Research Article

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Pharmacogenomics

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

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Aim: Pharmacoresistance is a major burden in epilepsy treatment. We aimed to identify genetic biomarkers in response to specific antiepileptic drugs (AEDs) in genetic generalized epilepsies (GGE). **Materials & methods:** We conducted a genome-wide association study (GWAS) of 3.3 million autosomal SNPs in 893 European subjects with GGE – responsive or nonresponsive 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. **Conclusion:** This first GWAS

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of AED response in GGE provides a comprehensive reference of SNP associations for hypothesis-driven candidate gene analyses in upcoming pharmacogenetic studies.

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Genetic generalized epilepsies (GGE) are common, affecting about a 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, 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 SNP variants [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 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.

Materials & 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 that was established in 2012 to identify genetic biomarkers of epilepsy treatment response and ADR. 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 were extracted from patient charts by trained personnel and collected in a common electronic case report form used by all consortium sites. Individuals included in the study were exposed to LTG, VPA and/or LEV. These three AEDs were the most frequent in our

Table 1. Clinical details for three antiepileptic drug cohorts.

AED group	Gender (% of females)		AOO (mean, SD)		Duration of epilepsy (mean in years)		Seizure frequency before and after initiation of AED (mean per month, SD)					Distribution of GGE subtypes (%)		
	R	NR	R	NR	R	NR	Seizure type	R		NR		Subtype	R	NR
								Pre	Post	Pre	Post			
VPA	61%	59%	12.3 (± 5.3)	11.6 (± 5.4)	21.2	22.9	GTCS nGTCS	1.8 (± 2.3) 20.4 (± 119.5)	0 0	1.8 (± 2.1) 40.1 (± 151.0)	3.1 (± 9.6) 30.1 (± 94.2)	JME AE EGTCS	35.6% 39.8% 24.4%	48.7% 35.0% 16.2%
LTG	67%	79%	12.9 (± 6.9)	12.2 (± 5.1)	20.9	19.4	GTCS nGTCS	1.9 (± 2.5) 20.3 (± 102.8)	0 0	2.0 (± 2.0) 72.0 (± 228.3)	2.8 (± 8.3) 44.3 (± 126.2)	JME AE EGTCS	33.6% 37.2% 29.2%	29.4% 35.1% 15.5%
LEV	85%	65%	13.1 (± 4.0)	11.4 (± 4.6)	22.0	22.1	GTCS nGTCS	1.8 (± 1.7) 14.3 (± 8.5)	0 0	2.0 (± 1.9) 12.8 (± 9.6)	2.3 (± 5.3) 22.9 (± 75.3)	JME AE EGTCS	61.7% 22.2% 16.0%	53.2% 33.9% 12.1%

Depiction of gender distribution, mean age of seizure onset, duration of epilepsy at the time of inclusion, seizure frequency for GTCS and non-GTCS before and after initiation of treatment with respective AED, and distribution of GGE subtypes for the three AED groups.
AE: Absence epilepsies (childhood and juvenile absence epilepsy); AED: Antiepileptic drug; AOO: Age of onset of first seizure; EGTCS: Generalized epilepsy with generalized tonic-clonic seizures only; GGE: Genetic generalized epilepsy; GTCS: Generalized tonic-clonic seizure; nGTCS: Seizures other than GTCS (absence seizures, myoclonic seizures); JME: Juvenile myoclonic epilepsy; LEV: Levetiracetam; LTG: Lamotrigine; NR: Nonresponder; Pre: Before initiation of treatment with AED; Post: After initiation of treatment with AED; R: Responder; SD: Standard deviation; VPA: Valproic acid.

cohort. Besides carbamazepine, they reflect the highest usage in Europe [29] and are broadly available [30]. We tested whether common genetic variants were significantly associated with drug response to one of these AEDs, to the combination therapy of LTG and VPA, which can provide additive benefits [31], or with drug response to at least one of these AEDs

Cohorts, phenotype definition, inclusion & exclusion criteria

From more than 12,000 individuals that are documented in the EpiPGx electronic case report form, only 893 individuals met our inclusion criteria. The cohorts exclusively consisted of individuals of non-Finnish European ancestry with an established diagnosis of GGE according to the current International League against Epilepsy diagnostic criteria [32]. Individuals were required to feature one of the four typical GGE syndomes: CAE, juvenile absence epilepsy (JAE), juvenile myoclonic epilepsy or GGE with generalized tonic-clonic seizures (GTCS) only. Diagnosis was based on patient history, seizure semiology, electroencephalogram (EEG) and cerebral imaging findings.

