Genome-wide association meta-analyses of drug-resistant epilepsy

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Summary

Background Epilepsy is one of the most common neurological disorders, affecting over 50 million people worldwide. One-third of people with epilepsy do not respond to currently available anti-seizure medications, constituting one of the most important problems in epilepsy. Little is known about the molecular pathology of drug resistance in epilepsy, in particular, possible underlying genetic factors are largely unknown.

Methods We performed a genome-wide association study (GWAS) in two epilepsy cohorts of European ancestry, comparing drug-resistant (N = 4208) to drug-responsive individuals (N = 2618) followed by meta-analyses across the studies. Next, we performed subanalyses split into two broad subtypes: acquired or non-acquired focal and genetic generalized epilepsy.

Findings Our drug-resistant versus drug-responsive epilepsy GWAS meta-analysis showed no significant loci when combining all epilepsy types. Sub-analyses on individuals with focal epilepsy (FE) identified a significant locus on chromosome 1q42.11-q42.12 (lead SNP: rs35915186, $P = 1{\cdot}51 \times 10^{-8}$, OR[C] = 0·74). This locus was not associated with any epilepsy subtype in the latest epilepsy GWAS (lowest uncorrected $P = 0{\cdot}009$ for FE vs. healthy controls), and drug resistance in FE was not genetically correlated with susceptibility to FE itself. Seven genome-wide significant SNPs within this locus, encompassing the genes *CNIH4*, *WDR26*, and *CNIH3*, were identified to protect against drug-resistant FE. Further transcriptome-wide association studies (TWAS) imply significantly higher expression levels of *CNIH3* and *WDR26* in drug-resistant FE than in drug-responsive FE. *CNIH3* is implicated in AMPA receptor assembly and function, while *WDR26* haploinsufficiency is linked to intellectual disability and seizures. These findings suggest that *CNIH3* and *WDR26* may play a role in mediating drug response in focal epilepsy.

Interpretation We identified a contribution of common genetic variation to drug-resistant focal epilepsy. These findings provide insights into possible mechanisms underlying drug response variability in epilepsy, offering potential targets for personalised treatment approaches.

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Introduction

Epilepsy is a burdensome neurological disorder affecting over 50 million people worldwide.[1] One-third of people with epilepsy experience ongoing seizures despite treatment with appropriate antiseizure medications (ASMs). The standard operational definition of drug resistance in epilepsy, formulated by the International League Against Epilepsy (ILAE), is "failure of adequate trials of two tolerated, appropriately chosen and used ASM schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom".[2] Drug-resistant epilepsy (DRE) is associated with reduced quality of life, treatment side effects, comorbidities, lowered socioeconomic status, stigmatisation, and premature mortality.[3–9] Despite the availability of more than 25 registered ASMs, the proportion of people with DRE has remained steady over time.[10] A single-centre 30-year longitudinal cohort study found a similar proportion of people with DRE over the study period, despite a marked increase in the use of newer ASMs.[11]

The causes of DRE are unknown. Evidence suggests the existence of general mechanisms of drug resistance that act regardless of epilepsy syndrome or specific drug.[12] Several hypotheses have arisen as putative explanations for DRE, including the target,[13] multidrug transporter,[14] intrinsic severity,[15] epigenetic,[16] network,[17] and others.[18] However, evidence for these hypotheses remains limited.[12] While genetic factors have been suspected to play a role in drug resistance, definitive evidence has been limited. Only one epilepsy GWAS on drug response has been published (N = 889), which did not find any genome-wide significant loci.[19] More recently, a familial aggregation of a history of uncontrolled seizures (≥ 4 tonic-clonic seizures per year) was demonstrated, suggesting a genetic component of seizure outcomes.[20]

We hypothesised a common-variant genetic component to DRE. We performed genome-wide SNP-based association studies (GWAS) in two independent international epilepsy cohorts (EpiPGX and Epi25) with drug

Research in context

Evidence before this study

The causes of drug resistance in epilepsy are not well understood, leading to a stagnation of drug therapy in epilepsy in the last 40 years. We searched PubMed with the terms 1.) "epilepsy" and "common variants", 2.) "seizure outcomes" and "genetics", 3.) "drug-resistant epilepsy" and "genetics" OR "association" OR "GWAS" for reports published before 1st July 2024, with no language restrictions. While rare genetic variants have been established as causal factors in epilepsy, and evidence suggests their potential overlap with drug resistance, this primarily applies to rare/monogenic epilepsy syndromes. These syndromes represent only a small fraction of all epilepsy cases. The majority of epilepsy cases exhibit a complex/polygenic genetic architecture, well-characterized by numerous successful genome-wide association and polygenic risk-scoring studies. However, to date, no study has successfully identified genome-wide significant common genetic factors influencing drug response in all forms of epilepsy.

Added value of this study

Prior studies, although inconclusive, suggested the involvement of common genetic variants in drug response and a potential heritable component to drug resistance in epilepsy. This study provides evidence for common genetic variants associated with drug response in focal epilepsy, confirming these earlier suggestions. To investigate the genetic basis of drug resistance, we leveraged data from two large-scale initiatives: EpiPGX, an international multicenter research project on epilepsy pharmacogenetics, and Epi25, the largest sequencing study in epilepsy. In the combined cohort of 6826 individuals with drug-resistant and -responsive epilepsy, we identified a locus on chromosome 1q42.11-q42.12, encompassing the genes *CNIH4*, *WDR26*, and *CNIH3*, associated with protection against drug resistance in focal epilepsy. Additionally, we observed significantly higher predicted expression levels of *CNIH3* and *WDR26* in individuals with drug-resistant focal epilepsy compared to those with drug-responsive focal epilepsy.

Implications of all the available evidence

The present study provides two key insights into understanding drug resistance in epilepsy. First, we demonstrate that drug resistance in focal epilepsy has a common genetic component, which may enable quantification of each individual's polygenic risk for drug resistance in (focal) epilepsy and, thus, inform treatment strategies. The common genetic basis of drug resistance also suggests a future need to target multiple pathways rather than single molecules/genes. Second, fine-mapping of the association signal for drug response in focal epilepsy implicates three candidate genes: *CNIH4*, *WDR26*, and *CNIH3*. Pathogenic variants in *WDR26* have been shown to cause a drug-responsive seizure phenotype consistent with the protective effect observed in our meta-analysis and the higher expression levels in drug-resistant cases suggested by our transcriptome-wide association study. *CNIH3* acts as an auxiliary subunit that regulates AMPA receptor gating and trafficking, and abnormal AMPA receptor trafficking could contribute to seizure activity. The findings of this study provide a foundation for future research exploring the common genetic origins of drug resistance in epilepsy.

response phenotypes, followed by meta-analyses. Given existing evidence that focal and generalised epilepsies have distinctive biologies and that DRE is more common in focal than generalised epilepsies, we hypothesised that any genetic basis for DRE would differ between these two categories[21] and performed sub-analyses in focal and generalised epilepsies.

