Profs*7).

HSJ0627; *NUS1*: 1.3 kb del exon2: Individual HSJ0627 is a 29 years old woman and is the first child of Caucasian non-consanguineous parents of European descent. She was born at 8 months of pregnancy by C-section due to suspected fetal distress. The delivery was uneventful and the baby did not present apparent signs of a perinatal hypoxic-ischemic event. She presented at 2.5 years of age with febrile myoclonic status epilepticus. She than developed myoclonic absences with behavioural arrest and eyelid flutters as well as limb myoclonus, lasting less than 1 minute. She also developed sudden drop attacks, often with a premonitory feeling (malaise?), occurring 1-2 times per week. She received various combinations of valproic acid, levetiracetam, clobazam, felbamate, lamotrigine and clonazepam. At her last evaluation, her seizures were relatively well controlled with a combination of valproic acid, lamotrigine and clonazepam. Her EEGs revealed generalized spike-wave and poly-spike wave activity. This individual presented with a mild motor delay. She walked at 17 months of age. She can run, jump and swim but is unable to ride a bicycle. She is autonomous on fine motor skills: she can eat, dress and wash independently. Her writing is imprecise and clumsy. Her language skills seemed unaffected as she started talking around one year of age, has a fluent spontaneous speech with full sentences. She can read short sentences and functions at an equivalent of 1st grade. On examination at 29 year, the head circumference was at the 25th percentile. Eye pursuits were saccadic but saccades were normal. She had mild dysarthria, mild postural and kinetic tremors but no ataxia. Her reflexes were brisk. Brain MRIs were repeated 3 times and were unremarkable. An extensive metabolic screen was normal (including serum ammonia, lactate, amino acid screen, urinary organic acid screen and creatine/GUAC dosages). An array CGH was normal. Genome sequencing revealed a *de novo* intragenic deletion of ~1.3 kb in exon 2 of the *NUS1* (NM_138459.4).

6) Individuals with *de novo* variants in *RAB11A* (NM_004663.4):

HK055; *RAB11A* (c.71A>G: p.Lys24Arg): This male individual was born normally at term with normal birth weight 3710g, length 51 cm and occipitofrontal circumference (OFC) 35 cm (-0.5 SD); Apgar score 9/9. After the birth moderate muscular hypotonia was noticed and spinal muscular atrophy was excluded by molecular testing. Soon after the birth he showed good weight gain. At 5 months of age his length was 68 cm (0 SD), weight was 10.9 kg (+3 SD) and OFC 42.7 cm (-1 SD). His main clinical problem was muscular hypotonia. Brain MRI showed dilated lateral ventricles. At 13 months of age developmental delay became more evident. He turned and crawled, but did not sit, stand or walk. He said few words. He had 3 episodes of unconsciousness with perioral cyanosis in early infancy, but EEG showed normal background activity in awake and sleep state. Cardiac pathology was excluded by normal results in electrocardiography and ultrasound investigation. At 2 years and 1 month of age his height was 87 cm (0 SD) and weight 13.6 kg (0 SD). Acquired microcephaly was noticed – OFC 46 cm (-2.5 SD). He had muscular hypotonia, walked with the aid and had axial ataxia. He said only few words. Passive understanding of simple speech was evident. He showed aggressive behavior. He has rough and curly hair, hypertelorism, epicanthal folds, astigmatism, single palmar crease in the left side, abnormal fatty skin folds and inverted nipples. EEG and ENMG were normal. Griffith scale at 2years and 1 month showed delay in development corresponding to the age of 12 months. He is presently 5.5-year-old boy with moderate ID and microcephaly – his OFC was 49 cm (-2.5 SD). His height is 112 cm

(0 SD) and weight 30 kg (+4 SD), KMI=24 (obesity). He has a clumsy walk. Griffith scale-III at 5years and 2 months showed delay in development. His cognitive skills correspond to that of an age of 2 years and his social skill to that of an age of 2 years and 5 months. EEG showed abnormal background activity, but no epileptic charges. Brain MRI at 3 years of age showed bilateral widening of third and lateral ventricles, which indicate central brain atrophy and bilateral periventricular white matter damage; corpus callosum is relatively thin. Chromosomal microarray analysis showed no abnormal chromosomal copy number variations. Extensive metabolic investigations were done, which were in normal range (amino and organic acids in serum and urine, acylcarnitines and transferrine isoelectric focusing in serum, creatine and guanidinoacetate in urine and neurotransmitters in cerebrospinal fluid). Trio exome sequencing identified a novel de novo missense in *RAB11A* (NM_004663.4: c.71A>G: p.Lys24Arg).

