

Widespread genomic influences on phenotype in Dravet syndrome, a ‘monogenic’ condition

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Abstract

Dravet syndrome is an archetypal rare severe epilepsy, considered “monogenic”, typically caused by loss-of-function *SCN1A* variants. Despite a recognisable core phenotype, its marked phenotypic heterogeneity is incompletely explained by differences in the causal *SCN1A* variant or clinical factors. In 34 adults with *SCN1A*-related Dravet syndrome, we show additional genomic variation beyond *SCN1A* contributes to phenotype and its diversity, with an excess of rare variants in epilepsy-related genes as a set and examples of blended phenotypes, including one individual with an ultra-rare *DEPDC5* variant and focal cortical dysplasia. Polygenic risk scores for intelligence are lower, and for longevity, higher, in Dravet syndrome than in epilepsy controls. The causal, major-effect, *SCN1A* variant may need to act against a broadly compromised genomic background to generate the full Dravet syndrome phenotype, whilst genomic resilience may help to ameliorate the risk of premature mortality in adult Dravet syndrome survivors.

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12
13 **Keywords:** *SCN1A*; Dravet syndrome; polygenic risk scores; blended phenotypes; polymorphism

14 **Abbreviations:** ACMG-AMP=American College of Medical Genetics and Genomics-Association
15 for Molecular Pathology, ANNOVAR=ANNOtate VARIation, BP5= Alternate locus
16 observations, supporting evidence for benign, DEEs=Developmental and Epileptic
17 Encephalopathies, FCD=Focal Cortical Dysplasia, FLNA=Filamin A, FS=Febrile Seizures,
18 GEFS+=Genetic Epilepsy with Febrile Seizures Plus, GEL=Genomics England, gnomAD=The
19 Genome Aggregation Database, GWAS=Genome-Wide Association Study, HMC=Helena
20 Martins Custodio, HPO=Human Phenotype Ontology, ID=Intellectual Disability,
21 IGV=Integrative Genomics Viewer, ILAE=International League Against Epilepsy, JDM=James
22 D. Mills, LDSC=Linkage Disequilibrium Score Regression, LMC=Lisa M Clayton,
23 MRI=Magnetic Resonance Imaging, NHS=National Health Service, NIHR=National Institute for
24 Health Research, PDB=Protein Data Bank, PRS=Polygenic Risk scores, PT=P-value Threshold,
25 RB=Ravishankara Bellampalli, SABA=Structural Axis for Binding Arrangement, SB=Simona
26 Balestrini, SCN1A=voltage-gated sodium channel alpha subunit 1 gene, SD=Standard Deviation
27 , SHEN=Steric Hindrance for Enhancement of Nucleotidase activity, SIFT=Sorting Intolerant
28 From Tolerant, SKAT=SNP-set (Sequence) Kernel Association Test, SKAT-O=The optimal

1 sequence kernel association test, SMS=Sanjay M Sisodiya, SNPs=Single Nucleotide
2 Polymorphisms, SP=Susanna Pagni, SUDEP=Sudden Unexpected Death in Epilepsy,
3 TSC1=Tuberous Sclerosis 1, UK=United Kingdom, VEP=Ensembl Variant Effect Predictor,
4 VUS=Variant of Uncertain Significance, WGS=Whole-Genome Sequencing

6 **Introduction**

7 With the discovery of numerous monogenic epilepsies, our understanding of the genetic
8 architecture underlying developmental and epileptic encephalopathies (DEEs) has grown
9 immensely¹. The initial identification of monogenic epilepsies is usually made through genetic
10 studies of individuals with relatively homogeneous phenotypes. Subsequent characterisation of
11 additional cases with pathogenic variants in the same gene typically broadens the phenotypic
12 spectrum^{2,3}. This evolving breadth of clinical presentations, even with a core defining phenotype,
13 can become surprisingly wide and unexplained. One potential source of such phenotypic diversity
14 within a single monogenic epilepsy may be variation across the rest of the genome. This possibility
15 is rarely explored; typically, genetic investigations cease with the discovery of the first plausibly
16 culpable variant.

17 Pathogenic variants in the voltage-gated sodium channel alpha subunit 1 gene (*SCN1A*) are one of
18 the most frequent causes of monogenic epilepsies, though all are rare⁴. The archetypal phenotype
19 associated with pathogenic *SCN1A* variants is Dravet syndrome. The spectrum also includes
20 familial febrile seizures (FS), genetic epilepsy with febrile seizures plus (GEFS+), and other
21 *SCN1A*-related epilepsies that do not obviously fit these categories but may share some core
22 features, such as fever-provoked seizures⁵. Further, people with pathogenic variants in *SCN1A* may
23 also present with features beyond epilepsy, including mild to severe intellectual disability (ID),
24 behavioural problems and movement disorders⁵. Within *SCN1A*-related conditions, and even for a
25 given pathogenic variant, phenotypic heterogeneity can be observed: a given *SCN1A* variant may
26 segregate with epilepsy in a family, and cause GEFS+ in one individual, and Dravet syndrome in
27 another; individuals meeting a tight clinical definition for Dravet syndrome, harbouring identical
28 *SCN1A* variants, may show divergent phenotypes. This wide range of associated phenotypes
29 confounds prognostication for infants with *SCN1A*-related epilepsies and makes treatment

1 challenging. As a prototypic monogenic disorder, *SCN1A*-related epilepsies provide a model for
2 elucidating the potential contribution of background genetic architecture to the disease phenotype.
3 Additional genetic factors have been implicated in the phenotypic diversity seen in *SCN1A*-related
4 epilepsies. Disease severity could be modulated by genomic factors directly related to *SCN1A*,
5 such as variant class, mosaicism of the pathogenic *SCN1A* variant, or variants in non-coding
6 regulatory regions affecting the expression of the mutated or wild-type *SCN1A* allele^{6,7}.
7 Alternatively, variants in other genes may influence *SCN1A*-related epilepsy phenotypes,
8 constituting blended phenotypes that reflect an aggregation of distinct or overlapping features,
9 depending on the pathway or function of the gene(s) harbouring the additional variant(s)⁸. The
10 poly-genetic “background” of each individual may act as a phenotypic modifier. Evidence from
11 animal models suggests that genetic background may modulate Dravet-like phenotypes, whilst an
12 enrichment of rare variants in neuronal excitability genes has been reported in severe Dravet
13 syndrome compared to mild Dravet syndrome^{9,10}. Beyond genomic influences, clinical
14 management, including medication choices, may also affect outcomes¹¹, potentially through
15 interactions with individual genetic features.

16 To test the hypothesis that the background genetic architecture influences the phenotypic
17 presentation of individuals with monogenic epilepsy, we utilised whole-genome sequencing
18 (WGS) across a cohort of adults with clinically well-characterised *SCN1A*-related Dravet
19 syndrome. We studied several features of background genomic variation, including the
20 contribution of rare variants in epilepsy-related genes, and common variation across the genome,
21 including polygenic risk scores (PRS), aiming to elucidate whether these features influence Dravet
22 syndrome phenotypes.

23

24 **Materials and methods**

25 **Ethics statement**

26 This research was approved by the relevant ethics committee. For all cases, written informed
27 consent for research use of clinical and genetic data was obtained from patients, their parents, or
28 legal guardians in the case of those with ID. All individuals for whom detailed phenotypic

1 information is provided were recruited through a REC-approved study (REC 11/LO/2016), and all
2 phenotypic and genetic information was gathered under this approval.

3

4 **Cohort descriptions:**

5 ***SCN1A*-related Dravet syndrome cohort**

6 Thirty-four adults with *SCN1A*-related Dravet syndrome were recruited from epilepsy clinics at
7 the National Hospital for Neurology and Neurosurgery, London, UK through a REC-approved
8 study (REC 11/LO/2016). WGS was performed on DNA extracted from peripheral blood
9 (Supplementary Material 1). Detailed clinical phenotyping was undertaken by LMC after
10 comprehensive review of the medical records. The Dravet syndrome phenotype was re-evaluated
11 independently by LMC, SB and SMS with reference to the diagnostic criteria for Dravet syndrome
12 currently under review by the International League Against Epilepsy (ILAE)¹² (Supplementary
13 Table 1 and Supplementary Material 2).