Methods

Ethics

All individuals from the EpiPGX Consortium and Epi25 Collaborative gave written informed consent. Each centre's ethics committees/institutional review boards approved data collection and use. For the EpiPGX consortium, all participants provided written informed consent for appropriately coded use of their clinical data. Ethical approval for this study was obtained from the Camden and Kings Cross Research Ethics Committee (reference number: 11/LO/2016). Consent from parents or legal guardians was obtained from those unable to consent. For the Epi25 cohort, patients or their legal guardians provided signed informed consent/assent according to local IRB requirements[22]; as samples had been collected over 20 years in some centres, forms reflected standards at the time of collection. For Epi25 Collaborative samples collected after 25th January 2015, forms required specific language according to the NIH Genomic Data Sharing Policy.[23]

Study cohorts

Individuals were recruited from EpiPGX, an international multicenter research project on epilepsy pharmacogenetics, and Epi25, the largest sequencing study in epilepsy.[22]

The EpiPGX database contains coded demographic and clinical details of about 10,000 individuals with a diagnosis of epilepsy confirmed by an epilepsy specialist. The database includes detailed data on > 39,000 treatment regimens collected retrospectively from contemporary records. Participants were recruited mainly from tertiary referral centres in the UK, Ireland,

Belgium, the Netherlands, Germany, and Italy. Data collection spanned from 2012 to 2016. All individuals were classified for treatment response, following a modification of the International League Against Epilepsy (ILAE) definition[2] of DRE. According to the ILAE definition of DRE, individuals with very rare seizures (for example, one seizure in 12 months) may be classified as drug-resistant[24] and preclude the identification of clinically meaningful DRE phenotypes. Therefore, this study adopted a threshold of four or more seizures per year, consistent with established practice in pharmacogenetic and pharmacogenomic investigations. This modified DRE definition was: "seizures occurring at a frequency of ≥ 4/year during the year preceding the latest data entry, despite adequate trials of ≥ 2 tolerated and appropriately chosen (and used) ASM schedules, whether as monotherapies or in combination." It is important to note that the ILAE study advises adaptation of the definitions for particular circumstances and studies. Given that the phenotypic data for this study were collected retrospectively and that pre-intervention inter-seizure intervals were not consistently documented, drug-responsive epilepsy was defined as freedom from seizures for ≥ 12 months up to the latest recorded visit.[11] Consequently, individuals with 1–3 seizures in the 12 months preceding the latest data entry were excluded from the study. This usage is within the ILAE definition,[2] which categorises a treatment outcome as "seizure-free" (Category 1 response) if "the treatment results in seizure freedom for 12 months, or for a minimum of three times the longest pre-intervention inter-seizure interval, whichever is longer".[2] Our usage aligns with the seizure-free interval that often actually leads to changes in daily life (e.g., permitting reinstatement of driving privileges) and ensures that those who are considered drug-responsive have experienced a seizure-free interval of at least 12 months. Of note, none of the individuals classified as drug-responsive were seizure-free without medication (Table 1; an average of 1·9 adequate ASM trials). An ASM trial was considered adequate if administered at an appropriate dose for a sufficient duration. Appropriateness was determined by prior evidence of efficacy, ideally from randomised controlled trials. Minimum therapeutic doses for adults were established by a panel of EpiPGX principal investigators (SMS, JC, ND, CD, HL, AGM, JWS, GJS), informed by World Health Organization (WHO) defined daily doses (DDD) (atcddd.fhi.no/atc_ddd_index/). It is important to note that the agreed appropriate ASM daily doses only apply to monotherapy trials and that the list was used as guidance rather than a set of strict rules. Clinical judgment was required to evaluate the adequacy of ASM trials in the context of polytherapy, extremely low or high body weight, and for ASM trials taking place in an individual's childhood. Laboratory reports of ASM levels were taken into account if available. If the ASM levels were below the local reference range while the individual was taking a stable dose of the ASM and there were no signs indicating CNS toxicity, the ASM trial was considered inadequate. Individuals with non-epileptic seizures or known non-adherence were excluded from the study. Individuals who underwent epilepsy surgery were classified as drug-resistant if they met the DRE criteria before surgery and excluded from analysis if they achieved remission following epilepsy surgery. This classification approach required substantial efforts and resources. Of the ~10,000 individuals in the EpiPGX database, only those who could be robustly classified in one of the two response groups were included. We note that this level of phenotyping depth requires significant time and effort and is not generally feasible. The EpiPGX cohort thus represents a deeply phenotyped group nested within the broader framework of the Epi25 cohort. The deep phenotyping used for EpiPGX, designed as a pharmacogenomics study, was not undertaken for the second cohort from the Epi25 Collaborative, the primary purpose of which was gene discovery. Overall, the joint

Cohort name	All-EPI	FE	GE	DEE	Epilepsy-NOS	Males (%)	Mean age at epilepsy onset (years) (SD)	Mean age at last follow-up (years) (SD)	Mean number of adequate ASM trials
EpiPGX									
Drug-resistant	2105	1802	179	0	124	47·5%	15·5 (SD 13·6)[a]	45·6 (SD 13·4)	4·3 (SD 2·3)
Drug-responsive	1394	999	233	0	162	49·3%	23·1 (SD 17·2)[a]	44·4 (SD 17·7)	1·9 (SD 1·3)
Epi25									
Drug-resistant	2103	1429	327	337	10	49·2%	15·6 (SD 14·8)	33·6 (SD 17·0)	Not available
Drug-responsive	1224	579	518	107	20	45·1%	10·7 (SD 16·6)	31·0 (SD 19·6)	Not available
Meta-analysis	**6826**	**4809**	**1257**	**444**	**316**				

Study cohorts of individuals with drug-resistant or drug-responsive epilepsy. Epilepsy and epilepsy sub-syndromes were diagnosed in all cohorts according to clinical criteria (clinical interview, neurological examination, EEG, imaging data), following ILAE classifications.[25] Abbreviations: All-EPI: all epilepsies; FE: focal epilepsy; GE: generalised epilepsy; DEE: developmental and epileptic encephalopathy; Epilepsy-NOS: epilepsy, not otherwise specified; SD: standard deviation. [a]Due to ethical restrictions at the time of data collection, the average age at epilepsy onset was based on only 32% of the EpiPGX GWAS sample size.

