Genome-wide mega-analysis identifies 16 loci and highlights diverse biological mechanisms in the common epilepsies

Abou-Khalil, Bassel

2018-12-10

Int League Against Epilepsy Conso 2018, 'Genome-wide mega-analysis identifies 16 loci and highlights diverse biological mechanisms in the common epilepsies' Nature Communications, vol. 9, 5269. https://doi.org/10.1038/s41467-018-07524-z

http://hdl.handle.net/10138/287694
https://doi.org/10.1038/s41467-018-07524-z

Downloaded from Helda, University of Helsinki institutional repository.

This is an electronic reprint of the original article.

This reprint may differ from the original in pagination and typographic detail.

Please cite the original version.
Genome-wide mega-analysis identifies 16 loci and highlights diverse biological mechanisms in the common epilepsies

The International League Against Epilepsy Consortium on Complex Epilepsies

The epilepsies affect around 65 million people worldwide and have a substantial missing heritability component. We report a genome-wide mega-analysis involving 15,212 individuals with epilepsy and 29,677 controls, which reveals 16 genome-wide significant loci, of which 11 are novel. Using various prioritization criteria, we pinpoint the 21 most likely epilepsy genes at these loci, with the majority in genetic generalized epilepsies. These genes have diverse biological functions, including coding for ion-channel subunits, transcription factors and a vitamin-B6 metabolism enzyme. Converging evidence shows that the common variants associated with epilepsy play a role in epigenetic regulation of gene expression in the brain. The results show an enrichment for monogenic epilepsy genes as well as known targets of antiepileptic drugs. Using SNP-based heritability analyses we disentangle both the unique and overlapping genetic basis to seven different epilepsy subtypes. Together, these findings provide leads for epilepsy therapies based on underlying pathophysiology.
he epilepsies are a group of brain disorders characterized by recurrent unprovoked seizures affecting up to 65 million people worldwide\(^1\). There are many different types of epilepsy, and its classification has recently evolved, driven by advances in clinical phenotyping, imaging, and genetics\(^2\). Since the identification of CHRNA4 as a cause of autosomal dominant nocturnal frontal lobe epilepsy\(^3\), genes underlying many different rare monogenic forms of epilepsy have been characterized, and discovery in this area has accelerated with the application of next generation sequencing\(^4\). This is particularly true of the relatively rare but devastating infantile group of epileptic encephalopathies, which are now emerging as a genetically heterogeneous group of largely de novo dominant disorders\(^5\). In contrast, single gene causes of the more common forms of epilepsy appear to be relatively rare. The common forms broadly comprise generalized and focal epilepsies, with the former having the highest heritability, yet the lesser yield in single gene discovery\(^6\). These common forms are likely multifactorial, with a significant and complex genetic architecture\(^7\)–\(^9\).

Consistent with the experience from many other disease fields, early attempts to disentangle the genetic architecture of the more common, sporadic forms of epilepsy were limited by study power and scope\(^10\)–\(^14\). In 2011, the International League Against Epilepsy (ILAE) launched the Consortium on Complex Epilepsies, to facilitate meta-analysis in epilepsy genomics. In 2014, the first such meta-analysis was reported comprising 8696 cases and 26,157 controls. This led to the identification of \(2q24.3, 4p15.1\), and \(2p16.1\) as epilepsy loci\(^15\).

Here we present an expanded analysis involving 15,212 cases and 29,677 controls, which leads to identification of 16 genome-wide significant loci. Importantly, 11 of these loci are associated with the genetic generalized epilepsies; the group of epilepsies where despite having the highest heritability we have made the least genetic progress to date. We show that the genes associated with each locus are biologically plausible candidates, despite having diverse functions, particularly as there is a significant enrichment for known monogenic epilepsy genes and anti-epileptic drug targets.

## Results

### Study overview

We performed a genome-wide mega-analysis on the ILAE Consortium cohort now comprising 15,212 epilepsy cases, stratified into 3 broad and 7 subtypes of epilepsy, and 29,677 control subjects (Supplementary Table 1). The current study includes a further 6516 cases and 3460 controls in addition to the 8696 cases and 26,157 controls from our previously published analysis\(^15\). Thus, this mega-analysis is not a formal replication of our previously published meta-analysis. We do not attempt any formal replication of novel association signals detected in this analysis. Furthermore, 531 cases of Asian descent, and 147 cases of African descent were included through a meta-analysis. However, we refer to our GWAS as a mega-analysis as the vast majority of our samples (96%) were analyzed under that framework.

At the broadest level, cases were classified as (a) focal epilepsy where seizures arise in a restricted part of the brain, a form traditionally not regarded as genetic although a number of genes for monogenic forms have been identified; (b) genetic generalized epilepsy where seizures arise in bilateral networks and evidence for a genetic component is very strong, yet genes have been hard to identify, and (c) unclassified epilepsy\(^2\)–\(^6\).

Subjects were assigned to three broad ancestry groups (Caucasian, Asian and African-American) according to results of genotype-based principal component analysis (Supplementary Fig. 1). Linear-mixed model analyses were performed stratified by ethnicity and epilepsy subtype or syndrome, after which trans-ethnic meta-analyses were undertaken.

### Genome-wide associations

Our analysis of all epilepsy cases combined revealed one novel genome-wide significant locus at 16q12.1 and reinforced two previous associations at \(2p16.1\) and \(2q24.3\) (Fig. 1 and Supplementary Fig. 2)\(^15\). When conditioning on the top SNP within the \(2q24.3\) locus, we demonstrate the existence of a second, independent signal within that locus (Supplementary Fig. 3). This locus was also significantly associated with focal epilepsy. Our analysis of genetic generalized epilepsy uncovered 11 genome-wide significant loci, of which seven are novel (Fig. 2).

Considering that focal and generalized epilepsy are clinically broad and heterogeneous classifications, we next assessed whether loci are specifically associated with any of the seven most common focal epilepsy phenotypes and genetic generalized epilepsy syndromes (Supplementary Fig. 4 and 5). We found a novel genome-wide significant association with juvenile myoclonic epilepsy (JME) and two novel loci associated with focal epilepsy with hippocampal sclerosis. Moreover, we found two genome-wide significant associations with childhood absence epilepsy (CAE) in loci that were previously associated with absence epilepsy and generalized epilepsy\(^12\). We did not find any significant loci associated with generalized epilepsy with tonic-clonic seizures (GTCS) alone, juvenile absence epilepsy (JAE), lesional or lesional focal epilepsy (other than hippocampal sclerosis). Further analysis of the association signals for each locus in the different syndromes suggested that some signals display specificity for a single subtype, while others show evidence for pleiotropy (Supplementary Fig. 6). However, the relatively small sample sizes of these phenotype subsets warrant caution for over-interpretation.

In total, we found 11 novel genome-wide significant loci associated with the epilepsies and we replicated the association of five previous known loci\(^12\)–\(^15\) (Supplementary Fig. 7). Two previous reports of association did not reach our threshold for significance. This included a locus \((rs2292096; 1q32.1)\) for focal epilepsy detected in an Asian population\(^14\) \((p = 0.057 in our trans-ethnic fixed-effects meta-analysis)\), and \(rs12059546 (1q33)\) detected previously for JME\(^12\) \((p = 7.4 \times 10^{-5} in our Caucasian-only BOLT-LMM analysis)\).