HSJ0637; *RAB11A* (c.244C>T: p.Arg82Cys): Individual HSJ0637 is a 9.5 years old girl and the only child of a French-Canadian non-consanguineous couple. The prenatal ultrasounds revealed mild dilatation of the lateral ventricles (14-15 mm at 19 weeks of gestation), which stabilized to 13 mm at 21 weeks of gestation. A prenatal fetal MRI at 25 weeks of gestation was unremarkable, as was a prenatal fetal echocardiogram. The virology screens were negative but hematological investigations revealed maternal anti PLA1 antibodies, which were treated prenatally. The child was born by C-section at 37 weeks of gestation and did not display signs of perinatal distress (APGAR 8-8-9, birth weight and length at the 10th and 25th percentile, HC 33 cm (10th percentile). She presented a mild physiological jaundice, which resolved after a few days, as well as anemia and neutropenia that resolved spontaneously at a few weeks of age. She was referred for progressive post-natal microcephaly at 6 months of age (stabilized at the 2nd percentile since 10 month of age). The child presented seizures at 4 months of age consisting of erratic limb myoclonus followed by flexion spasms of the limbs and trunk. The initial EEG revealed abundant multifocal epileptic activity with sustained spike-wave discharges over both occipital areas. An EEG monitoring at 6 months revealed hypsarrhythmia and electrodecremential responses during spasms confirming the diagnosis of West syndrome. The epileptic spasms were refractory to therapy, including various combinations of nitrazepam, clobazam, vigabatrine (initiated at 6 months) and topiramate. The epileptic spasms subsided around 2 years of age. However, at 8 months of age, the child had developed focal seizures with behavioral arrest, horizontal nystagmus (towards the right), chewing automatisms and version of the head and trunk towards the right, lasting less than a minute. She was treated with combinations of topiramate, clobazam and valproic acid with partial responses. The seizures were controlled with the addition of levetiracetam at one year of age. Similar episodes of behavioral arrests with head deviation were noted after weaning the levetiracetam at 3 years of age, but an EEG monitoring during the episodes did not reveal concomitant epileptic activity and the episodes were considered non epileptic. Since the age of 3, this individual has had rare episodes of behavioral arrest and head deviation, lasting less than 2 minutes, and that seem to respond at least partially to stimulation. The parents have declined medication since that time. Of note, repeated EEGs (including one at 9.5 years of age) revealed diffuse slowing of the background rhythms with persistent multifocal epileptic activity with low amplitude spikes followed by large slow waves occurring in short bouts of 3-4 seconds at 2 Hz, in the frontal, temporal, occipital areas bilaterally and independently. Nonetheless, interictal EEGs at 2 and 5 years

old were unremarkable apart from diffuse background slowing. She had developmental regression at the onset of seizures around 4 months of age with reduced interest and hypotonia. She eventually learned to turn from back to front at 18 months of age, could sit with support at 2.5 years and sat independently at 3.5 years old. She has moderate to severe intellectual deficiency with autistic traits. She is non-verbal but uses a few communication signs. Her visual contact is limited. She likes musical games and action-reaction games but has relatively restricted interested otherwise. She brings objects to her mouth and can hold her bottle to drink. She has trouble swallowing and drools easily. She needs help for most daily tasks. On formal examination at 5 years of age, she had microcephaly (47 cm, 2^{nd} percentile) and a height of 109 cm (25^{th} percentile). There were no other dysmorphic traits. She has moderate axial hypotonia and tends to lean forward but can sit up if prompted. There were no signs of spasticity, the limb tone and reflexes were normal. She could lift her limbs against gravity and perform simple directed tasks. On investigation, a brain ultrasound at 3 months of age revealed increase subarachnoid spaces. A brain MRI at 6 months of age revealed progressive diffuse brain atrophy with enhanced lateral ventricles, partial agenesis of the corpus callosum (rostrum and splenium) and myelination delay. The spectroscopy revealed a decreased NAA signal. The karyotype and array CGH were normal as was an extensive metabolic screen (serum lactate, ammonia, CK, amino acid screen, acylcarnitine profile, glycosylation screen, and urinary purines and pyrimidines profile). The urinary organic acid screen revealed non-specific increases in oxalic and glyceric acids. A heart ultrasound and bone XR were normal. Sequencing of the MECP2, FOXG1 and CDKL5 genes were negative. An ophthalmology evaluation at 1 year of age revealed visual inattention (with absent P100 wave on visual evoked potentials). The auditory screen was unremarkable. Whole genome sequencing revealed a de novo missense in *RAB11A* (NM_004663.4:c.244C>T: p.Arg82Cys).