Table 1: **GWA meta-analysis cohorts after quality control.**

cohort achieves robust and aligned classification of seizure freedom (and thus drug responsiveness, as all patients achieving seizure freedom were on ASMs) and real-world usages for response to individual ASMs. This approach will facilitate both ease of independent replication and enlargement of the cohort in our own future work.

For the Epi25 Collaborative, the unmodified ILAE definitions of DRE (failure of adequate trials of two tolerated, appropriately chosen, and used ASM schedules)[2] and drug responsiveness ("seizure-free for a minimum of three times the longest pretreatment interseizure interval, or 12 months, whichever is longer") were used. This, too, ensures that individuals were seizure-free for at least 12 months (or longer). Across both cohorts, therefore, those deemed drug-responsive had been seizure-free for at least 12 months, a meaningful and consequential period of seizure freedom aligned across the two cohorts. Because detailed drug response data was only provided in a minority of Epi25 participants, we could only include a fraction of the whole Epi25 study. The study cohorts are detailed in Table 1. Both cohorts displayed similar demographics, apart from the mean age at epilepsy onset of drug-responsive individuals, which was higher in the EpiPGX compared to the Epi25 cohort. Age is, however, not considered a factor in the development of drug resistance.[12] The EpiPGX and Epi25 GWAS cohorts included individuals with possible genetic causes (EpiPGX: 3·7% of the drug-resistant and 5·9% of the drug-responsive individuals; Epi25: 17% of the drug-resistant and 13% of the drug-responsive individuals; Supplementary Tables S6 and S7), without a significant enrichment of individuals with a possible genetic cause in either of the drug response groups across both cohorts (P = 0·083 [Cochran-Mantel Haenszel test stratified for the two cohorts]). These individuals were included in the analyses following evidence that common genetic risk variants are enriched in individuals with a family history of the phenotype or unique causal variants.[26–30] Epilepsy type and epilepsy sub-syndromes were diagnosed in all cohorts based on the primary mode of seizure onset (generalised vs. focal), taking into account clinical interview data, neurological examination, EEG, and imaging data, following ILAE schemata.[25]

Single nucleotide polymorphism genotyping

All EpiPGX samples were genotyped at deCODE Genetics (Reykjavik, Iceland) using Illumina single nucleotide polymorphism (SNP) arrays (OmniExpress-12 v1.1, OmniExpress-24 v1.1, Human610-Quad, HumanHap550v3). SNP genotypes were called with the Genotyping Module of the GenomeStudio Software (Illumina, CA, USA). Epi25 samples were genotyped at the Broad Institute of Harvard and MIT (Cambridge, MA, USA) using the Illumina Global Screening Array with Multi-disease drop-in (GSA-MD v1.0). SNP genotypes were called using Illumina's genotyping analysis software Autocall. Rare SNPs (minor allele frequency, MAF<0.1) were called with the zCall software[31] into the Autocall output.

Data quality control and imputation–EpiPGX cohort

For the EpiPGX samples, data quality control (QC) and imputation were performed separately for each chip type and genotyping batch. Before imputation, we excluded genotyped individuals based on the following criteria: (1) genotyping call rate (CR) < 0·98; (2) heterozygosity rate outliers with > 5 standard deviations (SD) from the median of the whole sample, using a subset of uncorrelated SNPs (pairwise r^2 < 0·1 in 100 Kbp sliding windows with a step size of 25 SNPs); (3) missing, ambiguous, or sex mismatch between X-chromosome genotype and reported sex; (4) one individual from each pair of closely related individuals with > 0·9 identity by state; (5) individuals with < 90% European ancestry, as identified using STRUCTURE-v2.2,[32] with HapMap European samples as the reference population and 2766 ethnicity-sensitive SNPs. We then excluded SNPs based on the following criteria: (1) SNP-CR < 0·95; (2) MAF < 0·01; (3) deviation from the Hardy–Weinberg equilibrium (HWE) with $P < 10^{-6}$. We applied pre-imputation checks according to scripts available on the website of Will Rayner of the Wellcome Trust Centre for Human Genetics (Supplementary material, URLs) to align the QC-filtered dataset to the imputation reference (variant name, variant position, and strand orientation), remove all A/T and C/G SNPs to avoid strand issues, and to remove SNPs with allele frequencies deviating > 20% from the frequency in the 1000 Genomes phase 3 reference.[33] We then split genotypes up according to chromosome arms (and in the case of chromosome X, we split additionally into pseudo-autosomal regions, PAR, and non-PAR) and created phased haplotypes using SHAPEIT-v215[34] with recommended effective size setting (HapMap2 European, N = 11,418), and using the 1000 Genomes phase 1 integrated (v3) map files as reference. Following haplotype phasing, we imputed genotypes into our dataset using IMPUTE-v2.3.0[35] with recommended effective population size settings (20,000) and 1000 Genomes phase 1 integrated (v3) genotypes as reference.[36] The haplotype phasing and imputation were performed in separate batches for each genotyping dataset.

Post-imputation QC filters were applied first separately for every imputation batch to remove genotyped variants with low concordance between the observed genotype and masked, imputed genotype (IMPUTE2 r2_type0 score < 0·90, concordance_type0 < 0·90). We then performed further QCs on the merged datasets for GWAS cases and controls separately, removing variants based on the following criteria: (1) SNPtest v2[37] imputation quality info score < 0·97; (2) SNPtest average_maximum_posterior_call < 0·90; (3) MAF < 0·01;

(4) deviation from HWE with $P < 10^{-6}$ in controls only. QC-filtered imputed genotypes were converted for subsequent analyses to hard calls using GTOOL (Supplementary material, URLs). At the individual level, we removed duplicate samples across imputation batches (using the same parameter as in the pre-imputation step).

Data quality control and imputation–Epi25 cohort

Before imputation, genotyped Epi25 individuals were excluded based on the following sample-level QC filters: (1) heterozygous/homozygous SNP ratio outliers with > 4 SD from the mean of the whole sample; (2) individuals with missing, ambiguous, or mismatch between genetically inferred and reported sex; (3) one individual from each pair of closely related individuals with > 0·2 proportion of identity by descent; (4) population outliers not clustering with the 1000 Genomes Project[33] European samples in a principal component analysis (PCA). SNPs were filtered out with the following criteria: (1) SNP-CR < 0·98; (2) monomorphic SNPs; (3) SNPs with batch association ($P < 10^{-4}$); (4) deviation from HWE with $P < 10^{-10}$. The resulting QC-filtered SNPs were used for imputation to the Haplotype Reference Consortium reference r1.1[38] using Minimac4[39] and reference-based phasing with Eagle-v2.4,[40] as implemented on the Michigan Imputation Server.[39] All Epi25 samples were imputed as one single batch.