### Gene mapping and biological prioritization

The genome-wide significant loci from all analyses were mapped to a total of 146 genes (Supplementary Data 1) using a combination of positional mapping (±250 kb from locus) and significant distal 3D chromatin interactions of the locus with a gene promoter (FDR < 0.05). Considering that most loci encompass several genes, we devised criteria to systematically prioritize the most likely candidate genes per locus based on established bioinformatics methods and resources. This biological prioritization was based on six criteria (Fig. 2), similar to previous studies\(^17\)–\(^18\). Each gene was given a score based on the number of criteria that were met (range 0–6). The gene(s) with the highest score in each locus, with a minimum of 2, were defined as biological epilepsy risk genes. We validated this approach using established epilepsy genes within our data (Supplementary Table 2). Using this approach, we were able to refine these loci to the 21 most likely biological epilepsy genes (Fig. 2).

These prioritized genes include seven ion-channel genes (SCN1A, SCN2A, SCN3A, GABRA2, KCNN2, KCNAB1, and GRK1), three transcription factors (ZEB2, STAT4 and BCL11A), the histone modification gene BRD7, the synaptic transmission gene STX1B and the pyridoxine metabolism gene PNPO. Notably, a conditional
transcriptome-wide association study (TWAS) analysis suggests that the signal for genetic generalized epilepsy at 17q21.32, which was also observed in an earlier study, seems driven by regulation of expression of *PNPO* (Supplementary Fig. 8). This suggests that the biology behind pyridoxine (vitamin-B6)-responsive epilepsy could be relevant to common genetic generalized epilepsies. Biological prioritization implicates *SCN1A*, *SCN2A*, *SCN3A*, and *TTC21B* as the most likely genes underlying the signal at 2q24.3 for.

**Fig. 1** Manhattan plots for epilepsy genome-wide association analyses. Genome-wide association analyses of (a) all epilepsy, (b) focal epilepsy, and (c) genetic generalized epilepsy. Negative log₁₀-transformed P-values (Y-axis) are plotted against chromosomal position (X-axis). P-values were calculated with METAL using fixed-effects trans-ethnic meta-analyses. The red line represents the genome-wide significance threshold (p < 5 × 10⁻⁸). Previously known loci are indicated in black; novel loci in red. The names above each locus represent the prioritized gene in the locus (see Fig. 2) or the name of the locus itself in case of multiple prioritized genes in the locus.
all epilepsy, focal epilepsy and genetic generalized epilepsy. Pathogenic variants in the sodium channels SCN1A, SCN2A and SCN3A are associated with various epilepsy syndromes and mutations in TTC21B impair forebrain development. Our analyses implicate STX1B as a potential gene underlying the association of JME at the 16p11.2 locus and the top variant in the locus is an eQTL that strongly correlates with expression of STX1B in the dorsolateral prefrontal cortex (Spearman’s correlation: Rs = 0.33, p = 3 × 10^{-14}). Interestingly, for one of the prioritized genes in genetic generalized epilepsy, PCDH7, an eQTL was recently detected in epileptic hippocampal tissue. Prioritized genes associated with focal epilepsy with hippocampal sclerosis include the gap-junction gene GJA1.

In addition we identified eight genes from Fig. 2 (BCL11A, GJA1, ATXN1, GABRA2, KCNAB1, SCN3A, PCDH7, STAT4) with evidence of co-expression in at least two independent brain expression resources, using a brain gene co-expression analysis with brain-coX25. These eight candidates are embedded in several established epilepsy gene co-expression modules (Supplementary Fig. 9; Supplementary Table 9).

**SNP annotation and tissue-specific partitioned heritability.** We functionally annotated all 492 genome-wide significant SNPs from all phenotypes (Fig. 3a–c) and found that most SNPs were either intergenic (29%) or intronic (46%); 78% were in open chromatin regions (as indicated by a minimum chromatin state of −1−−7−8,9), and 50% of SNPs showed some evidence for affecting gene transcription (RegulomeDB score ≤6). Four were coding SNPs of which two were nonsense variants.

To gain further biological insight into our results, we next used a partitioned heritability method to assess whether our genome-wide significant signals contained SNPs associated with enhanced transcription in any of the 88 tissues. We found significant enrichment of H3K4me1 markers in all epilepsy (stratified LD-score regression; p = 4 × 10^{-5}) and H3K27ac markers in genetic generalized epilepsy (stratified LD-score regression; p = 1.3 × 10^{-4}), specifically in the dorsolateral prefrontal cortex. Moreover, the distribution of heritability enrichment P-values was strongly skewed towards brain tissues for all epilepsy phenotypes (Fig. 3d, Supplementary Figs. 10–12).

---

**Table 1: Genome-wide significant loci of all analyses and prioritized biological epilepsy genes.** Genes were prioritized based on 6 criteria and scored based on the number of criteria met per gene (filled red boxes). The highest scoring gene, or multiple if they have the same score, in each locus is reported as ‘prioritized biological epilepsy gene(s)’. Similar to previous studies, we used a minimum score of 2 to define these genes and we noted ‘none’ if no gene in the locus reached this score. Filled blue boxes indicate overlap with known targets of anti-epileptic drugs and established monogenic epilepsy genes. The direction of the association of JME at the 16p11.2 locus and the top variant in the locus reached this score. Filled blue boxes indicate overlap with known targets of anti-epileptic drugs and established monogenic epilepsy genes. The direction of the association of JME at the 16p11.2 locus and the top variant in the locus reached this score.
H3K27ac and H3K4me1 are epigenetic markers associated with regulating gene transcription, suggesting that altered transcription in the dorsolateral prefrontal cortex could be one of the underlying mechanisms of epilepsy. This is further supported by a tissue-specific heritability enrichment analysis (using data from the GTEx Consortium), showing strongest enrichment for genetic generalized epilepsy with genes expressed in Brodmann Area 9 (stratified LD-score regression; \( p = 1.56 \times 10^{-6} \)), which encompasses the dorsolateral prefrontal cortex (Fig. 3e). These findings further corroborate our TWAS results (using data from the unrelated CommonMind Consortium database), which shows significant associations of epilepsy with gene expression of several

**Fig. 3** Functional annotation and heritability enrichment of epilepsy GWAS results. a Functional categories of all genome-wide significant SNPs in all phenotypes. b Minimum (most active) chromatin state across 127 tissues for all genome-wide significant SNP in all phenotypes; TSS - transcription start site. c The RegulomeDB score for all genome-wide significant SNPs in all phenotypes, where 7 represents no evidence for affecting regulation and lower scores represent increasing evidence; NA - the variant does not exist in RegulomeDB. d Heritability enrichment for genetic generalized epilepsy with 6 different chromatin markers in 88 tissues, calculated with stratified LD-score regression using data from the Roadmap Epigenomics Project. The main bar chart represent the 10 tissues with the strongest heritability enrichment and the inset shows the full distribution of all chromatin markers in all tissues. e Heritability enrichment of genes expressed in 53 tissues, calculated with stratified LD-score regression using data from the gene-tissue expression (GTEx) Consortium.
genes in the dorsolateral prefrontal cortex (Fig. 2; Supplementary Table 3). Although genetic generalized epilepsy has been regarded as a generalized process, anatomical, electrophysiological, cognitive, and functional imaging studies implicate dysfunction in the frontal lobes.\textsuperscript{20–34} Altogether, we have converging evidence from several unrelated methods and databases suggesting epigenetic regulation of gene expression in the dorsolateral prefrontal cortex as a potential pathophysiological mechanism underlying our epilepsy GWAS findings.

