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Genetic determinants of common epilepsies: a meta-analysis of genome-wide association studies

The International League Against Epilepsy Consortium on Complex Epilepsies*

Abstract

Background—The epilepsies are a clinically heterogeneous group of disorders. Despite strong evidence for heritability, genome-wide association studies in epilepsy have had limited success in identifying risk loci probably due to relatively small sample sizes and lack of power. Here we report three meta-analyses conducted for all epilepsy and its two largest clinical subtypes – genetic generalized epilepsy (GGE) and focal epilepsy.

Methods—We examined 12 case/control cohorts comprising 8,696 cases and 26,157 controls. Cases were predominantly Caucasian from the United Kingdom, Western Europe, Finland, USA and Australia, with some African-American and Han Chinese subjects. Controls were ethnically matched. Subjects were phenotyped into categories of GGE, focal epilepsy and unclassified epilepsy. A fixed-effects meta-analysis was performed after standardized imputation, to account for different genotyping platforms across sites. Standardized association protocols were applied locally, prior to combining summary statistics for meta-analysis. The genome-wide significance threshold was set at $p<1{\cdot}66\times10^{-8}$.

Findings—Meta-analysis of the 'all epilepsy' cohort identified loci at 2q24.3 ($p=8{\cdot}71\times10^{-10}$) implicating *SCN1A*, a well-established monogenic epilepsy gene encoding the alpha1 sodium channel subunit and at 4p15.1 ($p=5{\cdot}44\times10^{-9}$) harboring *PCDH7* as the lead candidate, which encodes a protocadherin molecule not previously implicated in epilepsy. For the GGE cohort, a single signal at 2p16.1 ($p=9{\cdot}99\times10^{-9}$) was observed, implicating *VRK2* or *FANCL* respectively encoding a protein kinase involved in signal transduction and apoptosis and a ubiquitin ligase involved in a DNA repair pathway. No single nucleotide polymorphism (SNP) achieved genome-wide significance for focal epilepsy.

Correspondence to: Epilepsy Research Centre, 245 Burgundy St, Heidelberg, VIC 3084, AUSTRALIA epilepsy-austin@unimelb.edu.au.
*The list of authors and affiliations appears at the end of the paper.

COMPETING FINANCIAL INTERESTS
The authors have no competing financial interests to declare.

DATA ACCESS
A complete list of p values for all SNPs analyzed can be found at the Epilepsy Genetic Association Database (epiGAD)http://www.epigad.org

Interpretation—This first meta-genome-wide association study in epilepsy has uncovered new loci for the common forms of epilepsy and is a further step in understanding the genetic architecture of the epilepsies with the ultimate aim of assisting in biological understanding, classification and prognostication. The data suggest thatspecific loci can actpleiotropically raising risk for epilepsy broadly, or have effects limited to a specific epilepsy subtype. This suggests that future genetic analysis may benefit from both “lumping”, where all epilepsies are grouped together, and “splitting”, where specific clinical subtypes are analyzed.

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INTRODUCTION

Epilepsy is a common disorder, affecting up to 4% of people at some time in life,[1] comprising heterogeneous syndromes defined by clinical, electroencephalographic (EEG) and brain imaging criteria.[2] Broadly, the epilepsies are divided clinically into generalized and focal forms. Genetic factors contribute to both, as shown by familial aggregation and twin studies.[3] Causative mutations in many genes, including some coding for ion channel subunits and others affecting synaptic function or brain development, have been discovered.[3,4] The majority of these discoveries are for relatively rare familial epilepsies segregating in a Mendelian fashion, or epilepsies (particularly the severe infantile epilepsies) arising from *de novo mutations*.[5-7]

The genetic determinants underlying the common epilepsies, where clinical genetic data suggest complex inheritance, remain largely unknown. There is some evidence to suggest a role for rare sequence and copy number variants,[8-10] whereas the contribution of common polymorphisms is still unclear,[11,12] partly reflecting the relatively small sample sizes analyzed to date.

The largest genome-wide association study (GWAS) in epilepsy so far, consisting of 3,445 focal epilepsy cases,[13] found no variants of genome-wide significance. More recently, a study of 1,018 cases of mesial temporal lobe epilepsy with hippocampal sclerosis, a subtype of focal epilepsy, implicated the 2q24.3 region around the sodium channel *SCN1A*,[14] and an independent study of Han Chinese patients with known or suspected lesional focal epilepsy found evidence for a risk allele at 1q32 based on a discovery sample of 504 cases.[15]

For generalized epilepsy, a GWAS incorporating 1,527 European cases with genetic generalized epilepsies (GGE) in the discovery analysis and 1,493 GGE cases in the replication cohort found evidence for common risk alleles at 2p16.1 and 17q21.32, and suggestive evidence at the *SCN1A* locus.[16] In addition, associations were reported for the GGE subtype juvenile myoclonic epilepsy at 1q43 and for generalized absence epilepsy at 2q22.3.[16]

Here we present the first large multicenter collaboration designed to discover variants that may increase risk for common epilepsies, consisting of a meta-analysis of (prior to quality control (QC)) 40,789 subjects comprising 10,064 epilepsy cases from twelve cohorts and 30,725 control subjects.

In view of clinical evidence that there may be genetic factors that raise risk for epilepsy broadly and in a syndrome-specific manner,[17-19] we pre-specified three analyses as part of the study. Variants were sought that influence risk for all epilepsies, for genetic generalized epilepsy (previously idiopathic generalized epilepsy)[2,20] and for focal epilepsy.

METHODS

Study Design and Participants

A meta-analysis was performed on twelve previously published and unpublished epilepsy cohorts from EPICURE,[16] EPIGEN,[13] Philadelphia, the Melbourne -Imperial - Liverpool Collaboration,[21] Finland[13] and Hong Kong[15] (see Supplementary Table 1 (pre-QC numbers)). These cohorts were identified from the literature (PubMed - using search terms of epilepsy, seizures, association studies) and through publicity via Chapters of the International League Against Epilepsy (ILAE) and during international conferences. All twelve case cohorts (and their associated controls) broadly aligned with European, Asian or African ancestry (see Table 1 (post-QC numbers) and Supplementary Fig.1).

