High rate of recurrent *de novo* mutations in developmental and epileptic encephalopathies

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ABSTRACT

Developmental and epileptic encephalopathies (DEE) are 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/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 of 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 life-time 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 may involve at least two non-exclusive mechanisms. In some cases, uncontrolled seizures can be detrimental to developing cortical networks, leading 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 slowing or regression, and can occur in a child with or without preexisting developmental delay.[5] In other instances, 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 prior to 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 characterized by both ID and epilepsy where both mechanisms may play a role.[5]

Recently, parent-child exome sequencing studies in sporadic DEE cases 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 novel 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. (2014) published a statistical framework that determined the rate of *de novo* variants per gene per class of variant [e.g., missense, nonsense, frameshift, canonical splice site (CSS)] that could be used to determine whether there is gene enrichment for a particular variant class in the studied cohort, thus providing evidence that the observed DNMs are likely implicated in the disease.[9] This strategy was recently successfully employed in meta-analyses of DNMs identified from various ID and/or developmental disorder sequenced trios to identify 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/deletions (indels)] and copy number variations (CNVs) affecting coding or splice site regions). To identify genes implicated in DEE, we performed meta-analyses combining the DNMs identified in our series along with 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 cases with DNMs in some of our prioritized genes, thus providing additional support for their implication in the disease. Based on these collective approaches, we provide herein evidence implicating DNMs in 8 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 approval by the ethics research boards and obtaining informed consent 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 AEDs over a 6-month period and an average of at least one focal, generalized tonic-clonic, myoclonic, epileptic spasms, tonic, atonic or absence seizure per month during the period of poor control; 2) intellectual disability or global developmental delay (GDD); 3) absence of malformations or focal/multifocal structural abnormalities on brain MRI; and 4) absence of parental consanguinity and family history of epilepsy, intellectual disability or autism in first-degree relatives. Each individual was classified into a specific epilepsy syndrome when possible (Table S1). The majority (~90% of cases) 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 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 comprised of 595 individuals with EEs 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 described.[12] We were also able to recruit, through various collaborations, additional subjects with DNMs in candidate genes identified from clinical or research exomes. Informed consent was similarly obtained from these individuals or their legal guardians.

Whole genome sequencing. WGS sequencing was performed at the McGill University and Genome Quebec Innovation Center as part of the Illumina Genome Network (IGN) and according to the IGN standard procedure. Briefly, genomic DNA extracted from blood samples was subjected to an additional cleaning step using ZR-96 DNA Clean & ConcentratorTM-5 Kit (Zymo) and then used to generate sequencing libraries using the TrueSeq DNA PCR-free library preparation kit and according to the manufacturer's procedure. Sequencing was done either on the HiSeq2000 (100 bp paired-end; 1 genome/ 3 lanes) or the HiSeq2500 (125 bp paired-end; 1 genome/ 2 lanes) such that a minimum final coverage of 30x was attained after data processing.

WGS data processing, variant calling and analyses. The Illumina sequencing reads were generated using bcl2fastq v1.8.4. Trimmomatic v0.32 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) using BWA-mem v0.7.10 to create a Binary Alignment Map file (.bam). Read set BAM files from different sequencing lanes for the same sample were merged into a single global BAM file using Picard v.1.123. Regions containing multiple base mismatches were realigned locally using Picard. Once local regions were realigned, Picard was also used to recalculate the read mate coordinates and to mark duplicates for removal. Individual base quality values were recalibrated using GATK v.3.3-0. Genotypes were called using GATK Haplotype-Caller and all variant calls were merged and recalibrated in three different sensitivity tranches using GATK and according to its recommended best practices. All variant sites were annotated using a custom version of Annovar.[13] Only variants whose positions were covered at ≥10x and supported by at least 4 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 vs 0.3, EVS (NHLBI Exome Sequencing Project; ESP) or ≤ 2% in the unaffected parents from the entire trio dataset. A variant segregation analysis (child-parents) was performed using an in-house script. Putative DNMs were identified by excluding those present in the genomes of the parents and those with a MAF ≥ 0.001 in ExAC. Potential *de novo* variants outside the exonic and splice consensus regions were further excluded if present in small-repeat regions (for SNVs and indels), in Alu regions (for indels) or had a SNV variant quality recalibration score (VQSRT) of ≥ 99.90-to-100.00 or an indel VQSRT different than PASS. The sequencing reads carrying putative DNMs were inspected visually in each trio 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 using 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, 5kb bin scans of the genome were used. CNV calls were filtered to exclude those with a size <1kb and qv (PopSV) or evidence set scores (Lumpy) ≤ 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 data set or in population controls from the 1000 Genomes or from the CNV map 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 1 algorithm, and thus likely enriched in 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 using standard qPCR (Taqman assay) and/or by Sanger sequencing.

Targeted sequencing using the Molecular Inversion Probes (MIPs) technique. Seven of our initially prioritized genes (*DHDDS, RYR2, HECW2, GABRB2, NUS1, NTRK2* and *CLTC*) were selected for MIPs 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 five base pairs of flanking sequence) in each of the seven genes. Single molecule molecular inversion probes (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. (2012)[19] except the ratio of probe to genomic DNA was adjusted to 2,000:1, a tenfold increase than previously reported. Sequencing was performed on an Illumina HiSeq2500 to generate 100 base pair pair-end reads. Raw read mapping and processing were performed as previously described.[12] Private variants (absent from SNP public databases: ExAC v0.3, EVS, and 1000 Genomes) predicted to affect the protein sequence (missense, nonsense, indels, and canonical splice sites) were validated using Sanger sequencing in the proband and the parents.

Gene-specific DNM enrichment. We used DenovolyzeR open access program to assess whether a specific gene is enriched in DNMs in subjects with DEE and/or GDD/ID.[20] This *R* package program is

based on gene-specific mutation rates.[9] DNM gene specific *p*-values for loss-of-function variants (LoF: nonsense, CSS, frameshift indels) and functional variants (missense + LoF) calculated by DenovolyzeR were further corrected for multiple testing based on the 19 618 genes with available mutation rates on which Denovolyzer based its calculation (Bonferroni correction) and the number of tests (2; for LoF and functional categories) (i.e, 2 x 19 618 = 39 236). A corrected *p*-value (c.*p*-value) <0.05 was considered statistically significant. To increase statistical power, a meta-analysis was performed combining DNMs identified herein along with those previously reported from trio whole exome sequencing (WES) done on other DEE cohorts.[8,21,22] We also performed another meta-analysis combining 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 10 trios were included in these meta-analyses (Table S3). To further increase the power to detect DNM-gene enrichment in genes whose mutations are not yet an established cause of DEE, we applied a similar strategy as Lelieveld et al (2016)[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 enriched with DNMs by Lelieveld et al. (2016) 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* used in the DDD Study[10,29] to calculate the probability of the proximity of *de novo* missense variants in genes of interest based on 1 million simulations weighted by the context trinucleotide rates. We considered a *p*-value < 0.01 as statistically significant.