Finally, we leveraged the Brainspan database, as implemented in FUMA\textsuperscript{35}, to assess whether the genes implicated by our GWAS are differentially expressed in the brain at various prenatal and post-natal ages. These analyses were performed for the genes prioritized in any epilepsy phenotype (21 genes), any focal epilepsy subtype (8 genes) or any genetic generalized epilepsy syndrome (15 genes). The results suggest that the expression of genes associated with focal epilepsy is up-regulated in late-infancy and young adulthood, whereas expression of those genes associated with genetic generalized epilepsy is down-regulated in early childhood and differentially expressed prenatally and at adolescence (Supplementary Fig. 13).

Enrichment analyses. A previous exome-sequencing study found an association for common epilepsies with ultra-rare variants in known monogenic epilepsy genes.\textsuperscript{36} To assess whether common epilepsies are also associated with common variants in monogenic epilepsy genes (see Methods), we pooled the 146 genes that were mapped to our genome-wide significant loci and performed a hypergeometric test. Results illustrated an enrichment of known monogenic epilepsy genes within the genes mapped to our genome-wide significant loci (6 genes overlapped; hypergeometric test: odds ratio \( \text{OR} = 8.45, p = 1.3 \times 10^{-5} \)). This enrichment is considerably more significant when limited to the 21 genes with the highest biological priority from Fig 2 (5 genes overlapped; hypergeometric test: \( \text{OR} = 61.4, p = 9.9 \times 10^{-10} \)). We did not find a bias for gene size in our enrichment analyses when using a conservative method to correct for this (see Methods). This suggests that both common and rare variants in monogenic epilepsy genes contribute to common epilepsy susceptibility, corroborating and further extending previous observations.\textsuperscript{8,37} Further studies are required to establish whether the signals from common and rare variants are independent of each other.

Using public databases of drug-targets, we found that 13 out of 24 currently licensed anti-epileptic drugs target genes that are implicated in our GWAS. Using the same list of 146 genes as described above, we performed a hypergeometric test which shows a significant enrichment of genes that are known targets of anti-epileptic drugs (8 genes overlapped; hypergeometric test: \( \text{OR} = 19.6, p = 1.3 \times 10^{-5} \)). This enrichment is considerably more significant when limited to the 21 most biologically plausible candidate genes (5 genes overlapped; hypergeometric test: \( \text{OR} = 101.2, p = 5.7 \times 10^{-11} \)). This observation suggests that other drugs that target genes from our GWAS could also have potential for the treatment of epilepsy. The Drug-Gene interaction database (http://dgidb.org) lists 166 drugs that target biologically prioritized genes from our GWAS (see Supplementary Data 2 for a full list), that may be further investigated for their anti-seizure potential.

Next, we used a complementary approach\textsuperscript{38} to search for repurposable drugs. By comparing GWAS-imputed and drug-induced transcriptomes, we predicted drugs capable of rectifying epilepsy-associated gene expression changes (see Methods). Our predictions are enriched with licensed antiepileptic compounds (permutation based \( p \)-value \( <1.0 \times 10^{-8} \)) and with other licensed compounds that have proven antiepileptic efficacy in animal models (permutation based \( p \)-value \( <1.0 \times 10^{-8} \)). We list 30 of our predicted drugs that are licensed for other conditions and have published evidence of efficacy in animal models of epilepsy (Supplementary Table 4).

Heritability analyses. Twin-based and genetic heritability studies have suggested that while epilepsy is strongly heritable,\textsuperscript{8,39} there is a substantial missing heritability component.\textsuperscript{40,41} We used LD4K to estimate \( h^2_{SNP} \), the proportion of heritability that can be attributed to SNPs.\textsuperscript{42,44} We estimate \( h^2_{SNP} = 32.1\% \) (95\%CI: 29.6–34.5\%) for genetic generalized epilepsy and \( h^2_{SNP} = 9.2\% \) (8.4–10.1\%) for focal epilepsy (estimates are on the liability scale, assuming a prevalence of 0.002 and 0.003, respectively) which are consistent with previous estimates.\textsuperscript{8} These results indicate that SNPs explain a sizeable proportion of the liability of genetic generalized epilepsy syndromes, but less so for focal epilepsy phenotypes (Fig. 4). To delineate the heritability of the different epilepsy phenotypes, we used LD4K to perform genetic correlation analyses between the different forms. We found evidence for strong genetic correlations between the genetic generalized epilepsies, whereas we found no significant correlations between the focal epilepsies (Fig. 4). Interestingly, we found a significant genetic correlation between JME and lesion-negative focal epilepsy (LD4K genetic correlation: \( R^2 = 0.46, p = 8.77 \times 10^{-4} \)), suggesting either pleiotropy and/or misclassification. It is known that focal EEG features can be seen in JME.\textsuperscript{45}

In view of the increasing data on comorbidities with epilepsy, we next used LD-score regression to analyze the genetic correlation between epilepsy and various other brain diseases and traits from previously published GWAS (Fig. 5; see Supplementary Table 5 for values). Perhaps surprisingly, we did not find significant correlations with febrile seizures. Similarly, we did not find any significant genetic correlations between epilepsy and other neurological or psychiatric diseases. However, we did observe significant correlations for all epilepsy and genetic generalized epilepsy with cognitive ability. We then used the method Multi-Trait Analysis of GWAS (MTAG)\textsuperscript{46} to leverage the larger sample size of the genetically correlated GWAS of cognitive ability (\( n = 78,308 \)) in order to boost the effective sample size of our all and genetic generalized epilepsy GWAS to 53,244 and 41,515 respectively. Using this approach, we found a novel genome-wide significant locus at 10q24.32 in all epilepsy (MTAG \( p = 2.2 \times 10^{-9} \)) and genetic generalized epilepsy (MTAG \( p = 4.0 \times 10^{-6} \)) which encompasses the \( K_{\text{v}} \)-channel-interacting protein 2 (\( KCNIP2 \)) gene (Supplementary Fig. 14), loss of which is associated with seizure susceptibility in mice.\textsuperscript{47}

Discussion

The increased sample size in this second ILAE Consortium GWAS of common epilepsies has resulted in the detection of 16 risk loci for epilepsy and illustrates how common variants play an important role in the susceptibility of these conditions. But compared to other common neurological diseases our sample size is modest. For example the latest GWAS in schizophrenia considered 36,989 schizophrenia cases and 113,075 controls, resulting in the identification of 108 risk loci.\textsuperscript{48} Larger efforts would deliver further insight to the genetic architecture of the common epilepsies.

