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FULL-LENGTH ORIGINAL RESEARCH

Epilepsia®

Testing association of rare genetic variants with resistance to three common antiseizure medications

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Abstract

Objective: Drug resistance is a major concern in the treatment of individuals with epilepsy. No genetic markers for resistance to individual antiseizure medication (ASM) have yet been identified. We aimed to identify the role of rare genetic variants in drug resistance for three common ASMs: levetiracetam (LEV), lamotrigine (LTG), and valproic acid (VPA).

Methods: A cohort of 1622 individuals of European descent with epilepsy was deeply phenotyped and underwent whole exome sequencing (WES), comprising 575 taking LEV, 826 LTG, and 782 VPA. We performed gene- and gene set–based collapsing analyses comparing responders and nonresponders to the three drugs to determine the burden of different categories of rare genetic variants.

Results: We observed a marginally significant enrichment of rare missense, truncating, and splice region variants in individuals who were resistant to VPA compared to VPA responders for genes involved in VPA pharmacokinetics. We also found a borderline significant enrichment of truncating and splice region variants in the synaptic vesicle glycoprotein (*SV2*) gene family in nonresponders compared to responders to LEV. We did not see any significant enrichment using a gene-based approach.

Significance: In our pharmacogenetic study, we identified a slightly increased burden of damaging variants in gene groups related to drug kinetics or targeting in individuals presenting with drug resistance to VPA or LEV. Such variants could thus determine a genetic contribution to drug resistance.

KEYWORDS
burden analysis, lamotrigine, levetiracetam, pharmacogenomics, rare variants, valproic acid

1 | INTRODUCTION

Drug resistance is a major challenge in the care of people with epilepsy. The International League Against Epilepsy (ILAE) defines drug resistance as the failure of at least two tolerated and appropriate antiseizure medications (ASMs) to achieve ongoing seizure freedom.[1] The odds that people with drug-resistant epilepsy will eventually reach seizure freedom are marginal.[2] Several new ASMs have been licensed in recent years, but the proportion of people with epilepsy who are drug-resistant has not changed significantly.[3]

The response rates of the more than 20 approved ASMs do not seem to differ considerably, although head-to-head comparisons are few.[4,5] The superiority of ethosuximide and valproic acid (VPA) compared with lamotrigine (LTG) in

Key Point

- Pharmacogenetic markers for response to antiseizure medication (ASM) response could improve treatment quality and patient safety
- We performed burden analyses of 1622 whole exome sequenced individuals to determine the role of rare variants in resistance to specific ASMs
- Rare missense and truncating variants in genes involved in valproic acid pharmacokinetics are enriched in individuals who are resistant to valproic acid
- Truncating variants in synaptic vesicle glycoprotein (*SV2*) family genes are enriched in individuals who are resistant to levetiracetam

people with childhood absence epilepsy (CAE),[6] or of VPA compared with LTG and topiramate in people with genetic generalized epilepsy (GGE)[7] or juvenile myoclonic epilepsy (JME),[8] are exceptions to this rule. Considering the retention rate of ASMs, other studies favor LTG and levetiracetam (LEV) because of their superior tolerability.[9] In light of the growing evidence of the teratogenicity of VPA, its use in women of child-bearing age has been widely restricted.[10] Usually, clinical practitioners recommend an ASM based on various factors such as age, gender, comorbidities, seizure type, and potential drug interactions or adverse drug reactions (ADRs). Finding an effective and well-tolerated ASM is, however, often the result of an arduous trial-and-error process.

The aim of pharmacogenomics is to promote personalized medicine by means of genetic markers that allow the prediction of drug response or ADRs. Whereas in other medical fields notable advancements have led to the incorporation of pharmacogenomic findings in clinical decision-making,[11] findings relevant to epilepsy therapy, so far, have fallen short of expectations.[12] Several studies report association of genetic polymorphisms with cutaneous ADRs in people receiving treatment with aromatic ASMs[13,14]; however, the practical meaning of these findings remains controversial.[15] The evidence of genetic markers for ASM response is even more scant. One study that examined common variants in candidate genes reported the *ABCB1* drug transporter as well as in *CACNA1H* and *CACNA1I,* subunits of T-type calcium channels, to be associated with response to ethosuximide and LTG.[16] In previous studies, we aimed to identify common genetic variants via genome wide association studies (GWAS) based on single nucleotide polymorphism (SNP) chip data but failed to produce significant association signals for response to lacosamide,[17] VPA, LTG, and LEV.[18]

Here, based on exome sequencing data, we aimed to investigate the genetic risk of rare variants for drug response to three frequently used ASMs—LEV, LTG, and VPA—by assessing the burden of variants at the gene level as well as in sets of candidate genes in a large cohort.