A combination of population-based datasets were employed as controls. These cohorts were both screened and unscreened for neurological conditions. Further details are available in Table 1 and Supplementary Table 2.

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

Procedures

Phenotyping—Seizures and epilepsy syndromes were classified according to the ILAE terminology.[2,20] For all cases, epilepsy specialists evaluated their phenotype at the source center. Patients with epilepsy were assigned to one of three phenotypic categories: 1) GGE, 2) focal epilepsy or 3) unclassified epilepsy. Cases for each were defined as follows:

– GGE: Criteria were tonic-clonic, absence or myoclonic seizures with generalized spike-wave discharges on EEG and no evidence of an acquired cause. In rare instances the criterion for a diagnostic EEG was waived when there was clear clinical evidence of myoclonic or absence seizures *with* tonic-clonic seizures, and no evidence for an acquired cause. The ILAE has adopted the term “GGE” for syndromes previously known as “idiopathic” or “primary” generalized epilepsies in view of strong evidence for a genetic basis from genetic epidemiological and twin studies and an absence of identified acquired factors.[2,20]

– Focal epilepsy: This comprised patients with a confirmed diagnosis of focal epilepsy, including cases with focal structural brain lesions. The samples were predominantly comprised of adults, so cases of benign epilepsy of childhood with centro-temporal spikes were not specifically included.

– Unclassified epilepsy: This group consisted of patients in whom there was neither electro-clinical evidence for generalized epilepsy, nor evidence for a focal seizure onset, or patients with evidence for generalized and focal epilepsy.

The phenotyping committee curated patient phenotypes into a single database. Details relating to individual case cohorts are provided in Supplementary Methods. Analyses were performed on three phenotypic groups – GGE, focal epilepsy and 'all epilepsy' consisting of all patients with a confirmed diagnosis of epilepsy, including GGE, focal and unclassified epilepsy.

Statistical analysis

Imputation—As contributing sites had employed different genotyping platforms, we conducted imputation to infer genotypes for common genetic variants that were not directly genotyped. This allowed us to combine results across sites. Each of the five sites imputed their study datasets according to a standardized protocol. This used IMPUTE2 to infer haplotypes and impute, usingthe 1000 Genomes Phase I (interim) June 2011 reference panel (see Supplementary Methods).

Association analysis—Every site performed linear mixed model (LMM) association analysis for each of their datasets, using the software FaSTLMM (version 1.09).[22] This performs linear regression, including a polygenic term designed to account for the contributions of population stratification and causal variants aside from the one being tested. Although evaluating a binary trait, it is valid to use linear regression (rather than logistic regression) because effect sizes are expected to be small.

This analysis was performed separately for each of the pre-selected phenotypic categories of epilepsy: 1) all epilepsy; 2) GGE; 3) focal epilepsy. Gender was included as a covariate.

Meta-analysis—Fixed-effects meta-analysis was conducted using the software METAL.[23] As the vast majority of the epilepsy cases considered were of European descent (see Table 1), we chose a fixed-effects model to optimize power. SNPs showing significant amounts of heterogeneity ($p<0.05$) were removed before applying the fixed-effects analysis. Genomic correction was applied to the association analysis results for each dataset before combining for meta-analysis. Again, these steps were performed separately for each of the three phenotypic tests.

Significance threshold for meta-analysis—We set our genome-wide threshold for statistical significance at $1{\cdot}66\times10^{-8}$, reflecting an empirical Bonferroni correction of the 5×10^{-8} genome-wide significance threshold for three tests. We regarded signals between $1{\cdot}66\times10^{-8}$ and 5×10^{-7} as suggestive evidence of association.

Power calculations—We calculated the proportion of heritability a variant must explain for the detection power to be at least 80%; we considered variance explained on the liability scale,[24] for which we assumed a point prevalence of 0·5% for all epilepsy, 0·2% for GGE and 0·3% for focal epilepsy[25] (Supplementary Fig.2).

Logistic regression—In addition to the main association analysis, we also performed logistic regression for variants in a 1Mb window centered on each variant that showed suggestive evidence of association ($p<5\times10^{-7}$) from any of the three meta-analyses (all epilepsy, GGE and focal). The purpose of this analysis was 1) a technical validation and 2)

to estimate odds ratios. For this we analyzed the dosage data, including gender and the first 20 principal components, using PLINK,[26] and then combined the results from each site again using a fixed effect meta-analysis.

Conditional analysis—Conditional analysis was performed using FaSTLMM on variants in the same regions as defined for logistic regression. The purpose of the conditional analysis was to determine if any other genetic variants in the region associated with the disease phenotype, independent of the strongest signal from that region. We conditioned on the most significant variants within each of the three regions, i.e. rs6732655 and rs28498976 for all epilepsy, and rs2947349 for GGE. Gender was included as a covariate in the conditional analysis.

Significance threshold for conditional analysis—We applied Bonferroni correction to control for multiple testing in the conditional analysis and set the threshold for significance at 5×10^{-6} (each 1Mb region contained approximately 10,000 SNPs).

Confirmatory genotyping—To examine the accuracy of the imputation across regions showing signals satisfying genome-wide significance, we conducted genotyping in a subset of patients included in the meta-analysis and compared hard genotypes with imputation dosage files. We selected a subset of individuals to represent each of the three broad ethnicities included in our analysis (i.e. Caucasian, African-American and Han Chinese). Genotyping was conducted using TaqMan®(Life Technologies) for rs28498976, Sanger sequencing for rs6732655 and Kasper KASP™ (LGC Genomics) for rs2947349. Results are shown in Supplementary Table 3.

Enrichment analysis—Enrichment analysis was conducted using the interval-based enrichment analysis tool as integrated in the package INRICH.[27] Briefly, INRICH takes a set of independent, nominally associated genomic intervals and tests for enrichment of predefined gene sets using permutation. We considered variants with a p value $< 1\times10^{-5}$ and defined the interval around index SNPs using an r^2 threshold of 0·2. Gene sets as defined by GO ontology pathways were tested for enrichment.

