Pharmacoresponse in genetic generalized epilepsy: a genome wide association study.

Stefan Wolking[1,2*], MD, Herbert Schulz[3], PhD, Anne T. Nies[4], PhD, Mark McCormack[5], PhD, Elke Schaeffeler[4], PhD, Pauls Auce[6], PhD, Andreja Avbersek[7], MD, Felicitas Becker[1], MD, Karl Martin Klein[8], PhD, Martin Krenn[9], MD, Rikke S Møller[10,11], PhD, Marina Nikanorova[10], MD, Sarah Weckhuysen[12,13,14], PhD, EpiPGx Consortium[#], Gianpiero L. Cavalleri [5,15], Norman Delanty[5,16,17], FRCPI, Chantal Depondt[18], PhD, Michael R. Johnson[15], FRCP, Bobby P.C. Koeleman[19], PhD, Wolfram S. Kunz[20], PhD, Anthony G. Marson[21], FRCP, Josemir W. Sander[7,22], FRCP, Graeme J. Sills[21], PhD, Pasquale Striano[23,24], PhD, Federico Zara[23], PhD, Fritz Zimprich[9], PhD, Yvonne G. Weber[1], MD, Roland Krause[25], PhD, Sanjay Sisodiya[7], FRCP, Matthias Schwab[4,26], PhD, Thomas Sander[3], PhD, Holger Lerche[1], MD

[1]Neurology and Epileptology, Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany, [2]Department of Neurosciences, CHUM Research Center, University of Montreal, Montreal, Canada, [3]Cologne Center for Genomics, University of Cologne, Cologne, [4]Dr Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany, [5]Molecular and Cellular Therapeutics, Royal College of Surgeons in Ireland, Dublin, Ireland, [6]Walton Centre NHS Foundation Trust, Liverpool, UK, [7]Department of Clinical and Experimental Epilepsy, UCL Queen Square Institute of Neurology, London and Chalfont Centre for Epilepsy, London, UK, [8]Epilepsy Center Frankfurt Rhine-Main, University Hospital Frankfurt, Goethe University Frankfurt, Frankfurt, Germany, [9]Department of Neurology, Medical University of Vienna, Vienna, Austria, [10]Danish Epilepsy Centre - Filadelfia, Dianalund, Denmark, [11]Department of Regional Health Research, University of Southern Denmark, Odense, Denmark, [12]Neurogenetics Group, VIB-University of Antwerp, Antwerp, Belgium, [13]Laboratory of Neurogenetics, Institute Born-Bunge, University of Antwerp, Antwerp, Belgium, [14]Department of Neurology, Antwerp University Hospital, Antwerp, Belgium, [15]Division of Brain Sciences, Imperial College Faculty of Medicine, London, UK, [16]Division of Neurology, Beaumont Hospital, Dublin, Ireland, [17]The FutureNeuro Research Centre, Royal College of Surgeons in Ireland, Dublin, Ireland, [18]Department of Neurology, Hôpital Erasme, Université Libre de Bruxelles, Brussels, Belgium, [19]Department of Genetics, University Medical Center Utrecht, Utrecht, Netherlands, [20]Institute of Experimental Epileptology and Cognition Research and Department of Epileptology, University of Bonn, Bonn, Germany, [21]Department of Molecular and Clinical Pharmacology, Institute of Translational Medicine, University of Liverpool, Liverpool, UK, [22]Stichting Epilepsie Instellingen Nederland (SEIN), Heemstede, Netherlands, [23]IRCCS "G. Gaslini" Institute, Genova, Italy, [24]Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genova, Genova, Italy, [25]Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Esch-sur-Alzette, Luxembourg, [26]Department of Clinical Pharmacology, Institute of Experimental and Clinical Pharmacology and Toxicology, University Hospital of Tübingen, Tübingen, Germany

*Corresponding Author:
Stefan Wolking, MD
Department of Neurology and Epileptology
Hertie Institute for Clinical Brain Research
University Hospital of Tübingen
Hoppe-Seyler Str. 3
72076 Tübingen

+1 514 778 6260
stefan.wolking@med.uni-tuebingen.de

#EpiPGx Consortium group members are listed in Appendix 1.