Post-imputation, we randomly removed one individual from each pair of individuals with 3rd-degree relationships and higher (kinship coefficient > 0·0442) using KING.[41] Imputed genotypes were converted to hard calls using PLINK-v1.9[42] and filtered for high quality based on the following criteria: (1) Minimac4 imputation quality score, $R^2 \geq 0{\cdot}3$; (2) Minimac4 squared correlation value between masked genotypes of genotyped SNPs and the imputed dosages, Emp-$R^2 \geq 0{\cdot}3$.

Detection of overlapping individuals across the EpiPGX and Epi25 cohorts

To identify individuals who were ascertained in the EpiPGX and the Epi25 study without sharing individual-level data between sites, we used a protocol inspired by the one-way cryptographic hash function.[43] One-way cryptographic hashes are a security algorithm form that alters input data so that the resulting output data cannot be reverted feasibly to the original form. We first generated ten batches of SNPs, which did not have missing genotypes in any of the studies. We then computed hash values (checksums) for each of the ten batches for each individual, using the Linux "cksum" command. The "cksum" command will always generate the same unique hash value when using the same SNPs, with the same information (same non-missing genotype), and in the same order (sorted by physical position). We then marked every pair of individuals with one or more identical hash values (out of the ten) as duplicate and excluded the corresponding individual from the Epi25 cohort. The procedure is implemented in Perl and is freely available (Supplementary material, URLs). We removed 22 samples from the Epi25 cohort duplicated between the EpiPGX and Epi25 cohorts before generating the GWAS statistics.

Genetic correlation analyses

We used LDSC to calculate the genetic correlation (Rg) of the drug response phenotype in focal epilepsy with epilepsy and the two main subtypes (focal and generalised epilepsy) (Supplementary Table S5). The summary statistics for epilepsy vs. (healthy) controls were obtained from the most recent GWAS in epilepsy.[44] We used pre-computed LD scores suitable for GWASs based on European individuals, generated as described in Bulik-Sullivan et al. (2015).[45]

Genome-wide association and meta-analysis

We used logistic regression adjusted for sex and the first ten principal components of ancestry in PLINK-v1.9[42] to perform separate GWASs in the EpiPGX and Epi25 cohorts. We did not adjust our analysis for potential non-genetic predictors of drug resistance. We performed three GWASs for each cohort in drug-resistant vs. drug-responsive individuals with (1) any type of epilepsy, 'all-EPI'; (2) non-acquired or lesional focal epilepsy, 'FE'; or (3) generalised epilepsy, 'GE'. SNPs for GWASs were selected based on the following criteria: (1) CR $\geq$ 0·98 in the combined case/control dataset; (2) MAF $\geq$ 0·01; (3) deviation from HWE with $P > 10^{-5}$. Sample and SNP QC procedures were performed using PLINK-v1.9.[42] To minimise confounding due to population stratification, we performed a stringent, post-imputation selection of individuals clustering exclusively with Western European and British individuals from the 1000 Genomes Project[33] in a PCA using GCTA.[46] Of note, as well as excluding individuals with Finnish ancestry, as is standard (best) practice for GWASs in the European population, we also excluded European individuals that clustered with Tuscan[47] and Iberian[48] individuals to avoid population stratification within the largely Western and Central European GWAS cohort.

Next, we performed *P*-value-based fixed-effects meta-analyses with GWAMA[49] for each of the three epilepsy phenotypes (all-EPI, FE, and GE). The threshold for genome-wide significance in the meta-analyses was set to the commonly used $\alpha = 5 \times 10^{-8}$. Fine-mapping of the meta-analysis association signals was performed using FUMA,[50] LocusZoom,[51] and Haploview.[52] Gene-based association analyses were performed using MAGMA[53] as implemented in FUMA. The Bonferroni-corrected threshold for a significant association in the MAGMA analysis was set to $\alpha = 2{\cdot}63 \times 10^{-6}$ (19,005 tested protein-coding genes).

Transcriptome-wide association analysis (TWAS) was performed using the S-MultiXcan framework[54] on all available brain-specific GTEx v8 transcriptome datasets (N = 13). S-MultiXcan[54] leverages the substantial sharing of quantitative trait loci (QTL) across tissues to increase the power of identifying associated gene expression or alternative splicing variation.[55] Expression and splicing predictions were generated using multivariate adaptive shrinkage (mash) models[56] for GTEx v8 expression QTL (eQTL) and splicing QTL (sQTL) data.[57] We then applied the S-MultiXcan framework on all brain-specific GTEx v8 transcriptome datasets (N = 13). The Bonferroni-corrected thresholds for a significant association were set to $\alpha = 2{\cdot}69 \times 10^{-6}$ (18,562 tested genes) in the eQTL-based TWAS and $\alpha = 3{\cdot}78 \times 10^{-7}$ (132,272 tested splicing events) in the sQTL-based TWAS. Power calculations were performed *post hoc* using the PGA Power Calculator,[58] assuming a disease prevalence of 0·1%, an additive risk model, and linkage disequilibrium (LD) $r^2 = 0{\cdot}9$ between a causal variant and a genotyped marker.

Role of funders

The funding institutions had no role in the design and conduct of the study, including data collection, analysis, and interpretation of results, or the preparation, review, and decision to submit the manuscript for publication.

Results

Genome-wide association meta-analysis reveals one locus associated with drug resistance in focal epilepsy

To test for a possible genetic basis of DRE, we performed European ancestry-focused genome-wide association (GWA) meta-analyses in 4208 individuals with DRE vs. 2618 individuals with drug-responsive epilepsy. We did not identify any genome-wide significant loci in the all-EPI analysis (Fig. 1) despite 80% power to detect a genetic predictor of relative risk ≥ 1·33 (Supplementary Figs. S1 and S2). Subanalyses were performed in drug-resistant vs. drug-responsive individuals with FE or GE (see cohorts in Table 1). The sample size for drug-resistant GE was underpowered to detect common risk factors and SNPs showing association trends did not overlap with 'all-EPI' or FE associatiation signals (Fig. 1, Supplementary Fig. S1). Fixed-effects GWA meta-analysis for drug resistance in FE identified seven genome-wide significant SNPs in a region of strong linkage disequilibrium on chromosome 1q42.11-q42.12 encompassing *CNIH4*, *WDR26*, and *CNIH3* (lead SNP: rs35915186, $P = 1{\cdot}51 \times 10^{-8}$ [logistic regression], odds ratio OR[C] = 0·74, 95% confidence interval [95%-CI]: 0·66 – 0·82) (Fig. 1). Interestingly, all associated SNPs at the identified locus had OR < 1, indicating that the minor allele (MAF = 0·22) protects against drug resistance. This locus was not significantly associated with any epilepsy subtype in the most recent epilepsy GWAS[44] (lowest uncorrected $P = 0{\cdot}009$ [linear mixed model] for FE vs. healthy controls, Supplementary Tables S2 and S3). The GWAS Catalog listed 86 associations with $P < 5 \times 10^{-8}$ within ± 500 Kb of the lead SNP, of which 33 were in strong LD with rs35915186 ($r^2 > 0{\cdot}8$), but none were related to neurological or psychiatric traits (Supplementary Table S4). Notably, we did not find any genetic correlation between drug resistance in FE and susceptibility to FE itself, based on genetic correlation analyses with the ILAE 2023 GWAS for FE[44] (linkage disequilibrium score regression genetic heritability = –0·22, standard error = 0·38, $P = 0{\cdot}28$ [regression]; Supplementary Table S5).