Role of the funding source

The sponsors of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The Strategy and Analysis committee members had full access to all data in the study. The Strategy committee takes final responsibility for the decision to submit for publication.

RESULTS

All epilepsy

After application of our quality control (QC) criteria (see Supplementary Methods), we included a total of 34,853 individuals (8,696 epilepsy cases and 26,157 controls) across twelve cohorts in the ‘all epilepsy’ meta-analysis (see Table 1). We estimated 80% power to

detect a variant explaining 0·07%, or greater, of the liability variance (see Supplementary Fig.2).

Principal component analysis indicated that patient cohorts clustered in three broad ethnicities (European, Asian and admixed African-American) as expected (see Supplementary Fig.1). We observed an inflation factor of 1·031, suggesting adequate control for possible cryptic stratification (see Supplementary Fig. 3a).

We identified two loci at genome-wide significance ($p<1{\cdot}66\times10^{-8}$, see Fig. 1a). The first signal was located at 2q24.3 (Fig. 2). This signal was centered on the voltage-gated sodium channel SCN1A gene, which is a knowngene for certain monogenic epilepsies.[28,29] The most strongly associated variant in this interval was rs6732655 ($p=8{\cdot}71\times10^{-10}$, OR 0·89 (CI: 0·86-0·93), see Table 2 and Supplementary Fig.4), located in intron 16 of SCN1A. Seventy other variants in this region satisfied the threshold for genome-wide significance. Logistic regression validated the association with 2q24.3 (Supplementary Fig.5). The direction of effect was consistent across most cohorts, and there was no evidence of significant heterogeneity.

Given the extent of linkage disequilibrium (LD) between the variants associated with ‘all epilepsy’ in the 2q24.3 region (Fig. 2), we conducted logistic regression conditioned on the most significant variant identified from the univariate analysis (rs6732655). Results indicated a tentative independent signal, coming from rs13406236, an intronic variant in *SCN9A* ($p=1{\cdot}39\times10^{-4}$ on conditioning, see Supplementary Fig.6). No further significant signals were identified.

A second signal for the ‘all epilepsy’ phenotype was located at 4p15.1 and included the 3’ end of the protocadherin gene, *PCDH7* (Fig. 3). The most strongly associated variant in this region was rs28498976 ($p=5{\cdot}44\times10^{-9}$, OR=0·90 (CI: 0·87-0·94), see Table 2), located 2·5kb from the 3’-end of *PCDH7*. Logistic regression across PCDH7 supported the association with this locus (see Supplementary Fig.5). There were no additional significant signals from 4p15.1 on conditioning for rs28498976 (see Supplementary Fig.6). The direction of effect was consistent across all cohorts and there was no evidence of heterogeneity. Although only achieving genome-wide significance for the ‘all epilepsy’ phenotype, the *PCDH7* signal appeared stronger in GGE compared to focal epilepsy (see Supplementary Fig.7).

PCDH7 encodes a calcium-dependent adhesion protein, a member of the cadherin gene family, not previously associated with epilepsy. The gene is expressed in the central nervous system, specifically in thalamocortical circuits and the hippocampus,[30,31] and expression of *PCDH7* is controlled by *MECP2*,[32]mutations in which cause Rett Syndrome. The cytoplasmic domain of the PCDH7 protein binds to protein phosphatase 1α (PPP1CA), which is enriched in dendritic spines and is important in learning and memory,[33] and template activation factor 1 (TAF1), which along with PCDH7 plays a role in neurite extension.[34,35]

Suggestive signals of note ($p<5\times10^{-7}$) for the ‘all epilepsy’ phenotype were detected at 3q26.2 ($p=4{\cdot}42\times10^{-7}$) and 4p12 ($p=1{\cdot}71\times10^{-7}$) (see Table 2). The 3q26.2 region contained the 5’ end of *GOLIM4* (see Supplementary Fig. 8), encoding Golgi internal membrane

protein 4, which is degraded when manganese increases above normal levels, suggesting a role for this protein in manganese regulation.[36] The vast majority of brain manganese is in glutamine synthase, an enzyme playing a key role in producing or degrading the neurotransmitters glutamate, glutamine, and gamma-aminobutyric acid (GABA). Decreased brain levels of glutamine synthase and of manganese have been reported in epilepsy.[37,38] The 4p12 region contained the 3' end of the gamma-aminobutyric acid receptor, α2-subunit gene (*GABRA2*, see Supplementary Fig.9). Mutations in other GABA receptors have been found to cause epilepsy.[39]

Genetic generalized epilepsy (GGE)

After QC, we considered a total of 21,596 individuals (2,606 cases and 18,990 controls) across six cohorts in the GGE meta-analysis (see Table 1). Individuals considered were a subset of those included in the 'all epilepsy' analysis. For the GGE analysis we estimated 80% power to detect a variant explaining 0·17% or greater of the liability variance (see Supplementary Fig.2). Results from the GGE meta-analysis indicated an inflation factor of 1·05 (see Supplementary Fig. 3b).

A single signal achieved the threshold of genome-wide significance (see Fig. 1b). Located at 2p16.1, the interval contained genes encoding vaccinia-related kinase 2 (*VRK2*) and Fanconi Anemia, Complementation Group L (*FANCL*) (see Fig. 4). The most strongly associated variant in this region was the intergenic variant rs2947349 ($p=9{\cdot}99\times10^{-9}$, OR=1·23 (CI: 1·16-1·31), see Table 2). Logistic regression analysis supported the association with 2p16.1 (see Supplementary Fig.5). There were no additional significant signals from 2p16.1 on conditioning for rs2947349 (see Supplementary Fig.6). The direction of effect was consistent across all cohorts, and the association appeared to be specific to GGE (see Supplementary Fig.10).