2 | METHODS

2.1 | Ethics statement

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

2.2 | Study design

The epilepsy cohort was derived from the EpiPGX Consortium (https://www.epipgx.eu/), which was established in 2012 to identify genetic biomarkers of epilepsy treatment response and ADRs. EpiPGX is a European-wide epilepsy research partnership under the European Commission Seventh Framework Protocol (FP7). Recruitment sites are listed in Appendix S2.

This case-control study is based on the retrospective evaluation of individual data. Relevant patient data were extracted from medical records by trained personnel and collected in a common electronic case report form (eCRF) used by all consortium sites. Our cohorts consisted exclusively of individuals of non-Finnish European ancestry.

We included individuals that were exposed to LTG, VPA, or LEV. Besides carbamazepine (CBZ), these are the most commonly used ASMs in Europe[19] and are broadly available.[20] They are approved for use in both focal epilepsy (FE) and GGE.

2.3 | Cohort description and phenotype definition

Individuals were selected according to our inclusion criteria from more than 12 000 individuals who were documented in the eCRF. Our cohort comprised 1622 individuals, of which 975 were female (60%), with a median age at onset of epilepsy of 15 years (±15.6). A total of 847 individuals (52%) had the diagnosis of FE; the remainder were diagnosed with GGE. Epilepsy diagnosis was based on current ILAE criteria.[21] The GGE group comprised individuals with JME (259), CAE (131), juvenile absence epilepsy (JAE, 111), and GGE with bilateral tonic-clonic seizures only (EGTCS, 274). EGTCS diagnosis required the absence of other seizure types, electroencephalography (EEG) showing generalized epileptic discharges, and normal magnetic resonance imaging (MRI). The FE cohort comprised individuals with structural epilepsy (259) and nonacquired focal epilepsy (NAFE, 578). Individuals with an unknown type of epilepsy, a known genetic cause of epilepsy, or a classic syndrome of developmental and epileptic encephalopathy (DEE) were excluded.

We based our drug response categories on the EpiPGX phenotype definitions: Response to a given ASM was defined as seizure freedom under ongoing treatment for at least 1 year and prior to initiation of any other treatment. ASM resistance was defined as recurring seizures at ≥50% of pretreatment seizure frequency given adequate dosage. Dosage requirements for the classification of drug resistance 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 neurologist (eg, 100 mg LTG). Individuals with recurrent noncompliance were excluded from the analysis. Several individuals fulfilled inclusion criteria for more than one of the three ASM groups and were therefore included in more than one analysis. The breakdown per ASM is shown in Table 1.

TABLE 1 Sample data

	Levetiracetam	Lamotrigine	Valproic Acid
Responder Status			
R	226	267	430
NR	349	559	352
Gender			
M	233	308	327
F	342	518	455
Epilepsy Type			
GGE	162	374	513
FE	413	452	269
Site			
EpiPGX	330	549	639
CENet	245	277	143
Total	575	826	782

Note: Number of included individuals for the three ASM analyses grouped by response status, gender, epilepsy type, and sequencing site.

Abbreviations: CENet, Canadian Epilepsy Network; EpiPGX, Epilepsy Pharmacogenomics Consortium; F, female; FE, focal epilepsy; GGE, genetic generalized epilepsy; M, male; NR, nonresponders; R, responders.

2.4 | Sequencing and genotyping

Samples were sequenced at two sites: 1157 at DeCODE genetics (Reykjavik, Iceland) using the Illumina Nextera target enrichment platform, 465 at the Genome Quebec Innovation Center (http://gqinnovationcenter.com/index.aspx?l=e) using the Roche Nimblgen SeqCap EZ Exome target enrichment platform in the framework of the Canadian Epilepsy Network (CENet). Individual FASTQ files were aligned to human genome reference b37 with Burrows-Wheeler Aligner. Resultant binary alignment map files were then processed through the genome analysis toolkit (GATK) best practice pipeline to remove duplicate reads, align indels, and recalibrate base quality scores to generate individual genomic variant call format (GVCF) files.

