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Genetic convergence of developmental and epileptic encephalopathies and intellectual disability

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ABBREVIATIONS

ASD	Autism spectrum disorder
DEE	Developmental and epileptic encephalopathy
NDD	Neurodevelopmental disorder
P/LP	Pathogenic/likely pathogenic

AIM To determine whether genes that cause developmental and epileptic encephalopathies (DEEs) are more commonly implicated in intellectual disability with epilepsy as a comorbid feature than in intellectual disability only.

METHOD We performed targeted resequencing of 18 genes commonly implicated in DEEs in a cohort of 830 patients with intellectual disability (59% male) and 393 patients with DEEs (52% male).

RESULTS We observed a significant enrichment of pathogenic/likely pathogenic variants in patients with epilepsy and intellectual disability (16 out of 159 in seven genes) compared with intellectual disability only (2 out of 671) ($p<1.86\times10^{-10}$, odds ratio 37.22, 95% confidence interval 8.60–337.0).

INTERPRETATION We identified seven genes that are more likely to cause epilepsy and intellectual disability than intellectual disability only. Conversely, two genes, *GRIN2B* and *SCN2A*, can be implicated in intellectual disability without epilepsy; in these instances

intellectual disability is not a secondary consequence of ongoing seizures but rather a primary cause.

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Genetic Overlap of DEEs and Intellectual Disability *Gemma L Carvill et al.*

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What this paper adds

- A subset of genes are more commonly implicated in epilepsy than other neurodevelopmental disorders.
- *GRIN2B* and *SCN2A* are implicated in intellectual disability and epilepsy independently.

[Main text]

Neurodevelopmental disorders (NDDs) encompass a broad spectrum of conditions that include intellectual disability, epilepsy, and autism spectrum disorder (ASD). These disorders are not necessarily mutually exclusive and comorbidity of NDDs is well established.[1]

Large exome-sequencing studies in NDDs have revealed that many of the genes that harbor de novo variants are commonly implicated in different cohorts of patients with NDD.[2,3] These findings mirror the overlap observed for NDD-associated copy number variants,[4,5] and collectively, led to the suggestion of shared genetic etiology for these conditions. To date, nearly 2000 genes have been implicated in intellectual disability and/or epilepsy. However, a fundamental question remains as to whether this shared genetic etiology is due to the comorbidity of intellectual disability and epilepsy in a single individual, and designation to a particular NDD cohort is due to recruitment bias, or whether certain genes are more commonly associated with a specific NDD. Some progress towards differentiating

between these two possibilities had been made. A targeted analysis of de novo variants in approximately 11 000 patients with ASD/intellectual disability demonstrated a subset of eight genes with increased burden in ASD, while 17 genes were enriched in intellectual disability and/or developmental delay.[6] In the same analysis, seizures and microcephaly were more likely to occur in patients with de novo variants in genes primarily associated with intellectual disability, while macrocephaly was probably a feature of patients with de novo variants in genes primarily associated with ASD. In contrast, a similar exome-based study in another cohort of approximately 11 000 patients with NDDs failed to identify de novo variants in genes enriched for ASD and only five genes enriched in intellectual disability.[7] In addition, a recent thorough evaluation of all evidence from large cohort studies of ASD and intellectual disability and/or developmental delay concluded that there is very little evidence for ASD-specific genes.[8] These discrepancies at least in part are accounted for by size of gene panel (targeted vs exome) and constraints of multiple testing corrections, as well as the heterogeneity of these conditions and the phenotypic heterogeneity and ascertainment of the cohort studied. Similar studies to identify epilepsy-specific genes are rare, with just a single meta-analysis of de novo variants in 6753 individuals (with some overlapping patient–parent trios with those reported in Coe et al.[7]) which identified rare variants in four genes (*SCN1A*, *SCN2A*, *KCNQ2*, and *SMC1A*) enriched in patients having NDD with epilepsy compared with those without epilepsy.[3]