The majority of the loci are associated with genetic generalized epilepsy. This observation is a welcome partial explanation for the high heritability of genetic generalized epilepsy, in light of the relative lack of rare variant variants discovered to date. We also show that there is substantial genetic correlation between the generalized syndromes. We speculate that the subtypes share a large part of the genetic susceptibility for generalized epilepsies, with specific modifying factors determining the specific syndrome.
**Table 1**

<table>
<thead>
<tr>
<th>Epilepsy Syndrome</th>
<th>CAE</th>
<th>JAE</th>
<th>JME</th>
<th>GTCS alone</th>
<th>Focal HS</th>
<th>Focal lesion negative</th>
<th>Focal other lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CAE</strong></td>
<td><img src="h2SNP58.438.578.4" alt="h^2_{SNP} = 58.4% (38.5-78.4%)" /></td>
<td>*</td>
<td>*</td>
<td>#</td>
<td>#</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>JAE</strong></td>
<td><img src="h2SNP73.636.6111" alt="h^2_{SNP} = 73.6% (36.6-111%†)" /></td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>JME</strong></td>
<td><img src="h2SNP46.232.659.6" alt="h^2_{SNP} = 46.2% (32.6-59.6%)" /></td>
<td></td>
<td></td>
<td>#</td>
<td>#</td>
<td><img src="h2SNP10760.7153" alt="h^2_{SNP} = 107%† (60.7-153%)" /></td>
<td></td>
</tr>
<tr>
<td><strong>GTCS alone</strong></td>
<td><img src="h2SNP180036.3" alt="h^2_{SNP} = 18.0% (0-36.3%)" /></td>
<td></td>
<td></td>
<td>#</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Focal HS</strong></td>
<td><img src="h2SNP7.21.712.8" alt="h^2_{SNP} = 7.2% (1.7-12.8%)" /></td>
<td></td>
<td></td>
<td>#</td>
<td></td>
<td><img src="h2SNP4.109.1" alt="h^2_{SNP} = 4.1% (0-9.1%)" /></td>
<td></td>
</tr>
<tr>
<td><strong>Focal lesion negative</strong></td>
<td><img src="h2SNP7.21.712.8" alt="h^2_{SNP} = 7.2% (1.7-12.8%)" /></td>
<td></td>
<td></td>
<td>#</td>
<td></td>
<td><img src="h2SNP4.109.1" alt="h^2_{SNP} = 4.1% (0-9.1%)" /></td>
<td></td>
</tr>
<tr>
<td><strong>Focal other lesion</strong></td>
<td><img src="h2SNP4.109.1" alt="h^2_{SNP} = 4.1% (0-9.1%)" /></td>
<td></td>
<td></td>
<td>#</td>
<td></td>
<td><img src="h2SNP4.109.1" alt="h^2_{SNP} = 4.1% (0-9.1%)" /></td>
<td></td>
</tr>
</tbody>
</table>

*: P < 0.0024 (Bonferroni threshold)
#: P < 0.05
†: h^2_{SNP} SNP-based heritability on liability scale (95% CI)

**Fig. 4** Heritability estimates and genetic correlations between epilepsy syndromes, calculated using LDAK. Subjects with a diagnosis of both CAE and JAE were excluded from both phenotypes. The genetic correlation coefficient was calculated with LDAK and is denoted with a color scale ranging from 0% (white) to 100% (red). #: P < 0.05; *: P < 0.0024 (Bonferroni threshold); h^2_{SNP} SNP-based heritability on liability scale (95% CI); †: Heritability estimate exceeded 100%, possibly due to small sample size and large SD; CAE - childhood absence epilepsy, JAE - juvenile absence epilepsy, JME - juvenile myoclonic epilepsy, GTCS alone - generalized tonic-clonic seizures alone, focal HS - focal epilepsy with hippocampal sclerosis.

**Fig. 5** Genetic correlations of epilepsy with other phenotypes. The genetic correlation coefficient, calculated using LD-score regression, is denoted with a color scale ranging from -100% (blue) to 100% (red). #: P < 0.05 *: P < 0.001 (Bonferroni threshold; 0.05/48).
Some syndrome-specific associations were detected, such as the relatively strong signal for STX1B in JME, and the association of GJA1 with focal epilepsy-hippocampal sclerosis. Interestingly, although the association signal for STX1B was only significant in the JME analysis, rare pathogenic variants in STX1B have been recently found in a spectrum of epilepsies, including genetic epilepsy with febrile seizures plus (GEFS+), genetic generalized epilepsies (including JME), epileptic encephalopathies and even some focal epilepsies19,20 (Wolking et al., Manuscript submitted (2018)). Further, mutations in the gap-junction gene GJA1 are associated with impaired development of the hippocampus21 and different expression has been reported in epileptic hippocampal and cortical tissue22,25. These findings represent a tantalizing glimpse of the different biological mechanisms underlying epilepsy syndromes that may guide us to the introduction of genetics for improved diagnosis, prognosis and treatment for these common epilepsies. However, the relatively low sample size of our subtype analyses warrants a conservative interpretation and follow-up with a larger cohort.

At least three association signals are shared between focal epilepsy and genetic generalized epilepsy. The clearest overlapping signal remains the 2q24.3 locus, as we reported previously25. However, this association signal is complex and we demonstrate that the locus consists of at least two independent signals (Supplementary Fig. 3). Our Hi-C chromatin analysis suggests the complexity includes levels of functional association to SCN2A and SCN3A, that are located more distally to the SCN1A locus. Mutations in SCN2A and more recently SCN3A are established monogenic causes of epileptic encephalopathy that, like SCN1A, cause dysfunction of the encoded ion-channels, which is believed to disturb the fine balance between neuronal excitation and inhibition. This may involve independent variation that either affects regulation of SCN1A, SCN2A, or SCN3A independently. However, the complex association may also reflect multiple rare risk variations, and large resequencing studies will shed further light on this issue.

The number of association signals we detected and increased power relative to our previous meta-analysis15 allowed us to explore the biological mechanisms behind the observed genetic associations. We show that the signals converge on the dorso-lateral prefrontal cortex as the tissue in which most functional effect is observed; this is broadly consistent with the importance of the frontal lobes in generalized epilepsies. Indeed, our analyses of the epigenetic markers H3K27ac and H3K4me1, TWAS, and tissue-specific heritability enrichment analysis all point towards epigenetic regulation of gene expression in the dorsolateral prefrontal cortex as a potential pathophysiological mechanism underlying our epilepsy GWAS findings.