14 The full cohort of 34 individuals with Dravet syndrome was utilised for the blended phenotype
15 analysis. For PRS and burden analyses, only individuals of European ancestry (28/34) were
16 included (Supplementary Fig. 1; Supplementary Material 3). A cohort including 13 individuals
17 with Dravet syndrome of European ancestry who have missense *SCN1A* variants was used for
18 post-hoc analyses.

19 **Control cohorts**

20 All control cohorts were compiled from participants recruited to the Genomics England (GEL)
21 100,000 genomes project (Supplementary Fig. 2). Only individuals of European ancestry were
22 considered in the control cohorts (Supplementary Fig. 1; Supplementary Material 3).

23 **GEL Epilepsy controls**

24 The GEL Epilepsy control cohort consisted of 772 adults with epilepsy recruited from clinics at
25 the National Hospital for Neurology and Neurosurgery, London, UK, through a REC-approved
26 study (REC 11/LO/2016), and genotyped by the GEL 100,000 genomes project. All individuals
27 fell within the GEL “epilepsy and other features” disease group. The human phenotype ontology

1 (HPO) terms used for these individuals when recruited to the GEL 100,000 genomes project can
2 be found in Supplementary Table 2. To minimise the possibility that individuals within this cohort
3 had *SCN1A*-related epilepsies, individuals with unique variants in *SCN1A* (i.e. not present in The
4 Genome Aggregation Database (gnomAD) (version 3.1.1)) were excluded (Supplementary Fig.
5 2).

6 **GEL controls**

7 The GEL control cohort consisted of 1,187 unaffected relatives of probands from GEL disease
8 categories considered to be unrelated to epilepsy (Supplementary Table 3)^{13,14}. Medical
9 information regarding these individuals is unknown, and a proportion, likely reflective of the
10 prevalence of active epilepsy in the UK (5-10 per 1000), may have epilepsy, which would serve
11 only to reduce the power of our comparisons. To minimise the number of individuals with potential
12 “monogenic” epilepsies in this cohort, individuals with unique variants (i.e. not present in
13 gnomAD) in epilepsy-related genes were excluded (Supplementary Fig. 2).

14 **GEL *SCN1A* controls**

15 Following testing of the primary hypotheses, it became clear that a further post-hoc investigation
16 would be useful, examining individuals bearing ultra-rare *SCN1A* variants, but without epilepsy.
17 The GEL *SCN1A* control cohort consisted of 45 GEL probands of European ancestry (median age
18 at recruitment 37 years (range 4-71)) from disease categories considered to be unrelated to epilepsy
19 (Supplementary Table 3)¹³, who were also identified as having unique/ultra-rare *SCN1A* missense
20 variants (i.e. not present in gnomAD) (Supplementary Fig. 2). No individuals in the disease
21 categories considered to be unrelated to epilepsy had truncating *SCN1A* variants. HPO terms and
22 medical history timelines were reviewed for all identified cases and no individuals were found to
23 have phenotypes that are known to be associated with *SCN1A* variants (see Supplementary
24 Material 4 and Supplementary Table 4).

26 **Epilepsy-related gene selection and annotation**

27 To test the hypothesis that phenotypic heterogeneity seen in Dravet syndrome could be partly
28 explained by variation in other epilepsy-related genes, in addition to *SCN1A*, samples were

1 screened for rare variants across the canonical coding sequences of 190 monoallelic or X-linked
2 epilepsy-related genes in the GEL Genetic Epilepsy Syndromes (Version 2.489) panel
3 (Supplementary Table 5; Supplementary Material 5). Only genes designated by GEL with a
4 “green” rating, (i.e. those in which there is a high level of evidence for gene-disease association),
5 were included and are referred to as “epilepsy-related genes”^{13,15}. Rare variants were defined as
6 those with an allele frequency in gnomAD ≤ 0.0005 , which is in line with previously defined “rare”
7 variant allele frequencies^{16,17}. The region of each epilepsy-related gene was extracted from variant
8 call format and annotated using ANNOtate VARIation (ANNOVAR) (version 2019Oct24). Stop-
9 gains, frameshift-deletion, frameshift-insertion, in-frame-deletion, in-frame-insertion, splicing,
10 and missense variants with a read coverage ≥ 8 were selected as qualifying variants. All variants
11 were confirmed manually using the Integrative Genomics Viewer (IGV) (version 2.9.4).

12

13 **Gene and gene-set based collapsing analyses of rare variants**

14 An enrichment of rare variants in known epilepsy-related genes confers risk for the common and
15 rare epilepsies¹⁶. To test the hypothesis that there was an excess of rare variants in epilepsy-related
16 genes in individuals with Dravet syndrome compared with GEL Epilepsy controls, we performed
17 a gene-based and gene-set collapsing analyses for rare variants across 190 epilepsy-related
18 genes^{13,15}. The optimal sequence kernel association test (SKAT-O) as implemented in SKAT R
19 package version 2.0.1 was used¹⁸. *SCN1A* variants were excluded in both gene-based and gene-set
20 collapsing analyses, to avoid the overestimation of enrichment of rare variants. The variants in
21 these 190 genes were identified using region extraction and Ensembl Variant Effect Predictor
22 (VEP) annotation¹⁹. Variants that were observed < 3 times in each cohort were included in the
23 SKAT-O analysis. Gender was included as a covariate. A small sample size adjustment by SKAT-
24 O was used. To determine if X chromosome gene variants were driving enrichment of rare variants
25 in Dravet syndrome cases, we performed a rare variant burden analysis for the 153 epilepsy-related
26 genes on autosomal chromosomes. To explore whether the burden of rare variants in epilepsy-
27 related genes may influence the expressed phenotype in the setting of a unique *SCN1A* variant, a
28 post-hoc analysis was performed estimating the gene and gene-set based rare variant enrichment
29 across the Dravet syndrome and GEL *SCN1A* control cohorts²⁰. Bonferroni correction was applied
30 to P-values to correct for multiple testing.

1

2 **Blended phenotypes**

3 Several large patient series have shown that 3.2 – 7.2% of those in whom a molecular diagnosis
4 has been identified have multiple molecular diagnoses, i.e., a pathogenic variant at more than one
5 genetic locus, each associated with a distinct clinical disease, and each segregating independently⁸.
6 Each independent clinical-molecular diagnosis may have distinct or overlapping phenotypic
7 features which together result in a “blended phenotype”, representing the complex interaction
8 between effects of pathogenic variants in multiple genes within one individual⁸. To test the
9 hypothesis that phenotypic heterogeneity could be explained by “blended phenotypes” in some
10 individuals with Dravet syndrome, rare variants in additional epilepsy-related genes were
11 evaluated for “potential clinical relevance” (see Fig. 1, Supplementary Material 6). All variants
12 that met the “potential clinical relevance” criteria were evaluated by three clinicians (LMC, SB
13 and SMS), and the published phenotypes associated with each epilepsy-related gene were
14 compared with the phenotype of the individual harbouring that gene variant, to determine its
15 potential contribution. Additional variants were determined to potentially contribute to blended
16 phenotypes when aspects of the individual’s phenotype were better explained by the additional
17 epilepsy-related gene variant than the *SCN1A* variant (Fig. 1). Variants that were deemed to
18 contribute to blended phenotypes were subsequently classified using American College of Medical
19 Genetics and Genomics/Association for Molecular Pathology (ACMG-AMP) criteria, excluding
20 the criterion “BP5 alternate locus observations” due to the known presence of the *SCN1A* variant²¹;
21 and were included if they were classified as pathogenic, likely pathogenic, or variants of uncertain
22 significance (VUS).

23

24 **Polygenic risk scores**

25 To test the hypothesis that common genetic variation also influences the phenotype, PRS were
26 calculated for epilepsy, intelligence and longevity in the Dravet syndrome, GEL Epilepsy and GEL
27 control cohorts. PRS for intelligence, longevity and epilepsy were estimated using GWAS
28 summary statistics generated by the ILAE Consortium on Complex Epilepsies, Savage JE et al.,
29 and Deelen J et al., respectively^{22–24}. To investigate the formal genetic correlation between

1 intelligence, longevity and epilepsy, we performed Linkage Disequilibrium Score Regression
2 (LDSC) comparing the GWASs used for each PRS estimation (Supplementary Fig. 3). Genetic
3 correlation rates were calculated using the LDSC tool²⁵ (see Supplementary Material 7).