Individual GVCF files were then jointly genotyped and underwent recalibration and filtering steps using GATK version 3.8 and following the GATK best practice guidelines. We selected only biallelic variants with a genotyping quality >20 using GATK. We removed genomic positions with >2% missingness using VCFtools [22] to eliminate positions that were only present in one of the two sequencing sets.

2.5 | Variant selection and annotation

Annotation and filtration of variant consequences were performed using Ensembl's Variant Effect Predictor (VEP)[23] for human genome assemble GRCh37.

We defined the following variant groups:

- Ultra-rare variant 1 (URV1): missense variants, ≤1 in gnomAD (http://gnomad-old.broadinstitute.org)
- Ultra-rare variant 2 (URV2): missense variants, ≤3 in gnomAD; with the following subgroups:
  a. Deleterious variants: SIFT[24] predicts "deleterious," and PolyPhen-2[25] predicts "damaging"
  b. Benign variants: SIFT matches "tolerated," and PolyPhen-2 matches "benign"
  c. Synonymous variants
- INDELs: insertions and deletions with one of the following consequences:
  a. Inframe deletion/insertion
- Protein truncating variants (PTVs): Variants that fulfilled one of the following consequences:
  a. Stop gain variant or frameshift variant
- PTVs and rare missense variants: variants either fulfilling the PTV criteria or missense variants with a minor allele frequency of ≤0.01 in the gnomAD database
- PTVs and splice region variants: variants either fulfilling the PTV criteria or variants annotated as splice acceptor variant, splice donor variant, or splice region variant
- PTVs, splice region, and rare missense variants: variants either fulfilling the PTV criteria, splice region criteria, or variants with a minor allele frequency of ≤0.01 in the gnomAD database

2.6 | Principal component analysis (PCA)

For the PCA, we selected variants with a minor allele frequency (MAF) >0.05 (using Plink 1.9). After pruning (--indep-pairwise 50 5 0.2), we performed PCA using the smartpca package from Eigensoft software.[26] At first, we observed a batch effect driven by the sequencing site. We then performed a logistic regression with the sequencing site as the dependent variable and the genotype as the independent variable in analogy to,[27] to identify variants that were associated with the sequencing site and thus presumably spurious. By selecting a *P*-value threshold of 0.01, we excluded 2876 variants and were able to eliminate the batch effect (Figures S1 and S2).

2.7 | Gene-based collapsing analysis for all coding variants

To assess whether nonresponders harbor a higher burden of coding variants, we performed gene-based collapsing analyses for the three ASM groups. After further filtering for missingness >2%, and Hardy-Weinberg *P*-value < .001 across all samples using Plink 1.9, a total of 1622 individuals and 808 583 variants remained in the analysis.

Nonresponders were defined as cases; responders were defined as controls.

Kernel regression–based association tests were performed using the SKAT-O function of the SKAT R package to determine an enrichment of variants on the gene level.[28] ANNOVAR software[29] was used to annotate gene names. Gene names were used to designate variant sets, defining 22 541 sets. Small sample size adjustment by SKAT-O was used. The first 10 principal components, gender, epilepsy type (GGE/FE), and the sequencing site were used as covariates. Bonferroni correction was applied to *P*-values to correct for multiple testing, defining a *P*-value threshold for significance of 2.3e-06.

2.8 | Gene-based collapsing analysis for rare variants

To evaluate the role of rare variants, we performed a gene-based collapsing analysis using the SKAT-O function as described earlier for eight variant groups (INDELs; PTVs; PTVs and rare missense; PTVs and splice region; PTVs, splice region and rare missense; PTVs; URV1; URV2 deleterious; URV2 benign). The tests were performed separately for the three ASMs. The number of variants remaining after filtering for each test is depicted in Table S1. To determine whether our model was performing correctly, we ran the same analysis for URV2 synonymous variants as well, for which no biological effect would be expected.