Altogether, we found 16 loci that are associated with the common epilepsies. Our heritability analyses show that collectively, common genetic variants explain a third of the liability for genetic generalized epilepsy. Our analyses suggest that the associated variants are involved in regulation of gene expression in the brain. The 21 biological epilepsy candidate genes implicated by our study have diverse biological functions, and we show that these are enriched for known epilepsy genes and targets of current antiepileptic drugs. Our analyses provide evidence for pleiotropic genetic effects that raise risk for the common epilepsies collectively, as well as variants that raise risk for specific epilepsy syndromes. Determining the shared and unique genetic basis of epilepsy syndromes should be of benefit for further stratification and eventually lead to possible applications for improved diagnosis, prognosis, and treatment. Future studies including pharmacoresponse data, imaging, and other clinical measurements have the potential to further increase the benefit of these studies for people with epilepsy. In combination, these findings further our understanding of the complex genetic architecture of the epilepsies and could provide leads for new treatments and drug repurposing.

Methods

Ethics statement. We have complied with all relevant ethical regulations. All study participants provided written, informed consent for use of their data in genetic studies of epilepsy. For minors, written informed consent was obtained from their parents or legal guardian. Local institutional review boards approved study protocols at each contributing site.

Cohorts and phenotype definition. A list of the sites included in this study is described in Supplementary Table 6. We classified seizures and epilepsy syndromes according to the classification and terminology outlined by the ILAE21,26. For all diagnoses, epilepsy specialists assessed each phenotype at the contributing site. Individuals with epilepsy were initially assigned to one of three phenotypic categories: genetic generalized epilepsy, focal epilepsy, or unclassified epilepsy. Based on EEG, MRI and clinical histories we further classified our cohort into the epilepsy subtypes listed in Supplementary Table 1. We use a combination of population-based datasets as controls. Some control cohorts were screened by questionnaire for neurodevelopmental disorders. 53.4% of cases were female compared to 51.6% of controls.

Study design. We conducted a case-control study in subjects of Caucasian, Asian (Han Chinese) and African-American ethnicities. Our primary analyses were structured to test common genetic variants for association with epilepsy according to broad epilepsy phenotypes. We pooled cases from cohorts of the same ethnic group to perform linear mixed model analysis, followed by subsequent meta-analyses of regression coefficients across the three ethnic groups. Our secondary analyses tested for associations with specific syndromes of genetic generalized epilepsy (childhood absence epilepsy, juvenile absence epilepsy, juvenile myoclonic epilepsy, and generalized tonic-clonic seizures alone) and phenotypes of focal epilepsy (focal seizure positive, focal epilepsy with hippocampal sclerosis, and focal epilepsy with other lesions). The secondary analyses were limited to Caucasian subjects due to sample size. We prioritized the results of the GWAS by incorporating eQTL information, transcriptome-wide analysis, and biological annotation. Finally, we estimated the genetic correlation of epilepsy phenotypes using Linkage-Disequilibrium Adjusted Kinship (LDK).

Genotyping. The EpiPGX samples were genotyped at deCODE Genetics on Illumina OmniExpress-12 v1.1 and OmniExpress-24 v1.1 single nucleotide polymorphism (SNP) arrays. The EPGP samples were genotyped on Illumina HumanCore beadchips at Duke University, North Carolina. The remainder of the samples were genotyped on various SNP arrays, as previously published15.

Genotyping quality control and imputation. Quality control of genotyping was performed separately for each cohort using PLINK 1.99,27. Each genotype cohort was temporarily merged with a genetically similar reference population from the 1000 Genomes Project (CEU, CHB, or YRI). A test for Hardy–Weinberg equilibrium (HWE) was performed and SNPs significant at p < 1 × 10−10 were removed. All samples and all SNPs with missing genotype rate >0.05 and all SNPs with minor allele frequency (MAF) <0.01 were removed. Next, we performed PLINK –indep-pairwise command (settings: window size 100 kb, step size 25, R2 > 0.1). Using this subset of SNPs, we removed samples with outlying heterozygosity values (>5 SD from the median of the whole cohort). Identity by descent (IBD) was calculated in PLINK to remove sample duplicates (>0.9 IBD) and to identify cryptic relatedness. We removed one from each pair with IBD>0.15% with the exception of the EPGP familial epilepsy cohort. Subjects were removed if sex determined from X-chromosome genotype did not match reported gender. Array-specific maps were used to update all SNPs positions and chromosome numbers to the Genome Reference Consortium Human Build 37 (GRCh37), and remove all A/G SNPs to avoid strong strand issues. We applied pre-imputation checks according to scripts available on the website of Will Rayner of the Wellcome Trust Centre for Human Genetics (www.well.ox.ac.uk/~wray/genetics/) to remove SNPs with allele frequencies deviating >20% from the frequency in the Haploptype Reference Consortium. Samples were submitted to the Sanger Imputation Service (https://imputation.sanger.ac.uk/)28. We selected the Human Reference Consortium (release 1.1; n = 32470) dataset as reference panel for Caucasian and Asian datasets and the African Genome Resources (n = 4956) for the African-American datasets. Post-imputation quality control filters were applied to remove SNPs within each imputed cohort with an imputation info score <0.9 or HWE p<1e-6. Imputed genotype dosages with a minimum certainty of 0.9 per subject were converted to hard-coded PLINK format after which all samples were pooled into a single cohort. We performed a principal components analysis using GCTA. From the PCA results we stratified our subjects into three broad ethnic groups (Caucasian, Asian and African) while removing extreme outliers. After stratifying by ethnicity, we removed SNPs with HWE p<1e-6, all the <0.05 or MAF<0.01. In total 816 subjects out of 45705 subjects were filtered out by quality control procedures, leaving 44889 subjects for analyses.
Study power. We estimated using PGA\textsuperscript{57} that the study had 80% power to detect a genetic predictor of relative risk for all epilepsy (approximated to odds ratio) $P \leq 1.45$ with MAF = 1% and an alpha level of $5 \times 10^{-8}$. We estimated that our meta-analyses had 80% power to detect genome-wide significant SNPs of MAF = 1% with relative risks $\geq 1.5$ and $\geq 2.1$, for focal and generalized epilepsy respectively (see Supplementary Figure 15). Our analysis of generalized epilepsy sub-phenotypes had 80% power to detect genome-wide significant SNPs of MAF = 1% with relative risks $P \leq 2.6$, $P \leq 3.3$, and $P \leq 2.4$ for CAE, JE, and JME respectively. Our analysis of focal epilepsy sub-phenotypes had 80% power to detect genome-wide significant SNPs of MAF = 1% with relative risks $P \geq 1.9$, $P \geq 2.8$, and $P \geq 2.9$ for focal epilepsy lesion negative, focal epilepsy with hippocampal sclerosis and focal epilepsy with lesion on hippocampal sclerosis, respectively.