4 Following quality control steps (Supplementary Material 8), we calculated PRS based on the
5 overlap of the study groups' remaining quality-controlled SNPs²⁶. PRS for each individual was
6 obtained using the clumping and thresholding method implemented by PRSice-v2.3.3 across a set
7 of P-value thresholds ($PT= 10^{-4}, 10^{-3}, 10^{-2}, 5 \times 10^{-2}, 10^{-1}, 0.5, 1$)²⁷. PT with the best fit for the target
8 trait across the thresholds was identified (Supplementary Material 9; Supplementary Fig. 4-10). R^2
9 was used to measure the variance explained by the PRS and was produced directly from PRSice²⁷.

10 To compare PRS between the three cohorts for the selected best-fit PT , a one-way ANOVA was
11 applied (Supplementary Material 10). The analysis of variance model was adjusted for sex and the
12 first four principal components of ancestry, which further controls for ancestry bias²⁸. Differences
13 in the means between each pair of groups were assessed for significance using a post-hoc multiple
14 pairwise comparison (Tukey's test). To correct for multiple testing across three PRS analyses
15 Bonferroni correction was applied to P-values and the significance set to $\alpha=0.05/3$.

16 To further demonstrate that a potentially "causal" *SCN1A* variant is acting against a genomic
17 background that may influence the expressed phenotype, we performed a set of post-hoc analyses.
18 We estimated the same three PRS across the Dravet syndrome and GEL *SCN1A* control cohorts.
19 Differences in the PRS between cohorts were calculated as above. There is evidence that the most
20 significantly associated SNP from the epilepsy GWAS may exert regulatory control over *SCN1A*²²
21 and, therefore, may influence the outcome of PRS for epilepsy in Dravet syndrome. Therefore, we
22 also performed a localised PRS for epilepsy, intelligence and longevity, where we separated out
23 from the GWAS of common epilepsies the genome-wide significant SNPs which mapped to
24 2q24.3 and corresponded to the *SCN1A*-related locus. Although the 2q24.3 signal consisted of two
25 independent sub-signals, as shown by the ILAE Consortium on Complex Epilepsies in 2018²², the
26 insufficient number of genome-wide significant SNPs corresponding to the two sub-signals made
27 performing separate PRS analyses for the two signals impossible; therefore, the genome-wide
28 significant 2q24.3 SNPs across the two regions were considered as a single *SCN1A*-related signal.
29 Localised PRS for epilepsy, intelligence and longevity were performed both for only the 2q24.3
30 SNPs and excluding the 2q24.3 SNPs and compared across the three cohorts.

1

2 **Data availability**

3 Data will be made available on publication. The data can be requested by emailing the
4 corresponding author. Data will be shared with bona fide researchers after approval of proposals
5 with signed data access agreements as required by, and subject to, institutional and national
6 regulations.

7

8 **Code Availability**

9 No bespoke code was used for this study. All code used in the manuscript is in the public domain
10 already and has been appropriately referenced.

11

12 **Results**

13 ***SCN1A*-related Dravet syndrome cohort and variant description**

14 Thirty-four adults with *SCN1A*-related Dravet syndrome were included; 28 were of European
15 ancestry. Mean age at last follow-up was 32.5 years (SD \pm 13.6; range 16–70); mean age at genetic
16 diagnosis was 25.8 years (SD \pm 15.3; range 3–59); mean age at seizure onset was 6.5 months
17 (SD \pm 3.1; range 2–16); 18 (52.9%) were female. Further information is given in Supplementary
18 Table 1.

19 All pre-identified *SCN1A* variants were validated in the WGS data. Across the 34 individuals, 34
20 unique *SCN1A* variants were identified including one whole gene deletion. Details of the *SCN1A*
21 variants can be found in Fig. 2, Supplementary Material 11, and Supplementary Table 1. The
22 variant distribution is comparable to published cohorts of individuals with *SCN1A*-related
23 syndromes^{4,29,30}. No obvious association between variant class (i.e. missense or null) and specific
24 phenotypes was observed (Supplementary Table 1). In addition, divergent phenotypes were seen
25 in two unrelated individuals (1-105287 and 1-105683) who shared the same *SCN1A* variant
26 (Supplementary Table 6). The WGS mean read coverage of the *SCN1A* gene region across the
27 samples was 43.5 (excluding the *SCN1A* gene deletion). Visual inspection of the aligned reads

1 using IGV showed an average alternate allele fraction of the known pathogenic *SCN1A* variants of
2 47.81%, confirming heterozygosity (excluding the homozygous *SCN1A* variant and whole gene
3 deletion). None of the individuals showed evidence for mosaicism of the pathogenic *SCN1A*
4 variant (P -value >0.05 ; Chi-squared test) (Supplementary Table 1; Supplementary Material 12).

5 We explored whether particular differences between ultra-rare *SCN1A* missense variants identified
6 in the Dravet syndrome and GEL *SCN1A* control cohorts might explain differences in phenotype
7 between these groups. No difference in the *SCN1A*-encoded variant residue location within the
8 protein sequence was seen between missense variants identified in the Dravet syndrome cohort
9 compared with the GEL *SCN1A* control cohort (Supplementary Table 1, Supplementary Table 4,
10 Supplementary Material 13). Five GEL *SCN1A* controls carried *SCN1A* missense variants that
11 have previously been reported in association with epilepsy syndromes, including Dravet
12 syndrome^{31–35} (Supplementary Table 4).

13

14 **Rare variant analyses**

15 **Gene and gene-set based collapsing analyses of rare variants: enrichment of** 16 **rare variants in Dravet syndrome cohort**

17 All individuals with Dravet syndrome were first assessed for the presence of additional rare
18 variants, meeting a frequency cut off ≤ 0.0005 in gnomAD, across 190 epilepsy-related genes: 95
19 additional rare variants across 59 epilepsy-related genes were identified (Supplementary Table 7).
20 Individuals had a median of 3 (range 0-7; interquartile range 2-3) additional rare variants
21 (Supplementary Table 1).

22 To evaluate if individuals with Dravet syndrome harbour a higher burden of additional rare
23 variants compared to the control cohorts, we performed gene-based and gene-set collapsing
24 analyses for rare variants across 190 epilepsy-related genes, excluding *SCN1A*^{13,15}. Each gene was
25 considered individually for the gene-based analysis, while all 190 genes were considered as a set
26 for the gene-set collapsing analysis. In the gene-set collapsing analysis, there was an enrichment
27 ($P=0.0006$) of rare variants in epilepsy-related genes in Dravet syndrome (78 qualifying rare
28 variants in 28 cases; 2.78 variants per individual) compared to the GEL Epilepsy controls (1251
29 qualifying rare variants in 772 cases; 1.62 variants per individual), in concordance with a previous

1 study reporting an excess of rare variants in (different but overlapping) epilepsy-related genes in
2 individuals with Dravet syndrome³⁶. The gene-based collapsing analyses suggested a higher rare
3 variant burden in the genes *EHMT1*, *CHD2*, *FLNA*, *TSC1*, *PRICKLE1*, *SETBP1*, *NRXN1*, *SPTAN1*
4 and *ARID1B* ($P < 0.05$) in Dravet syndrome compared to GEL Epilepsy controls (Supplementary
5 Fig. 11A), but after correction for multiple comparisons, none of the adjusted P-values were
6 significant. Of the 78 rare variants identified in these individuals with Dravet syndrome, a
7 significant proportion (11/78 variants; 14.10%) overlapped with the 1251 rare variants identified
8 in the GEL Epilepsy controls ($P = 0.0001$, Fisher's exact test). The results of burden analysis for
9 rare variants across 153 autosomal genes showed the same direction of enrichment as in the main
10 analysis for rare variants across all 190 genes (Supplementary Material 14). Though we
11 investigated whether the observed variant enrichment in Dravet syndrome was driven by
12 individuals with missense *SCN1A* variants, but were underpowered to formally report this outcome
13 (Supplementary Material 15; Supplementary Material 16).