Abstract

Background: Pharmacoresistance is a major burden in epilepsy treatment. We aimed to identify genetic biomarkers for response to specific antiepileptic drugs (AEDs) in genetic generalized epilepsies (GGE).
Methods: We conducted a genome-wide association study (GWAS) of 3.3 million autosomal SNPs in 893 European subjects with GGE - responsive or non-responsive to lamotrigine, levetiracetam, and valproic acid.
Results: Our GWAS of AED response revealed suggestive evidence for association at 29 genomic loci ($P < 10^{-5}$) but no significant association reflecting its limited power. The suggestive associations highlight candidate genes that are implicated in epileptogenesis and neurodevelopment.
Conclusions: This first GWAS of AED response in GGE provides a comprehensive reference of SNP associations for hypothesis-driven candidate gene analyses in upcoming pharmacogenetic studies.

Keywords

Pharmacoresistance, antiepileptic drugs, GWAS, genetic generalized epilepsy, valproic acid, levetiracetam, lamotrigine

Summary Points

- **Drug resistance to antiepileptic drugs is a common challenge in the clinical management of patients with epilepsy**
- **There are no pharmacogenetic markers for drug response in epilepsy are so far**
- **We conducted a GWAS of 893 European subjects with genetic generalized epilepsy for drug response to lamotrigine, levetiracetam, and valproic acid.**
- **We identified 29 genomic loci ($P < 10^{-5}$) with suggestive evidence for association with AED response but did not find significant genetic association ($p < 5\times10^{-8}$) of responder status with common variants**
- **A gene set and gene level analysis for genes involved in drug absorption, distribution, metabolization, and excretion (ADME) revealed no significant association.**
- **The replication of a previously reported marker for lamotrigine response in *ABCB1* was not significant.**

Introduction

Genetic generalized epilepsies (GGE) are common, affecting about one third of all patients with epilepsy. Most GGE follow a complex mode of inheritance, supposedly involving a multitude of common and rare genetic variants [1]. Unlike developmental and epileptic encephalopathies (DEE), monogenic forms of GGE account for only a small fraction of cases: culpable genes include *GABRG2* [2], *GABRA1* [3] or *SLC2A1* [4]. Furthermore, a small percentage of GGE is associated with common copy number variations (CNVs) such as 15q13.3 [5–8] as well as rare CNVs [9]. Recent studies highlighted the complexity of GGE genetics by underpinning the role of common single nucleotide variants (SNPs) [1] as well as the enrichment of rare deleterious missense variants in known epilepsy genes and the group of $GABA_A$ receptor encoding genes [10–12].

Resistance to antiepileptic drugs (AEDs) is a widespread problem in the treatment of epilepsies. Drug resistance is defined by the International League against Epilepsy (ILAE) as ongoing seizures despite treatment with two correctly chosen AEDs in a sufficient dose [13]. As a rule, response to the first AED is achieved in about 50% of patients [14]. In the case of ongoing seizures, the addition of or exchange with another AED will result in seizure freedom in further 15% of patients. Patients refractory to two AEDs have a chance of less than 5% to reach seizure freedom - with a shrinking likelihood of success with an increasing number of drug trials [14]. Despite the approval of various novel AEDs in recent years, the proportion of patients who are drug resistant has remained more or less unchanged [15].