2.9 | Gene set–based collapsing analysis for selected rare variants

We limited the gene set–based tests to five variant groups (PTVs; PTVs and rare missense; PTVs and splice region; PTVs; splice region and rare missense; URV2 deleterious) that were the most likely to harbor functional consequences. We tested one or two gene sets per ASM (Table 1). The target gene sets and ADME (absorption, distribution, metabolism, excretion) gene sets (Table S2) were compiled based on a literature research in PubMed. We did not find enough evidence to create an ADME set for LEV. For the VPA target gene set, we found no sufficient

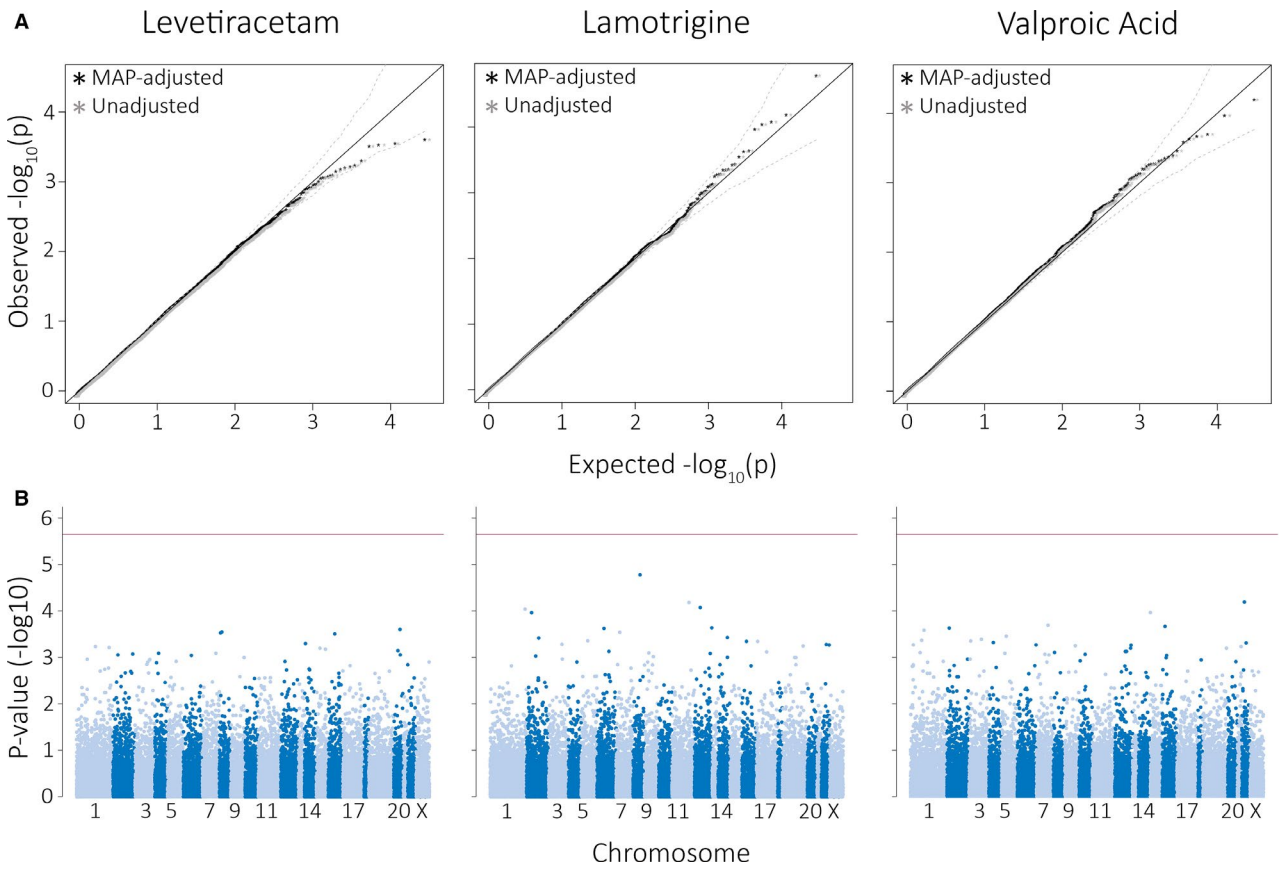

FIGURE 1 Gene-based enrichment analysis for all variants. A, Quantile-quantile (QQ) plots of SKAT-O analyses of all coding variants for response to three antiseizure medications: levetiracetam, lamotrigine, and valproic acid. MAP-adjusted depicts the QQ plot adjusted for minimum achievable *P*-values. B, Corresponding Manhattan plots. Red line shows the threshold for significance

evidence for the inclusion of genes coding for ion channels. However, VPA is also an inhibitor of histone deacetylase (*HDAC*) genes of group 1[30] that were included in the target gene set.