24631; *RAB11A* (c.461C>T: p.Ser154Leu): This male individual was born at term by caesarean section as breech presentation, following a normal pregnancy apart from borderline gestational diabetes. His birth weight and OFC were 4.3kg and 36.5cm, respectively. He had a urinary tract infection at 7 months, found to have a dilated left calix and ureter. He had numerous hearing tests due to concerns about speech delay, but all were normal. He has language delay; at age 4 he had some 2 word phrases and an array of single words. He rolled at 12 months and walked from 31 months. He was diagnosed with moderate developmental delay. He has limited attention span and is highly distractible. He did not have hypotonia or seizures. He was found to have a mildly raised phenylalanine level. Genetic analysis identified 2 variants in PAH, c.117C>G & c.805A>C, but these were not judged to be contributing to his delay. On examination aged 3 years & 11 months, height was 104.4cm (60th centile) and OFC was 50cm (18th centile). He shows bilateral frontal cowlicks, thin upper lip and single palmar crease on the left. Standard karyotype, Fish for 22q11, CGH array, all normal. MRI aged 6 showed partial agenesis of the corpus callosum, but was otherwise normal. Trio exome sequencing as part of the DDD study revealed a *de novo* missense variant in *RAB11A* (NM_004663.4:c.461C>T: p.Ser154Leu).

84049; *RAB11A* (c.461C>T: p.Ser154Leu): This female individual was born to non-consanguineous family. Possible poor fetal movements were noted but she was delivered at term by Caesarean (birth weight 3.43kg, OFC 36cm). She sat at 8 months but never crawled. She pulled to stand at 20 months

and walked at the age of 3 years. Her language skills are delayed. She had moderate GDD. At the age of 9 years, 11 months she was coping at mainstream school with one to one support. She had obesity from early on despite the fact that her parents were very strict as to intake and that she was active and not food-seeking. She has no history of seizures. Brain MRI was not done. aCGH, PWS methylation, UPD14 studies were all normal. Trio exome sequencing as part of the DDD study revealed a *de novo* missense variant in *RAB11A* (NM_004663.4:c.461C>T:p.Ser154Leu).

7) Individuals with *de novo* variants *GABBR2* (NM_005458.7) or *SNAP25* (NM_130811.3):