The entire cohort of 893 individuals (587 females and 306 males) comprised 359 individuals with juvenile myoclonic epilepsy, 194 patients with CAE, 191 patients epilepsy GTCS and 149 patients with JAE. Median age of seizure onset was 12 years (±5.6). Altogether, 589 patients originated from Central Europe (Austria, Belgium, Denmark, Germany and The Netherlands), 218 from the British Isles (UK and Ireland), and 86 from Southern Europe (Italy). Recruitment sites are listed in the Supplementary data. More detailed clinical information is shown in Table 1.

Individuals with a diagnosed monogenetic cause of epilepsy, severe developmental or intellectual deficits, epileptogenic lesions on cerebral imaging, seizures other than GTCS, myoclonic or absence seizures, or recurrent noncompliance were excluded from the analysis.

Furthermore, individuals were required to fulfill response or nonresponse criteria for at least one AED. The following are our definitions in EpiPGx: response was defined as seizure freedom under continuous treatment for at least 1 year and prior to initiation of any other treatment. The period of seizure-freedom did not have to be ongoing at the time of inclusion. Nonresponse was defined as recurring seizures at ≥50% of pretreatment seizure frequency given adequate dosage. The assignment to the response or nonresponse groups was based on the evaluation of at least one epilepsy specialist 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

Table 2. Sample size of genome-wide association study cohorts.

Cohort	Responders	Nonresponders
Overall	608	278
VPA	410	155
LTG	137	250
LEV	82	127
LTG and VPA	31	73

Number of responders and nonresponders in each of the five genome-wide association study cohorts.
LEV: Levetiracetam; LTG: Lamotrigine; VPA: Valproic acid.

and five nonresponders) and seven patients with zonisamide (three responders and four nonresponders). Dosage requirements for the classification of nonresponse were a minimal daily dose of 150 mg for LTG, and 1000 mg for VPA and LEV, respectively. For response classification, lower doses were accepted on a case-by-case evaluation left to the discretion of the specialist (e.g., 100 mg LTG).

Imputation & genotyping quality controls

Genome-wide association studies (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 [33]. Per-individual QC: we included unrelated individuals (pairwise identity-by-descent [IBD]: PI_HAT <0.06) with European ancestry, and an SNP genotype missingness rates less than 2%. Cohort consistency was controlled via principal component analysis using the EIGENSOFT software [34]. Outlier subjects in the five datasets (Table 2) were identified and removed using a sigma of >5 standard deviations from the first ten principal components. A European ancestry of the remaining cohort of 893 individuals was verified by a principal component analysis comparison to 1000 Genomes data (Supplementary Figure 1). Per-SNP QC: SNPs were included by the following QC criteria: autosomal annotation, IMPUTE2 info-score >0.9 [35], genotype missingness rate less than 2% and minor allele frequency more than 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 [36] to correct for confounding by population stratification or cryptic relatedness. The spectral decomposition matrix was calculated using a linkage disequilibrium (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, CA, USA) 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 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 November 2014 reference data.

Gene-set analysis & gene-level analysis for ADME genes

To test whether genes involved in pharmacokinetics, in other words, ADME, were associated as a group with pharmacoresponse, we created a gene set of 307 genes (Supplementary Table 3). 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 [37].

Study power estimates

We performed power analyses, using the power calculator for case–control genetic association analyses PGA2 version 2.0 [38]. 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 minor allele frequency (MAF) = 5% with relative risks ≥1.48, ≥1.54, ≥2.51, ≥2.93, ≥4.65 for overall, VPA, LTG, LEV, and LTG and VPA, respectively (Supplementary Figure 2).

Functional annotation of SNPs & gene-level analysis

We applied the FUMA webtool [39] to our summary statistics to perform a genome-wide gene-level analysis. Given about 14,000 genes interrogated in our GWASs, p-values $<3.6 \times 10^{-6}$ 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 2. The overlap of the cohorts is shown in Supplementary Figure 3. Comparing the ratio of responders to nonresponders for the different groups, we saw more responders than nonresponders for VPA, whereas for LTG and LEV the nonresponders outweighed the responders (Table 2). Regarding the seizure rate before treatment with the respective AED, we saw for LTG and VPA a higher frequency of seizures other than GTCS (i.e., myoclonic and absence seizures) in nonresponders compared with responders. We did not observe this effect for LEV and for GTCS for all AEDs.