RESULTS

We performed WGS on 197 individuals with DEE and their unaffected parents. The average coverage of the genomes was 37.9x, with 99% of the genome (GRCh37) bases covered at ≥10x (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, 5 indels) that passed IGV inspection (~75% of total DNMs calls) per individual, translating into a mutation rate of ~$1.2x10^{-8}$ DNMs per diploid genome/generation, which is

in the range reported from other WGS trio studies.[24,30-32]

We next focused our attention on the putative DNMs affecting the coding and the CSS that passed IGV inspection. We were able to validate by Sanger sequencing 95% of these calls. In total, 288 DNMs were validated (1.46 DNM/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 DNM in the coding or CSS regions of 39 probands (20%) (Figure S2A). Considering only *de novo* SNVs, 7.8% are predicted to cause a loss of function (nonsense and CSS variants) while 72% 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 autism spectrum disorders (ASD; 66.5% missense, 4.8% LoF)[33] or in Icelandic controls (82% missense, 2.7% LoF).[32] We found an excess of LoF SNVs in our EE subjects when compared to these control sibling exomes (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, all of which 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; 6 of these putative CNVs were confirmed *de novo* by qPCR, 17 were inherited and 12 were false positives. In total, 10/18 validated *de novo* CNVs affected exonic regions, including 5 deletions and 5 duplications (Table S6).

Likely pathogenic variants identified in the CENet series

For all DNMs and rare recessive variants (bi-allelic, X-linked hemizygous) affecting the coding regions or CSS, we assessed the involvement of the corresponding genes in epilepsy or related neurodevelopmental disorders by searching Pubmed [Gene name and ("epileptic encephalopathy" or "epilepsy" or "seizure" or "mental retardation" or "intellectual disability")] and verifying the gene's OMIM description. Using the ACMG 2015 guidelines for sequence variant interpretation,[34] we initially identified pathogenic or likely pathogenic variants in 50/197 subjects (25%) in genes which, 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 3 individuals, including a 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-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 MIPs sequencing

From the WGS results of our first 120 DEE trios, we prioritized 7 of our best candidate genes (*CLTC, DHDDS, GABRB2, HECW2, NTRK2, RYR2* and *NUS1*) for targeted resequencing in 595 unsolved DEE cases. These genes were selected based on the documentation of predicted-damaging DNMs in at least 2 unsolved individuals from the CENet series or in 1 unsolved individual from the CENet series and in at least 1 previously reported case 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 ≥10x), possibly due to its high GC content. On average, 90% of the target bases were covered at ≥10x in 476 samples. Reduced coverage was obtained in the remaining 119 cases such that only 70% of the target bases reached the ≥10x, probably due to poor DNA quality. Four predicted-damaging missense variants absent from ExAC were identified, each in a single DEE subject, in *NTRK2* (NM_006180.4:c.1301A>G: p.Tyr434Cys), *GABRB2* (NM_021911.2:c.730T>C:p.Tyr244His and c.911C>T: p.Ala304Val), and *HECW2* (NM_020760.1:c.4484G>A: p.Arg1495Lys). These variants were validated as *de novo* by Sanger sequencing. Interestingly, 2 of the DNMs affecting GABRB2 (p.Tyr244His) and NTRK2 (p.Tyr434Cys) were also recurrent in the CENet series. The missense (p.Arg1495Lys) in HECW2 is also recurrent as 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 individuals with DEE or ID who carry DNMs in the candidate genes that were prioritized for MIPs sequencing by mining GeneMatcher[36] and DDD/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 trio WGS and targeted sequencing strategy led to the identification of 3 individuals with DEE carrying *de novo* predicted-damaging missense variants in *NTRK2* (NM_006180.4), including an individual with c.2159C>T (p.Thr720Ile) variant and 2 unrelated individuals with the same c.1301A>G (p.Tyr434Cys) variant. In addition, we identified 2 other individuals with the *de novo* p.Tyr434Cys missense through clinical WES.

In total, we identified 4 individuals with the p.Tyr434Cys missense. All subjects with this missense variant had severe GDD/ID and optic nerve hypoplasia with visual impairment, and 3 had

significant feeding impairment (Table 2; Supplemental Note). Three of them presented with epileptic spasms in the first few months of life and subsequently developed intractable seizures of various types associated with multifocal epileptic activity on EEG whereas the remaining individual had startle-like myoclonic events at 12 hours of life and developed, at 5 years of age, focal seizures with impaired awareness occasionally evolving towards bilateral tonic clonic seizures. Clustering analysis using the *Denovonear* algorithm indicated that the presence of the p.Tyr434Cys variant in 4 individuals with similar phenotypes is statistically significant ($p = 0.0001$).

The subject with the p.Thr720Ile missense mutation had moderate-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, she had hyperphagia and early-onset obesity from the age of 3 years. Interestingly, Yeo et al. (2004) reported an individual carrying the *de novo* c.2165A>G (p.Tyr722Cys) variant, affecting an amino acid residue adjacent to Thr720, who presented with a similar phenotype as that of our subject, including 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 the brain derived neurotrophic factor (BDNF) and for neurotrophin-4 (NT4). BDNF-TrkB signalling is a critical regulator of neuronal development and function.[40] The p.Tyr434Cys variant is located at the beginning of the transmembrane domain (TM) of NTRK2 (Figure 1A). The fact that this *de novo* variant has been identified in 4 cases 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 signalling.[38] It is currently unknown whether the p.Thr720Ile affects NTRK2's function in a similar way but its proximity to p.Tyr722Cys and the similarity in the phenotype of both individuals carrying these mutations suggest that this could be the case. Interestingly, mice expressing 25% of TRKB levels are hyperphagic and have excessive weight.[41] Altogether, our findings unequivocally show that DNMs in *NTRK2* cause DEE.

GABRB2. Our WGS and MIPs screens identified 3 individuals with DEE carrying DNMs in *GABRB2* (NM_021911.2), including the c.911C>T (p.Ala304Val) variant in one subject and the recurrent c.730T>C (p.Tyr244His) variant in 2 subjects. Two other individuals with DNMs in *GABRB2* were identified by the DDD study[10], one with the c.830T>C (p.Leu277Ser) variant and another with the c.373G>A (p.Asp125Asn) variant. We also identified from WES and targeted gene panel sequencing 6