So far, the choice of an AED is guided by several factors such as age, gender, epilepsy type as well as by potential drug interactions or side effects, and personal experience. Recommendations for AED choice can be found in national and international guidelines [16]. Substantial pharmacogenetic findings that resulted in the adaptation of treatment guidelines are sparse and exist only for cutaneous adverse drug reactions (ADR) of different severity associated with sodium channel blockers that share an aromatic ring structure [17–20]. The overall usefulness of pharmacogenetic screenings in reducing the frequency of ADR remains, however, controversial [21]. For AED responder status, pharmacogenetic findings in childhood absence epilepsy (CAE) showed an association of common variants in the *ABCB1* drug transporter as well as in *CACNA1H* and *CACNA1I,* subunits of T-type calcium channels, with responder status for the drugs ethosuximide and lamotrigine (LTG) [22].

Genes involved in drug absorption, distribution, metabolization and excretion (ADME) have been in the focus of pharmacogenetic research of AEDs for some time [23–25]. Influence of variants in genes encoding drug transporters have been shown to influence pharmacokinetic parameters of LTG or valproic acid (VPA) [26–28]. Therefore, ADME genes represent prospective locations of genome-wide association.

This study aimed to test whether common genetic variants predict drug response to LTG, levetiracetam (LEV), VPA, the combination of VPA and LTG or overall drug response in a cohort of 893 people with GGE that were deeply phenotyped regarding clinical presentation and pharmacoresponse.

Methods

Ethics Statement

All study participants provided written, informed consent for genetic analysis. Local institutional review boards reviewed and approved study protocols at each contributing site.

Study Design

The epilepsy cohort derived from the EpiPGx Consortium which was established in 2012 to identify genetic biomarkers of epilepsy treatment response and adverse drug reactions. EpiPGx (https://www.epipgx.eu/) is a European-wide epilepsy research partnership under the European Commission Seventh Framework Protocol (FP7). This case-control study is based on the retrospective evaluation of patient data. Relevant patient data was extracted from patient charts by trained personnel and collected in a common electronic case report form (eCRF) used by all consortium sites. Our cohorts exclusively consisted of individuals of non-Finnish European ancestry with an established diagnosis of GGE according to current ILAE diagnostic criteria [29]. Individuals included in the study were exposed to LTG, VPA and/or LEV. The 3 AEDs were chosen because they were the most frequent in our cohort and represent the highest use in GGE [30]. We tested whether common genetic variants were significantly associated with drug response to one of these AEDs, to the combination therapy of lamotrigine and valproic acid, which can provide additive benefits [31], or with drug response to at least one of these AEDs

Cohorts and Phenotype Definition

The individuals in this study were selected according to our inclusion criteria from more than 12.000 individuals that were documented in the EpiPGx eCRF. Our cohort included 893 patients with GGE (587 females & 306 males) comprising 359 patients with juvenile myoclonic epilepsy (JME), 194 patients with CAE, 191 patients with GGE with bilateral tonic-clonic seizures only displaying generalized epileptic EEG discharges (EGTCS), and 149 patients with juvenile absence epilepsy (JAE). Median age of seizure onset was 12 years [± 5.6]. Altogether, 589 patients originated from Central Europe (Austria, Belgium, Denmark, Germany, Netherlands), 218 from the British Isles (UK, Ireland), and 86 from Southern Europe (Italy). Recruitment sites are listed in the supplement information.

GGE patients were classified as responders or non-responder to the specific AEDs or groups of AEDs, controls were defined as non-responders. The following are our definitions in EpiPGx: Response was defined as seizure freedom under ongoing treatment for at least one year and prior to initiation of any other treatment. Non-response was defined as recurring seizures at ≥ 50% of pretreatment seizure frequency given adequate dosage. Individuals with recurrent non-compliance for AED intake were excluded. The assignment to the response or non-response groups was based on the evaluation of one or more epilepsy specialists at the source center. For the overall response analysis of the entire GGE cohort, in the case of exposure to multiple AEDs, patients were defined as responders if they fulfilled the responder criteria for at least one AED. We also included patients in the overall response cohort that fulfilled the criteria based on their response profile for other AEDs. This included 43 patients with ethosuximide (38 responders, 5 non-responders) and 7 patients with zonisamide (3 responders, 4 non-responders).