The analysis was performed using the SKAT-O function for the three ASM groups separately, that is, a total of 25 separate tests was performed. Because the gene sets were not entirely independent, we chose a false discovery rate (FDR) correction to account for multiple testing. A significant enrichment was defined at an FDR <0.05.

3 | RESULTS

3.1 | Gene-based enrichment analyses

We tested the burden of all coding variants using the SKAT function for LEV, LTG, and VPA separately (Figure 1). We could not identify any genes that surpassed the significance threshold after correction for multiple testing.

We then tested whether different variant groups showed a gene-based enrichment for the three ASM cohorts (for definition of variant groups, see above). After correction for multiple testing, we could not identify any significant associations (Figure 2, S3-S5). The full results of the enrichment analysis are shown in Table S3.

3.2 | Gene set–based enrichment analysis

We next tested whether specific variant types showed an enrichment in ASM-specific sets based on ASM target and ASM ADME genes (Table 2).

For the VPA cohort, we found a marginally significant enrichment of all types of rare variants, including PTVs, rare missense (MAF <0.01), and ultra-rare deleterious variants in ADME genes in individuals with resistance to VPA. We found no association for variants in the VPA target gene set.

For the LEV cohort, we observed a significant enrichment of PTVs in conjunction with splice-region variants in the *SV2* gene group in association with drug resistance. However, we did not observe this effect for PTVs alone, as only one variant in the nonresponder group remained