individuals with *de novo* missense variants in *GABRB2*, including one with the same c.830T>C (p.Leu277Ser) as that 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) adjacent to c.911C>T (p.Ala304Val) identified in our MIPs 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 with a *de novo* frameshift in *CHAMP1* (NM_032436.2: c.1876_1877delAG:p.Ser626Leufs), which also likely contributes to the cognitive impairment of the subject (F3-II.1 in Isidor et al. 2016).[42] All of these *de novo* missense variants are predicted 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; 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 cases had microcephaly (n=7/11), which was acquired in 6 individuals and congenital in the 7th. Most individuals developed refractory seizures within the first year of life, with a preponderance of myoclonic seizures and absences, sometimes evolving towards myoclonic status epilepticus or non-convulsive status epilepticus. Some individuals developed focal seizures with impaired awareness or autonomic seizures, tonic, atonic seizures, and rarely generalized tonic-clonic seizures. In half of the cases, the epilepsy remained refractory despite multiple drug trials. Two individuals were trialled on vigabatrin with marked deterioration. Responses were observed to lamotrigine, valproate, levetiracetam or high dose steroids in 5 individuals. Axial hypotonia, spasticity, dystonia and choreoathetosis appear to be common features. Cortical visual impairment was present in 3/11 cases. Brain MRIs were usually normal, except for delayed myelination or diffuse T2 hypersignal in the subcortical white matter, as noted in 3 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] Mutations in other $GABA_A$ receptor subunits encoding *GABRA1* (OMIM 137160), *GABRB1* (OMIM 137190), *GABRB3* (OMIM 137192) and *GABRG2* (OMIM 137164) are established causes of DEE. Three cases with DNMs in *GABRB2* and detailed phenotypic information have been previously published: one of these subjects, who carries the DNM p.Met79Thr, also found in one of our cases, 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] [45,46] Four additional cases with *de novo*

missense in *GABRB2*, including c.845T>C (p.Val282Ala), c.863T>G (p.Ile288Ser), c.909G>T (p.Lys303Asn), 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 mutated in our series. These 4 individuals appear to show ID/GDD and epilepsy but no detailed clinical information was available.

Out of the 13 DNMs in *GABRB2* previously reported or described herein, 10 are clustered within a stretch of 60 amino acids (positions 244-304) encompassing 3 transmembrane domains and/or their boundaries (*p*-value = 0.000002, *Denovonear*) (Figure 1B). These clustering mutations appear to be mostly associated with severe GDD/ID and, with the exception of p.Arg293Pro, intractable generalized seizures and DEE. So far only 1 of these *de novo* missense variants, p.Thr287Pro, has been functionally tested in transfected HEK293 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 may confer specific property to the protein such as gain-of-function or dominant negative effects. Collectively, the cases with DNM in *GABRB2* reported here along with the previously published ones confirm that *de novo* missense mutations in *GABRB2* can cause a DEE phenotype.

CLTC. Our WGS trio screen identified a *de novo* frameshift variant in the *CLTC* gene (NM_004859.3), c.4575dupA (p.Val1526fs*18), in an individual with moderate ID associated with severe refractory seizures (absences, myoclonic, tonic, generalized tonic-clonic and focal seizures). We obtained detailed clinical information on 11 additional cases with DNMs in *CLTC*, 4 of which were identified by the DDD study[10], while the other 7 we identified through clinical WES (Table 4; Supplemental Note). We were able to obtain detailed clinical information for all of these 12 individuals. Most individuals presented with early-onset hypotonia and GDD, evolving towards mild to severe ID (or borderline intelligence). Four individuals also developed ataxia. When performed, neuromuscular investigations (EMG, biopsy) were negative. Two individuals had pharmaco-resistant epilepsy, with 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/ID with seizures, starting between the ages of 1 and 2 years, that were well controlled with valproate or levetiracetam. Interestingly, 3 of the ID cases (one sequenced by the CAUSES Study, a second in the context of the Undiagnosed Patient Program [UPP] at OPBG, Rome) had a recurrent *de novo* missense (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 4 independent cases 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] Two additional DNMs were also reported by Leliveld et al. (2016)[11] from their study of 800 probands with ID, including c.4615C>T (p.Glu1539*) and c.3621_3623del (p.Asp1207del), the latter being also identified in one of our DEE cases. *CLTC* is predicted intolerant to LoF mutations with a pLi score of 1.00 according to ExAC.[54] The phenotypic spectrum associated with these cases is heterogeneous ranging from mild ID or learning disability to severe ID or DEE. Interestingly, individuals with refractory epilepsy were found to carry mutations in the first section of the clathrin LC binding domain, whereas *de novo* truncating mutations at the C-terminus of CLTC tended to be associated with hypotonia, GDD and ID (Figure 1C).

DHDDS. WGS identified a *de novo* missense variant (c.110G>A: p.Arg37His) in *DHDDS* (NM_024887.3) in of one of our DEE individuals (HSJ0762). We identified, by clinical WES, another individual with EE who carries the same *de novo* p.Arg37His. Interestingly, this missense lies adjacent to p.Arg38His, which has been reported in a DEE case from the Epi4K study.[7] Clustering analysis indicated that the presence of these two *de novo* mutations in 3 individuals is statistically significant (p=0.0005, *Denovonear*). In addition, we identified by clinical/research WES two other individuals with DEE and a *de novo* missense 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 DNMs in DHDDS are shown in Figure 1D and their associated phenotype are summarized in Table 5 and detailed in the Supplemental Note.

These 5 individuals with DNMs in *DHDDS* presented with a generalized epilepsy disorder with myoclonic seizures, either as myoclonic absences or as isolated cortical myoclonus, sometimes with light-sensitivity or fever susceptibility. Two of these individuals also presented other generalized seizure types, including atonic seizures or generalized tonic-clonic seizures. In 3 cases, the EEG revealed clear generalized spike-wave discharges (in one case with additional photosensitivity). The seizures were aggravated by levetiracetam in 2 cases, but favourable responses to valproic acid were observed. Interestingly, all cases presented with marked hypotonia and 4 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 evolutionary conserved stretch of 5 amino-acids (pos. 34-38) corresponding to the catalytic domain of the enzyme (Figure 1D). Based on the crystal structure and mutagenesis studies done on the bacterial Dhdds enzyme (*M. luteus* ndecaprenyl diphosphate synthase, UDPS), 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 with retinitis pigmentosa.[58,59] In addition, bi-allelic truncating/splicing variants in *DHDDS* were reported in a case of type I congenital disorder of glycosylation with severe GDD and refractory seizures.[60] We hypothesize that null alleles of *DHDDS* disrupt brain development only in in the context of a recessive genotype whereas the DNMs documented in our study cause DEE via a dominant-negative or gain-of-function mechanism.

NUS1. We identified 2 DNMs in *NUS1* (NM_138459.3), each in individuals from our WGS trio study, including a frameshift variant in exon-1 (c.128_141dup: p.Val48Profs*7) and a ~1.3 kb deletion encompassing the entire exon-2 of *NUS1* in the other case. 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; Figure 1E). These cases with DNMs in *NUS1* all presented with GDD (or isolated motor delay), evolving towards mild to severe ID. Furthermore, they all presented generalized myoclonic seizures (in one case with myoclonic status epilepticus), combined with myoclonic absences in 2 cases. In all cases, other generalized seizure types were observed, including atonic seizures (drop attacks) or generalized tonic-clonic seizures. EEGs revealed either generalized epileptic activity or bi-frontal epileptic discharges. Movement disorders were also common, including tremor (2 cases) and ataxia (one case). Together, this clinical phenotype is highly reminiscent of the one we observed in cases with DNMs in *DHDDS*.