Although these large studies present a powerful approach to study the overall burden and heterogeneity of NDDs, one of the major limitations is the lack of detailed clinical information. Moreover, patients are often assigned to ASD/intellectual disability/developmental delay/epilepsy cohorts based on the interest of the clinician recruiting the patient to the study, such as an epileptologist versus a geneticist. This does not take into account the natural history of NDDs which evolve over time, as patients often develop epilepsy and ASD. For instance, the developmental and epileptic encephalopathies (DEEs) are the most severe infant or childhood-onset epilepsies, where ongoing seizures and epileptiform activity cause developmental plateauing or regression, resulting in severe cognitive and behavioral problems.[9] Yet many individuals have typical development before seizure onset and develop cognitive impairment (e.g. intellectual disability/developmental delay and/or ASD) later. Therefore, in this study we assessed the frequency of pathogenic variants in a set of 18 genes more commonly or first associated with DEEs, in a cohort of 830 patients with intellectual disability and 393 patients with DEEs. Rather than focus on de novo variants more broadly as previously,[3,6,7] our primary aim was to perform detailed clinical

analysis of patients with pathogenic and likely pathogenic variants in these 18 genes and to determine the genotypic and phenotypic overlap between epilepsy, DEEs, and intellectual disability.

METHOD

We selected 18 genes for targeted resequencing on the basis of the following criteria: (1) are commonly implicated in patients with DEEs; (2) are regarded as established DEE genes; or (3) are currently only described in individuals with DEE (Table S1, online supporting information). We assessed the presence of rare variants in these genes in two patient cohorts, one comprising 830 intellectual disability patients (of whom 159 also had epilepsy) and one comprising 393 DEE patients. This study was approved by local institutional review boards of the University of Washington, the University of Melbourne, and Radboud University Medical Center. Informed written consent was obtained from all individuals or was provided by a parent or legal guardian in the case of minors and/or individuals with intellectual disability.

For both cohorts, targeted resequencing was performed using molecular inversion probes spanning all coding exons and five base pairs of flanking intronic sequence on an Illumina Hiseq2500 (Illumina, Inc., San Diego, CA, USA).[10] Only samples that had a mean depth of coverage of more than 60% were included for further analysis. Data analysis, including read mapping and variant calling, was performed as described previously.[10] We also integrated data from a previously published exome-sequencing cohort (*n*=6753), extracting de novo variants in these same 18 DEE genes.[3]

Variants that fulfilled the following criteria were prioritized for inclusion: (1) non-synonymous, (2) altering the acceptor/donor splice sites, (3) indels that disrupted the coding frame, which (4) were absent from the 114 704 'non-neuro' individuals in GnomAD (http://gnomad.broadinstitute.org/). After segregation analysis where parental DNA was available, variants were classified according to the American College of Medical Genetics guidelines.[11] In essence, rare variants for which familial segregation could not be determined were classified as a variant of unknown significance. Variants that arose de novo, were inherited from an affected parent, or were previously reported as causing disease (e.g. recurrent) were classified as likely pathogenic or pathogenic. All statistical analyses and odds ratio (OR) calculations were performed in R (version 3.6.1; R Foundation for Statistical Computing, Vienna, Austria) and Fisher's exact test was used to test enrichment of pathogenic/likely pathogenic (P/LP) variants in each cohort.

RESULTS

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P/LP variants in 18 DEE genes

We performed targeted resequencing of 18 established DEE genes in 830 patients with intellectual disability to identify variants that were likely to explain disease. In total, we identified 22 candidate variants in 11 of 18 genes (Fig. 1 and Table S2, online supporting information). Eighteen of the 22 candidate variants were classified as P/LP and four as variants of unknown significance. For 10 out of 18 of the P/LP variants we were able to perform segregation analysis; eight variants arose de novo, one *STXBP1* missense variant was inherited from a mosaic (10% in lymphocyte DNA) father, and a *GRIN2A* truncation was maternally inherited and detected in an affected sibling (Fig. S1, online supporting information). For the remaining eight likely pathogenic variants for whom parental DNA samples were unavailable for segregation analysis, five were previously reported as recurrent variants and three were predicted to result in a premature protein truncation. We did not have access to parental DNA for the four variants of unknown significance and they were not previously reported; these included variants in *SCN2A* (n=2), *STXBP1* (n=1), and *SLC6A1* (n=1) in patients with intellectual disability without epilepsy (Table S3, online supporting information).