Genome-wide association study 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 and VPA, Figure 1 & Supplementary Figure 4). 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 ($p < 5 \times 10^{-8}$). However, we identified 29 loci with lead SNPs that were suggestive for an association with AED response ($p < 10^{-5}$). The strongest association was found in the LEV response group for rs17676256 (4q25), an intronic SNP in the *ANK2* gene ($p = 1.07 \times 10^{-7}$) (Figure 1 & Supplementary Figure 8). Among the other loci several represented genes involved in neuronal development or associated with neurodevelopmental disorders: *CACNB2* and *CNTNAP2* for the overall response, *CELF2* for LTG response, *LRRTM4* and *MAGI2* for the response to LTG plus VPA. The top results for all GWASs are depicted in Table 3 and Supplementary Table 1. Regional genomic plots are shown in Supplementary Figures 5–9. We also did not observe an enrichment of SNPs at the gene-level (Supplementary Table 4 shows hits with $p < 1 \times 10^{-4}$, Supplementary Figure 10 presents the quantile-quantile plots).

Gene-level & 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 LTG and 0.99 for VPA) (Supplementary Table 2). The gene-level analysis for the 307 genes showed no significant results (Supplementary Table 3) with a p-value threshold of 1.6×10^{-4} after Bonferroni correction.

Replication analysis of SNP associations predicting LTG 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 LTG response in CAE showed an association with LTG responder status in our cohort. We tested our entire GGE LTG cohort (Table 2) as well as the fraction of CAE patients that were responders or nonresponders to LTG (26 responders, 41 nonresponders; 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, odds ratio [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. They are the most frequently used AEDs in GGE and are considered as the first line of treatment [40]. The ratio of responders to nonresponders was higher in the VPA group compared with LTG and LEV for which nonresponders prevailed. This observation could reflect the superiority of VPA in the treatment of