WDR26, *CNIH3*, *and CNIH4* are candidate drivers of drug response in focal epilepsy

Fine-mapping of the region associated with drug response in FE narrowed down the critical region to a 161 Kb LD block of 106 SNPs in high LD with at least one of the seven genome-wide significant SNPs ($r^2 \geq 0{\cdot}8$ using 1000 Genomes Phase 3 EUR data, Fig. 2). The identified LD block featured three genes: *CNIH4*, *WDR26*, and the first two exons of a *CNIH3* transcript variant (ENST00000471578.5). All three genes emerged as genome-wide significant after Bonferroni correction for multiple testing ($P < 2{\cdot}63 \times 10^{-6}$ [multiple regression with F-test]) in a MAGMA[53] gene-based association analysis of drug-resistant FE (Supplementary Fig. S3 and Table S1).

We then performed two multi-tissue TWASs for eQTL and sQTL GTEx v8 data using S-MultiXcan[54] to identify expression or splicing events associated with drug response in FE. eQTL-based TWAS across 13 GTEx v8 brain tissues implied significantly higher expression levels of *CNIH3* and *WDR26* in drug-resistant compared to drug-responsive FE ($P_{CNIH3} = 1{\cdot}10 \times 10^{-6}$, $Z_{MEAN} = 3{\cdot}55$; $P_{WDR26} = 1{\cdot}60 \times 10^{-6}$, $Z_{MEAN} = 3{\cdot}44$; multivariate regression with F-test; Table 2) at a Bonferroni-corrected significance threshold $\alpha = 2{\cdot}69 \times 10^{-6}$. sQTL-based TWAS across the same brain tissues revealed 18 unique splicing events associated with drug response in FE, mapping exclusively to the three candidate genes at a Bonferroni-corrected significance threshold $\alpha = 3{\cdot}78 \times 10^{-7}$ (*CNIH3*, *WDR26*, and *CNIH4*, Supplementary Table S9).

CNIH3 is one of two members of the cornichon family of transmembrane proteins coassembled with AMPA receptors (along with *CNIH2*)[59] and a brain-specific expressed gene that shows the highest expression in the frontal cortex (BA9).[60] Upon successful assembly, *CNIH3* increases the surface expression of AMPA receptors and slows deactivation and desensitisation kinetics.[59,61] *Cnih3* knock-out in mice depresses AMPA receptor synaptic transmission only when