Imputation and Genotyping Quality Controls

GWASs were conducted separately for each AED-response cohort using imputed best-guess genotypes. Genotyping and imputation methods have been described previously [20]. We applied stringent per-individual and per-SNP quality controls (QC) using PLINK 1.9 [32]. Per-individual QC: We included unrelated individuals (pairwise IBD: PI_HAT < 0.06) with European (EUR) ancestry, and a SNP genotype missingness rates < 2%. Cohort consistency was controlled via principal component analysis (PCA) using the EIGENSOFT software [33]. Outlier subjects in the 5 datasets (table 1) were identified and removed using a sigma of > 5 standard deviations from the first 10 principal components. A European (EUR) ancestry of the remaining cohort of 893 individuals was verified by a PCA comparison to 1000 Genomes data (figure S1). Per-SNP QC: SNPs were included by the following QC criteria: 1) autosomal annotation, 2) IMPUTE2 info-score > 0.9 [34], 3) genotype missingness rate < 2%, and 4) minor allele frequency > 1%. After SNP QC-filtering, between 3,287,443 and 3,347,871 SNPs remained for GWAS analysis.

Statistical Association Analyses

Single marker association analyses were performed using the linear mixed model application FaST-LMM [35] to correct for confounding by population stratification or cryptic relatedness. The spectral decomposition matrix was calculated using a LD-pruned SNP dataset (LD $r^2 < 0.2$ and a window size of 100 SNPs) under exclusion of the major histocompatibility complex cluster on 6p22.3-p21.2. The covariates gender, age-of-onset and array-type (Illumina, Affymetrix) were included in a linear mixed model. P-values below 5×10^{-8} or 10^{-5} were considered significant or suggestive respectively. Given the exploratory approach of this pilot-GWAS, we did not correct for multiple testing of five AED response traits - accepting a slightly higher false positive rate in order to present a comprehensive list of candidate loci for each AED response trait for follow-up studies. Manhattan and quantile-quantile (QQ) plots were created using the R package qqman. Genomic inflation factors were calculated using the R package GenABEL. Regional plots were created using the LocusZoom webtool (http://locuszoom.org) based on the hg19/1000 Genomes Nov 2014 reference data.

Gene-set Analysis and Gene Level Analysis for ADME Genes

To test whether genes involved in pharmacokinetics, i.e. absorption, distribution, metabolization, and excretion (ADME), were associated as a group with pharmacoresponse, we created a gene-set of 307 genes (table S3). We applied MAGMA version 1.04 using the entire set of SNPs and GWAS P-values to run the gene-set and gene level analysis [36].

Study Power Estimates

We performed power analyses, using the power calculator for case-control genetic association analyses PGA2 version 2.0 [37]. For an alpha level of $P \leq 5 \times 10^{-8}$ our analysis of the five AED response cohorts had 80% power to detect genome-wide significant SNPs of MAF = 5% with relative risks ≥ 1.48, ≥ 1.54, ≥ 2.51, ≥ 2.93, ≥ 4.65 for overall, VPA, LTG, LEV and LTG & VPA respectively (figure S2).

Functional Annotation of SNPs, and gengene level analysis

We applied the FUMA webtool [38] to our summary statistics to perform a genome wide gene-level analysis. Given about 14.000 genes interrogated in our GWASs, P-values < 3.6×10^{-6} were considered significant after Bonferroni correction.

Results

Cohort description

After per-individual QC, 893 persons were included in the GWASs. There was a substantial overlap between the different analysis cohorts since various patients were treated with two or more AEDs. The breakdown of the different AED-response cohorts is shown in table 1. The overlap of the cohorts is shown in figure S3.