NUS1 encodes the 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, thus having the potential of inducing 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 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 2 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 as well as 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. (2015) reported cases with early onset seizures and microdeletions of chromosome 6q22.1, which are centered on a 250 kb critical region that only includes *NUS1* and the promoter of *SLC35F1*.[63]

Collectively, our finding of DEE cases with 2 truncating DNMs and 1 *de novo* whole exon deletion in *NUS1*, the reported DEE cases 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 dataset where no such LoF mutations were reported (pLi = 0.87).[54] The more severe phenotype previously observed in the siblings with the homozygous p.Arg290His mutation could be due to a more dramatic reduction in NUS1 activity due to the recessive nature of a potentially hypomorphic mutation. Failure to identify other cases with *NUS1* truncating mutations from the MIPs screen or other EE published trios maybe in part due to reduced capture efficiency of exon-1, which encodes 137/293 amino acids of NUS1 (~ 47%). Indeed, in the ExAC database, exon-1 of *NUS1* is, on average, poorly covered by WES compared to the rest of exons of this 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 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 DEE trio studies (combined DEE trios = 624; Table S3). In total, 12 genes were found to be statistically enriched for LoF and/or functional DNMs (Table 6); mutations in all of these genes are now considered causative of DEE.

As epilepsy is a frequent co-morbidity 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: 5948 trios: 7778 DNMs). In total, 111 genes were found enriched for functional and/or LoF DNMs, 37 of which were found mutated in at least one DEE case (Table S8). Interestingly, DNM enrichment has not been previously documented for 22/111 genes, including 9 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), *SF1* (OMIM 601516)] or have been described only in single or few cases, therefore representing candidates pending additional evidence [(*CLTC* (OMIM 118955),[52] *GABBR2* (OMIM 607340),[12,27] *PHIP* (OMIM 612870)].[64] Among these only *GABBR2, PHIP* and *CLTC* had some DNMs in DEE cases, while the rest had DNMs only in the ID cohorts (Table S8).

Lelieveld et al. (2016) recently showed increased power to detect novel 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 here a similar strategy to both the DEE-ID cohort and excluded individuals with DNMs in any of the genes mentioned in the list established by these authors.[11] We also removed the cases with DNMs in genes that showed DNM enrichment from the recent meta-analyses done on ID/developmental disorder trios.[10,11] This retained 4424 trios from the combined DEE-ID cohorts. As a result, 3 additional genes from the DEE-ID cohort showed modest but significant functional DNM enrichment, including *GABRB2* (OMIM 600232; *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, SNAP25* in DEE

Our meta-analyses of EE-ID trios showed significant enrichment of DNMs in *GABBR2, PHIP, CLTC, RAB11A, SNAP25* and *GABRB2*, genes whose mutations have not yet been confirmed as a cause 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 cases in the context of our MIP screen or other WGS/WES studies (see above). As shown below, we also provide additional evidence for the involvement of *RAB11A*, and *GABBR2* and *SNAP25* in DEE.

RAB11A **(**NM_004663.3). We found a *de novo* predicted-damaging missense variant in *RAB11A* (c.244C>T: p.Arg82Cys) in a CENet case with refractory epileptic spasms and erratic

myoclonus with developmental regression. She subsequently developed focal seizures and severe ID. We also identified by WES another predicted-damaging *de novo* missense in *RAB11A* (c.71A>G: p.Lys24Arg) in an individual with moderate GDD and an abnormal EEG but with no seizures reported so far. Three additional individuals with DNMs in *RAB11A* were identified in the context of the DDD study[10], including 2 individuals with the same variant (c.461C>T: p.Ser154Leu) and another individual with a different variant (c.39A>C: p.Lys13Asn). We were able to obtain detailed clinical information on the cases with the p.Ser154Leu variant that both showed moderate GDD without epilepsy. The other individual from the DDD study had abnormalities of the nervous system according to the Decipher database 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; 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 mutations do not affect any of the nucleotide-sensitive switch domains of RAB11A (Figure 1F). The fact that *RAB11A* is enriched in DNMs in the DEE-ID cohorts and found with a recurrent *de novo* missense in 2 cases 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 in *GABBR2* (NM_005458.7), c.2077G>T (p.Gly693Trp), in one subject of our CENet cohort 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; supplemental Note). The Epi4K consortium reported 2 *de novo* predicted-damaging missense variants (c.2114T>A: p.Ile705Asn and c.2084G>T: p.Ser695Ile) in *GABBR2* in two unsolved cases with infantile spasms.[8] Lopes et al. (2016) recently reported a *de novo* missense 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, present both at the presynaptic and post-synaptic membrane where it regulates neurotransmitter release and the activity of ion channels.[71] The receptor is the target of baclofen, a medication often used to treat spasticity. The hypotonia and hyporeflexia

observed in our case might therefore reflect underactivation at the neuromuscular junction or in spinal motor control centers. Interestingly, unlike the missense identified by Lopes et al., which affects transmembrane domain 3 (TM3) of GABBR2, the 3 DNMs in cases with infantile spasms cluster together in the TM6 of the protein (p = 0.001, *Denovonear*), suggesting that these TM6 mutations are specific for DEE. Meta-analysis of DNMs from DEE-ID cohorts showed an enrichment in 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 2 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 their location in the protein.

SNAP25. We identified from our WGS a *de novo* missense in *SNAP25* (NM_003081.3/ NM_130811.2:c.496G>T: p.Asp166Tyr) in a male with DEE. He presented with apneas, GDD, nocturnal generalized tonic-clonic seizures and focal seizures with impaired awareness and progressed towards moderate ID (Table 7, Supplemental Note). SNAP25 is a member of the SNARE complex that is required for the exocytosis of neurotransmitters during synaptic transmission by mediating synaptic vesicle fusion.[72,73] Developmentally regulated alternative splicing of 2 similar exon 5 sequences of *SNAP25* generates 2 isoforms (a and b), differing only by 9 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 mutations are known to cause DEE.[26,76] So far, only 2 single cases have been published with *de novo* mutations in *SNAP25*, including one with a missense affecting both isoforms (NM_003081.3/ NM_130811.2: c.142G>T: Val48p.Phe) found in a case with DEE[77] and another with a missense affecting a conserved residue in exon 5 of only *SNAP25b* (NM_130811.2: c.200T>A: p.Ile67Asn) 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 mutation identified in our cohort (p.Asp166Tyr) is predicted damaging (SIFT, polyphen-2, 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* were recently reported in the DDD study [NM_130811.2: c.118A>G p.(Lys40Glu); c.127G>C (p.Gly43Arg); c.520C>T: p.(Gln174*)] but no detailed clinical information were 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 LoFs (n= 56 DNMs; missense = 43, LoF = 13 ratio; missense/LoF = 3.3).[8] Interestingly, these observed *de novo* missense/LoF ratios of pathogenic/likely pathogenic variants in both the CENet (p = 0.004, 2-tailed Fischer's exact test) and Epi4K (p=0.0004, 2-tailed Fischer's Exact test) series were significantly increased when compared to those similarly observed in 192 moderate-to severe ID published trios (WES and WGS) with detailed phenotypic and pathogenic variant information (36 missense/42 LoF = 0.85).[23,24,26,28] Remarkably, out of all the pathogenic/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/likely pathogenic DNMs was significantly higher in the CENet DEE series compared to that identified from the exomes/genomes of the 192 previously published moderate-to severe ID trios used in the above comparison [out of 80 pathogenic/likely pathogenic variants only 19 (13 missense, 6 LoF) were also reported independently in ClinVar] (p = 0.0012, 2-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 cases (27%), including 50 cases with point mutations in genes previously found to be causally linked to DEE/ID, 1 with a recurrent pathogenic CNV (15q11-q13 duplication) and 2 cases with CNVs encompassing genes previously associated with ID or DEE (*PCDH19* and *DNMT3A*). Moreover, we were able to explain DEE in 10 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 likely been higher in an unbiased series since many of our subjects had previously been screened by clinical targeted sequencing and/or array genomic hybridization. Interestingly, 2 of the 4 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), the remaining cases being 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 individuals from other series that were primarily ascertained because of ID. 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. (2017) recently showed that genes with LoF mutations compared to genes with missense mutations were associated with different pathways across neuro-developmental disorders such as ID, autism spectrum disorder 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 when compared to genes with LoFs. Altogether, this data raises the possibility that the genetic landscape of DEE is enriched for gene products that act as protein hubs. It would be important to understand why these hubs are specifically associated with DEE.