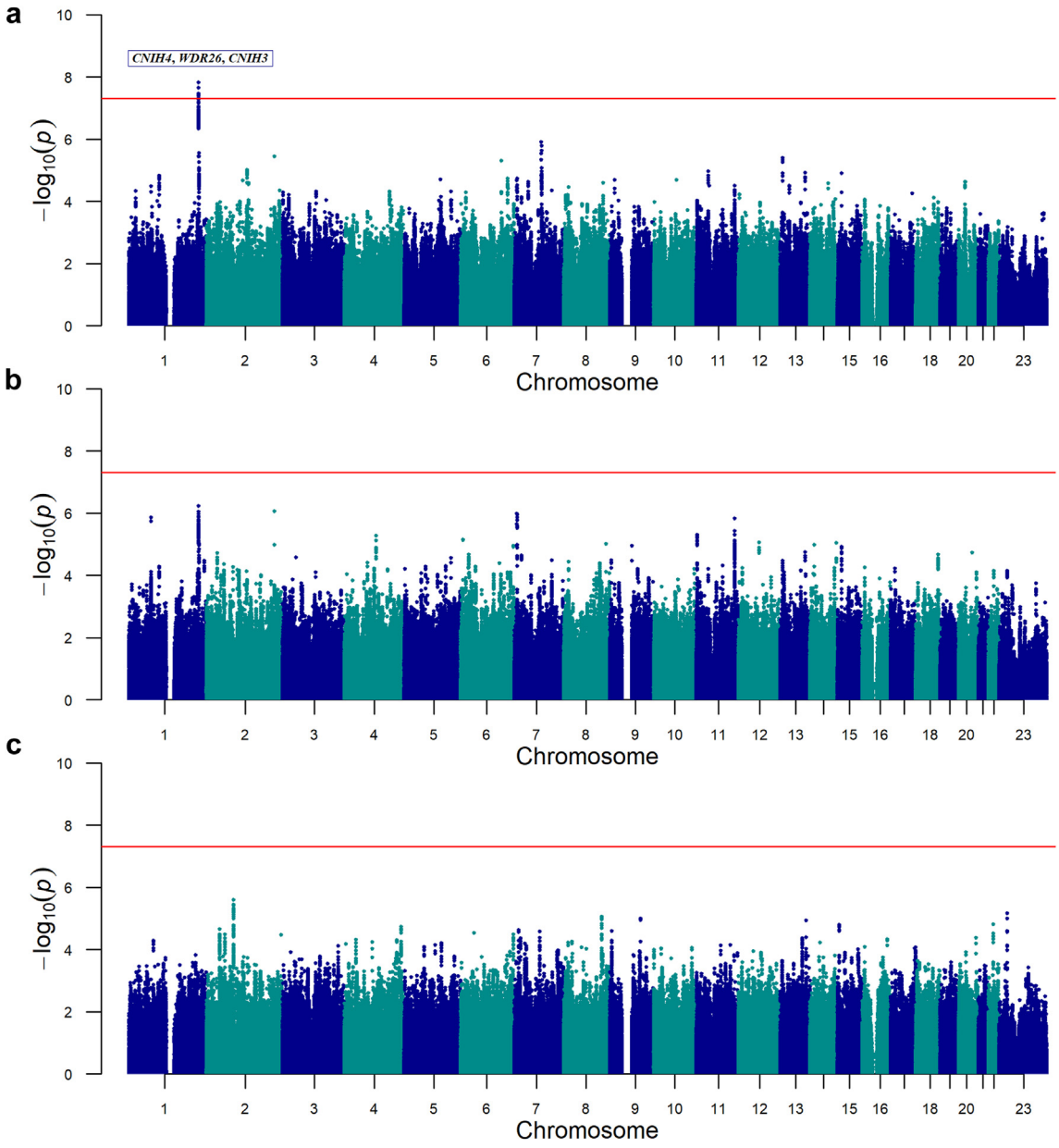


Fig. 1: **Manhattan plot of the GWAS meta-analyses in drug-resistant vs. drug-responsive individuals with epilepsy.** The red line shows the threshold for genome-wide significance ($P < 5 \times 10^{-8}$). Chromosome and position are displayed on the x-axis and -$\log_{10}$ (*P*-values) [logistic regression] on the y-axis. **a**: GWAS meta-analysis in 3231 drug-resistant vs. 1578 drug-responsive individuals with focal epilepsy (FE). Annotated genes were tagged by SNPs in high linkage disequilibrium with the lead SNP rs35915186 ($r^2 \geq 0{\cdot}8$). **b**: GWAS meta-analysis in 4208 drug-resistant vs. 2618 drug-responsive individuals with epilepsy (all-EPI). **c**: GWAS meta-analysis in 506 drug-resistant vs. 751 drug-responsive individuals with generalised epilepsy (GE).

combined with *Cnih2* knock-out, suggesting that *CNIH2* can compensate for the lack of *CNIH3*.[62] All four genes encoding AMPA receptors have been reported to cause monogenic autosomal dominant neurodevelopmental disorders with seizures (*GRIA1*,[63] *GRIA2*,[64] *GRIA3*,[65] *GRIA4*[66]). *CNIH4* is a brain-expressed but not brain-specific gene that shows the highest expression in cultured fibroblasts[60] and is a distantly related member of the cornichon family,[67] which lacks key residues responsible for binding to AMPA receptors.[68] *Cnih4* knock-out mice were reported as viable without any "overt" developmental

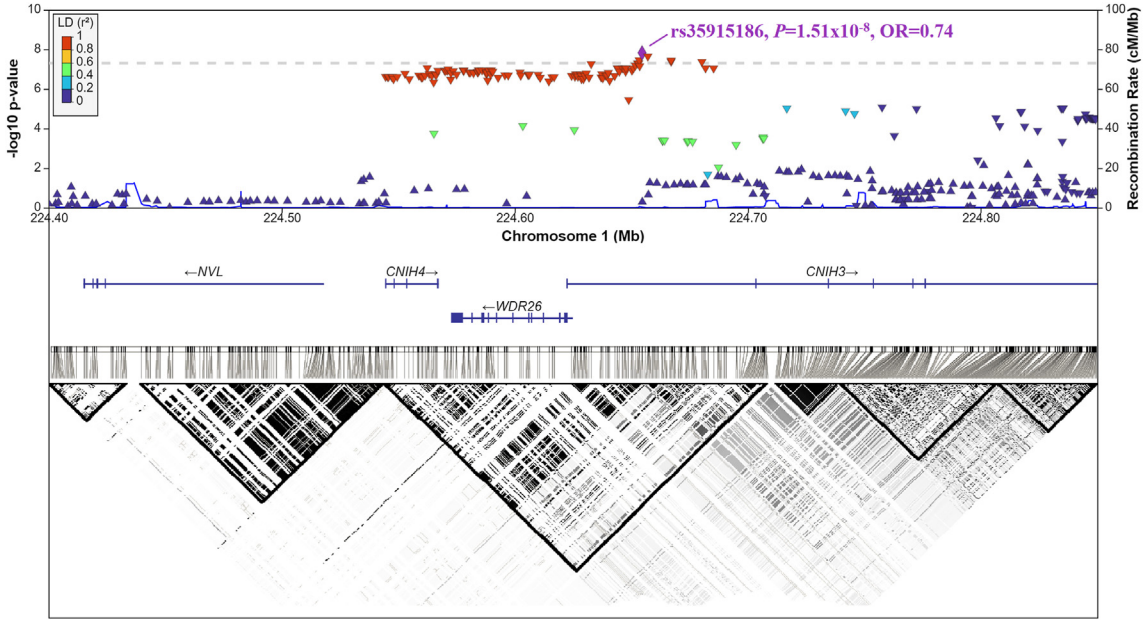


Fig. 2: **Chromosome 1q42.11-q42.12 locus associated with drug response in FE.** The SNPs in the upper plot are coloured according to their linkage disequilibrium (LD) r^2 value with the lead SNP rs35915186. The linkage disequilibrium pattern with corresponding LD blocks (black triangles) is shown in the lower plot. The pairwise LD values are displayed in shades of grey, with black representing SNP pairs in full LD ($r^2 = 1$).

abnormalities.[68] *WDR26* is a brain-expressed but not brain-specific gene with the highest expression levels in the skin.[60] *WDR26* haploinsufficiency is known to cause an (ultra-rare) distinct clinical phenotype characterised by intellectual disability and seizures (*WDR26*-related intellectual disability/Skraban-Deardorff syndrome).[69] The exact biological function of *WDR26* is not established; studies suggest roles in MAPK signalling,[70] PI3K/AKT signalling,[71] and the negative regulation of β-catenin degradation within the Wnt signalling pathway[72] (among other possible functions[73–76]). Notably, the seizure types described in affected individuals were self-limited or responded well to standard treatments.[77] Upon screening samples that also had whole-exome

Ensemble ID	Gene name	*P*-value	N	P_i_best	T_i_best	ZMEAN
ENSG00000143786.7	*CNIH3*	1·10E-06	2	2·13E-07	Brain_Amygdala	3·55
ENSG00000162923.14	*WDR26*	1·60E-06	8	2·13E-07	Brain_Frontal_Cortex_BA9	3·44
ENSG00000143771.11	*CNIH4*	7·68E-06	13	2·37E-07	Brain_Spinal_cord_cervical_c-1	2·85
ENSG00000085563.14	*ABCB1*	5·09E-05	13	4·50E-04	Brain_Cerebellum	0·26
ENSG00000225924.2	*RP1-111D6.4*	2·28E-04	2	5·94E-05	Brain_Hippocampus	3·24
ENSG00000254480.1	*RP11-23F23.2*	2·63E-04	10	2·03E-04	Brain_Putamen_basal_ganglia	−1·74
ENSG00000114446.4	*IFT57*	2·80E-04	13	4·58E-03	Brain_Hippocampus	−1·01
ENSG00000166268.10	*MYRFL*	3·46E-04	13	2·07E-04	Brain_Amygdala	−3·52
ENSG00000247970.2	*RP11-543C4.1*	5·96E-04	9	0·18	Brain_Cerebellar_Hemisphere	0·35
ENSG00000173465.7	*SSSCA1*	6·19E-04	5	3·07E-03	Brain_Anterior_cingulate_cortex_BA24	−1·07
ENSG00000088930.7	*XRN2*	7·34E-04	10	8·76E-03	Brain_Cerebellum	−0·31
ENSG00000251562.7	*MALAT1*	7·51E-04	3	5·39E-03	Brain_Amygdala	1·51
ENSG00000139168.7	*ZCRB1*	7·83E-04	13	1·33E-03	Brain_Hippocampus	−1·78
ENSG00000006634.7	*DBF4*	8·00E-04	12	2·54E-03	Brain_Anterior_cingulate_cortex_BA24	−0·89