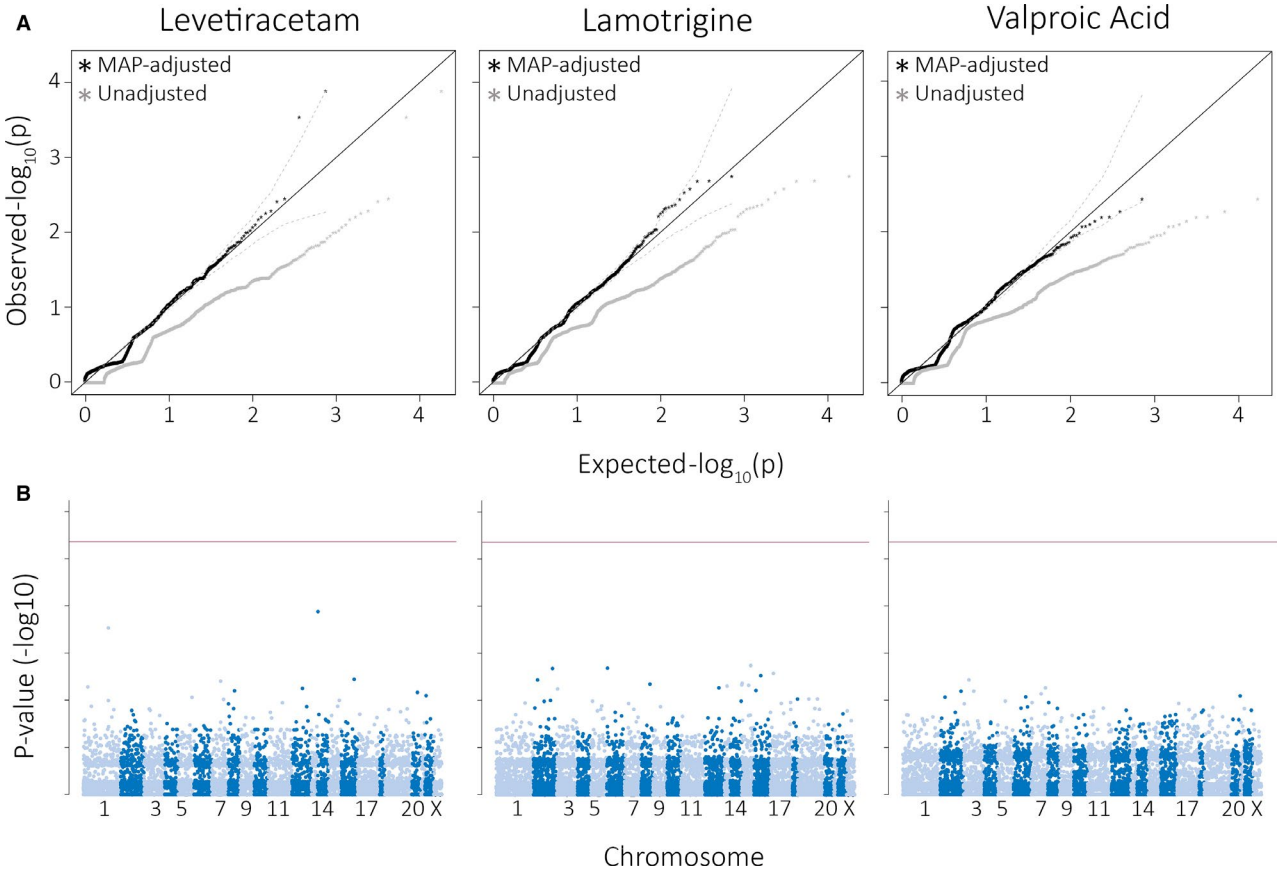

FIGURE 2 Gene-based enrichment analysis for ultra-rare deleterious missense variants (URV2). Quantile-quantile (QQ) plots of SKAT-O analyses of ultra-rare deleterious missense variants, that is, all variants with a frequency ≤3 in gnomAD, for response to three antiseizure medications: levetiracetam, lamotrigine, and valproic acid. MAP-adjusted depicts the QQ plot adjusted for minimum achievable *P*-values. B, Corresponding Manhattan plots. Red line shows the threshold for significance

TABLE 2 Results of gene set analyses

Gene sets (n of genes)	PTV	PTV/splice region	PTV/rare missense	PTV/rare missense/ splice region	Ultra-rare deleterious missense
Levetiracetam					
SV2 group (2)	1 (1)	**0.04 (9.5E-03)**	0.82 (6.5E-01)	0.05 (5.1E-02)	0.34 (2.0E-01)
Lamotrigine					
ADME genes (6)	0.79 (2.5E-01)	0.79 (4.0E-01)	0.79 (4,9E-01)	0.79 (4.2E-01)	0.79 (4.1E-01)
Target genes (17)	0.79 (7.5E-01)	0.79 (5.9E-01)	0.79 (7.1E-01)	0.79 (7.8E-01)	0.47 (4.6E-02)
Valproic Acid					
ADME genes (12)	**0.03 (1.3E-02)**	0.07 (3.8E-02)	**0.03 (1.4E-02)**	**0.02 (3.5E-03)**	**0.02 (3.9E-03)**
Target genes (4)	0.70 (6.2E-01)	0.93 (9.3E-01)	0.65 (4.3E-01)	0.70 (6.1E-01)	-

Note: Results of gene set–based SKAT-O analysis based on one gene set for LEV and two gene sets LTG and VPA for five variant annotation groups. The table shows the FDR-adjusted *P*-values and the raw P-values in parentheses. After correction for multiple testing the SV2 group showed a marginally significant association with LEV resistance for PTV and splice region variants; VPA-specific ADME genes showed a marginally significant association for all variant groups but splice region variants. We found no variant fulfilling the ultra-rare deleterious missense criteria in the VPA target gene set.

Significant findings are depicted in bold.

after filtering. For LTG, we did not find any significant association with the respective gene sets containing target or ADME genes.

4 | DISCUSSION

In this exome-based pharmacogenomic study, we analyzed the influence of common and rare genetic variants on pharmacoresponse for three commonly used ASMs. Although we did not identify an enrichment of variants in single genes, we found some evidence for enrichment of variants in our gene set–based approach. We selected our gene sets based on different hypotheses for the emergence of drug resistance—the involvement of drug transporters and other ADME genes,[31,32] and of ASM target genes.[31] For VPA, we also included a set of *HDAC* genes. This set reflects the methylation hypothesis of drug resistance.[33] Variants in HDACs could possibly alter the interaction with VPA and thus confer resistance to VPA via epigenetic mechanisms.[33]

We detected a marginally significant enrichment of PTVs, rare missense variants and splice region variants in ADME genes in individuals resistant to VPA. We also found some evidence for an enrichment of PTV in conjunction with splice region variants in the *SV2* gene group in individuals resistant to LEV.