Of the 8 genes highlighted herein for their involvement in DEE, we were not able to show *de novo* gene-enrichment for 3 of them, *NTRK2, DHDDS* and *NUS1*. However, the multiple occurrences of DNMs affecting the same conserved amino acid residues in *NTRK2* and *DHDDS* in cases with a similar phenotype, nonetheless, represents a strong evidence implicating these 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 variants that can induce haploinsufficiency. It is thus likely that meta-analyses involving larger number of subjects will be necessary to identify these rare forms of DEE. No DNM enrichment was observed for *NUS1* in our meta-analysis, possibly due to the poor capture of its exon-1, which represents almost half of the entire coding sequence of this gene. However, the identification of 3 DNMs in *NUS1* (including 2 truncating variants and a microdeletion) in DEE cases 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. This is the case for: 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, GABRB2 and GABBR2 both belong to the family of GABAergic receptors, which include other members involved in epilepsy [*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 raise the possibility that many of the major pathways involved in DEE have been identified. Stratification of genetic forms of EE based on the involvement of these pathways may facilitate the development of tailored therapies.

Supplemental Data

Supplemental data include a Supplemental Note, 2 figures, and 8 tables.

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WEB RESOURCES

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
1000 Genomes Project, http://browser.1000genomes.org/index.html
ExAC Browser, http://exac.broadinstitute.org/
GoNL browser, http://www.nlgenome.nl/search/
GATK Best Practices, https://software.broadinstitute.org/gatk/best-practices
ESP exome variant server (EVS): 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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Figure 1. Localisation of *de novo* mutations in protein domains of the genes of interest. A) GABRB2, B) CLTC, C) NUS1, D) NTRK2, E) DHDDS, SNAP25, GABBR2 and RAB11A. Recurrent DNMs are in italics and red font. The transmembrane domains of GABRB2 and GABBR2 are labeled 1-4 and 1-7, respectively. TM, transmembrane domain; TD, trimerization domain; SP, Signal Peptide; LRRNT, Leucine Rich Repeat N-Terminal domain; LRR, Leucine-rich Repeats; 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; CC, prenylation residues.

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)*
CSS	*SCN1A(1), SCN8A (1)*	-
de novo CNVs (n=4)	**(n = 3)**	**(n = 1)**
deletions	*del(exons 21-23) of DNMT3A (1), del-encompassing PCDH19 (1)*	*del(exon 2) of NUS1 (1)*
duplications	*dup-encompassing UBE3A (1)*	-
Inherited recessive SNVs/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 parenthesis; CSS, canonical splice site variant.

Table 2. Summary of the clinical features in cases with DNMs in *NTRK2* (NM_006180.4)

Individual/Gender/Age[a]	De novo variant (detection)	Cognitive and behavioral	Epilepsy diagnosis	Age at Seizure onset	Seizure types	AEDs	EEG	Brain MRI	Associated neurological features /seizure outcome
HSC0103/M/2y9m	c.1301A>G (p.Tyr434Cys) (WGS[b])	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/M/6y3m	c.1301A>G: p.Tyr434Cys (cWES[c])	Severe ID; ASD;	DEE	12hrs with recurrence at 5 y	M, FIA	OXBZ, DZP	DS and TIRDA	Optic nerve hypoplasia	Hypotonia; lower limbs spasticity; visual impairment; seizures controlled on OXBZ for one month.
T25821/F/4y7m	c.1301A>G: p.Tyr434Cys (MIPs)	Severe GDD and severe ID;	IS	4 m	ES, To	Prednisol., 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/M/4y3m	c.1301A>G: p.Tyr434Cys (cWES[c]; WGS[d])	Severe GDD, suspected severe ID; ASD;	IS	4 m	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/F/9y	c.2159C>T : p.Thr720Ile (WGS[b])	GDD; mod.-severe ID; ASD	DEE	2.5 y (febrile sz at 23 m)	Febrile, FIA, GTC, SE	CLB, *LEV*, TPM, VPA, CBZ	Normal; DS post status	Delayed myelination, reduced WM, ventriculomegaly, thin CC	Swallowing difficulties; hyperphagia after 3 years of age; seizure-free for 2 years under CBZ.

[a]age at last examination (years, y; months, m). [b]CENet. [c]GeneDx. [d]HudsonAlpha Study. cWES, clinical WES. IS, infantile spasms; seizures: Fo, focal; FIA, focal impaired awareness; ES, epileptic spasms; M, myoclonus; To, tonic; GTC, generalized tonic-clonic.; SE, status epilepticus. AEDs, anti-epileptic therapies: 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; Prednisol., prednisolone; RFN, rufinamide; TPM, topiramate; VGB, vigabatrin; VPA, valproic acid; ZNS, zonisamide; underlined, treatment with clinical response (decreased seizure frequency or severity); in italics, negative response (aggravation of seizure frequency/severity). EEG: Hyps., hypsarrhythmia; DS, diffuse slowing; MF, multifocal; TIRDA, temporal intermittent rhythmic delta frequency activity;. MRI, magnetic resonance imaging: WM, white matter tracts; CC, corpus callosum.