VRK2 is a serine/threonine protein kinase involved in signal transduction and apoptosis.[40,41] Variation in *VRK2* has previously been suggested as a risk factor for epilepsy[16] and schizophrenia.[42-44] Indeed, the schizophrenia risk variant (rs2312147)[43] shows a strong signal for GGE ($p=2{\cdot}3\times10^{-6}$, OR=1·22 (CI:1·14-1·30)) and is in high LD with the strongest variant for GGE ($r^2=0{\cdot}82$) – although the direction of the effect is opposite. The EPICURE cohort in which 2p16.1 was originally proposed as a risk factor for GGE was included in our meta-analysis. Excluding the EPICURE cohort, the top SNP (rs13026414)[16] remains nominally significant at $p=7\times10^{-3}$. These results provide further support to the suggestion that *VRK2* is a risk locus for both epilepsy and schizophrenia. The other gene in the region, FANCL, is a RING-type E3 ubiquitin ligase of the Fanconi anemia pathway. FANCL mono-ubiquitinates FANCD2 and FANCI, proteins involved in DNA repair and homologous recombination.[45] FANCL has not been previously implicated in epilepsy or any seizure-related phenotype.

Suggestive evidence for association with GGE was detected at 4p15.1 ($p=1{\cdot}87\times10^{-7}$), 5q22.3 ($p=6{\cdot}34\times10^{-8}$) and 11q22.2 ($p=2{\cdot}37\times10^{-8}$)(see Table 2). The 4p15.1 *PCDH7* signal is the same as that appearing genome-wide significant for the 'all-epilepsy' phenotype (see Fig. 3 and Supplementary Fig.11). The 5q22.3 signal is intergenic (see Supplementary Fig. 12). The 11q22.2 signal contained the 5' end of the matrix metallopeptidase gene *MMP8*

(see Supplementary Fig. 13). The direction of effect was consistent across all cohorts and appeared specific to GGE (see Supplementary Fig. 14). With a p value of $2{\cdot}37\times10^{-8}$, it reached the conventional threshold for genome-wide significance ($p<5\times10^{-8}$), but not our more stringent one ($p<1{\cdot}66\times10^{-8}$). Matrix metaloproteases are zinc-dependent endopeptidases involved in the breakdown of the extracellular matrix in normal physiological processes and of the blood-brain barrier in inflammation.[46] Increased expression of MMPs have been recorded in various neurological disease states,[47] and epileptogenesis is decreased in MMP9 knockout mice but increased in transgenic rats overexpressing MMP9.[48]

Focal

Post-QC, 28,916 individuals (5,310 cases, 23,606 controls), across eightcohorts, were included in the 'focal' epilepsy analysis. No signal achieved genome-wide significance. For the focal analysis we estimated 80% power to detect a variant explaining 0·10% or greater of the liability variance (see Supplementary Fig.2). Results from the focal meta-analysis indicated an inflation factor of 1·014 (see Supplementary Fig. 3c). We observed one sub-threshold signal of note (rs12987787, $p=1{\cdot}45\times10^{-7}$) from 2q24.3, the region containing *SCN1A* (see Table 2 and Supplementary Fig. 15).

Targeted genotyping of the three GWAS-significant signals confirmed that imputation was accurate with a minimum correlation of 0·98 observed between experimentally determined and imputed genotypes (see Supplementary Table 3).

An assessment of enrichment of gene ontology (GO) terms for regions containing variants with nominally significant p values ($p<1\times10^{-5}$) for each of the three phenotypes found enrichment in several pathways (see Supplementary Table 4). Although none of these survived correction for multiple testing, the results highlight pathways of biological plausibility.

Finally, we investigated whether any of the four susceptibility loci at nominal genome-wide significance ($p<5\times10^{-8}$) were associated with outcome of newly treated epilepsy using data from Speed et al., 2014.[21] We considered both the index SNP (Table 2) and SNPs within a 20Kb window around each of the 5 genes (*SCN1A, PCDH7, VRK2/FANCL, MMP8*) (see Supplementary Table 5). The minimum p value of association with outcome of newly treated epilepsy for any susceptibility locus was $8{\cdot}14\times10^{-4}$ (*MMP8*). We found no evidence for an association between *SCN1A* (the target for sodium channel-blocking class anti-epileptic drugs) and epilepsy outcome.

DISCUSSION

This first GWAS meta-analysis in the common epilepsies identified three loci with genome-wide significance and suggests some loci may show specificity for epilepsy type.

In the whole cohort consisting of all epilepsy, the region of the sodium channel subunit *SCN1A* was clearly implicated. This gene is a well established cause of genetic epilepsy with febrile seizures plus (GEFS+),[28,29] a generally mild, familial form of epilepsy, and Dravet

syndrome, a severe epileptic encephalopathy usually arising from *de-novo* mutation.[7] *SCN1A* was implicated in a recent GWAS of mesial temporal lobe epilepsy and hippocampal sclerosis with febrile seizures (mTLEHS+FS)[14] and a meta-analysis of *SCN1A* rs3812718 in all epilepsy.[49] *SCN1A* mutations are also observed in a spectrum of paroxysmal neurological disorders including familial hemiplegic migraine[50] and, more rarely, in certain focal epilepsies.[51] It is therefore unclear whether this robust association with all epilepsy is a true common variant association or a synthetic association due to tagged rare variants in cases with GEFS+. Whilst it is possible the cohorts could have included individuals from monogenic GEFS+ families with *SCN1A* mutations of large effect, review of the phenotyping data suggested inclusion of more than a few such cases is unlikely; moreover *SCN1A* variants are only found in about 10% of large GEFS+ families.[52]

Our ‘all epilepsy’ analysis identified a second locus (4p15.1), which also satisfied our threshold for genome-wide significance. This locus is novel for epilepsy and implicates the gene *PCDH7*. This protocadherin gene is a plausible candidate for common forms of epilepsy with mutations in another protocadherin gene, *PCDH19*, causing epilepsy and mental retardation limited to females (EFMR).[53]

For the specific category of GGE, we again observed the association at 2p16.1, previously reported in the EPICURE cohort,[16] which comprised approximately half of our GGE cohort (see Table 1). The association maintained nominal significance after removal of EPICURE casesfor this locus, where the genes *VRK2* and *FANCL* are within close proximity. With our additional samples, we did not observe significance for the 17q21 locus reported by EPICURE for GGE (see Supplementary Fig. 16).