14 **Rare variants in additional epilepsy-related genes: blended phenotypes may** 15 **explain some phenotypic heterogeneity in Dravet syndrome**

16 Across all individuals with Dravet syndrome, 51 rare variants in 38 epilepsy-related genes met
17 pre-specified "potential clinical relevance" criteria and underwent a detailed phenotype-genotype
18 review (Supplementary Table 7). Five variants across four epilepsy-related genes (*DEPDC5*,
19 *CHD2*, *SCN8A*, and *IQSEC2*), all VUS by ACMG-AMP criteria alone, were considered to offer
20 an independent molecular diagnosis, alongside the known *SCN1A* variant, resulting in blended
21 phenotypes including features of both Dravet syndrome and the additional epilepsy-related genetic
22 disorder. Parental samples were not available for these five adults, so we were unable to determine
23 if the additional variants were *de novo*. For each of the five individuals, the variant and phenotype
24 are discussed in detail (see Case 1 below, and Supplementary Material 17).

25 **Case 1: Blended phenotype due to *SCN1A* and *DEPDC5* variants (Case ID: 1-102398)**

26 This individual with Dravet syndrome and a likely pathogenic splicing variant in *SCN1A*
27 (NM_001165963:exon22:c.3706-2A>G), has left temporal lobe focal cortical dysplasia (FCD)
28 (Fig. 3A), and ictal scalp EEG recordings consistently demonstrating that many of his seizures are
29 of left temporal onset (see Supplementary Material 17 for full details). He was found to have a

1 *DEPDC5* missense variant (NM_001242896.3:c.G4183A:p.A1395T) that met pre-specified
2 “potential clinical relevance” criteria.

3 The identified *DEPDC5* missense variant replaces a highly conserved alanine with threonine at
4 codon 1395 of the *DEPDC5* protein (Fig. 3B and C), with a Genomic Evolutionary Rate Profiling
5 score of 4.1, indicating the site is under evolutionary constraint³⁷. Computational evidence (SIFT,
6 PolyPhen2, MutationTaster) suggests the variant is damaging (Supplementary Table 7). Whilst
7 most pathogenic variants in *DEPDC5* are truncating, some missense variants are also established
8 as disease-causing, and have been identified in individuals with FCD³⁸⁻⁴¹. This variant is
9 encountered in seven individuals in gnomAD, corresponding to an allele frequency of 0.00005,
10 considered to be within the pathogenic range⁴², and is absent from an ancestry-matched population
11 database ($n=800$)⁴³. The penetrance of *DEPDC5*-related epilepsies is estimated to be around
12 60%⁴⁴, and therefore the presence of this variant at low numbers within a population database
13 would not be unexpected. This variant is considered a VUS according to a classification framework
14 specifically adapted to GATOR1 genes⁴⁵, by ACMG-AMP criteria, and reported as a VUS in
15 ClinVar. To further explore its potential pathogenicity, *in silico* modelling was undertaken.
16 Ala1395 lies at an internal inter-domain interface between the N-terminal, SABA and C-terminal
17 domains of *DEPDC5* (domains as defined by Shen *et al.*⁴⁶), in close proximity to residues within
18 those domains (Fig. 3D-G and Supplementary Fig. 12A-C). The effect of the variant was examined
19 in both published structures for *DEPDC5*, PDB 6ces (GATOR1 complex bound to Rag GTPases)
20 and 6cet (GATOR1 complex alone), with similar, though not identical, results (for details, see Fig.
21 3H, Supplementary Fig. 12D, and Supplementary Material 18). In summary, the Ala1395Thr
22 variant has a deleterious impact either on the folding and/or stability of *DEPDC5*, or impairs the
23 ability of the GATOR1 complex to respond to Rag GTPases, in both cases likely leading to loss
24 of function, the most commonly recognised mechanism of disease causation associated with
25 *DEPDC5* variants.

26 FCD is a malformation of cortical development. We explored the potential contribution of the
27 *SCN1A* and *DEPDC5* variants to the FCD by examining the dynamic expression patterns of those
28 genes in the human temporal neocortex. FCD is thought to arise at 8-20 weeks post-conception⁴⁷,
29 the time frame in which *DEPDC5* has a peak in expression; conversely, at this time expression of
30 *SCN1A* is minimal (Supplementary Fig. 13 and Supplementary Material 19). Therefore, the variant
31 in *DEPDC5* is temporally more likely to be causative of the FCD, in keeping with known

1 consequences of *DEPDC5* loss of function variants^{41,48}. However, we acknowledge that this
2 finding is an association only, that is, we do not know and cannot establish when the FCD arose
3 in the individual. Eight individuals with Dravet syndrome and *SCN1A* variants with FCD, six with
4 histopathological confirmation, have been described (Supplementary Table 8)^{49–53}. To our
5 knowledge, in these reports, only *SCN1A* sequencing was undertaken.

6 Overall, in the context of the visualised FCD, concordant electroclinical onset for many of his
7 seizures, the *in silico* analysis and the temporal expression, we consider this variant to likely be
8 contributory, thus potentially responsible for generating a blended phenotype in this individual.
9 To confirm this finding a full exploration with model systems would be required.

10 **Polygenic Risk Score Analyses**

11 In Dravet syndrome, phenotypic heterogeneity encompasses many elements, including seizure
12 severity and type, degree of intellectual disability, risk of sudden unexpected death in epilepsy
13 (SUDEP) and comorbidities. Common genetic variation that confers risks for these traits may
14 influence the phenotypic expression. We utilised two PRS analyses to explore key characteristics
15 of Dravet syndrome for which there is known phenotypic heterogeneity: “epilepsy” and
16 “intelligence”. In addition, recognising that our adult Dravet syndrome cohort represents self-
17 selected survivors, we also performed a PRS for “longevity”. All PRS were performed on
18 individuals of European ancestry only.

19 **PRS for Intelligence: common genetic variation may influence severity of ID in** 20 **Dravet syndrome**

21 ID is almost universal in adults with Dravet syndrome, but the severity of impairment can range
22 from borderline to severe^{29,54,55}, although, rarely, adults and adolescents with Dravet syndrome
23 have near-normal intellect^{54–56}. Identical *SCN1A* variants can present with a range of cognitive
24 phenotypes even within families⁵⁷. Factors impacting cognitive outcomes in people with Dravet
25 syndrome are debated^{11,29,55,58–60}. We hypothesised that the common variant load for intelligence
26 would be lower in individuals with Dravet syndrome compared with GEL Epilepsy and GEL
27 controls. PRS for intelligence was significantly lower in the Dravet syndrome cohort than in GEL
28 Epilepsy (Adjusted $P=0.0024$, at $PT=10^{-4}$, Tukey’s test), and GEL controls (Adjusted $P=0.003$,
29 at $PT=10^{-4}$, Tukey’s test). There was no significant difference in the intelligence PRS between

1 GEL Epilepsy and GEL controls (Adjusted $P=0.69$, at $PT=10^{-4}$, Tukey's test) (Fig. 4A,
2 Supplementary Material 9; Supplementary Fig. 4 and 5). The intelligence PRS explained
3 approximately 3% ($R^2=0.03$) of the total phenotypic variance in the Dravet syndrome group
4 (derived from PRSice; Supplementary Fig. 6A).

5 **PRS for longevity: common genetic variation may contribute to survival in** 6 **Dravet syndrome**

7 An estimated 10-20% of children with Dravet syndrome die before reaching adulthood, mostly
8 due to SUDEP and status epilepticus^{61,62}. We hypothesised that the longevity PRS would be *higher*
9 in this cohort of individuals with Dravet syndrome who have survived into adulthood (mean age
10 32.5 years), especially as many had received a late diagnosis and had unknowingly had what in
11 retrospect was suboptimal antiseizure medication (e.g. sodium channel-blocking medications)
12 (Supplementary Table 1). PRS for longevity was significantly higher in the Dravet syndrome
13 cohort than in GEL Epilepsy controls (Adjusted $P=0.011$, at $PT=10^{-2}$, Tukey's test), and higher
14 than, but not significant, in GEL controls (Adjusted $P=0.024$, at $PT=10^{-2}$, Tukey's test). No
15 significant difference was seen in the longevity PRS comparing GEL controls with GEL Epilepsy
16 controls (Adjusted $P=0.68$, at $PT=10^{-2}$, Tukey's test) (Fig. 4B, Supplementary Material 9;
17 Supplementary Fig. 7 and 8). The longevity PRS explained around 2% ($R^2=0.02$) of the total
18 phenotypic variance in the Dravet syndrome cohort (Supplementary Fig 6B).