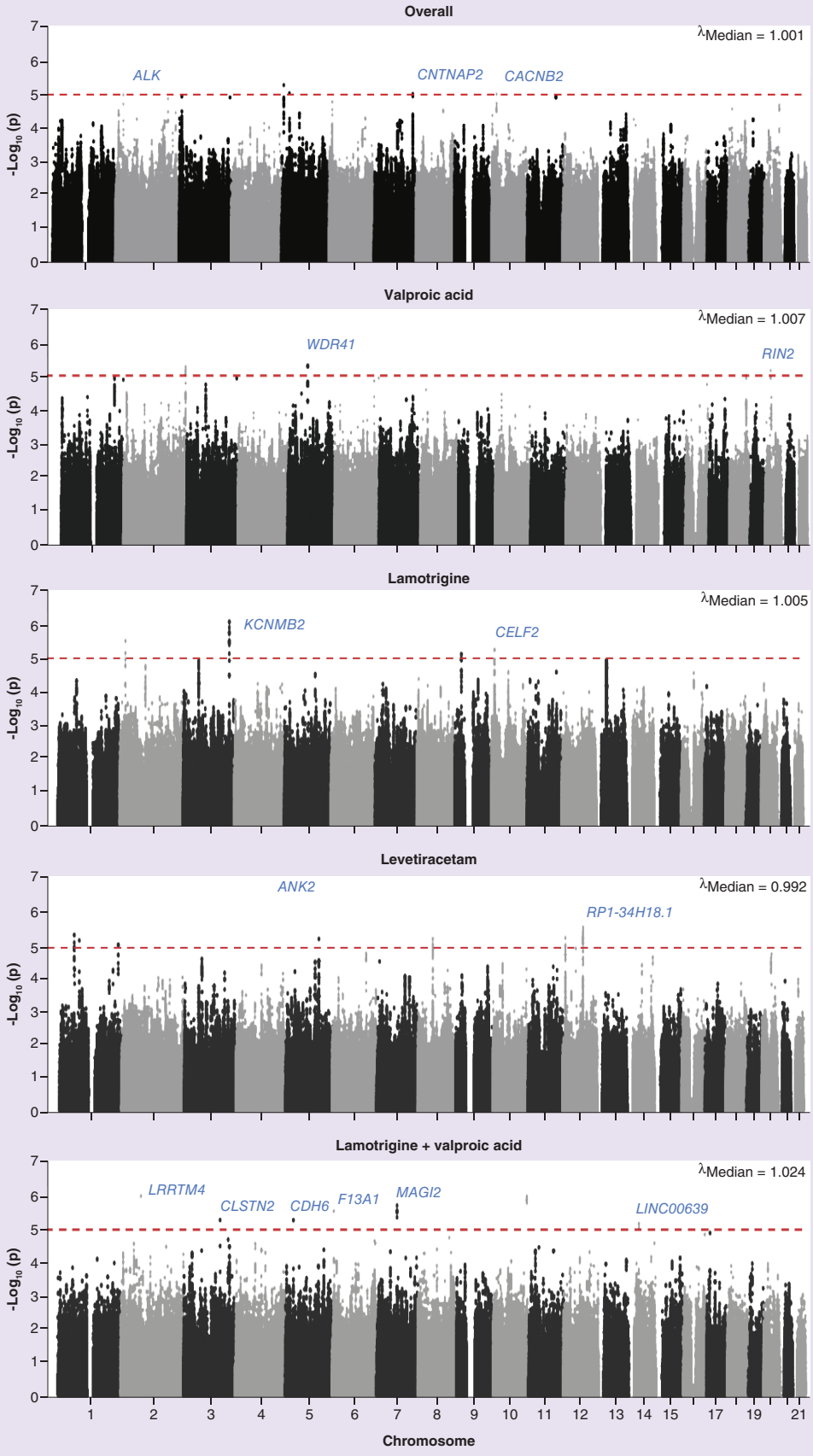

Figure 1. Manhattan plots and genomic inflation factors (λ) for the five genome-wide association study analyses. Dashed line represents the p-value threshold for suggestive association (linear mixed model $p = 10^{-5}$).

Table 3. Top genome-wide association study analysis results ($p < 10^{-5}$) for therapy response studies of five antiepileptic treatments.

SNP	Location (hg19)	p-value	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*

Genome-wide association study lead SNPs ($p < 10^{-5}$) associated with response to respective antiepileptic drugs or overall response, 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.

GGE [40]. The observation that nonresponders had a higher seizure frequency before the start of treatment with the respective AED reflects the common clinical observation that individuals with severe epilepsies are less likely to achieve seizure freedom – the cornerstone of the intrinsic severity hypothesis of pharmacoresistance [41].

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 response and pharmacoresistance [23]. Other mechanisms for the development of drug resistance have been proposed [42]. Novel antiseizure agents are in development that aim to overcome drug resistance [43]. However, there is compelling evidence that multiple genetic factors influence AED response [44].

Assuming a complex genetic trait, one would expect 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.

Among them, we identified several loci associated with genes of interest: *ANK2* encodes a 440 kDa polypeptide that is exclusively expressed in brain tissue [45] and has been identified as a high-confidence autism spectrum disorder (ASD) gene [46]. A recent study showed that *ANK2* mutations lead to increased axon branching and ectopic connectivity [47]. Deletions of *MAGI2* that encodes a scaffold protein, which interacts with several pre- and postsynaptic proteins in inhibitory and excitatory synapses [48], have been described in association with infantile spasms [49]. *CELF2*, which is involved in alternative RNA splicing in the brain [50], has been recently implicated as a modifier gene for individuals with *KCNQ1*-associated epilepsy [51]. *CACNB2* encodes a L-type calcium channel subunit, which has also been associated with ASD [52] as well as Brugada syndrome [53]. *CNTNAP2*, also known as *CASPR2*, encodes a neuronal transmembrane protein that is involved in neuron–glia interaction and the clustering of potassium channels [54]. It has been associated with ASD and epilepsy [55] and Cntnap2$^{-/-}$ mice show seizures and abnormal EEG patterns [56]. *LRRTM4* is implicated in synaptogenesis [57] and in the organization of excitatory and inhibitory synapses [58]. As in the nature of GWAS, these findings should not be considered as causal variants, but as markers for regions, where the actual causal variant has yet to be identified.

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 LTG 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 that 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, for example, 50 or 75% seizure reduction compared with 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.

Conclusion

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. Future hypothesis-driven association studies should attempt to reproduce our top findings, freed from the threshold ($p < 5 \times 10^{-8}$) 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 [10–12,59], will shed more light on drug response. More novel analysis techniques such as the polygenic risk score [60] or the polygenic transmission disequilibrium test [61] could also help to elucidate the role of common variants in future analyses.

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 so far.
- We conducted a genome-wide association study 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 antiepileptic drug response but did not find significant genetic association ($p < 5 \times 10^{-8}$) of responder status with common variants.
- A gene-set and gene-level analysis for genes involved in drug absorption, distribution, metabolization and excretion revealed no significant association.
- The replication of a previously reported marker for lamotrigine response in *ABCB1* was not significant.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/pgs-2019-0179

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Appendix

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