TWAS *P*-values were calculated using S-MultiXcan[54] with MASHR models for GTEx v8 eQTLs across 13 brain-specific tissues. Shown are all genes with $P < 10^{-3}$ [multivariate regression with F-test] in a TWAS in drug-resistant vs. drug-responsive FE. The threshold for significant associations after Bonferroni correction was set to $\alpha = 2{\cdot}69 \times 10^{-6}$ (18,562 tested genes). Significant associations are highlighted in bold. Legend: N: number of "tissues" available for this gene, P_i_best: best *P*-value of single-tissue S-PrediXcan association (plotted in Supplementary Fig. S4), T_i_best: name of best GTEx v8 single-tissue S-PrediXcan association, Z_{MEAN}: mean z-score among single-tissue S-PrediXcan associations.

Table 2: **Gene-based TWAS in drug-resistant vs. drug-responsive FE.**

sequencing, we identified 10 individuals with FE, eight individuals with GE and one individual with DEE who carried rare variants in the candidate genes (N_{WDR26} = 10, N_{CNIH3} = 7, and N_{CNIH4} = 2). Only one of these variants was classified as likely pathogenic according to ACMG criteria (without considering gene-disease relationships), while all others were classified as variants of uncertain significance. There was no clear over-representation of rare variant carriers in either group (drug-resistant or drug-responsive) (Supplementary Table S8).

Discussion

We performed case–case GWAS meta-analyses for drug response in the EpiPGX Consortium and the Epi25 Collaborative cohorts. Following evidence from previous studies that showed significant differences between the genetic architectures of epilepsy sub-syndromes,[78,79] we performed additional GWAS meta-analyses for drug resistance in focal (FE) and generalised epilepsy (GE). We found a genome-wide significant locus at 1q42.11-q42.12 associated with protection against drug resistance in FE. This common risk locus driving drug response in FE was not previously reported as a risk factor for FE itself or any other epilepsy type.[44] We had insufficient power to identify genetic factors associated with drug-resistant GE. In line with our hypothesis that different mechanisms drive drug response in FE compared to GE, we found no significant risk factors when combining FE and GE in an 'all epilepsies' (all-EPI) analysis. This study and one of our previous GWAS studies in mesial temporal lobe epilepsy with febrile seizures[80] demonstrate the value of focusing on more narrowly defined subtypes to identify common risk factors for traits of interest in FE.

Fine-mapping the association signal for drug response in FE revealed three candidate genes: *CNIH4*, *WDR26*, and *CNIH3*. Among these, pathogenic variation in *WDR26* has been shown to cause a drug-responsive seizure phenotype[77] consistent with the protective effect from drug-resistant epilepsy we observed from the meta-analysis, and the higher expression levels for *CNIH3* and *WDR26* in drug-resistant cases suggested by the TWAS. Although *CNIH3* has not been identified as a monogenic epilepsy gene, common *CNIH3* variants could plausibly act as a modifier of drug response. CNIH3 acts as an auxiliary subunit that regulates AMPA receptor gating and trafficking,[59,61,81] and abnormal AMPA receptor trafficking could contribute to seizure activity.[82] Our result should spark further research to uncover novel therapies, as no drug–gene interactions are currently reported for the three candidate genes.[83] Our eQTL- and sQTL-based TWAS framework could not conclusively prioritise between the three candidate genes. However, as the underlying gene expression and splicing variation predictions are based on GTEx post-mortem bulk transcriptomics data, our analyses may suffer from sensitivity limitations and not fully capture cell-type-specific expression and transcriptional patterns of living tissues or under disease-specific conditions.[84]

While we identified common variants predicting drug response in FE, additional genetic (and environmental) factors are likely to play a role in DRE. There is accumulating evidence that rare genetic variation is important in epilepsy causation, and such variation can overlap with poor response to ASMs.[85] Rare variants known to cause monogenic forms of epilepsy can also influence drug response. For example, sodium channel blockers aggravate seizures in most people with Dravet syndrome due to loss-of-function *SCN1A*[86] mutations or epilepsy due to loss-of-function variants in *SCN2A*[87] or *SCN8A*.[88] Conversely, sodium channel blockers are an effective treatment for people with epilepsy due to gain-of-function variants in *SCN1A*,[89] *SCN2A*,[87] or *SCN8A*.[88]

Further research in larger cohorts is needed to detect the causal genes and mechanisms for drug resistance in epilepsy. Our GWA meta-analyses were underpowered to capture significant single-SNP associations with drug-resistant GE. We focused on overall drug resistance in large epilepsy subgroups. Testing in larger cohorts that allow drug-specific sub-analyses, drug-matched control usage, and stratification for comorbid disorders may help uncover biomarkers for drug-specific resistance in epilepsy. For example, a recent study suggested rare variants underlie resistance to two common ASMs[85]: rare variants in ADME (absorption, distribution, metabolism, and excretion) genes were associated with resistance to valproic acid and rare variants in drug target genes were associated with resistance to levetiracetam. We opted for a very stringent selection of individuals with Western and Central European-like ancestry to reduce potential confounding of association statistics by population sub-structure.[90] Therefore, the generalizability of these results to individuals beyond European-like ancestry remains to be determined. Operational definitions, typically applied at a single point in time to define drug-resistant and drug-responsive cases, cause additional challenges in drug-resistance research in epilepsy. Such definitions do not consider the dynamic relationship between drug resistance and seizure remission and recurrence. Most people with epilepsy attain remission early, later in their disease history, or never, with only a minority fluctuating between periods of seizure freedom and relapse.[91] Because a dynamic course is more common in individuals with infrequent seizures,[92] the EpiPGX definition of DRE (which requires a minimum of four seizures in the past 12 months) partially addresses this issue. Continued efforts are needed in the field to reach a consensus on addressing the temporal course of drug resistance in epilepsy for research purposes. Finally, phenotyping and clinical information collection for the

EpiPGX cohort was completed over a decade ago, utilising terminology and classifications predating the current definition of Developmental and Epileptic Encephalopathies (DEEs).[93] Consequently, the presence of individuals with DEE within the FE GWAS meta-analyses cannot be entirely ruled out. However, we note that even if there were an over-representation of individuals with DEE in the drug-resistant cohorts, and even if these individuals have a monogenic basis for their epilepsy and the drug-resistant nature of that epilepsy, this would serve only to reduce the power of our current analysis. The same applies to the possibility that any focal epilepsies might have been monogenic.