19 **PRS for epilepsy: no common genetic variant contribution to the epilepsy** 20 **phenotype in individuals with Dravet syndrome**

21 Variants in *SCN1A* are associated with a spectrum of disorders in which the seizure phenotype is
22 variable, from simple, self-remitting febrile seizures at the mild end, to drug-resistant epilepsy in
23 people with Dravet syndrome at the severe end. Even amongst family members segregating one
24 pathogenic *SCN1A* variant, the severity of the seizure phenotype can be wide-ranging, suggesting
25 a contribution of additional genetic variation to the phenotype⁶³. Therefore, we hypothesised that
26 the PRS for epilepsy would be *higher* in individuals with Dravet syndrome compared to GEL
27 Epilepsy and GEL controls. The epilepsy PRS was higher in the Dravet syndrome cohort compared
28 with the GEL Epilepsy and GEL controls, although this did not reach statistical significance
29 (Adjusted $P=0.89$, at $PT=10^{-2}$, and Adjusted $P=0.11$, at $PT=10^{-2}$, Tukey's test, respectively). As

1 expected, the epilepsy PRS was significantly higher in GEL Epilepsy compared with GEL controls
2 (Adjusted $P < 2.22 \times 10^{-16}$, at $PT = 10^{-2}$, Tukey's test) (Fig. 4C, Supplementary Material 9;
3 Supplementary Fig. 9 and 10). The epilepsy PRS explained around 0.05% ($R^2 = 0.0005$) of the total
4 phenotypic variance in the Dravet syndrome cohort (Supplementary Fig 6C).

5 **Post-hoc Analyses**

7 **Localised PRS: Common variation in *SCN1A* does not influence the difference** 8 **in PRS for intelligence and longevity observed in Dravet syndrome**

9 To further investigate the influence of *SCN1A*-related common variation on the PRS results, we
10 selected the genome-wide significant SNPs from the largest published GWAS of common
11 epilepsies, which mapped to 2q24.3, corresponding to the *SCN1A*-related locus²². We then
12 performed a localised PRS for intelligence, longevity and epilepsy first excluding the 2q24.3
13 SNPs, and then evaluating *only* the 2q24.3 SNPs²². Exclusion of the *SCN1A* signal did not modify
14 the findings from the full PRS analysis, confirming that common variation in *SCN1A* is not driving
15 the lower PRS for intelligence and higher PRS for longevity in the Dravet syndrome cohort
16 compared with GEL Epilepsy and GEL control cohorts (Supplementary Fig. 14). PRS performed
17 considering only the 2q24.3 *SCN1A*-related SNPs did not show a significant difference across the
18 cohorts, further supporting the finding that the *SCN1A* signal is not driving differences in PRS
19 (Supplementary Fig. 15).

20 **PRS and burden analyses of GEL *SCN1A* control cohort: variants beyond** 21 ***SCN1A* may be required for the full phenotypic expression of Dravet syndrome**

22 To further evaluate the hypothesis that additional rare and common genetic variation may be
23 necessary for the Dravet syndrome phenotype in some individuals with *SCN1A* variants, a post-
24 hoc exploration with PRS and burden analysis was undertaken, comparing individuals with Dravet
25 syndrome with a GEL *SCN1A* control cohort composed of 45 GEL probands with unique *SCN1A*
26 missense variants, but without epilepsy (Supplementary Table 4). Five GEL *SCN1A* controls
27 carried unique *SCN1A* variants that have previously been reported in association with epilepsy
28 syndromes^{31–35} or sudden unexpected death⁶⁴ (Supplementary Table 4).

1 PRS for intelligence was lower but not significant (Adjusted $P=0.033$, at $PT=10^{-4}$, Tukey's test)
2 (Fig. 5A), PRS for longevity was higher but not significant (Adjusted $P=0.049$, at $PT=10^{-2}$,
3 Tukey's test) (Fig. 5B), and PRS for epilepsy was higher but not significant (Adjusted $P=0.28$, at
4 $PT=10^{-1}$, Tukey's test) in the Dravet syndrome cohort compared with the GEL *SCN1A* controls
5 (Fig. 5C). We also compared PRS for intelligence, longevity, and epilepsy between GEL *SCN1A*
6 controls and the 13 Dravet syndrome cases with *SCN1A* missense variants. No significant
7 difference was identified, though the direction of effect was maintained in comparison to the main
8 analysis (Supplementary Fig. 16).

9 The gene-set collapsing analysis revealed an enrichment ($P=0.010$) of rare variants in Dravet
10 syndrome (78 variants in 28 individuals; 2.78 variants per individual) compared with GEL *SCN1A*
11 controls (81 variants in 45 individuals; 1.8 variants per individual). None of the variants identified
12 in Dravet syndrome overlapped with variants in the GEL *SCN1A* controls. A gene-based collapsing
13 analysis highlighted an increased variant burden in *CHD2*, *FLNA* and *TSC1* ($P<0.05$) in Dravet
14 syndrome compared with GEL *SCN1A* controls (Supplementary Fig. 11B) that was not significant
15 after correction for multiple comparisons.

16

17 Discussion

18 Dravet syndrome is the archetypal DEE and amongst the most common of the rare epilepsies^{1,4}.
19 Understanding of Dravet syndrome pathophysiology is amongst the most advanced for any DEE,
20 reflected in the range of targeted therapies now in development⁶⁵⁻⁶⁷. The core phenotype is
21 sufficiently distinct that the diagnosis is usually made clinically, followed by genetic testing
22 anticipating a causal *SCN1A* variant, reflecting the very strong association between phenotype and
23 causal gene. Nevertheless, the currently understood full phenotypic spectrum of Dravet syndrome
24 is very broad, to the extent that in the absence of the telling early clinical history, the diagnosis
25 may be missed clinically, especially in adulthood, and only considered on revelation of a putatively
26 pathogenic *SCN1A* variant⁶⁸. Moreover, even given the distinct core phenotype, there is marked
27 phenotypic heterogeneity within the syndrome³⁰, which is not fully explained by differences
28 between causal pathogenic variants^{29,69}, and unexplained heterogeneity (not always due to
29 mosaicism) within families segregating one pathogenic variant⁶³ and between unrelated

1 individuals carrying the same variant⁷⁰. “Incomplete penetrance” and “variable expressivity” are
2 useful operational constructs in clinical practice to accommodate such heterogeneity. As with the
3 concept of a “syndrome”, the undoubted utility of the terms “penetrance” and “expressivity”
4 presumably reflects their basis in biology and pathophysiology. Some of the heterogeneity
5 captured by these terms is probably due to genetic variation beyond the causal *SCN1A* variant.
6 Digenic, oligogenic, polygenic, dual molecular diagnoses, mutational burden and double-hit
7 contributions to disease phenotypes are well established as concepts⁸. Discovering real examples
8 in epilepsy is complicated both by the many syndromes and conditions that constitute this umbrella
9 term, and by the known common variant contribution to the epilepsies overall. Controlling for the
10 main genetic contributor of a genetic condition can allow additional genetic contributions to the
11 phenotype to be discovered, as has been shown for example in Huntington’s disease^{71,72}. Here, we
12 adopted the same approach to Dravet syndrome, exploring WGS from a small group of adults with
13 Dravet syndrome due to variation in *SCN1A*. We show that in clinically-distinct cases of Dravet
14 syndrome, with a known *SCN1A* variant (classified as pathogenic or likely pathogenic in 33/34
15 cases, and published as pathogenic in the remaining case⁷³), there are examples of blended
16 phenotypes, an excess of rare variants in epilepsy-related genes, and polygenic contributions to the
17 overall phenotype, with additional evidence for genomic resilience (significantly elevated PRS for
18 longevity). We show that beyond the causal coding or genic *SCN1A* variant, enrichment of rare
19 variants in epilepsy-related genes and common variation in both *SCN1A* and across the genome
20 are present and may have an impact. The presence of two disease-causing rare variants can lead to
21 blended phenotypes, as shown by the presence of symptomatic FCD and a *DEPDC5* variant in one
22 individual with a clear Dravet syndrome phenotype due to a causal variant in *SCN1A*, with
23 additional examples in other genes (*CHD2*, *IQSEC2*, and *SCN8A*). PRS analyses demonstrate that
24 the causal *SCN1A* variant is acting against particular backgrounds. The effect size (as demonstrated
25 by the explained variance) is limited, a common observation in studies of polygenic risk using
26 current tools. However, evidence shows that the polygenic background may have a more
27 substantial and clinically relevant effect in individuals with a monogenic disease^{74,75},
28 demonstrating the principle that the rest of the genome is not inert in monogenic epilepsies, as
29 recently demonstrated in unselected DEEs⁷⁶.