HSJ0048; *GABBR2* (c.2077G>T: p.Gly693Trp): Individual HSJ0048 is a 14 years old boy and the second child of a non-consanguineous French-Canadian couple. He was born at term after an uneventful pregnancy. There were no perinatal complications, the APGAR score was 8-9-9 and the birth head circumference was 34.5 cm (P50). He presented at 11 months of age with severe global developmental delay and seizures. The initial seizures were brief focal seizures with impaired awareness characterized by behavioral arrest and perioral cyanosis, which sometimes progressed to become bilateral tonic-clonic seizures, lasting 20-60 seconds. He was initially treated with carbamazepine but within a month he had clusters of epileptic spasms with bouts of flexion spasms of the trunk and limbs. The EEG at 1 year of age revealed modified hypsarrhythmia. Carbamazepine was therefore discontinued and vigabatrin was initiated, with complete resolution of the epileptic spasms. The seizures recurred around 4.5 years of age, with focal seizures with impaired awareness presenting as behavioral arrest, visual fixation, forced laughter, sometimes with a myoclonic jerk of the axial musculature, lasting less than 10 seconds. These seizures were refractory to valproic acid, but responded to topiramate. The seizures recurred after two years and were now longer (30 seconds to 1 minute), occurring 1-2 times per day, together with primary generalized tonic-clonic seizures lasting 1-3.5 minutes and occurring up to 8 times per day. The seizures did not respond to serial combinations of topiramate, carbamazepine, clobazam, phenytoin, levetiracetam and lacosamide. However, partial seizure control was achieved with the addition of lamotrigene at 13 years of age. At last follow-up, the child was receiving a combination of lamotrigene, lacosamide, clobazam and phenytoin and was still presenting brief generalized tonic-clonic seizures (<30 seconds) once per week, mostly during sleep. He presented a severe global developmental delay. He had axial hypotonia from the first few months of age. He started rolling from back to belly at 17 months of age and could sit with support around 2 years of age. He cannot sit on his own, does not crawl or stand. He has profound intellectual deficiency, remains non-verbal, has no communication skills, poor visual contact, grunts but does not point. He presents very limited interests for objects and people in his environment and does not reach for objects placed in front of him. He presents frequent bouts of aggressiveness and self-inflicted injuries despite treatment with benzodiazepines, antipsychotic medications (risperidal and clozapine) and clonidine. On examination at 12 years of age, the head circumference was 56 cm (90^{th} percentile). There were no dysmorphic traits but he presented occipital plagiocephaly. He had no eye contact, was drooling and had a tendency to keep his mouth open. He presented major axial hypotonia, necessitating truncal support to sit up, and thoracic scoliosis. He also had moderate limb hypotonia with hyporeflexia, but could lift his limbs against gravity. On investigation, a brain CT scan and a brain MRI at 13 months of age revealed increased sub-arachnoid spaces, mostly bi-frontal, as well as *ex vacuo* dilatation of the

lateral ventricles. The karyotype, sub-telomeres, Fragile X and 15q13 FISh studies were negative. A metabolic screen, including serum lactate, ammonia, amino acid VLCFA and transferrin glycosylation screens as well as the urinary organic acid screen were negative. An electromyogram was performed and was non-contributory, but a muscle biopsy revealed signs of underuse myopathy. Whole genome sequencing revealed a *de novo* variant in *GABBR2* (NM_005458.7: c.2077G>T: p.Gly693Trp).

HSJ0745; *SNAP25* (c.496G>T: p.Asp166Tyr): Individual HSJ0745 is a 23 years old male. He was born at term after an uneventful pregnancy from a non-consanguineous French-Canadian couple. He presented mild respiratory distress at birth, requiring nasopharygeal aspirations of meconium-tainted amniotic fluid, which resolved quickly (APGAR score 5-6-9). He was hospitalized in the first few months of life for recurrent apneas with cyanosis and bradycardia. The investigation, including EEG, eosophageal pH monitoring, ECG and Holter monitorings, were normal. The apneas resolved spontaneously at 6 months of age. He also presented with severe constipation in the first months of life, for which an extensive gastroenterological investigation was conducted and was found to be normal (including a rectal manometry, rectal biopsy and contrast imaging of the intestines). The child was followed in neurology since the first year of life for global developmental delay which evolved towards a moderate ID. He started crawling at 14 months of age and took his first steps at 2 years of age. He is now active and autonomous: he can run, climb, plays hockey and basketball and drives a bicycle. He has no major issues with fine motor skills: he eats, dresses and writes independently. He had a moderate language delay (first words at 2 years) but he now speaks fluently with full sentences. His speech is sometimes imprecise with some degree of speech dyspraxia. His receptive abilities are intact. He received special education and now continues training in a special school for adults with cognitive impairment. He recognizes written words but cannot read sentences. He can write his name and most letters but cannot write sentences. He can count but cannot do arithmetical calculations (no additions or subtractions). He developed epilepsy at 18 months of age with nocturnal tonic-clonic seizures and a few brief episodes of arrest of activity with confusion during daytime, suggestive of focal seizures with impaired awareness. The seizures were controlled with valproic acid but recurred upon weaning. He eventually required a combination of valproic acid and clobazam to control seizures, and the clobazam was weaned at the age of 18 years. He remains on valproic acid and has been seizure-free for the last 2 years. His initial EEGs revealed generalized spike-wave discharges that became almost constant during sleep (continuous spike-wave seizures during slow-wave sleep). The last available EEG at age 15 years revealed intermittent generalized discharges during drowsiness. On examination, he presents a few minor dysmorphic traits with upslanted short palpebral fissures, telecantus and short thumbs. He has speech dyspraxia. The neurological exam is otherwise unremarkable. A brain MRI at 20 years of age revealed mild diffuse cortical atrophy. The genetic investigation, including array CGH, Fragile X screen and *GRIN2A* gene sequencing was negative. His genome sequencing revealed a *de novo* missense in *SNAP25* (NM_130811.3:c.496G>T: p.Asp166Tyr).