To compare the distribution of P/LP variants in intellectual disability with a cohort of individuals with DEEs under the same study design, we performed targeted resequencing in 393 patients with DEEs to a comparable depth across these 18 genes, although a subset of the DEE cohort had lower mean coverage than other cohorts (intellectual disability/DEE) (Table S1). Although it is possible that, as a result, variants may have been missed in this cohort, this does not change the conclusions of this study, as increasing our detection rate in DEE does not affect the goals of this study to detect P/LP variants in individuals with intellectual disability/epilepsy. Using the same American College of Medical Genetics criteria, we identified a total of 32 P/LP variants in 11 out of 18 genes (Fig. 1).

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P/LP variants enriched in individuals with epilepsy and intellectual disability

Overall, 16 out of 159 (10%) patients with intellectual disability and epilepsy carried P/LP variants in these genes compared with 2 out of 671 (0.2%) from the group of patients with intellectual disability only. We observed a significant enrichment of P/LP variants in patients with the comorbidity of epilepsy and intellectual disability compared with intellectual disability only ($p<1.86\times10^{-10}$, OR 37.22, 95% confidence interval [CI] 8.60–337.0) (Fig. 2).

The individuals without epilepsy were 8 and 15 years of age; thus, these two individuals (in particular the 8-year-old) could still have developed epilepsy, as epilepsy most commonly begins in infancy or childhood, with some epilepsies developing after puberty. Similarly, we observed a significant enrichment for P/LP in patients with DEEs (32 out of 393, 8%) compared with intellectual disability only (2 out of 671, 0.2%) ($p<1.54\times10^{-12}$, OR 29.57, 95% CI 7.47–254.43). In contrast, there was no difference in the frequency of P/LP variants in these genes between the DEE and epilepsy and intellectual disability cohorts ($p=0.5$). We contrasted these results with a recent meta-analysis of exome sequencing data from 6753 individuals with a variety of NDDs.[3] Analysis of the P/LP variants in the 18 target genes in this meta-analysis confirmed the same statistically significant enrichment of P/LP variants in patients with epilepsy and intellectual disability (168 out of 1942, 8.6%) compared with individuals with intellectual disability only (50 out of 4811, 1%) ($p<2.20\times10^{-16}$, OR 9.01, 95% CI 6.5–12.68) (Table S4, online supporting information).

A total of nine genes harbored P/LP variants in the epilepsy and intellectual disability cohort that did not overlap with the intellectual disability only cohort (Fig. 1). While in our targeted resequencing cohorts we did not have sufficient power to identify enrichment for specific genes, in combination with exome-sequencing cohort data[3] we identified six genes (*CDKL5*, *CHD2*, *GRIN2A*, *SCN1A*, *SCN8A*, *STXBP1*) that were enriched in epilepsy and intellectual disability compared with intellectual disability only (Fig. 3 and Table S5, online supporting information).

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P/LP variants present in individuals with intellectual disability only cohort

Only 2 out of 671 individuals in the intellectual disability-only cohort carried P/LP variants in any of the 18 genes studied. Probands 7 and 11 did not have epilepsy and carried truncating variants in *GRIN2B* (Leu973Cysfs*3) and *SCN2A* (Glu1211Lys) respectively. Proband 7 was an 8-year-old male presenting with global developmental delay; an electroencephalogram (EEG) had never been performed. Proband 11 was a 15-year-old male with severe intellectual disability and ASD, with a normal EEG. To test whether these two genes were more common in those with intellectual disability only, we integrated our results with the exome-sequencing cohort data and showed that this was not the case. In fact, in the cohorts studied here, *SCN2A* is also enriched in those with epilepsy and intellectual disability compared with intellectual disability only, and *GRIN2B* P/LP variants were identified in both cohorts equally (Fig. 3 and Table S5).

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DISCUSSION

Exome sequencing and copy number variant studies in cohorts of patients with intellectual disability, epilepsy, and ASD have suggested a shared genetic etiology for NDDs. However, whether this shared genetic etiology is due to frequent comorbidity of these conditions or shared genetic causes is unknown. We show here that overall P/LP variants in 18 genes are enriched in individuals with epilepsy and comorbid intellectual disability compared with intellectual disability only. Moreover, seven genes (*CHD2*, *CDKL5*, *GRIN2A*, *SCN1A*, *SCN2A*, *SCN8A*, and *STXBP1*) were individually more common in the individuals with epilepsy and intellectual disability compared with intellectual disability only. Finally, we show that P/LP variants in these 18 genes account for a similar proportion of cases of both DEE (8%) and epilepsy with intellectual disability (10%), while only 0.2% of the intellectual disability only cohort had pathogenic variants in these genes.