30 For example, in two unrelated individuals with Dravet syndrome from this cohort, who share the
31 same *SCN1A* splicing variant, the milder seizure and cognitive phenotype in one may in small part

1 be explained by their lower epilepsy, and higher intelligence, PRS, respectively (Supplementary
2 Table 6), demonstrating how a more (or less) favourable genetic background may contribute to
3 explaining intra-familial and variant-specific phenotypic heterogeneity, and have bearing on our
4 understanding of disease biology in “monogenic” epilepsies. Of particular interest, the
5 significantly lowered PRS for intelligence in our cohort could imply that even with symptomatic
6 treatment leading to seizure freedom, or with disease-modifying treatment increasing *SCN1A*
7 expression, the full phenotype of Dravet syndrome may not be entirely reversible. All these
8 additional rare and common variants are obviously present independently of the observed *SCN1A*
9 variant. Our results demonstrate that there is value in exploring additional genomic variation even
10 when a “causal”, plausible and compatible pathogenic variant is identified, but clearly challenges
11 remain in such work. Gathering and sequencing a cohort large enough to explore additional
12 genomic variation, such as *SCN1A*-independent common (for example, through a genome-wide
13 SNP-based association study) and rare variation (for example, through gene burden testing) is
14 challenging. Functional validation for multiple variants will be complex, especially when, in most
15 cases, there is no functional validation in clinical practice for the *SCN1A* variant itself found in an
16 individual with Dravet syndrome: individual-based induced programmable stem cells and
17 organoids may offer a way forward⁷⁷. More tools are being developed that will allow integration
18 and joint analysis of the contributions of different types of variation (e.g. category-wise association
19 studies), but many potentially useful existing tools, especially those devised for clinical
20 application, such as the ACMG-AMP system, are not intended to be used for additional variants²¹:
21 our mindset is still largely centred on monogenic causation.

22 Nevertheless, we demonstrate that pathogenic variants in *SCN1A* do not necessarily act alone to
23 produce the final phenotype: *SCN1A* may be the gene of major effect in Dravet syndrome, but it
24 is not always the only gene, or only variant, of relevance. Moreover, Dravet syndrome-causing
25 pathogenic variants may need to act against a broadly compromised genomic background (with,
26 for example, a lower PRS for intelligence) to generate the full Dravet syndrome phenotype, whilst
27 on the other hand genomic resilience may ameliorate some serious outcomes, such as premature
28 mortality in Dravet syndrome, as shown by the elevated PRS for longevity in our adult Dravet
29 syndrome survivors, most of whom had received a diagnosis in adulthood, and had been exposed
30 to contraindicated medication. That a causal *SCN1A* variant inevitably acts within the context of
31 the rest of the genome, some variation within which is relevant to the final phenotype, is perhaps

1 unsurprising, but has not been demonstrated across a range of *SCN1A* variants before, and has not
2 been addressed using the range of variation that can be examined using WGS data. Such work may
3 help define the true phenotypic breadth of DS and other “monogenic” conditions, and constrain
4 the often bewildering expansion of phenotype in any given condition. Finally, the revelation of
5 additional influential genomic variation in individual cases may have relevance to individual
6 prognostication, and to treatments currently in development (e.g. gene-based therapies), informing
7 realistic outcomes to be expected from new and existing treatments, and point the way to novel
8 treatments, for example by using information from genomic variants in individuals with mild
9 phenotypes to generate therapies to lessen severity in those with more severe phenotypes.

10 There are limitations to this study, primarily the limited size of the cohort, the cohort only
11 consisting of adults and the lack of experimental validation using appropriate model systems.
12 Despite these limitations, the results suggest that there may be occasions when stopping at the first
13 plausible causal variant is premature⁸, with additional biological information of value identifiable
14 by more extensive interrogation of the rest of an individual’s genome. Non-genomic factors will
15 undoubtedly also modulate phenotype, but genomic variation may contribute more than is
16 currently believed.

17

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10

11 **Competing interests**

12 The authors declare the following competing interests: AB has received honoraria for presenting
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11

12 **Supplementary material**

13 Supplementary material is available at *Brain* online.

14 **Appendix 1**

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16 Full details are provided in the Supplementary material.

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1 **Figure legends:**

2 **Figure 1 Method for selection of variants in epilepsy-related genes:** Method for selection of
 3 variants in epilepsy-related genes with “potential clinical relevance” that may contribute to
 4 blended phenotypes. GEL = Genomics England.

5
 6 **Figure 2 Distribution of *SCN1A* variants found in the Dravet syndrome cohort.** A schematic
 7 diagram of the *SCN1A* gene. Exons are indicated by vertical black boxes (1-29) and introns by the
 8 horizontal black line (not to scale). Missense (purple), splicing (dark blue), frameshift insertion
 9 (light blue), frameshift deletion (green), and stop-gain (red) variants are shown. The whole gene
 10 deletion is not shown. Variants are shown according to the NM_001165963.4 reference sequence.

11
 12 **Figure 3 Focal cortical dysplasia (FCD) and details of *DEPDC5* variant.** (A) Brain MRI
 13 showing FCD. Coronal T1-weighted brain MRI from case 1-102398, with *DEPDC5* variant
 14 NM_001242896.3:c.G4183A:p.A1395T, showing left temporal lobe FCD (right of patient is on
 15 left of image in these images, following radiological convention), with blurred grey-white interface
 16 and cortical thickening apparent in the left temporal lobe across several consecutive slices. (B)
 17 MetaDome map of regional constraint in *DEPDC5*. Grey bar below the graph represents the
 18 protein, pink bars showing Pfam domains: PF12257, Vacuolar membrane-associated protein Iml1
 19 domain; PF00610, Domain found in Dishevelled, Egl-10, and Pleckstrin (DEP); A1395 is marked
 20 by a vertical green line, with a reported tolerance score of 0.28 (“intolerant”). (C) VarSite sequence
 21 logo for *DEPDC5* residues 1375-1414, based on alignment of structural homologues; below the
 22 logo is the sequence of *DEPDC5* itself, with A1395 boxed; sequence conservation score for this
 23 residue was 0.92 (range 0(low)-1(high)); alanine was observed at this position in 31/33 aligned
 24 sequences. (D) Structure of the GATOR1-Rag GTPases complex and context of *DEPDC5*
 25 Ala1395. PDB 6ces, the structure of the heterotrimeric GATOR1 complex
 26 (*DEPDC5*:NPRL2:NPRL3) bound to RagA and RagC GTPases; protein surfaces shown by colour
 27 as indicated (except *DEPDC5*, shown as a ribbon and coloured by structural domains as annotated
 28 by⁴⁶: bright green=N-terminal domain (NTD) (residues 38-165); cyan=structural axis for binding
 29 arrangement (SABA) domain (166-425); orange=steric hindrance for enhancement of nucleotidase

1 activity (SHEN) domain (721-1010); dark green=DEP domain (1175-1270); violet=C-terminal
 2 domain (CTD) (1271-1600); Ala1395 is pink with sidechain atoms shown as spheres. **(E, F)**
 3 Ala1395 lies at an inter-domain interface in DEPDC5. The figure shows selected residues of
 4 DEPDC5 from PDB 6ces (chain D); residues of the NTD, SABA domain and CTD are shown as
 5 separate surfaces; residues of the SHEN domain and DEP domain are shown as ribbons. **(F)** shows
 6 the same structure as **(E)** with SHEN and DEP domains removed; residues Tyr108 (bright green),
 7 Phe326 (blue) and Ala1395 (rose pink) lie in close proximity at a 3-way interface between the
 8 NTD, SABA and CTD. **(G)** Zoomed DEPDC5 structure (PDB 6ces, chain D) as in **(E)** and **(F)**,
 9 zoomed to show detail around the 3-way interface between the NTD, SABA and CTD; **(H)** The
 10 Ala1395Thr substitution results in reduced space at the inter-domain interface in 6cesD. This
 11 figure shows the same structure as **(G)** after introduction of the Ala1395Thr variant by *in silico*
 12 mutagenesis. Quantitative results are given in Supplementary Material 18. Analysis of DEPDC5
 13 from PDB 6cet is shown in Supplementary Fig. 12.