Supplemental figures

Figure S1: % of the complete coding sequence (CCDS) and genome bases covered at ≥ 10x in the CENet DEE trios

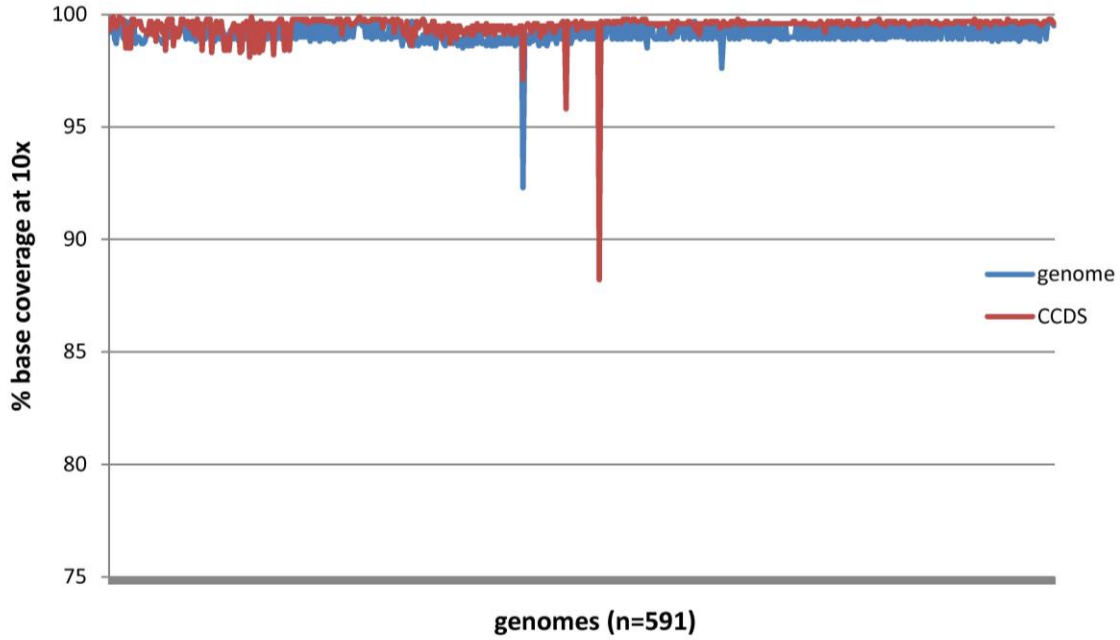
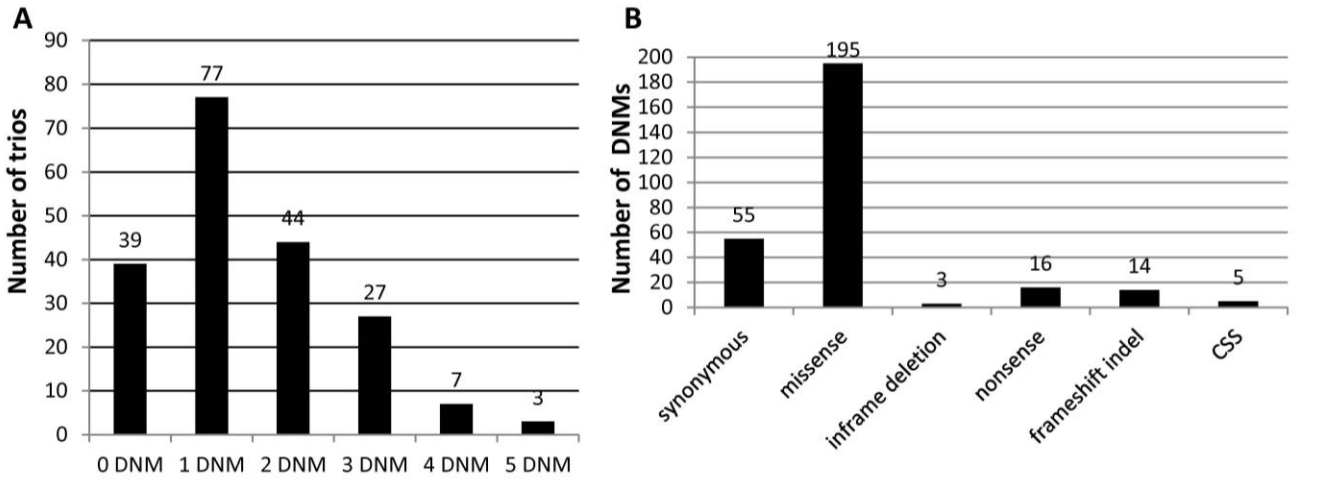