For the larger subcategory of focal epilepsy we did not find any locus at genome-wide significance, consistent with the EPIGEN study of focal epilepsy that was negative (samples included here).[13] However, a signal at 2q24.3 (containing *SCN1A*) in focal epilepsy approached, but did not achieve significance (see Supplementary Fig.15). The focal signal was in high LD with that observed for ‘all epilepsy’ ($r^2 = 0{\cdot}85$). Importantly, the 2q24.3 signal for focal epilepsy observed here is different to that reported in a recent study of the narrow focal epilepsy phenotype of mTLEHS+FS.[14] rs7587026 (the previously reported mTLEHS+FS variant) is not significant in our analysis of a broader focal epilepsy phenotype consisting of all focal epilepsies ($p=0{\cdot}01$) (see Supplementary Fig. 17). We also failed to observe the previously reported association at 1q32.1, implicating *CAMSAP1L1*, in the Hong Kong cohort[15] (included here, see Supplementary Fig.18), where the majority of cases had focal epilepsy due to known lesions.

Consistent with experience of GWAS analyses in other neuropsychiatric disorders, and common disorders in general, this study reinforces the value of large sample sizes. In the epilepsies, electro-clinical and imaging data permit the identification of clinical syndromes that share common clinical features. Our study suggests that an experimental design that includes fractionation of samples into clinical subtypes may reveal syndrome-specific risk alleles, but the identification of these alleles will be facilitated by the collection and genotyping of larger sample sizes. While this “lumping” versus “splitting” debate in genetic

analyses is not unique to the epilepsies, there has been long-standing controversy about this in clinical epileptology[54] that genetics will help to inform.

Limitations of our study include sample size; although ours is large, even larger samples have yielded increasing discoveries in other disorders.[55-57] Larger samples would enable further analysis of epilepsy subtypes and the ILAE Consortium now provides a useful vehicle for this. Second, our meta-analysis relied on separately generated genotypes on a variety of platforms, an issue common to most meta-analyses. Third, it would be ideal to extend the phenotyping data to include treatment outcome but, in a cross-sectional cohort, this has methodological problems. Finally, we did not have an independent replication sample. However, stringent criteria for statistical significance were set a *priori* and, for loci achieving our threshold of genome-wide significance, the direction of effects were uniform across the cohorts, and extended over multiple variants in high LD.

Taken together, these data show that, given sufficient sample size, susceptibility loci for common epilepsies can be identified through the analysis of common variation. The role of rare variants of large effect is also well established, particularly in rarer Mendelian epilepsies.[3-7] The role of rare variants in the common epilepsies is currently being explored by deep sequencing approaches.[11,58,59] A dual approach of identifying both rare and common variation will result in a greater understanding of the genetic architecture for the overall epilepsy population, necessary for precision medicine. While these findings will not be of immediate clinical utility, they are an important first step to understanding the genetic architecture of epilepsies which may lead to clinically relevant markers of prognosis and outcome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix

AUTHOR CONTRIBUTIONS

Analysis committee:*Association analysis:* J.P.B., S.S.C., C.G.F.dK., H.G., C.L., D.S., Z.W., C.W.*Coordination*:G.L.C.*Imputation:*J.P.B.,S.S.C., C.G.F.dK.,H.G., D.S.,C.W.*Protocol development*:J.P.B., G.L.C., C.L., D.S., Z.W.

Phenotyping committee: C.D., D.J.D., W.S.K., P.K., D.H.L., A.G.M., M.R.S., P.S.

Strategy committee: L.B., S.F.B., R.J.B., H.Hak, E.L.H, M.R.J., B.P.C.K., P.K., H.L., T.J.O'B., K.L.O. (*ex-officio*), S.Sis.

Governance committee: S.F.B., A.C., A-E.L., D.H.L.

Writing committee: S.F.B., G.L.C., M.R.J.

Patient recruitment and phenotyping: A.A., F.B., R.J.B., C.B.C., K.C., P.C., G-J.dH., P.DJ., N.D., C.D., D.J.D., C.P.D., C.E.E., T.N.F., M.F., J.F., V.G., H.Hja, I.H., M.J., J.J-K., D.J., M.R.J., R.K., A-M.K., D.K-N.T., W.S.K., P.K., H.L., D.L., W.Lo., A.G.M., M.Moe, R.S.M., H.M., M.N., P-W.N., T.J.O'B., M.P., R.R., P.S.R., F.R., T.San, T.Sca, St.Sc, Su.Sc,S.Sis, C.S., I.E.S., B.S., D.F.S., P.E.S., M.R.S., U.S., P.S., H.S., R.S., K.M.T, M.T., A.T., R.T., F.V., S.V., N.M.W., Y.G.W., C.W., F.Zim.

Genotyping and bioinformatics: R.J.L.A., D.B., L.B., J.P.B., G.L.C., S.S.C., A.J.C, C.G.F.dK, D.B.G., H.G., Y.G., H.Hak, E.L.H., I.H., D.K., C.L., A.M., M.McC, P.N., S.P., AK.R., T.San, P.C.S., D.S., M.S., H.T., Z.W., C.W., F.Zar.

Control cohorts: L.C.B., A.F., H.Hak, Y-L.L., W.Li., J.L.M., A.M.M., M.M.N, A.P., F.P., H.S., G.N.T., The KORA study group, W.Y.