Statistical analyses. Association analyses were conducted within the three ethnic subgroups using a linear mixed model in BOLT-LMM\textsuperscript{58}. A subset of SNPs, used to correct for (cryptic) relatedness and population stratification by BOLT-LMM, were derived by applying SNP imputation into score $P \geq 0.001$, MAF $\geq 0.01$, call rate $> 99.9$ before pruning the remaining variants using LDARK with a size of 1 MB and $R^2 \geq 0.245$. All analyses included gender as a covariate and the threshold for statistical significance was set at $5 \times 10^{-8}$. We compared $R^2$ values of the BOLT-LMM output between all pairs of SNPs in high LD ($R^2 \geq 0.4$) and removed pairs of SNPs with extreme $x^2$ differences using a formula that scales exponentially with magnitude of $x$ and $R^2$: $x^2 \text{difference} = \frac{x^2}{\sqrt{\frac{M}{n}} \left(1 - R^2 \right)}$

where $SNP_1 - x^2$ and $SNP_2 - x^2$ are the $x^2$-statistic of the two SNPs in each pair and $R^2$ is their squared correlation (LD). We tested the homogeneity of all SNPs by splitting the pooled cohort into 13 distinct clusters of ethnically matched cases and controls and removed SNPs showing significant heterogeneity of effect ($P_{\text{meta}} < 1 \times 10^{-8}$). Fixed effects, trans-ethnic meta-analyses were conducted using the software package METAL\textsuperscript{59}. Manhattan plots for all analyses were created using qman. Considering that our study had unequal case-control ratios, we calculate the effective sample size per ethnicity using the formula recommended by METAL: $N_{\text{eff}} = 4/(1/N_{\text{cases}} + 1/N_{\text{controls}})$. Since $> 95\%$ of all cases were Caucasian, we included all SNPs that were present in at least the Caucasian dataset (−5 million).

Conditional association analysis was performed with PLINK on loci containing significant SNPs to establish whether other variants in the region (500 kb upstream and downstream) were independently associated with the same phenotype. The conditional threshold for significance was set at $2 \times 10^{-5}$, based on approximately 2500 imputed variants per 1 MB region.

Assessment of inflation of the test statistic. Potential inflation of the test statistic was assessed by ethnicity and phenotype by calculating the genomic inflation factor ($\lambda$; the ratio of the median of the empirically observed distribution of the test statistic to the expected median) and the mean $\chi^2$. Since $\lambda$ is known to scale with sample size, we also calculated the $\lambda$ for an equivalent sample size of 1000 cases and 1000 controls\textsuperscript{60}. We observed some inflation of the test statistic ($\lambda$ > 1) across the different phenotypes (Supplementary Table 7), suggesting either polygenicity or confounding due to population stratification or cryptic relatedness. Therefore, we applied LD score regression\textsuperscript{61}, estimating LD scores using matched populations from the 1000 GP (EUR for Caucasians (n = 669), AFR for African-Americans and EAS for Asians). These LDSC results suggested that inflation of the test statistic was primarily due to polygenicity for most analyses (Supplementary Table 7). Only the Caucasian focal and all epilepsy analyses had LDSC intercepts $< 0.7$ across the different phenotypes (Supplementary Table 8). Supplementary Fig. 9 shows the gene co-expression pattern using the weighted average gene co-expression across all seven datasets for candidate genes from the GWAS that show significant gene co-expression with any of the 10 known epilepsy genes. The complete results are shown in Supplementary Table 9.

Gene co-expression analysis for epilepsy with brain-coX. In silico gene prioritization was performed using brain-coX\textsuperscript{52}. brain-coX uses a combindium of seven large-scale normal brain gene expression data resources to identify co-expressed genes with a set of given genes (known, or putative disease causing epilepsy candidate genes). Multiple genes in a locus were selected if they had an equally high score whilst no genes were selected in a locus if all genes within it had a score <2, similar to previous studies\textsuperscript{17,18}.

Functional annotations. We annotated all genome-wide significant SNPs ($P \leq 5 \times 10^{-8}$) from all phenotypes using the Variant Effect Predictor of ENSEMBL\textsuperscript{67} and the RegulomeDB database\textsuperscript{68}. We annotated chromatin states using epigenetic data from the NIH Roadmap Epigenomics Mapping Consortium\textsuperscript{29} and ENCODE\textsuperscript{31}. We used FUMA\textsuperscript{65} to annotate the minimum chromatin state (i.e. the most active state) across 127 tissues and cell lines for each SNP, similar to a previous study\textsuperscript{27}.
We supplemented the list of 43 known dominant epilepsy genes with an additional 59 monogenic epilepsy genes (as described above). The top 10% enrichment of monogenic epilepsy genes became more significant (Wallenius noncentral hypergeometric test, p = 5.3 × 10−9). When limiting our results to the 21 biological prioritized genes, the enrichment of monogenic epilepsy genes became more significant (Wallenius’ noncentral hypergeometric distribution p = 1.0 × 10−8).

Connectivity mapping. Connectivity mapping was performed using our GWAS results in order to identify drugs which can potentially be repurposed for the treatment of epilepsy, enabling significant savings in the time and cost of anti-epileptic drug development. Recently, So et al. identified candidate drugs that could be repurposed for the treatment of schizophrenia by using GWAS results to impute the gene expression changes associated with the disease, and then, identifying drugs that change gene-expression in the opposite direction in cell lines. Interestingly, the set of candidate drugs they identified was significantly enriched with antipsychotics. We adopted a similar strategy.

Gene-expression changes associated with epilepsy were imputed from the all epilepsy GWAS summary statistics using the FUSION software package and dorsolateral prefrontal cortex tissue RNA-sequencing data (n = 452, CommonMind Consortium). We calculated z-scores for the association between epilepsy and changes in expression of all 5261 significantly heritable genes, using default settings of the FUSION software package as described above. The top 10% of the gene-expression changes most strongly correlated with the disease were identified to construct the disease signature. Then, we identified drugs that change gene-expression in the opposite direction in cell lines, using the Connectivity Mapping Bioconductor package and the Library of Integrated Network-Based Cellular Signatures (LINCS) data. This package utilizes cosine distance to determine if drugs change gene-expression differences more strongly opposed to those associated with the disease. A lower (more positive) cosine distance value indicates that the drug induces gene-expression changes more strongly opposed to those associated with the disease. A lower (more positive) cosine distance value indicates that the drug induces gene-expression changes more strongly opposed to those associated with the disease. A lower (more positive) cosine distance value indicates that the drug induces gene-expression changes more strongly opposed to those associated with the disease. A lower (more positive) cosine distance value indicates that the drug induces gene-expression changes more strongly opposed to those associated with the disease. A lower (more positive) cosine distance value indicates that the drug induces gene-expression changes more strongly opposed to those associated with the disease. A lower (more positive) cosine distance value indicates that the drug induces gene-expression changes more strongly opposed to those associated with the disease.

To demarcate the set of drugs predicted to significantly reverse epilepsy-associated gene-expression changes, the threshold of statistical significance for cosine distance values was determined. For this, we performed 100 permutations of the disease gene-expression z-scores and compared them to drug gene-expression signatures. We combined the distribution of cosine distance values across all permutations, such that the null distribution was derived from 2,405,100 cosine distance values under H0. The cosine distance value corresponding to α of 0.05 was −0.386. Of the drugs with a cosine distance less than −0.386, thirty were experimentally-validated drug repurposing candidates from the Prescribable Drugs with Efficacy in Experimental Epilepsies (PDE) database—a recently published systematic and comprehensive catalogue of licensed drugs with published evidence of antiepileptic efficacy in animal models. We determined whether this is more than expected by chance, by creating 1,000,000 random drug-sets of the same size as drugs with a significant cosine distance. Next, we counted the number of subsets containing an equal or higher number of experimentally-validated drug repurposing candidates than in our list. We determined whether the experimentally-validated drug repurposing candidates were significantly overrepresented compared to the random drug-sets.