Genome-wide Association Analysis

To test the hypothesis that genetic markers predispose to pharmacoresponse, a linear mixed model analysis of the AED subgroups as well as of the overall cohort was performed. We observed no evidence for a substantial GWAS P-value inflation (lambda-range between 0.99 for LEV and 1.02 for LTG & VPA, figures 1 and S4). We did not detect any genome-wide markers for any of the AEDs or the overall cohort (figure 1) that exceeded the threshold of significance (P-value < 5×10^{-8}). However, we identified 29 loci with lead SNPs that were suggestive for an association with AED response (P-value < 10^{-5}). The strongest association was found in the LEV response group for rs17676256 (4q25), an intronic SNP in the *ANK2* gene (P = 1.07×10^{-7}) (figures 1, S8). Among the other loci several represented genes involved in in neuronal development or associated with neurodevelopmental disorders: *CACNB2,* and *CNTNAP2* for the overall response, *CELF2* for lamotrigine response, *LRRTM4* and *MAGI2* for the response to lamotrigine plus valproic acid. The top results for all GWASs are depicted in table 2 and table S1. Regional genomic plots are shown in figures S5-S9. We also did not observe an enrichment of SNPs at the gene level (table S4 shows hits with P < 1×10^{-4}, figure S10 presents the QQ plots).

Gene-level and Gene-set Analysis of the ADME Gene Panel

The gene-set analysis using MAGMA on a set of 307 ADME candidate genes revealed no significant result (the P-values ranged between 0.41 for lamotrigine and 0.99 for valproic acid) (table S2). The gene-level analysis for the 307 genes showed no significant results (table S3) with a P-value threshold of < 1.6×10^{-4} after Bonferroni correction.

Replication Analysis of SNP Associations predicting Lamotrigine Response

We aimed to test whether the SNPs described by *Glauser et al*. [22] (rs2032582 for *ABCB1,* rs2753325, and rs2753326 for *CACNA1H*) that were reportedly associated with lamotrigine response in CAE showed an association with lamotrigine responder status in our cohort. We tested our entire GGE LTG-cohort (table 1) as well as the fraction of CAE patients that were responders or non-responders to LTG (26

responders, 41 non-responders; 20 males, 47 females; median age of seizure onset 6 years [± 2.3]). rs2032582 revealed no significant association for the whole group (p = 0.35, OR = 1.17) and the CAE group (p = 0.45, OR = 0.70) by Fisher's exact test. The two synonymous SNPs, rs2753325 and rs2753326, were neither present in our imputed SNP set, nor did we find SNPs in LD.

Discussion

No pharmacogenetic marker for drug response to specific AEDs has been reproducibly identified to date. In this pilot study we aimed to explore common genetic variants associated with drug response in three common AEDs: LEV, LTG, and VPA. Our GWAS approach did not reveal evidence that strong genetic effects contribute to the genetic variance of therapy response of the most common AEDs used in the treatment of GGE. The lack of significant findings in this study rules out single variants with large effect size. This underlines that there is no simple answer to the question of the causes of pharmacoresistance [23]. Drug resistance either constitutes a complex trait that is driven by many genetic factors or does not have a significant genetic contribution. The former assumes the presence of multiple genetic variants with small effect sizes – a hypothesis that cannot be dismissed by our study due to insufficient power. Our power to detect variants with small effect sizes was too low due to the limited sample size. Nonetheless, we identified several suggestive loci.

Amongst them, we identified several loci associated with genes of interest: *ANK2* encodes a 440kDa polypeptide that is exclusively expressed in brain tissue [39] and has been identified as a high confidence autism spectrum disorder (ASD) gene [40]. A recent study showed that *ANK2* mutations lead to increased axon branching and ectopic connectivity [41]. Deletions of *MAGI2* that encodes a scaffold protein, which interact with several pre- and postsynaptic proteins in inhibitory and excitatory synapses [42], have been described in association with infantile spasms [43]. *CELF2*, which is involved in alternative RNA splicing in the brain [44], has been recently implicated as modifier gene for individuals with *KCNQ1* associated epilepsy [45]. *CACNB2* encodes a L-type calcium channel subunit, which has also been associated with ASD [46] as well as Brugada syndrome [47]. *CNTNAP2,* also known as *CASPR2,* encodes a neuronal transmembrane protein that is involved in neuron-glia interaction and the clustering of potassium channels [48]. It has been associated with ASD and epilepsy [49] and Cntnap2$^{-/-}$ mice show seizures and abnormal EEG patterns [50]. *LRRTM4* is implicated in synaptogenesis [51] and in the organization of excitatory and inhibitory synapses [52].