We identified pathogenic variants in 10 genes (*CDKL5*, *CHD2*, *GABRA1*, *GRIN2A*, *KCNT1*, *SCN1A*, *SCN2A*, *SCN8A*, *SLC2A1*, *STXBP1*) in the epilepsy with intellectual disability cohort. Of these genes, six (*CDKL5*, *CHD2*, *GRIN2A*, *SCN1A*, *SCN8A*, *STXBP1*) were significantly enriched by combining our data with a large exome-sequencing study of patients with epilepsy with intellectual disability, versus intellectual disability only (Table S4).[3] Only *SCN1A* is exclusively implicated in those individuals with epilepsy and intellectual disability, including an individual in our targeted resequencing cohort (proband 9). This female presented with moderate intellectual disability and generalized tonic–clonic and absence seizures beginning in the first year of life, which evolved to drug-resistant tonic–clonic seizures. Although we have a limited history, the features are highly suggestive of Dravet syndrome.[12] In cohort studies in patients with epilepsy, *SCN1A* is the most frequently implicated gene, accounting for 3% to 18% of cases.[13–15] In contrast, P/LP *SCN1A* variants are extremely rare in intellectual disability only (Figs 2 and 3).

Five additional genes were enriched in the epilepsy and intellectual disability cohort including, most frequently, *STXBP1* and *CHD2*. We identified two P/LP *STXBP1* variants in probands 17 and 18. Both individuals had epilepsy that started in infancy and impaired development. However, proband 18 had delayed development before the first seizure at age 1 year and had mild intellectual disability at the age of 6 years, while proband 17 had their first seizure at the age of 2 weeks and severe intellectual disability at the age of 7 years. This is in keeping with a published study that showed 95% of patients with *STXBP1* pathogenic variants (n=147) present with epilepsy and intellectual disability; although rare, patients with intellectual disability only are reported.[16] We also detected two truncating P/LP *CHD2*

variants in probands 2 and 3. Both individuals presented with generalized tonic–clonic seizures, intellectual disability, and ASD, and one was refractory to antiseizure medications and showed regression. Pathogenic variants in this gene were first described in patients with DEEs.[17] Since then, the phenotypic spectrum has been expanded to include patients with intellectual disability and ASD with or without epilepsy, although to date P/LP variants are more common in patients with epilepsy.[17,18] The clinical features of these patients are consistent with *CHD2*-associated epilepsy. Overall, no apparent genotype–phenotype relationship exists with regards the position or type of P/LP variant for either *STXBP1* or *CHD2*.[16,17] Conversely, *SCN8A* P/LP variants have been described in individuals with intellectual disability without epilepsy, but in this instance these variants are thought to act in a loss of function manner compared with the gain-of-function mechanism that is known to underpin DEE-associated P/LP variants.[19] *GRIN2A* and *CDKL5* were identified only in the epilepsy and intellectual disability cohort in our targeted resequencing cohort. However, in the larger published intellectual disability only exome cohort, a single P/LP variant was identified in *GRIN2A*/*CDKL5*, although detailed clinical data were not available.[3] Collectively, these results demonstrate that, while these five genes are more common in individuals with comorbid epilepsy and intellectual disability, they can be identified in individuals with intellectual disability only. In some instances, this may be due to genotype–phenotype (variant type/position) correlations, but also suggests that, for some genes, intellectual impairment may be caused by the gene defect alone and is independent of epilepsy, although ongoing epileptic activity may have additional adverse effects.

In our target resequencing cohort, only two genes, *GRIN2B* and *SCN2A*, harbored P/LP variants in the intellectual disability only cohort (probands 7 and 11). These individuals were aged 8 and 15 years and had no history of seizures and one had a normal EEG. However, when we tested whether these genes were more commonly implicated in intellectual disability only in a larger cohort, this was not the case; indeed, *SCN2A* was found to be more commonly implicated in individuals with epilepsy and intellectual disability, while *GRIN2B* was found in both cohorts equally. Certainly, for *SCN2A*, these observations are likely to be due to a genotype–phenotype correlation where gain-of-function variants are more commonly associated with epilepsy and loss of function with intellectual disability and/or ASD.[20,21] Indeed proband 11 also had ASD. Similar correlations do not currently exist for *GRIN2B*. Thus, in summary we failed to identify a previously associated DEE gene that was more common in individuals with intellectual disability only.