Figure S2. Number (A) and type (B) of Sanger-validated DNMs in the coding and canonical splice sites (CSS) per trio



Supplemental Tables

Table S1. Composition of the CENet DEE cohort used for WGS

DEE phenotype	**number of cases**
Unclassified developmental and epileptic encephalopathies (DEE)	84
Lennox-Gaustaut syndrome (LGS)	16
Infantile spasms (IS)	18
Early-onset epileptic encephalopathy (EOEE)	63
Myoclonic astatic epilepsy (MAE)	5
Childhood Absence Epilepsy	3
Dravet syndrome (DS)	3
Ohtahara syndrome (OS)	2
CSWS/Landau-Kleffner spectrum disorders	2
Early myoclonic epilepsy (EME)	1
Total	**197**

Table S2. Composition of the DEE cohort used for MIPs screening.

DEE phenotype	**number of cases**
Devastating epileptic encephalopathy in school-aged children (DESC)	1
Dravet syndrome (DS)	7
Epilepsy-aphasia syndromes (EAS)	36
Unclassified developmental and epileptic encephalopathy (DEE)	210
Epilepsy of infancy with migrating focal seizures (EIMFS)	1
Early myoclonic encephalopathy (EME)	2
Early-onset absence epilepsy (EOAE)	3
Early-onset EE (EOEE)	41
Electrical status epilepticus during slow-wave sleep (ESES)	1
Febrile infection-related epilepsy syndrome (FIRES)	10
Hemiconvulsion-hemiplegia epilepsy (HHE)	4
Infantile spasms (IS)	111
Lennox-Gaustaut syndrome (LGS)	57
Late-onset spasms (LOS)	2
Myoclonic astatic epilepsy (MAE)	100
Migrating partial seizures in infancy (MPSI)	3
Ohtahara syndrome (OS)	4
Progressive myoclonus epilepsy (PME)	2
Total	**595**

Table S3. DEE and ID cohorts used in the DNM meta-analyses

cohort	PMID	phenotype	number of trios	sequencing	total trios	
CENeT (current study)	-	DEE	197	WGS	624	5948
Halvardson et al. (2016)	27334371	DEE	39	WES		
Hino-Fukuyo et al. (2015)	25877686	DEE	14	WES		
Epi4K Consortium (2014)	25262651	DEE	356	WES		
Michaud et al. (2014)	24781210	DEE	18	WES		
DDD (2017)	28135719	ID	4293	WES	5324	
Lelieveld et al. (2016)	27479843	ID	820	WES		
Lopes et al. (2016)	26740508	ID	19	WES		
Gilissen et al. (2014)	24896178	ID	50	WES		
Hamdan et al. (2014)	25356899	ID	41	WGS		
de Ligt et al. (2012)	23033978	ID	50	WES		
Rauch et al. (2012)	23020937	ID	51	WES		

Table S4. Variants called per genome

variant type	average number of variants/affected individual (n=197)	average number of variants/parent (n=394)
SNVs	3790550	3789423
insertion	180005	179822
deletion	212653	212528
total genomic variants	**4183208**	**4181772**
stopgain_SNV	97	97
stoploss_SNV	43	42
frameshift_deletion	112	113
frameshift_insertion	163	163
missense_SNV	11005	11013
nonframeshift_deletion	135	135
nonframeshift_insertion	94	94
synonymous_SNV	11873	11885
total variants in coding regions	**23521**	**23542**