In conclusion, we show that drug resistance in focal epilepsy has a common genetic component. More large-scale projects are needed to identify biomarkers for drug resistance in epilepsy. Potentially, such work could provide new clues to the aetiology and pathophysiology of drug-resistant epilepsy, especially focal epilepsy. The common polygenic nature of the genetic contribution to drug resistance could inform treatment strategies and may point to the need for alternative approaches focused broadly on pathways rather than single molecular targets.

Contributors

CL and SMS designed the study. AA, AGM, ALJ, BMN, BPCK, CAB, CD, FZ, GJS, GLC, HL, HS, JJC, JWS, KS, LJ, MRJ, ND, PM, PS, SFB, SMS, TJOB, UU, the EpiPGX Consortium, and the Epi25 Collaborative collected data. AA, CL, EH, HMC, LJ, MEO, PM, RS, SC, and UU analysed the data. AA, CL, DS, RS, and SMS accessed and verified the underlying data, interpreted the analyses, and were responsible for the decision to submit the manuscript. BMN, DL, HS, PM, RK, and SMS provided the computational infrastructure. SMS supervised the study. AA, CL, RS, and SMS wrote the manuscript. All authors interpreted the data, read and revised the manuscript, and approved the final version of the manuscript.

Data sharing statement

GWAS/meta-analysis summary statistics that support the findings of this study are available on application to the Data Access Committees of the EpiPGX Consortium and the Epi25 Collaborative through the corresponding authors. Due to ethical restrictions, we cannot publicly share individual-level raw data of the EpiPGX cohort. However, subject to data use agreements and collaborator approval, we can provide regulated access to bona fide researchers on our secure servers. Interested researchers can contact the corresponding authors for further details. Individual-level raw data of the Epi25 cohort are deposited in dbGaP (accession number: phs001489.v3.p2). Summary statistics of GWAS in epilepsy used in this study for correlation analyses are available from the open-access Epilepsy Genetic Association database (epiGAD, https://www.epigad.org/).

Declaration of interests

AA is an employee of Regenon and owns Regenon stocks. AGM received institutional consulting fees from Jazz Pharmaceuticals and UCB Pharma; institutional honoraria for lectures from Sanofi and GSK, institutional support for meeting attendance/travel from Angelini Pharma, and has unpaid leadership roles at the European Academy of Neurology and Epilepsy Research Institute. BMN is a member of the Neumora Scientific and Deep Genomics Scientific Advisory boards. CD received institutional honoraria from UCB Pharma, support for meeting attendance/travel from Angelini Pharma, and is a member of Angelini Pharma and Neuraxpharm Advisory Boards. DL received institutional research funding from the National Institutes of Health (NIH), National Institute of Neurological Disorders and Stroke (NINDS) under R01 NS117544. GC received research funding from Janssen Pharmaceuticals and consultancy fees from Ono Pharmaceuticals. GJS received personal consulting fees from Angelini Pharma, and personal honoraria for lectures from Angelini Pharma, Bial Pharma UK, and UCB Pharma. HL received personal consulting fees from Praxis Precision Medicine and institutional fees from Lario Therapeutics, personal lecture honoraria from Eisai and UCB Pharma, personal payment for expert testimony for Fondazione Telethon, and is an advisory board member of IntraBio. HS, KS, and UU are employees of deCODE genetics/Amgen Inc. JJC received honoraria from UCB-Pharma, Glaxo Smith Kline, Janssen-Cilag, Sanofi-Synthelabo, Pfizer, and Eisai to attend advisory boards, present lectures/tutorials. JWS received institutional research funding from the UK National Institute for Health Research, Angelini Pharma, UCB Pharma, Epilepsy NL, the Academy of Medical Sciences, and the Wellcome Trust Alliance, institutional consulting fees from the UK Competition & Markets Authority and Eisai, personal honoraria from Angelini Pharma, Eisai, and UCB, and personal fees for participation on an advisory board of Angelini Pharma, and holds unpaid roles as Medical Director of the UK Epilepsy Society and Editorial Board member for Lancet Neurology. LJ received institutional research funding from the Swedish state under the ALF agreement (ALFGBG-966370), Genomic Medicine Sweden (GMS K131050263), and the Söderström König Foundation. MRJ received research funding from the UK Research and Innovation (UKRI) Medical Research Council (MRC) (Award Nos. MR/S02638X/1 and MR/W029790/1). ND received consulting fees and honoraria from Angelini Pharma, Actiobio, Eisai, UNEEG Medical, and Jazz Pharma, and owns stocks from Actiobio. PS received consulting fees from Jazz Healthcare and UCB Pharma for participation on advisory boards, and honoraria from Proveca and UCB Pharma for presentation at congresses/workshops. SFB received institutional research funding from the National Health and Medical Research Council (NHMRC) (Grant IDs 1091593, 1196637, 2010562), UCB Pharma, Eisai, SEER, Chiesi, and LivaNova, personal consulting fees from Praxis Precision Medicines, personal honoraria from Eisai and DeltaMed, co-owns a patent held by Bionomics Inc. licensed to Athena Diagnostics, Genetics Technologies Ltd, and is the Chief Medical Officer of the Epilepsy Foundation (Victoria). SMS received institutional research funding from CURE Epilepsy, Epilepsy Society, and MRC, honoraria from Angelini Pharma, Eisai, Zogenix, UCB, Eisai, Jazz Pharmaceuticals, and UCB, travel support from UCB Pharma, and is a member of Advisory Boards of Biocodex, Stoke Therapeutics, and Takeda. TJOB received institutional research funding from NHMRC, MRFF, DOD, and NIH, institutional consulting fees from Kinoxis Therapeutics, Jazz Pharmaceuticals, and Livanova, institutional honoraria from UCB, institutional travel support from Longboard Pharmaceuticals, is a member of the Kinoxis Therapeutics Advisory Board, and hold an unpaid position as Cabrini Health Board Member. All other authors have no conflict of interests to declare.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2025.105675.

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