Validation of connectivity mapping results. Validation of the connectivity mapping results was performed using two non-overlapping sets of drugs with known antiepileptic efficacy. The first set comprised the drugs predicted to have antiepileptic efficacy in animal models. For the clinically-effective drug-set, we used the names of all recognized antiepileptic drugs, as listed in category N03A of the World Health Organization (WHO) Anatomical Therapeutic Chemical (ATC) Classification System. The second set comprised the drugs predicted to have antiepileptic efficacy in people, and (2) a set of ‘experimentally-validated’ drugs that have antiepileptic efficacy in animal models. For the experimentally-validated drug-set, we extracted drug names from the PDE3 database. We determined whether, in our results, clinically effective drugs are ranked higher than expected by chance. The median rank of clinically effective drugs was 7372. Hence, the median rank of experimentally-validated drugs was 8301 positions higher than that of all drugs. A permutation-based p-value was determined by calculating the median ranks of 1,000,000 random drug-sets, each in equal size to the number of clinically effective drugs. The median rank of experimentally-validated drugs was 6564 positions higher than that of all drugs. A permutation-based p-value was determined by calculating the median ranks of 1,000,000 random drug-sets, each in equal size to the number of experimentally-validated drug repurposing candidates in the LINCS database. This permutation-based p-value was <1.0 × 10−6.
assessed above) with a larger sample size (n=78,307). MTAG utilizes the fact that estimations of effect size and standard error of a primary GWAS, in this case epilepsy, can be improved by matching them to a genetically correlated secondary GWAS, in this case cognitive ability.

Data availability
The GWAS summary statistics data that support the findings of this study are available at http://www.epigad.org/gwas_ilae2018_16loci.html.

Received: 21 May 2018 Accepted: 30 October 2018
Published online: 10 December 2018

References
Acknowledgements

The International League Against Epilepsy Consortium on Complex Epilepsies

Peter Widdess-Walsh, Markus Wolff, Stefan Wolking, Wanling Yang, Federico Zara & Fritz Zimprich