14
 15 **Figure 4 Polygenic Risk Scores (PRS) applied across the cohorts. (A)** PRS for intelligence was
 16 lower in the Dravet syndrome cohort than in GEL Epilepsy (Adjusted $P=0.0024$) and GEL control
 17 cohorts (Adjusted $P=0.003$). The difference between GEL Epilepsy and GEL controls was not
 18 significant (Adjusted $P=0.69$). **(B)** PRS for longevity was significantly higher in the Dravet
 19 syndrome cohort than in GEL Epilepsy controls (Adjusted $P=0.011$), and higher than, but not
 20 significant, in GEL controls (Adjusted $P=0.024$) and not significantly different in GEL Epilepsy
 21 controls compared to GEL controls (Adjusted $P=0.68$). **(C)** PRS for epilepsy was not significantly
 22 different in the Dravet syndrome cohort compared with the GEL controls (Adjusted $P=0.89$) and
 23 GEL Epilepsy controls (Adjusted $P=0.11$). PRS for epilepsy was significantly higher in the GEL
 24 Epilepsy controls than in the GEL controls (Adjusted $P<2.22e-16$). The per-PRS P-values shown
 25 in the graphics are estimated using a post-hoc multiple pairwise comparison (Tukey's test). As
 26 multiple PRS analyses were performed, the final Adjusted P-value significance threshold was set
 27 to $\alpha=0.05/3$.

28
 29 **Figure 5 Polygenic Risk Scores (PRS) applied across the GEL SCN1A control and Dravet**
 30 **syndrome cohorts: (A)** PRS for intelligence was lower, but not significant, in the Dravet

1 syndrome cohort than in GEL *SCN1A* controls (Adjusted $P=0.033$). **(B)** PRS for longevity was
2 higher, but not significant, in the Dravet syndrome cohort than in GEL *SCN1A* controls (Adjusted
3 $P=0.049$). **(C)** PRS for epilepsy was not significantly different between the Dravet syndrome
4 cohort and GEL *SCN1A* controls (Adjusted $P=0.28$). Black circles = Individuals from the GEL
5 *SCN1A* control cohort with variants previously reported to be associated with disease. The per-
6 PRS P-values shown in the graphics are estimated using a post-hoc multiple pairwise comparison
7 (Tukey's test). As multiple PRS analyses were performed, the Adjusted P-value significance
8 threshold was set to $\alpha=0.05/3$.

9

10

11

ACCEPTED MANUSCRIPT

Rare variants in epilepsy-related genes:

GEL “green-rated” epilepsy genes with an allele frequency in gnomAD <0.0005

95 variants across 59 genes

“Potential clinical relevance” criteria:

- gnomAD allele count ≤ 8
- Deleterious according to at least one *in silico* tool (SIFT, PolyPhen, MutationTaster)
- Not reported as benign/likely benign in ClinVar

50 variants across 38 genes

Detailed genotype-phenotype review:

- Aspects of phenotype better explained by the additional variant

5 variants across 4 genes

Blended phenotypes:

SCN1A and *DEPDC5*, *CHD2*, *SCN8A*, *IQSEC2*

Figure 1
140x195 mm (x DPI)

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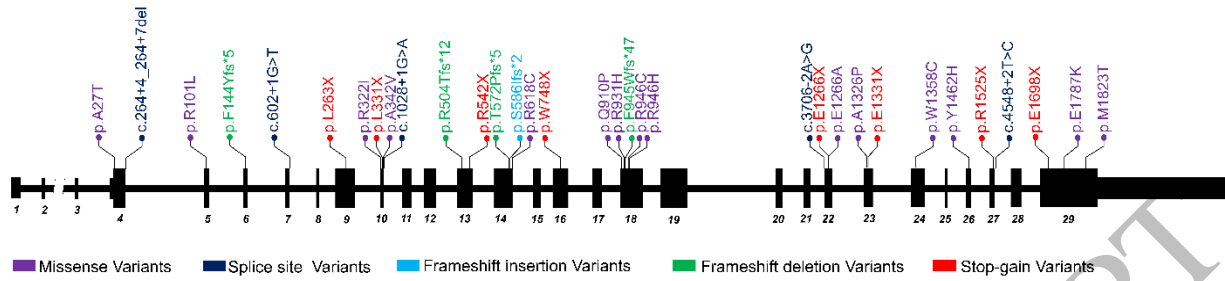
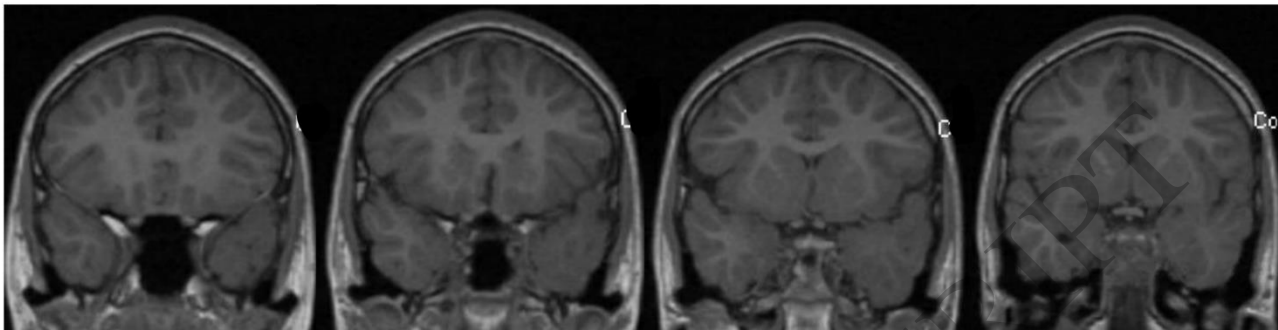


Figure 2
559x126 mm (x DPI)

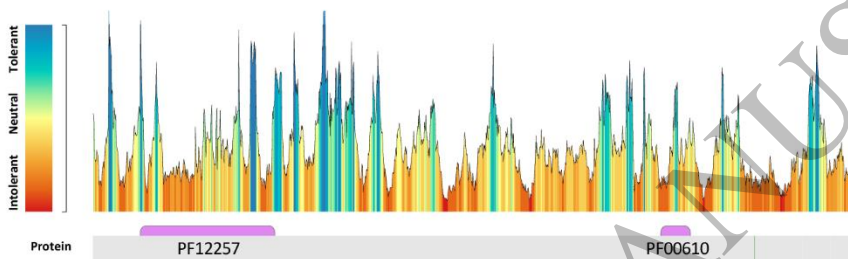
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ACCEPTED MANUSCRIPT

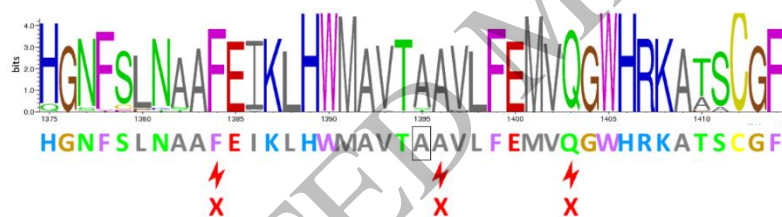
A



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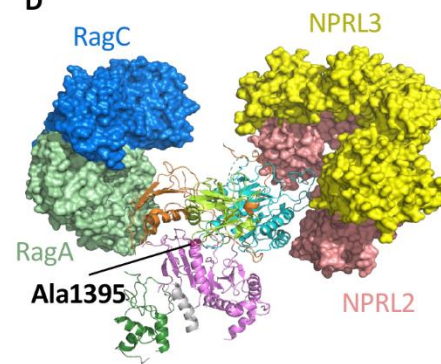


C

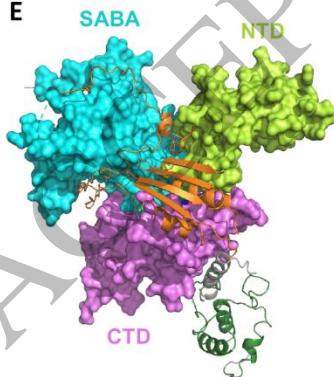


The amino acid codes are coloured by residue type: H, K, R = positive, D, E = negative, S, T, N, Q = neutral, A, V, L, I, M = aliphatic, F, Y, W = aromatic, P, G = Pro&Gly, C = cysteine