Our study emphasizes the complexity of NDDs and the genetic variation causing it. While several genes were related to epilepsy with intellectual disability, some could be related to intellectual disability only. The shared genetic etiology is not only implicated in intellectual disability and epilepsy, but also with other NDDs such as ASD, attention-deficit/hyperactivity disorder, as well as their related features such as an abnormal head circumference. Many patients suffer from more than one NDD, which suggests that these genes are NDD genes rather than epilepsy or intellectual disability genes. However, it is important to look for differences between the related phenotype to better inform patients and guide future neurobiological research.

In summary, our study used phenotypically well-defined cohorts of patients with intellectual disability and DEE to delineate the genetic convergence between epilepsy and intellectual disability. We identified seven genes (*CHD2*, *CDKL5*, *GRIN2A*, *SCN1A*, *SCN2A*, *SCN8A*, *STXBP1*) that were more likely to be related to epilepsy with intellectual disability than intellectual disability only. We provide evidence that two genes, *GRIN2B* and *SCN2A*, can be implicated in intellectual disability only, while the others appear to confer a high risk of epilepsy in individuals with pathogenic variants. Thus, these genes cause both intellectual disability and epilepsy, rather than the intellectual disability being a consequence of seizure activity alone. While further studies are needed to determine underlying genotype–phenotype correlations for each gene, our results are an important contribution to our understanding of the pathogenic mechanisms underlying NDDs.

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Pharmaceuticals, Atheneum Partners, Ovid Therapeutics, Care Beyond Diagnosis, Epilepsy Consortium, and UCB. She may accrue future revenue on pending patent WO61/010176 (filed 2008): Therapeutic Compound; has a patent for *SCN1A* testing held by Bionomics Inc and licensed to various diagnostic companies; has a patent molecular diagnostic/theranostic target for benign familial infantile epilepsy [PRRT2] 2011904493 and 2012900190 and PCT/AU2012/001321 (TECH ID 2012-009). The other authors have stated that they had no interests that might be perceived as posing conflict or bias.

SUPPORTING INFORMATION

The following additional material may be found online:

Table S1: Percentage of bases covered greater than variant calling threshold for 18 known DEE genes.

Table S2: Phenotypes of likely pathogenic variants in known epilepsy genes.

Table S3: Phenotypes of patients with variant of uncertain significance in known epilepsy genes.

Table S4: Pathogenic variants in 18 known DEE genes in meta-analysis of patients with NDDs1.

Table S5: Pathogenic variants in 12 known DEE genes in epilepsy and intellectual disability.

Figure S1: Pedigree for proband 5 with a likely pathogenic nonsense variant in *GRIN2A*.

REFERENCES

1. American Psychiatric Association. Diagnostic and statistical manual of mental disorders (5th edition). Washington, DC: American Psychiatric Association, 2013.
2. Vissers LE, Gilissen C, Veltman JA. Genetic studies in intellectual disability and related disorders. *Nat Rev Genet* 2016; **17**: 9–18.
3. Heyne HO, Singh T, Stamberger H, et al. De novo variants in neurodevelopmental disorders with epilepsy. *Nat Genet* 2018; **50**: 1048–53.
4. Girirajan S, Rosenfeld JA, Coe BP, et al. Phenotypic heterogeneity of genomic disorders and rare copy-number variants. *N Engl J Med* 2012; **367**: 1321–31.
5. Mullen SA, Carvill GL, Bellows S, et al. Copy number variants are frequent in genetic generalized epilepsy with intellectual disability. *Neurology* 2013; **81**: 1507–14.