Vanderbilt University Medical Center, Nashville, TN 37232, USA. 2Department of Molecular and Clinical Pharmacology, University of Liverpool, Liverpool L69 3GL, UK. 3The Walton Centre NHS Foundation Trust, Liverpool L9 7LJ, UK. 4Department of Clinical and Experimental Epilepsy, UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK. 5Population Health and Immunity Division, The Walter and Eliza Hall Institute of Medical Research, Parkville 3052, Australia. 6Department of Biology, University of Melbourne, Parkville 3010, Australia. 7School of Mathematics and Statistics, University of Melbourne, Parkville 3010, Australia. 8UCL Genetics Institute, University College London, London WC1E 6BT, UK. 9Melbourne Integrative Genomics, University of Melbourne, Parkville 3052, Australia. 10Medical Faculty of the University of Freiburg, Freiburg 79085, Germany. 11Centre for Genomic Sciences, The University of Hong Kong, Hong Kong, Hong Kong. 12Section for Translational Epilepsy Research, Department of Neuropathology, University of Bonn Medical Center, Bonn 53105, Germany. 13Department of Neurology and Epileptology, Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen 72076, Germany. 14Department of Neurology, University of Ulm, Ulm 89081, Germany. 15Stichting Epilepsie Instellingen Nederland (SEIN), Zwole 8025 BV, The Netherlands. 16Epilepsy Research Centre, University of Melbourne, Austin Health, Heidelberg 3084, Australia. 17Center for Applied Genomics, The Children’s Hospital of Philadelphia, Philadelphia, PA 19104, USA. 18Quantum Research LLC, San Diego, CA 92101, USA. 19National Human Genome Research Institute, National Institutes of Health, Bethesda, MD 20892, USA. 20Department of Biomedical Sciences, Cooper Medical School of Rowan University Camden, Camden, NJ 08103, USA. 21Department of Neurology, Thomas Jefferson University Hospital, Philadelphia, PA 19107, USA. 22Belfast Health and Social Care Trust, Belfast BT9 7AB, UK. 23Division of Epilepsy, Department of Neurology, Mayo Clinic, Rochester, MN 55902, USA. 24Department of Molecular and Preventive Medicine, School of Public Health, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 6997801, Israel. 25The Welcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA, UK. 26Department of Clinical Neurosciences, Cambridge Biomedical Campus, Cambridge CB2 OSL, UK. 27Institute of Neurosciences, Reproductive and Endodontomatosological Sciences, University Federico II, Naples 80138, Italy. 28Laboratory of Neurogenetics and Neurosciences, Institute G. Gaslini, Genova 16148, Italy. 29Department of Neurosciences, University of Montreal, Montreal CA 26758, Canada. 30Department of Neurology, Royal Victoria Hospital, Belfast Health and Social Care Trust, Grosvenor Road, Belfast BT12 6BA, UK. 31Stichting Epilepsie Instellingen Nederland (SEIN), Heemstede 2103 SV, The Netherlands. 32Neurogenetics Group, Center for Molecular Neurology, VIB and Laboratory of Neurogenetics, Institute Born-Bunge, University of Antwerp, Antwerp 2610, Belgium. 33Department of Neurology, University Hospital Uedegei, Edegem 2650, Belgium. 34Department of Genetics, University Medical Center Utrecht, Utrecht 3584 CX, The Netherlands. 35Division of Neurology, Beaumont Hospital, Dublin D09 FT51, Ireland. 36Department of Neurology, Hôpital Erasme, Université Libre de Bruxelles, Brussels 1070, Belgium. 37Comprehensive Epilepsy Center, New York University School of Medicine, New York, NY 10016, USA. 38Department of Neurology, The Children’s Hospital of Philadelphia, Philadelphia, PA 19104, USA. 39Neurology Department, St. James’s Hospital, Dublin D03 VX2, Ireland. 40Department of Epileptology, University of Bonn Medical Center, Bonn 53127, Germany. 41Department of Pharmacology and Psychiatry, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104, USA. 42Department of Pediatrics and Neonatology, Medical University of Vienna, Vienna 1090, Austria. 43Department of Biostatistics, University of Liverpool, Liverpool L69 3GL, UK. 44Institute of Clinical Molecular Biology, Christian-Albrechts-University of Kiel, University Hospital Schleswig Holstein, Kiel 24105, Germany. 45Department of Neurology, NYU School of Medicine, New York City, NY 10003, USA. 46Department of Neurology, Charité Universitätsmedizin Berlin, Campus Virchow-Clinic, Berlin 13353, Germany. 47Institute of Neurology and Neurosurgery at St. Barnabas, Livingston, NJ 07039, USA. 48Research Unit of Molecular Epidemiology, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg D-85764, Germany. 49Institute of Epidemiology, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg D-85764, Germany. 50Comprehensive Epilepsy Center, Division of Neurology, Cincinnati Children’s Hospital Medical Center, Cincinnati, OH 45229, USA. 51Department of Neurology, University of Michigan, Ann Arbor, MI 48109, USA. 52Center for Human Genome Variation, Duke University School of Medicine, Durham, NC 27710, USA. 53Institute for Genomic Medicine, Columbia University Medical Center, New York, NY 10032, USA. 54Division of Human Genetics, Department of Pediatrics, The Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA. 55Life and Brain Center, University of Bonn Medical Center, Bonn 53127, Germany. 56Montefiore Medical Center, Bronx, NY 10467, USA. 57Department of Neuropediatrics, University Medical Center Schleswig-Holstein (UKE), Kiel 24105, Germany. 58Danish Epilepsy Centre, Dianalund 49, Denmark. 59Department of Regional Health Services Research, University of Southern Denmark, Odense S000, Denmark. 60Department of Psychiatry and Applied Psychology, Institute of Mental Health, University of Nottingham, Nottingham NG7 2TU, UK. 61Faculty of Medicine, Imperial College London, London SW7 2AZ, UK. 62Kuopio Epilepsy Center, Neurocenter, Kuopio University Hospital, Kuopio 70029, Finland. 63Institute of Clinical Medicine, University of Eastern Finland, Kuopio 70029, Finland. 64Department of Neurology, University of California, San Francisco, CA 94143, USA. 65University of Alabama Birmingham, Department of Neurology, Birmingham, AL 35233, USA. 66Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Esch-sur-Alzette L-4362, Luxembourg. 67Department of Neurology, Medical University of Vienna, Vienna 1090, Austria. 68Department of Neurology, Zuckers Hofstra Northwell School of Medicine, New York, NY 10075, USA. 69Department of Medicine, University of Miami, Royal Hospital, Parkville, VIC 3050, Australia. 70Department of Neurology, Central Clinical School, Monash University, Melbourne, VIC 3004, Australia. 71Stamford Center for Psychiatric Research, Broad Institute of Harvard and M.I.T, Cambridge, MA 02142, USA. 72Department of Paediatrics and Adolescent Medicine, The University of Hong Kong, Hong Kong, Hong Kong. 73Folkhälsan Research Center and Medical Faculty, University of Helsinki, Helsinki 00290, Finland. 74Genomic Medicine Institute, Lerner Research Institute, Cleveland Clinic, Cleveland, OH 44195, USA. 75Institut für Epidemiologie Christian-Albrechts-Universität zu Kiel, Kiel 24105, Germany. 76Department of Pediatrics and Neurology, Ohio State University and Nationwide Children’s Hospital, Columbus, OH 43205, USA. 77Department of Medical Genetics, School of Medical Sciences, University of Campinas (UNICAMP), Campinas 13083-887 SP, Brazil. 78Brazilian Institute of Neuroscience and Neurotechnology (BRAININ), Campinas, SP 13083-970, Brazil. 79Istituti Clinici Scientifici Maugeri, Pavia 27100, Italy. 80Epilepsy Center Kleinwachau, Radeberg 01454, Germany. 81Division of Intramural Population Health Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA. 82Wilhelm Johannsen Centre for Functional Genome Research, Copenhagen, DK-2200, Denmark. 83School of Medicine, Trinity College Dublin, Dublin 2, Ireland. 84Department of Neurology, Austin Health, Heidelberg, VIC 3084, Australia. 85United Hospital Hong Kong, Hong Kong, Hong Kong. 86Department of Human Genetics, University of Bonn Medical Center, Bonn 53127, Germany. 87Cologne Center for Genomics, University of Cologne, Cologne 50931, Germany. 88Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Helsinki 0014, Finland. 89The Broad Institute of M.I.T. and Harvard, Cambridge, MA 02142, USA. 90AstraZeneca Centre for Genomics Research, Precision Medicine and Genomics, IMED Biotech Unit, AstraZeneca, Cambridge CB2 0AA, UK. 91Department of Neurology, Boston Children’s Hospital,
Harvard Medical School, Boston, MA 02115, USA. 98Department of Neurology, Neuroscience Institute, University of Cincinnati Medical Center, Cincinnati, OH 45220, USA. 99Department of Neurology, Duke University School of Medicine, Durham, NC 27710, USA. 100Epilepsy-Center Hessen, Department of Neurology, University Medical Center Giessen and Marburg, Marburg, Germany and Philipps-University Marburg, Marburg 35043, Germany. 101Epilepsy Center Frankfurt Rhine-Main, Center of Neurology and Neurosurgery, Goethe University Frankfurt, Frankfurt 60528, Germany. 102Chalfont Centre for Epilepsy, Chalfont-St-Peter, Buckinghamshire SL9 0RJ, UK. 103Department of Endocrinology, Hospital of The University of Pennsylvania, Philadelphia, PA 19104, USA. 104Departments of Neurology, Beth Israel Deaconess Medical Center, Massachusetts General Hospital, and Harvard Medical School, Boston, MA 02215, USA. 105Department of Neurology, Inselspital, Bern University Hospital, University of Bern, Bern 3010, Switzerland. 106Department of Neurology, Royal Children’s Hospital, Parkville, VIC 3052, Australia. 107Department of Neurosciences, University of California, San Diego, La Jolla, CA 92037, USA. 108The Royal College of Surgeons in Ireland, Dublin D02 YN77, Ireland. 109Rush University Medical Center, Chicago 60612 IL, USA. 110Department of Neurology, Alan Richens Epilepsy Unit, University Hospital of Wales, Cardiff CF14 4XW, UK. 111Aarhus Institute of Advanced Studies (AIAS), Aarhus University, Aarhus 8000, Denmark. 112Department of Neurology and Comprehensive Epilepsy Center, Thomas Jefferson University, Philadelphia, PA 19107, USA. 113Institute of Genetic Epidemiology, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Neuherberg D-85764, Germany. 114IBE, Faculty of Medicine, LMU Munich, Munich 80539, Germany. 115Pediatric Neurology and Muscular Diseases Unit, Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, G. Gaslini Institute, University of Genoa, Genova 16148, Italy. 116CWZ Hospital, Nijmegen 6532 SZ, The Netherlands. 117Department of Neurology, Washington University School of Medicine, St. Louis, MO 63110, USA. 118Institute for Applied Health Research, University of Birmingham, Birmingham B15 2TT, UK. 119C. Mondino National Neurological Institute, Pavia 27100, Italy. 120Departments of Neurology and Pediatrics, The Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA. 121Department of Neurology, Admiraal De Ruyter Hospital, Goes 4462, The Netherlands. 122Division of Medical Genetics, Department of Pediatrics, Duke University Medical Center, Durham, NC 27710, USA. 123Department of Computer Science, New Jersey Institute of Technology, New Jersey, NJ 07102, USA. 124Department of Pediatric Neurology and Developmental Medicine, University Children’s Hospital, Tübingen 72076, Germany.