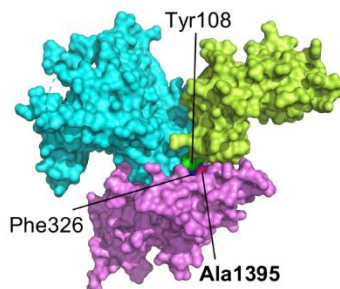
D



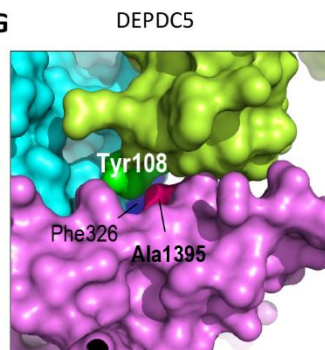
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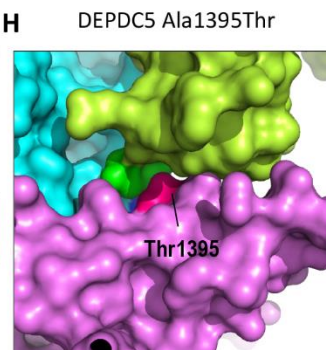
F



G

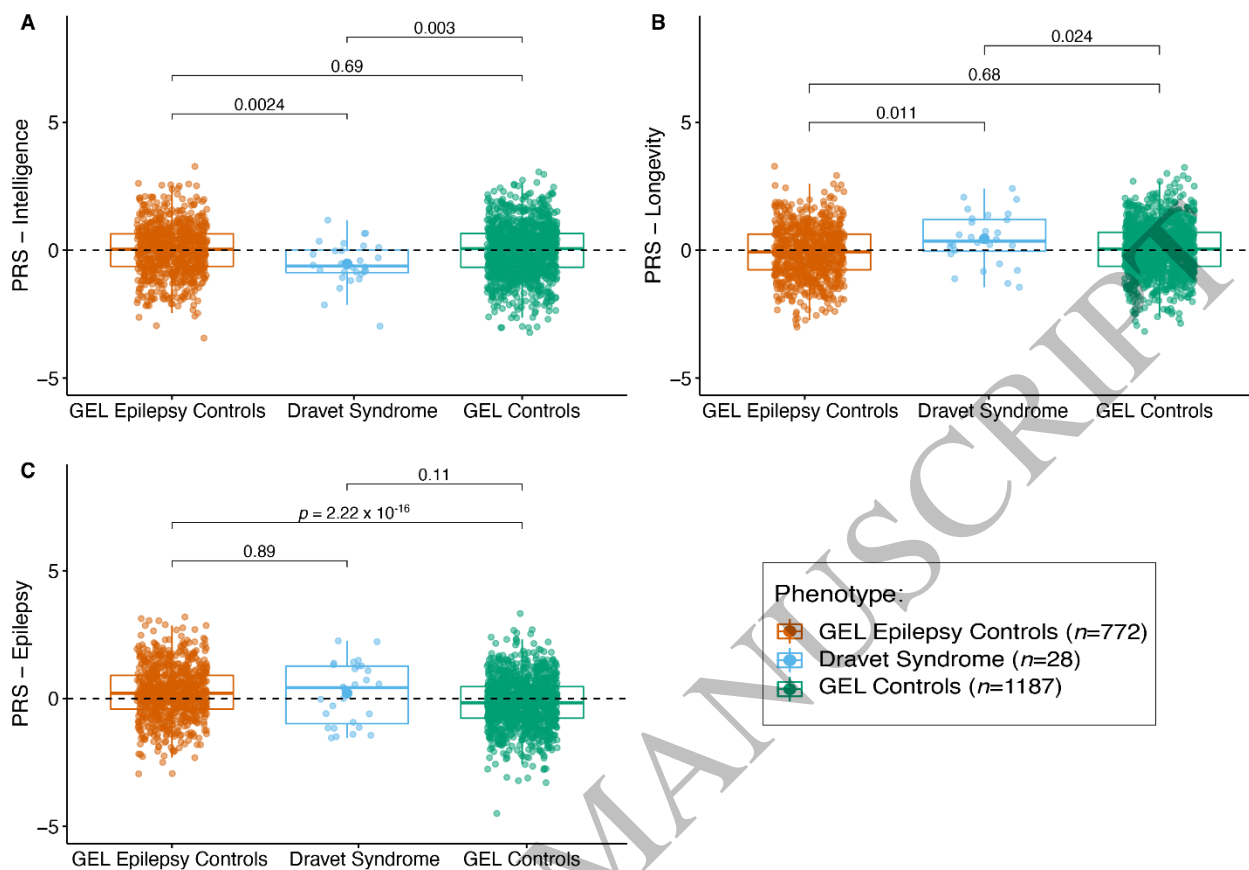


H



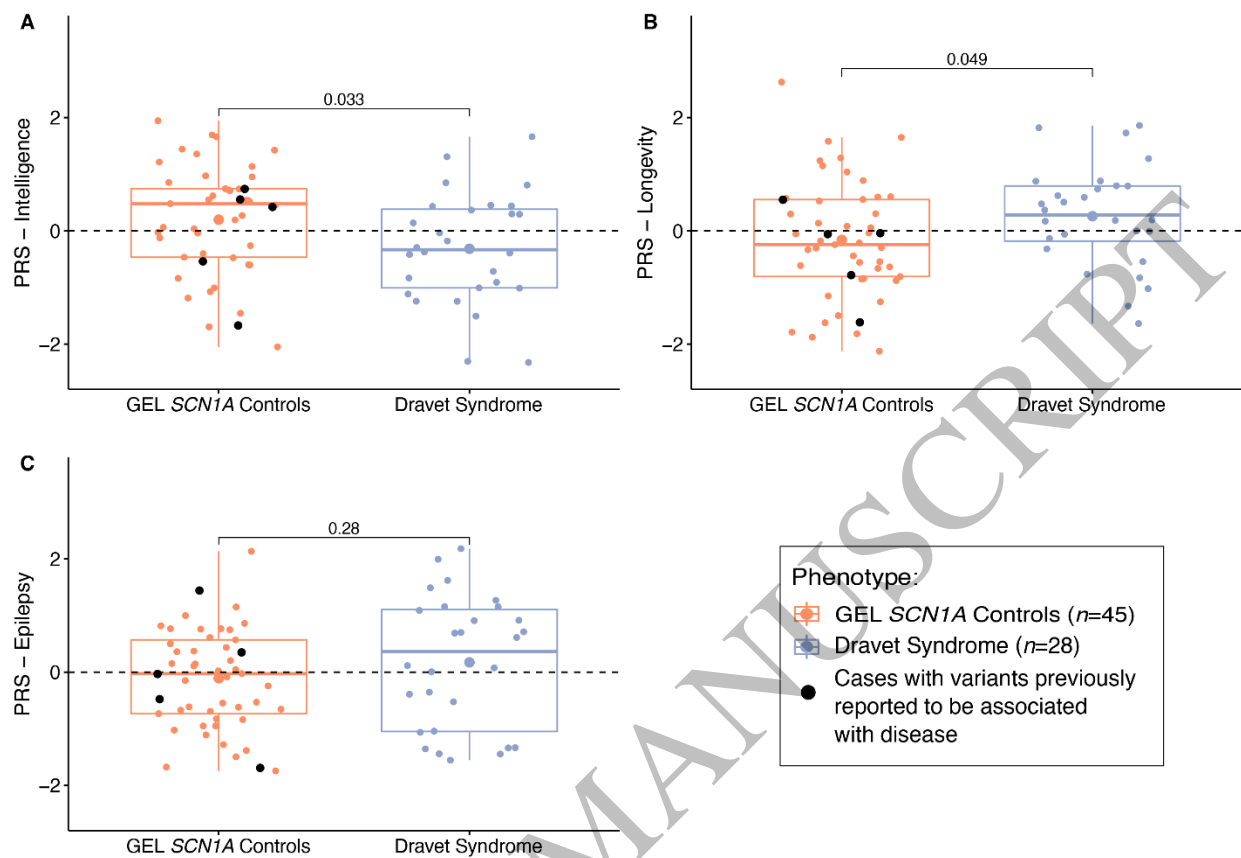
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Figure 3
186x200 mm (x DPI)



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Figure 4
293x200 mm (x DPI)



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Figure 5
297x209 mm (x DPI)