ADME genes represent a plausible mediator for ASM response.[31,32] Our VPA-specific ADME gene set comprised genes of the cytochrome P450 (CYP) group,[34,35] several UDP-glucuronosyltransferase (UGT) genes,[36,37] and transcriptional regulator genes of the former group.[38] The association of the ADME gene set with pharmacoresponse was driven mainly by the genes *UGT1A3* and *UGT1A4* (Table S3). Both are known to catalyze the glucuronidation of VPA in vitro.[36] Furthermore, common variants in these genes are correlated with the trough plasma concentration and the concentration to dose ratio of VPA.[39] *UGT1A4* has also been shown to be overexpressed in brain tissue of individuals with drug-resistant epilepsy.[40] To date, however, no studies link these genes directly to VPA resistance.

For LEV, we found an enrichment of PTVs in conjunction with splice region variants in the *SV2* family genes. The *SV2* family comprises the three paralogous proteins *SV2A*, *SV2B*, and *SV2C*, which are broadly expressed presynaptic proteins[41] that are involved in synaptic transmission via calcium-regulated exocytosis.[42] *SV2A* has been identified as an interacting protein and the potential main binding site of LEV in the brain.[43] Although LEV does not seem to bind to *SV2B* directly,[43] the latter seems to retain an important role for LEV function, nonetheless.[44] LEV appears to mediate *SV2A*-associated decrease of neurotransmitter release only in synapses that do not express *SV2B*.[44] The role of *SV2C* remains obscure given its expression pattern that differs from that of *SV2A* and *SV2B*,[45] and there is no evidence for involvement in LEV pharmacodynamics. Therefore, only *SV2A* and *SV2B* were included in this gene set.

Unlike the ADME set, the *SV2* set is based on the drug target hypothesis of pharmacoresistance,[31] which postulates that variation in ASM target proteins contributes to drug resistance. Previous candidate gene-based studies did not identify an association of common genetic variants in the *SV2* family with epilepsy[46] or LEV response,[47] but they did not cover rare truncating or splice region variants. Dibbens et al[48] reported no effect of genetic variants on LEV response in 158 individuals with epilepsy who underwent sequencing of *SV2A*, but the study did not cover the entire *SV2B* and included fewer individuals. The inclusion criteria for drug response were also less strict than in our study, admitting individuals as responders that had >75%

seizure reduction, whereas people with <75% were defined as nonresponders. A third group with an increase in seizure rate of >50% was defined as exacerbators but was a small group (n = 16). The less strict separation between responders and nonresponders may have obliterated any genetic differences. We applied stricter response definitions, acknowledging the trade-off of a smaller sample size.

The association between *SV2* variants and LEV resistance was only observable for PTVs in conjunction with splice region variants, indicating that the observed effect was mainly driven by splice region variants. The impact of splice region variants on gene expression is poorly understood. Therefore, our results for this gene group should be considered with caution and warrant evaluation in future studies.

Our study was limited by the lack of a replication cohort and a still relatively small sample size in the analyzed subgroups. Despite this, our results generate hypotheses for future studies that are required to confirm our findings. Obstacles for future larger studies are no longer the costs of sequencing but rather the costs and availability of manpower needed to collect and deeply phenotype a sufficiently large cohort of individuals.

In conclusion, our study sheds some light on the question of a genetic contribution to drug resistance in epilepsy treatment. In the light of our and previous studies, it can be concluded that single variants/genes of a large effect size are unlikely to drive drug resistance to LEV, LTG, or VPA. It seems more likely that the genetic basis of drug resistance is heterogeneous and, as our study implies, influenced by rare variants affecting pharmacokinetics and pharmacodynamics. Because many individuals with epilepsy do not respond to any ASM, regardless of its mechanism of action, it seems obvious that other factors are involved. Thus, pharmacoresistance may also be due to altered gene expression of target or ADME genes via epigenetic mechanisms such as DNA methylation,[33] seizure-induced alterations of neural networks,[49] or intrinsic factors mediating disease severity.[50]

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The computational analysis was performed on the high-performance computer system of the University of Luxembourg (https://hpc.uni.lu). CENet sequences were stored and processed on Compute Canada cluster Beluga.

CONFLICTS OF INTEREST

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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