6. Stessman HA, Xiong B, Coe BP, et al. Targeted sequencing identifies 91 neurodevelopmental-disorder risk genes with autism and developmental-disability biases. *Nat Genet* 2017; **49**: 515–26.
7. Coe BP, Stessman HAF, Sulovari A, et al. Neurodevelopmental disease genes implicated by de novo mutation and copy number variation morbidity. *Nat Genet* 2019; **51**: 106–16.
8. Myers SM, Challman TD, Bernier R, et al. Insufficient evidence for "autism-specific" genes. *Am J Hum Genet* 2020; **106**: 587–95.
9. Scheffer IE, Berkovic S, Capovilla G, et al. ILAE classification of the epilepsies: position paper of the ILAE Commission for Classification and Terminology. *Epilepsia* 2017; **58**: 512–21.
10. Carvill GL, Heavin SB, Yendle SC, et al. Targeted resequencing in epileptic encephalopathies identifies de novo mutations in *CHD2* and *SYNGAP1*. *Nat Genet* 2013; **45**: 825–30.
11. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015; **17**: 405–24.
12. Scheffer IE, Nabbout R. SCN1A-related phenotypes: epilepsy and beyond. *Epilepsia* 2019; **60** (Suppl 3): S17–24.
13. Lemke JR, Riesch E, Scheurenbrand T, et al. Targeted next generation sequencing as a diagnostic tool in epileptic disorders. *Epilepsia* 2012; **53**: 1387–98.
14. Allen AS, Berkovic SF, Cossette P, et al. De novo mutations in epileptic encephalopathies. *Nature* 2013; **501**: 217–21.
15. Gokben S, Onay H, Yilmaz S, et al. Targeted next generation sequencing: the diagnostic value in early-onset epileptic encephalopathy. *Acta Neurol Belg* 2016; **117**: 131–8.
16. Stamberger H, Nikanorova M, Willemsen MH, et al. STXBP1 encephalopathy: a neurodevelopmental disorder including epilepsy. *Neurology* 2016; **86**: 954–62.
17. Carvill G, Helbig I, Mefford H. *CHD2*-related neurodevelopmental disorders. In Pagon RA, Adam MP, Ardinger HH, et al., editors. GeneReviews®. Seattle, WA: University of Washington, 1993.
18. Carvill GL, Regan BM, Yendle SC, et al. *GRIN2A* mutations cause epilepsy-aphasia spectrum disorders. *Nat Genet* 2013; **45**: 1073–6.
19. Meisler MH, Helman G, Hammer MF, et al. *SCN8A* encephalopathy: research progress and prospects. *Epilepsia* 2016; **57**: 1027–35.

20. Wolff M, Johannesen KM, Hedrich UB, et al. Genetic and phenotypic heterogeneity suggest therapeutic implications in *SCN2A*-related disorders. *Brain* 2017; **140**: 1316–36.

21. Ben-Shalom R, Keeshen CM, Berrios KN, An JY, Sanders SJ, Bender KJ. Opposing effects on $Na_V1.2$ function underlie differences between SCN2A variants observed in individuals with autism spectrum disorder or infantile seizures. *Biol Psychiatry* 2017; **82**: 224–32.

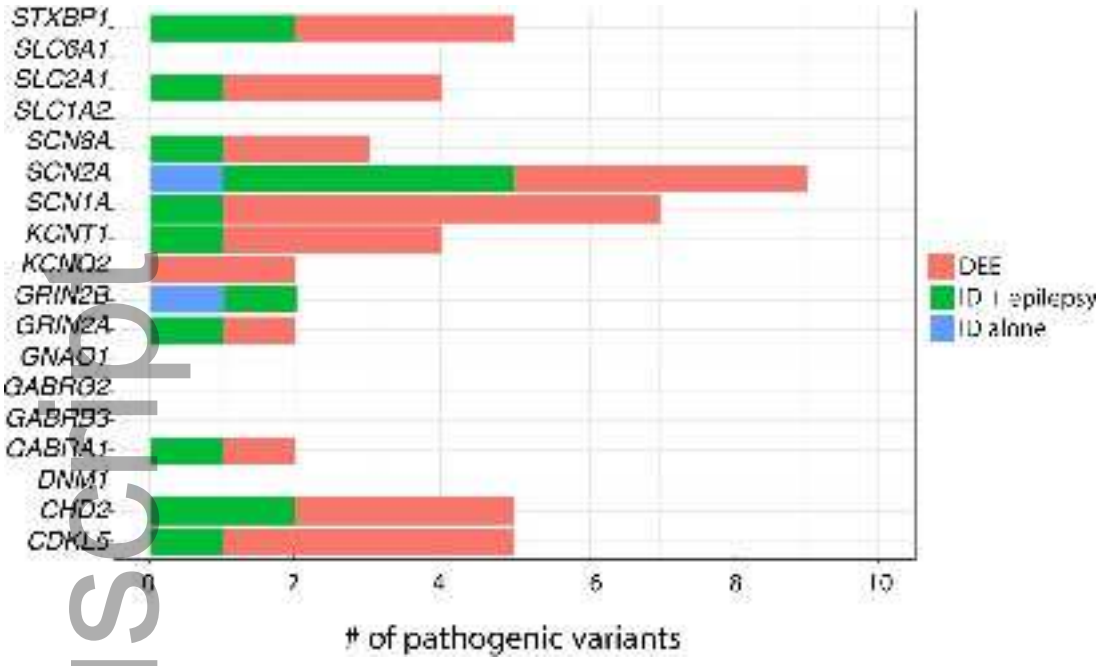
22.