Table 3. Summary of the clinical features in cases with DNMs in *GABRB2* (NM_021911.2)

Individual/Gender/Age[a]	De novo variant (detection)	Cognitive/ behavioral	Epilepsy diagnosis	Age at Seizure onset	Seizure types	AEDs	EEG	Brain MRI	Associated neurological features /seizure outcome
1242500 /F/9.3y	c.236T>C: p.Met79Thr (cWES[e])	GDD, Severe ID	DEE	11 m	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/ F/10y	c.373G>A: p.Asp125Asn (WES[f])	GDD, Mod. ID	DEE	6 y	Febrile, GTC	VPA	ND	Normal	Acquired microcephaly; seizure free (responded to VPA, off medication).
indvLB/ F/1.5y	c.878G>C: p.Arg293Pro (WES)	GDD	No seizures	NA	NA	NA	Normal	Normal	Severe psychomotor delay, generalized dyskinesia, dystonia, cortical visual impairment.
CNSA01 M/4y	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 WM hyper T2 at birth and 18m	Acquired microcephaly, neonatal feeding difficulties, nonambulatory, hypotonia, spasticity, dystonia; rare seizures under TPM.
T21213B F/ 14y 6m	c.911C>T: p.Ala304Val (MIPs)	GDD, Severe ID	DEE	4y	M, A, At, non-convulsive SE	CLB, *VGB*, Pred, TPM, HCT, VPA, LTG, LEV, CZP, SULTH	biF SW or sharp SW	Normal	Acquired microcephaly, nonambulatory, hypotonia, intractable seizures.
HSJ0753/ F/4y	c.730T>C: p.Tyr244His (WGS[b])	Severe GDD	DEE	4m	M, GTC, MSE	LEV, VPA, TPM, B6, DZP, CLB, PB, PHT, *CBD*, KD	biF SW; Hyps.; continuous diffuse SW	Normal (9d and 1y)	Acquired microcephaly, nonambulatory, axial hypotonia, spasticity, nystagmus, cortical visual impairment; intractable seizures.
T23211/ F/5y1m	c.730T>C: p.Tyr244His (MIPS)	GDD, Severe ID	DEE	< 5 m	To, Focal, 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.
HA076/ M/15y8 m	c.830T>C : p.Leu277Ser (WES)	GDD, Severe ID	DEE	4y8m	M, At, A, GTC	VPA, TPM, CZP, CLB, LEV, LTG	slow; rhythmic notched slow waves	At 2y: MF hyper T2 WM; normal at 4y and 9y	Spasticity, poor coordination, broad-base gait; seizure control with LVT + LTG.

G64518/ F/10 y	c.830T>C : p.Leu277Ser (WES[f])	GDD, Severe ID	DEE	2y	GTC, A, Febrile	VPA, LTG	high ampl. rhythmic slow waves	Mild incr. LV at 2y; N at 3 y	Acquired microcephaly, brisk reflexes, seizure control with LTG.
31841 M/17d	c.851C>A: p.Thr284Lys (WES)	Severe GDD	EME	7d	M, To	PB, LEV, MDZ, biotin, FOL, B6	BS	Normal	Hypotonia, jitteriness, back arching, apneas, intractable seizures; deceased at age 17 days.
3001866 /F/21m	c.946G>A: p.Val316Ile (cWES[e])	Language delay	DEE	12mo	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. 2016); [a]age at last examination (y, years; m, months; days, d). [b]CENet. [e]BCM-Miraca. [f]DDD Study. NA, not applicable. MAE, myoclonic astatic epilepsy. EME, early myoclonic encephalopathy. Seizures: A, absence; At, atonic, GTC, generalized tonic clonic; SE, status epilepticus; MSE, myoclonic status epilepticus. To, tonic, M, myoclonus. AEDs: CZP, clonazepam; FOL, folinic acid; HCT: hydrocortisone; LTG, lamotrigine; MDZ, midazolam; NZP, nitrazepam; P5P, pyridoxal 5-phosphate; Pred., prednisone; SULTH, sulthiam. EEG: biF, bi-frontal predominance; BS, burst-suppression; SW, spike wave. MRI: WM, white matter tracts; LV: lateral ventricles See footnote of Table 2 for other abbreviations.

Table 4. Summary of the clinical features in cases with DNMs in *CLTC* (NM_004859.3)

Individual/Gender/Age[a]	De novo variant (detection)	Cognitive/ behavioral	Epilepsy diagnosis	Age at Seizure onset	Seizure types	AEDs	EEG	Brain MRI	Associated neurological features /seizure outcome
PBSD F/11y2m	c.977_980delCAGT: p.Ser326Cysfs*8 (cWES[c])	GDD, borderline IQ at 5y	No seizures	NA	NA	NA	NA	hyperT2 WM (Hypomyelination)	ADHD, impulsivity and poor socialization skills; Mild hypotonia; wide-based gait.
5289183 M/20y5m	c.1660_1668del: p.Met554_Tyr556del (cWES[c])	Borderline IQ, learning disabilities	NA	14y	1 seizure	LEV	Normal	Normal	Progressive paraparesis with LL spasticity, ataxia, myoclonus. One seizure without recurrence under LEV. Seizure-free for 4 years off meds.
indvAA M/3y2m	c.2669C>T: p.Pro890Leu (cWES[g])	GDD	No seizures	NA	NA	NA	Normal	Normal	Mild ataxia and possibly myoclonus.
CAUSES1/M/ 4y7m	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/ F/30y	c.2669C>T: p.Pro890Leu (WES[h])	Mod. ID	No seizures	NA	NA	NA	Normal	Normal	Bradykinesia, bradypsychism, hypomimia, hypokinesia, clumsiness, attention instability.
indvPAR/M/ 16y	c.3140T>C: p.Leu1047Pro (trio cWES)	Severe ID	DEE	1 yr	Suspected FIA	VPA	Non specific irritative pattern, no foci	thin, short corpus callosum, with hypoplasia of its posterior part, wide Virchow-Robin spaces	Neonatal-onset hypotonia, noneverbal, acquired microcephaly, severe gastroinstestinal reflux; seizure-free under VPA.
273692 M/4y	c.3322T>C: p.Trp1108Arg (WES[f])	Severe GDD, suspected severe ID	DEE	2y	M, GTC, gelastic seizures?	LEV	abnormal	Pontocerebellar atrophy; delayed myelination	Non ambulatory; spasticity; dystonia; myoclonus; neonatal feeding difficulties; visual impairment; seizure control with LEV.
DDD261801 M/10y7m	c.3595C>T: p.Gln1199* (WES[f])	Mild GDD, Mild ID	No seizures	NA	NA	NA	NA	Normal	Neonatal-onset hypotonia, congenital ptosis, poor social skills.
indvMB F/7.5y	c.3621_3623del: p.Asp1207del (WES)	GDD, Severe ID	DEE	3y	Febrile GTC, M, To	VPA, LTG, CLB, CZP, LEV, TPM, LCM	M SW, biF	Thin CC; hyperT2 WM, enlarged LV	Acquired microcephaly, severe hypotonia; ataxia; oral and motor apraxia. Intractable seizures.

HSC0054 F/23y	c.4575dupA: p.Glu1526Argfs*18 (WGS[b])	GDD, mod. ID	DEE	5m	A, M, To, GTC, Fo	CLB, VPA,HCTZ, LEV, LTG, KD	gen SW and PSW	Delayed myelination. Normal at 20y	Neonatal hypotonia; scoliosis, Intractable seizures until puberty. Seizure-free under LEV and LTG.
LDKQS M/12y10m	c.4605+2T>C (c.WES[c])	GDD, mod. ID	No seizures	NA	NA	NA	NA	Normal	Hypotonia, neonatal feeding difficulties, sensorineural hearing loss.
DDD0280 F/6y	c.4663C>T p.Gln1555* (WES[f])	GDD, mod.-severe ID	No seizures	NA	NA	NA	NA	ND	Hypotonia.
281177 M/11y	c.4667G>A: p.Trp1556*(WES[f])	Mod. ID	No seizures	NA	NA	NA	NA	ND	Neonatal hypotonia.