Interestingly, whereas several of the top SNPs belong to genes that are associated with neurological development and neurodevelopmental disorders, none was found in ADME genes. This was further corroborated by the lack of significant findings in the gene-set analyses. Furthermore, we could not corroborate the finding by *Glauser et al.* who reported an association of lamotrigine response with a variant in the gene *ABCB1* [22]*.* However, our analysis did not allow to further elucidate the role of the two *CACNA1H* variants [22].

The major limitation of this study was its sample size which is reflected by the fact that of more than 12.000 individuals in our database only 893 fulfilled our inclusion criteria. There is an elemental trade-off between the need of a large sample size on the one side and accuracy and stringent phenotype definition on the other side. In our study, we decided to emphasize the latter. It could be argued that a looser definition of drug response, e.g. 50% or 75% seizure reduction compared to base level or 6 months of

seizure freedom would have resulted in a larger sample size. However, we assume that a less rigorous definition would have blurred potential genetic association. Thus, even though large cohorts of genotyped [1] and exome-sequenced [10] patients have recently become available, detailed clinical data and the personnel to collect and analyze these data are the main constraint to perform larger studies of this kind.

This is the first GWAS for individual AED response in GGE. While our study did not reveal significant association signals for drug response, we identified several suggestive loci that that warrant further scrutiny in subsequent pharmacogenetic studies. Future hypothesis-driven association studies should attempt to reproduce our top findings, freed from the threshold (P-value < 5×10^{-8}) for genome-wide correction for multiple testing. Furthermore, this study, by design, focused on SNPs. Possibly, the inclusion of rare variants and CNVs, in analogy to recent case-control studies on epilepsy risk factors [10–12,53], will shed more light on drug response. More novel analysis techniques such as the polygenic risk score [54] or the polygenic transmission disequilibrium test [55] could also help to elucidate the role of common variants in future analyses.

Acknowledgements

SW received funding from the German Research Foundation (DFG) (WO 2385/1-1), and the Clinician Scientist program of the University of Tübingen (418-0-0). HL and TS received funding from the FP6 Integrated Project EPICURE (LSHM-CT-2006-037315). TS received also funding from the DFG EUROCORES Program EuroEPINOMICS (SA434/5–1), and DFG Research Unit FOR2715 (SA434/6-1). SaW was supported by the BOF-University of Antwerp (FFB180053) and FWO (1861419N). The computational analysis was performed on the high-performance computer system of the University of Luxembourg (https://hpc.uni.lu).The EpiPGx Consortium was funded by FP7 grant 279062 “EpiPGx” from the European Commission.

Conflicts of interest

AA is employed by UCB Pharma SPRL, Belgium as Director. The other authors report no competing interests related to the article.