[Figure legends]

Figure 1: Distribution of pathogenic/likely pathogenic (P/LP) variants in patient cohorts in 18 genes. Overall, most patients with P/LP variants were identified in individuals with intellectual disability and epilepsy (green, *n*=16) rather than intellectual disability only (blue, *n*=2). Only two genes, *SCN2A* and *GRIN2B*, harbored P/LP variants in patients with intellectual disability only. Across the same set of 18 genes, 32 P/LP variants were identified in individuals with DEE (peach).

Figure 2: Enrichment for pathogenic/likely pathogenic (P/LP) variants in 18 DEE-associated genes in neurodevelopmental disorder (NDD) cohorts. For each cohort, i.e. developmental and epileptic encephalopathy (DEE) (*n*=393), intellectual disability and epilepsy (*n*=159), and intellectual disability only (*n*=671), the number of individuals with P/LP variants in one of 18 genes is shown in peach, whereas, when no variant was identified is in turquoise. Having epilepsy in the context of DEE or epilepsy and intellectual disability was associated with a significantly greater likelihood of identifying a P/LP variant. There was no difference between DEE and epilepsy with intellectual disability cohorts.

Figure 3: Replication analysis in a neurodevelopmental disorder (NDD) exome cohort confirms single-gene enrichment in individuals with epilepsy and intellectual disability. For the nine genes (top) where we identified individuals with pathogenic/likely pathogenic (P/LP) variants in individuals with epilepsy and intellectual disability, and not intellectual disability only, six genes (in bold type) were individually significantly associated with epilepsy and intellectual disability compared with intellectual disability only. For the two genes where we identified P/LP variants in our intellectual disability only cohort, inclusion of the larger cohort showed significant enrichment in epilepsy and intellectual disability (not intellectual disability only) for *SCN2A*. *GRIN2B* was not significantly associated with either cohort ($p<0.001$, individual *p*-values and odds ratios in Table S5, online supporting information).