The International League Against Epilepsy Consortium on Complex Epilepsies

Richard J L Anney, Andreja Avbersek, David Balding, Larry Baum, Felicitas Becker, Samuel F Berkovic, Jonathan P Bradfield, Lawrence C Brody, Russell J Buono, Claudia B Catarino, Gianpiero L Cavalleri, Stacey S Cherny, Krishna Chinthapalli, Alison J Coffey, Alastair Compston, Patrick Cossette, Gerrit-Jan de Haan, Peter De Jonghe, Carolien G F de Kovel, Norman Delanty, Chantal Depondt, Dennis J Dlugos, Colin P Doherty, Christian E Elger, Thomas N Ferraro, Martha Feucht, Andre Franke, Jacqueline French, Verena Gaus, David B Goldstein, Hongsheng Gui, Youling Guo, Hakon Hakonarson, Kerstin Hallmann, Erin L Heinzen, Ingo Helbig, Helle Hjalgrim, Margaret Jackson, Jennifer Jamnadas-Khoda, Dieter Janz, Michael R Johnson, Reetta Kälviäinen, Anne-Mari Kantanen, Dalia Kasperavičiūte, Dorothee Kasteleijn-Nolst Trenite, Bobby P C Koeleman, Wolfram S Kunz, Patrick Kwan, Yu Lung Lau, Anna-Elina Lehesjoki, Holger Lerche, Costin Leu, Wolfgang Lieb, Dick Lindhout, Warren Lo, Daniel H Lowenstein, Alberto Malovini, Anthony G Marson, Mark McCormack, James L Mills, Martina Moerzinger, Rikke S Møller, Anne M Molloy, Hiltrud Muhle, Mark Newton, Ping-Wing Ng, Markus M Nöthen, Peter Nürnberg, Terence J O'Brien, Karen L Oliver, Aarno Palotie, Faith Pangilinan, Katharina Pernhorst, Slave Petrovski, Michael Privitera, Rodney Radtke, Philipp S Reif, Felix Rosenow, Ann-Kathrin Ruppert, Thomas Sander, Theresa Scattergood, Steven Schachter, Christoph Schankin, Ingrid E Scheffer, Bettina Schmitz, Susanne Schoch, Pak C Sham, Sanjay Sisodiya, David F Smith, Philip E Smith, Doug Speed, Michael R Sperling, Michael Steffens, Ulrich Stephani, Pasquale Striano, Hans Stroink, Rainer Surges, K Meng Tan, The KORA study group, G Neil Thomas, Marian Todaro, Anna Tostevin, Rossana Tozzi, Holger Trucks, Frank Visscher, Sarah von Spiczak, Nicole M Walley, Yvonne G Weber, Zhi Wei, Christopher Whelan, Wanling Yang, Federico Zara, Fritz Zimprich

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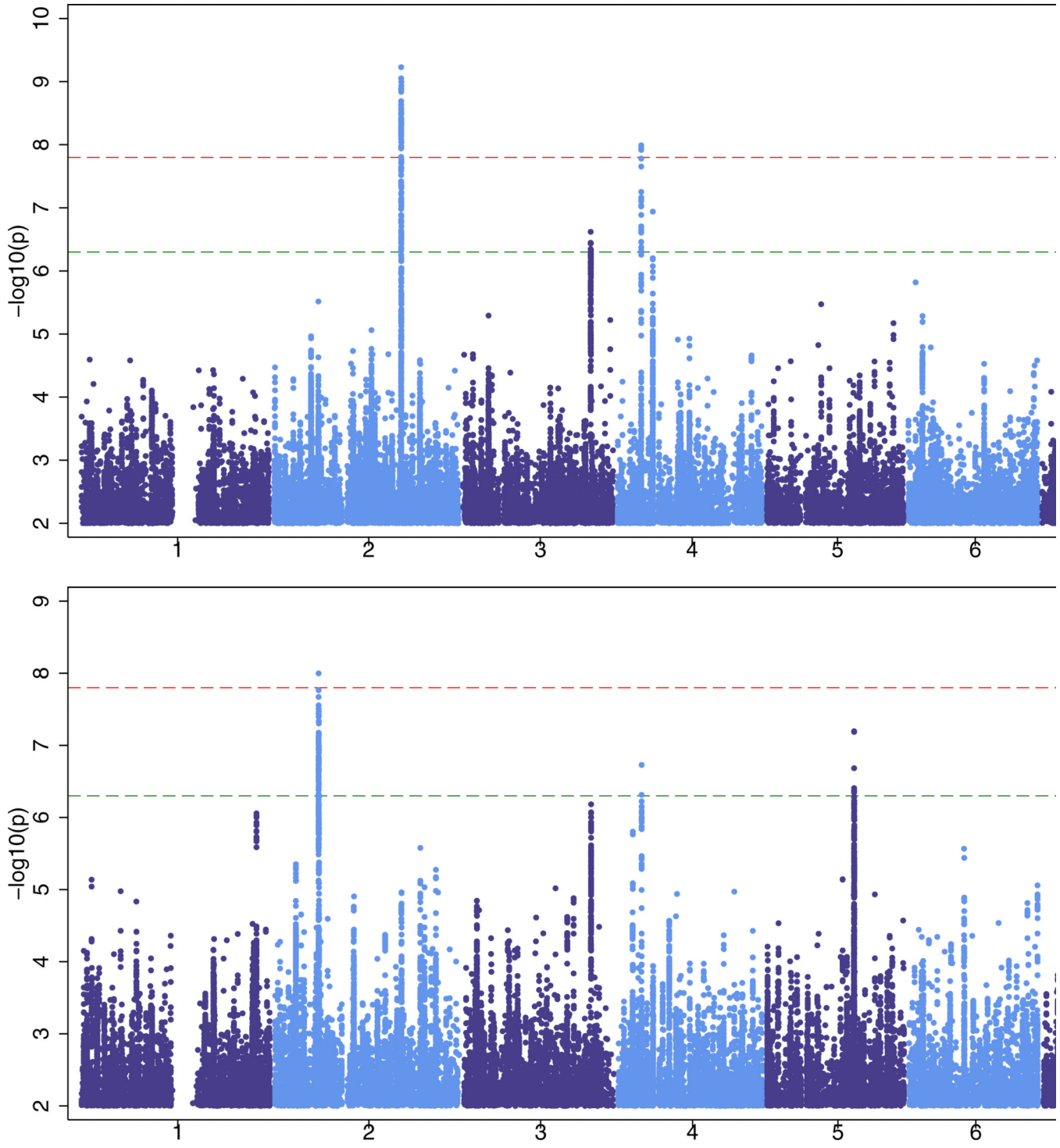