[a]age at last examination (y, years; m, months). [c]GeneDx. [g]Radboud UMC. [f]DDD Study. [b]CENet. [h]OPBG. NA: not-available or not-applicable. ND: not done. HCTZ: hydrochlorothiazide. EEG: MSW, multifocal spike-wave; biF, bi-frontal predominance, gen. SW, generalized spike-wave; PSW, poly-spike and wave. NA, not applicable. ND, not done. See footnotes of Tables 2, 3 for other abbreviations.

Table 5. Summary of the clinical features in cases with DNMs in *DHDDS* (NM_024887.3) and *NUS1* (NM_138459.4)

ID/Gender/Age[a]	Gene	De novo variant (detection)	Cognitive/ behavioral	Epilepsy diagnosis	Age at Seizure onset	Seizure types	AEDs	EEG	Brain MRI	Associated neurological features /seizure outcome
indvSG/ F/5y1m	*DHDDS*	c.110G>A: p.Arg37His (c.WES[e])	GDD, severe ID	DEE	18m	MA photo, GTC, febrile Fo	VPA, LTG, *LEV*, ETH, VPA	gen. SW, photo+	Normal	Hypotonia; short stature, intractable seizures.
HSJ0762/ M/5y6m	*DHDDS*	c.110G>A: p.Arg37His (WGS[b])	GDD	DEE	1y	M, A, At, fever-sensitive	*LEV*, VPA	gen. SW, diffuse slowing	Normal	Hypotonia, tremor, wide-based gate, ataxia. Seizure-free x 1 year on VPA.
indvEF/ F/5y6m	*DHDDS*	c.632G>A: p.Arg211Gln (c.WES[c])	GDD, borderline IQ	DEE	4y	MA	*LEV*, LTG, OXBZ	epileptiform	Normal; Chiari I malformation	Hypotonia, tremor, ataxia, inattention, obesity; seizures controlled with OXBZ.
MDB31882/M/35	*DHDDS*	c.632G>A: p.Arg211Gln (WES[h])	GDD, severe ID	DEE	6-9y	Myoclonic	VPA, benzodiazepines	gen. PSW	Normal	Generalized tremor, facial myokimia, bradykinesia, hypomimia, rigidity, freezing and impaired postural reactions, frontal lobe impairment features. Seizure free since the age of 9y. Current therapy: VPA, Clonazepam, Tetrabenazine. Normal glycosylation assay.
indvNCJ/ F/7y1m	*DHDDS*	c.632G>A: p.Arg211Gln (c.WES[g])	GDD, mod-severe ID	NA	7y	M	None	Normal	Normal	Ataxia, myoclonus, tremor, dystonia, short stature; no treatment initiated yet for cortical myoclonus. Normal glycosylation assay.

indvKW/M/7y11m	*NUS1*	c.743delA: p.Asp248Alafs (c.WES[c])	GDD, severe ID	DEE	12m	M, GTC	LEV	BiF epileptiform	Normal	Ataxia with LEV. Lack of coordination. Seizures controlled with LEV.
HSJ0623/M/15y	*NUS1*	c.128_141dup: p.Val48Profs*7 (WGS[b])	GDD, mod. ID, ASD	DEE	10m	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/CLB.
HSJ0627/F/20y	*NUS1*	exon2-deletion (WGS[b])	Motor delay, mild ID	DEE	2.5y	M status, MA, At	VPA, LEV, CLB, FEL, LTG, CZP	gen. SW and PSW	Normal	Tremor, dysarthria. Seizures controlled on VPA/LTG/CZP.

[1]age at last examination (years, y; months, m); [e]BCM, [b]CENet, [c]GeneDx, [g]Radboud UMC. Seizure types: MA, myoclonic absence; MA photo: myoclonic absences with photosensitivity; GTC: generalized tonic-clonic; Fo, focal; M, myoclonic; A. absences; At, atonic. AED: FEL, Felbamate; OXBZ, oxcarbazepine; Stiri, stiripentol. See footnotes of Tables 2, 3 for other abbreviations.

Table 6. Genes enriched in 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-09	**0.0003**	5	0	8.90E-10	**3.49E-05**
DNM1	0	0	1	1	6	0	1.03E-11	**4.04E-07**
GABRB3	0	0	1	1	4	0	7.58E-09	**0.0003**
GNAO1	0	0	1	1	4	0	4.47E-09	**0.0002**
IQSEC2	3	0	6.76E-09	**0.00026**	3	0	1.14E-05	0.45
KCNQ2	0	0	1	1	4	0	1.69E-07	**0.007**
KCNT1	0	0	1	1	4	0.1	9.12E-07	**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-07**
SCN8A	1	0	0.007	1	7	0.1	3.08E-12	**1.21E-07**
SLC35A2	2	0	4.74E-07	**0.018**	3	0	4.89E-07	**0.019**
STXBP1	1	0	0.006	1	6	0	1.27E-12	**4.98E-08**

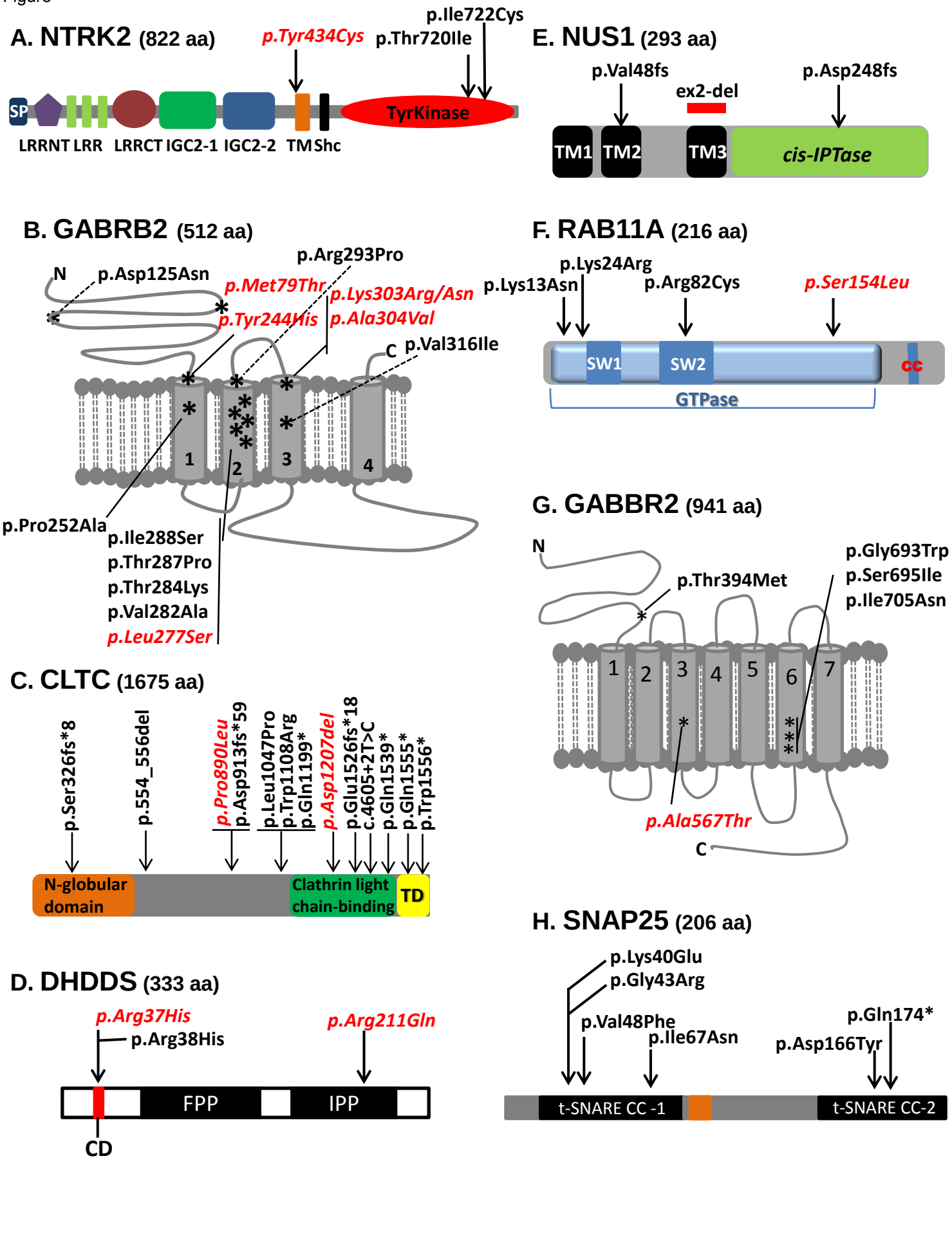
c.*p*-value, corrected *p*-value = *p*-value x 2 x 19618 (significant < 0.05, in bold). LoF: nonsense, frameshift and CSS *de novo* variants. Functional: LoF + missense variants