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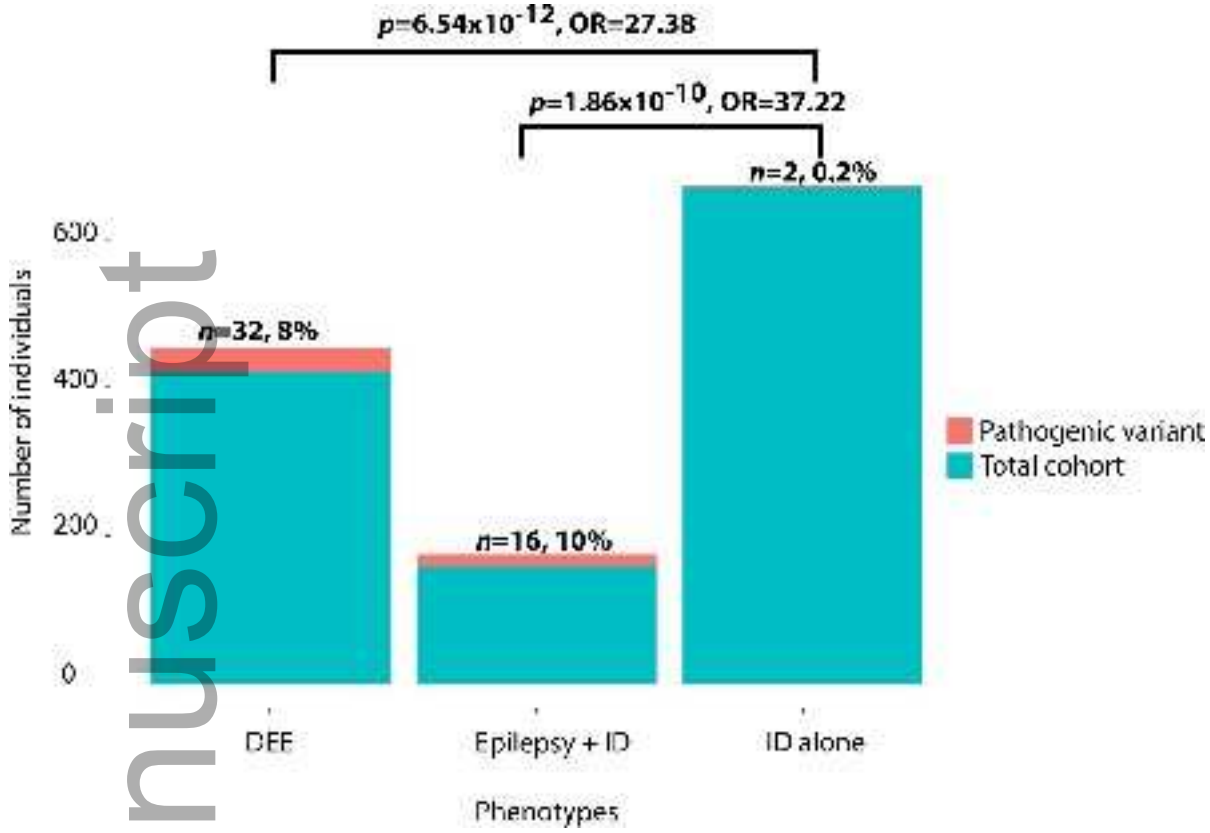

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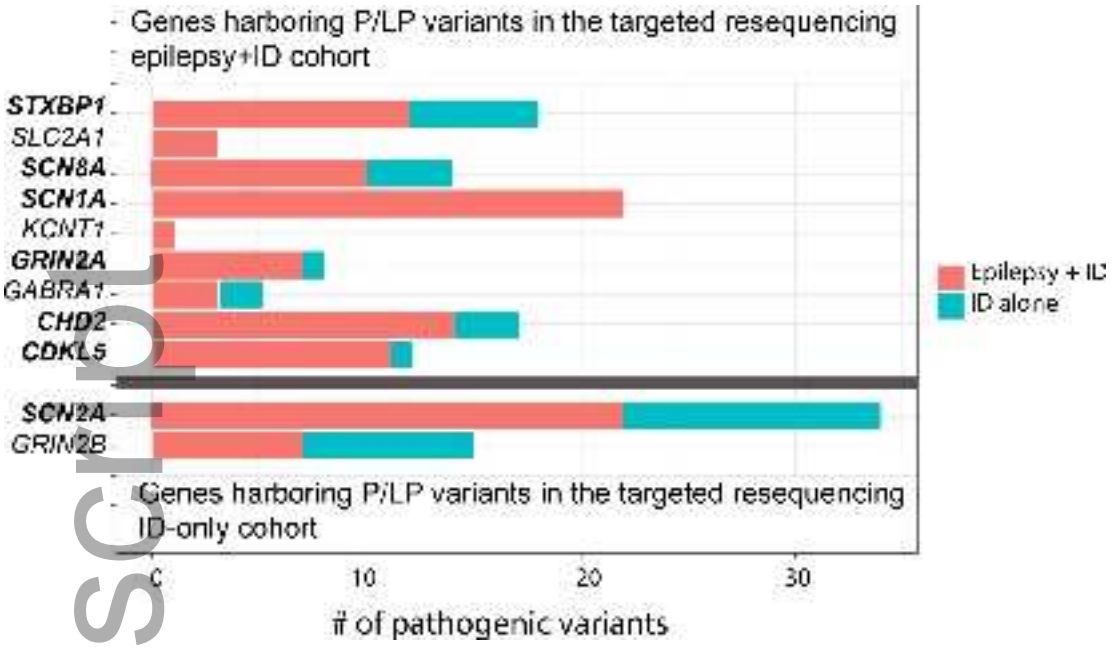

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