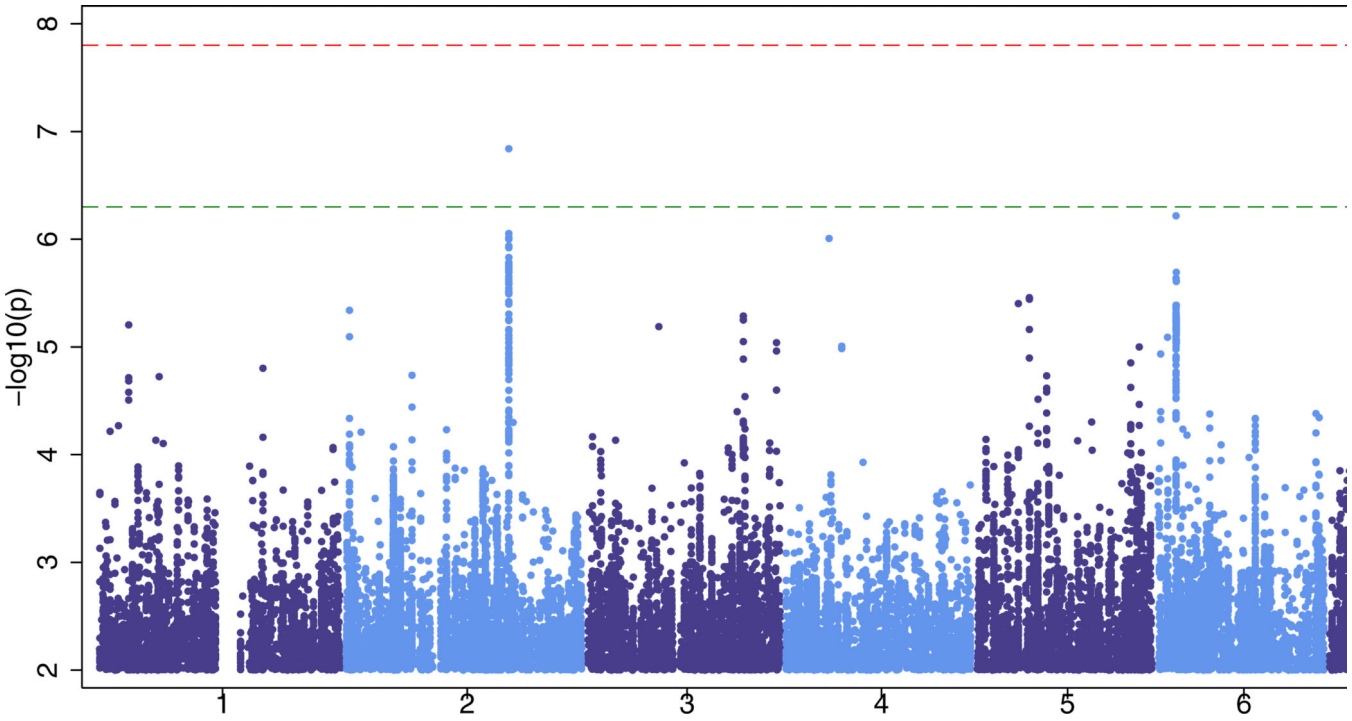

Figure 1. Manhattan plots for our three primary analyses

'all epilepsy' (Fig 1a), GGE (Genetic Generalized Epilepsy, Fig 1b) and focal epilepsy (Fig 1c). The red line shows our threshold of 'significance' set at $1{\cdot}66\times10^{-8}$ and the greenline indicates the 'suggestive' threshold of 5×10^{-7}.