Appendix

EpiPGx Consortium:
Andreja Avbersek, Costin Leu, Kristin Heggeli, Rita Demurtas, Joseph Willis, Douglas Speed, Narek Sargsyan, Krishna Chinthapalli, Mojgansadat Borghei, Antonietta Coppola, Antonio Gambardella, Stefan Wolking, Felicitas Becker, Sarah Rau, Christian Hengsbach, Yvonne G. Weber, Bianca Berghuis, Wolfram S. Kunz, Mark McCormack, Norman Delanty, Ellen Campbell, Lárus J. Gudmundsson, Andres Ingason, Kári Stefánsson, Reinhard Schneider, Rudi Balling, Pauls Auce, Ben Francis, Andrea Jorgensen, Andrew Morris, Sarah Langley, Prashant Srivastava, Martin Brodie, Marian Todaro, Slave Petrovski, Jane Hutton, Fritz Zimprich, Martin Krenn, Hiltrud Muhle, Karl Martin Klein, Rikke S Møller, Marina Nikanorova, Sarah Weckhuysen, Zvonka Rener-Primec, Gianpiero L. Cavalleri, John Craig, Chantal Depondt, Michael R. Johnson, Bobby P. C. Koeleman, Roland Krause, Holger Lerche, Anthony G. Marson, Terence J. O'Brien, Josemir W. Sander, Graeme J. Sills, Hreinn Stefansson, Pasquale Striano, Federico Zara and Sanjay M. Sisodiya

TABLES

Table 1: Cohorts for 5 GWAS.

Cohort	Responder	Non-responder
Overall	608	278
VPA	410	155
LTG	137	250
LEV	82	127
LTG & VPA	31	73

Table 1: Number of responders and non-responders in each of the 5 GWAS cohorts. VPA = valproic acid, LTG = lamotrigine, LEV = levetiracetam.

Table 2: Top GWAS results ($p < 10^{-5}$) for therapy response studies of five antiepileptic treatments

SNP	Location (hg19)	p	Gene
Overall Responder Status			
rs6871559	5:8047709	5.03×10^{-6}	-
rs13179734	5:29350681	8.82×10^{-6}	-
rs7457112	7:146876502	9.30×10^{-6}	*CNTNAP2*
rs1277731	10:18563985	9.41×10^{-6}	*CACNB2*
rs11681922	2:29442426	9.84×10^{-6}	*ALK*
Valproic acid			
rs78269837	5:76809481	5.03×10^{-6}	*WDR41*
rs4292046	2:238149704	5.29×10^{-6}	-
rs6046489	20:19945493	6.88×10^{-6}	*RIN2*
rs619889	18:62929316	9.65×10^{-6}	
Lamotrigine			
rs17650998	3:178313693	8.66×10^{-7}	*KCNMB2*
rs10206521	2:21420828	3.23×10^{-6}	-
rs1291861	10:11111799	5.93×10^{-6}	*CELF2*
rs11794033	9:25100016	7.97×10^{-6}	-
Levetiracetam			
rs17676256	4:114061536	1.07×10^{-7}	*ANK2*
rs12320526	12:77952683	1.59×10^{-6}	*RP1-34H18.1*
rs12734159	1:66185458	2.76×10^{-6}	-
rs7956831	12:9889157	3.36×10^{-6}	-
rs1014085	8:57643998	3.65×10^{-6}	-
rs3756744	5:128428722	3.70×10^{-6}	-
rs7515154	1:85704435	4.08×10^{-6}	-
rs72765466	1:236218004	5.69×10^{-6}	*NID1*
rs17124115	12:50305590	7.36×10^{-6}	*RP11-70F11.11*
Lamotrigine and Valproic acid			
rs1922809	2:77687101	7.77×10^{-7}	*LRRTM4*
rs4751538	10:129635908	8.00×10^{-7}	-
rs78723182	7:78521292	1.51×10^{-6}	*MAGI2*
rs4416719	6:6164208	2.24×10^{-6}	*F13A1*
rs1479876	3:140044009	4.23×10^{-6}	*CLSTN2*
rs7705566	5:31259129	4.28×10^{-6}	*CDH6*
rs8003775	14:39335815	5.54×10^{-6}	*LINC00639*

Table 2: GWAS lead SNPs ($p < 10^{-5}$), including SNP position (hg19 assembly) and gene for genic markers. For SNPs in linkage disequilibrium, only the SNP with the lowest P-value are depicted.

Figure Legends

Figure 1: Manhattan plots and genomic inflation factors (λ) for the 5 GWASs.

Dashed line represents the P-value threshold for suggestive association (linear mixed model P-value = 10^{-5}).

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