Table 7. Summary of the clinical features in cases with DNMs in *RAB11A* (NM_004663.4), *GABBR2* (NM_005458.7) *and SNAP25* (NM_003081.3)

Individual /Gender/ Age[a]	Gene	De novo variant (detection)	Cognitive/ behavioral	Epilepsy diagnosis	Age at Seizure onset	Seizure types	AEDs	EEG	Brain MRI	Associated neurological features /seizure outcome
HK055/M/ 5.5y	*RAB11A*	c.71A>G: p.Lys24Arg (WES)	GDD, Mod. ID	No seizures	NA	NA	NA	Abnormal background activity, but no epileptic charges	Central brain atrophy and bilateral periventricular white matter damage, thin CC	Acquired microcephaly, axial hypotonia, obesity, aggressive behavior.
HSJ0637/F /9.5y	*RAB11A*	c.244C>T: p.Arg82Cys (WGS[b])	GDD, Severe ID	IS	4m	M, ES, Fo	NZP, CLB, VGB, TPM, CLB, VPA, LEV	Modified Hyps.; M; Diffuse slowing with M spikes	Atrophy, partial agenesis of CC, delayed myelination. Decrease NAA	Acquired microcephaly, axial hypotonia.
24631/M/ 4y	*RAB11A*	c.461C>T: p.Ser154Leu (WES[f])	mod. GDD	No seizures	NA	NA	NA	NA	partial agenesis of the CC	Distractible, ADHD?
84049/F/9 y/11m	*RAB11A*	c.461C>T: p.Ser154Leu (WES[f])	mod. ID	No seizures	NA	NA	NA	NA	ND	Possible hyperactivity. Obesity.
HSJ0048/ M/14	*GABBR2*	c.2077G>T: p.Gly693Trp (WGS[b])	Severe GDD, severe ID	DEE, IS	11m	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/ M/23y	*SNAP25*	c.496G>T: p.Asp166Tyr (WGS[b])	GDD, mod. ID	DEE	18m	GTC, FIA	VPA, CLB	gen. SW, CSWS	mild diffuse cortical atrophy	Apneas, bradycardia, severe constipation. Minor dysmorphic traits. Seizure-free x 2y on VPA.

[a]age at last examination (years, y; months, m). [b]CENet. [f]DDD Study. CSWS, continuous spike and-wave during sleep. NAA, N-acetylaspartate. See footnotes of Tables 2, 3 for other abbreviations.

Figure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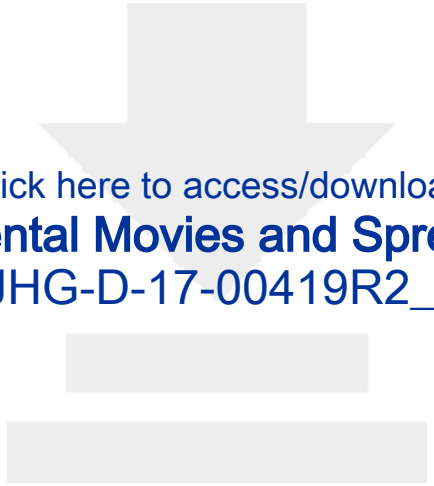
p.Tyr434Cys
p.Thr720Ile
p.Ile722Cys
SP
TyrKinase
LRRNT LRR LRRCT IGC2-1 IGC2-2 TM Shc
B. GABRB2 (512 aa)
N
p.Asp125Asn
p.Arg293Pro
p.Met79Thr
p.Lys303Arg/Asn
p.Tyr244His
p.Ala304Val
C
p.Val316Ile
1
2
3
4
p.Pro252Ala
p.Ile288Ser
p.Thr287Pro
p.Thr284Lys
p.Val282Ala
p.Leu277Ser
C. CLTC (1675 aa)
p.Ser326fs*8
p.554_556del
p.Pro890Leu
p.Asp913fs*59
p.Leu1047Pro
p.Trp1108Arg
p.Gln1199*
p.Asp1207del
p.Glu1526fs*18
c.4605+2T>C
p.Gln1539*
p.Gln1555*
p.Trp1556*
N-globular domain
Clathrin light chain-binding
TD
D. DHDDS (333 aa)
p.Arg37His
p.Arg38His
p.Arg211Gln
FPP
IPP
CD
E. NUS1 (293 aa)
p.Val48fs
ex2-del
p.Asp248fs
TM1
TM2
TM3
cis-IPTase
F. RAB11A (216 aa)
p.Lys24Arg
p.Lys13Asn
p.Arg82Cys
p.Ser154Leu
SW1
SW2
CC
GTPase
G. GABBR2 (941 aa)
N
p.Thr394Met
p.Gly693Trp
p.Ser695Ile
p.Ile705Asn
1
2
3
4
5
6
7
p.Ala567Thr
C
H. SNAP25 (206 aa)
p.Lys40Glu
p.Gly43Arg
p.Val48Phe
p.Ile67Asn
p.Gln174*
p.Asp166Tyr
t-SNARE CC -1
t-SNARE CC-2



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Supplemental Movies and Spreadsheets

Hamdan et al_AJHG-D-17-00419R2_tablesS5-S8.xlsx