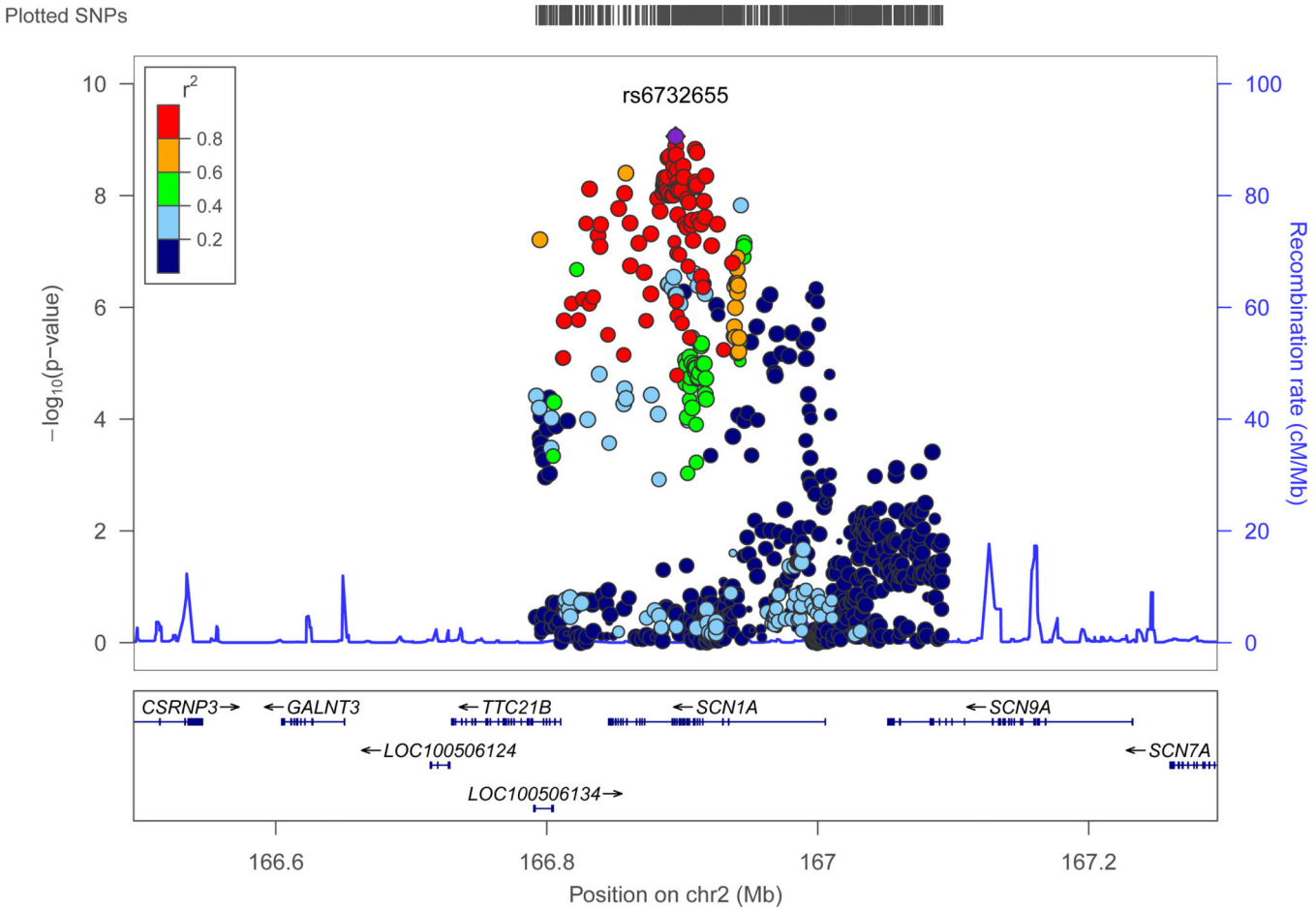

Figure 2. Genomic context of 2q24.3 signal from 'all epilepsy' analysis

Plot created using LocusZoom.[60] Linkage disequilibrium data are taken from 1000 Genomes Project, HG19, March 2012.

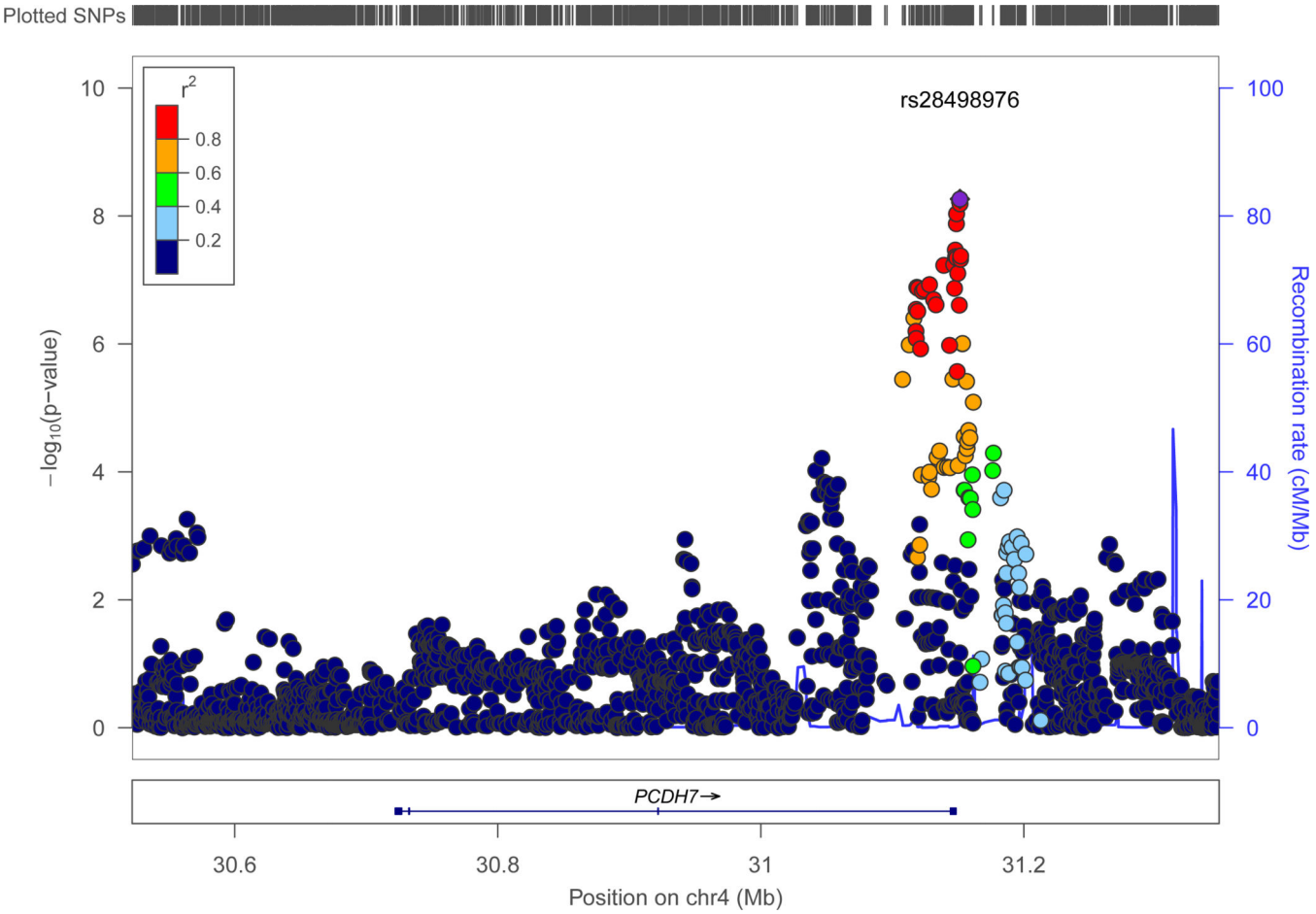

Figure 3. Genomic context of 4p15.1 signal from 'all epilepsy' analysis

Plot created using LocusZoom.[60] Linkage disequilibrium data are taken from 1000 Genomes Project, HG19, March 2012.

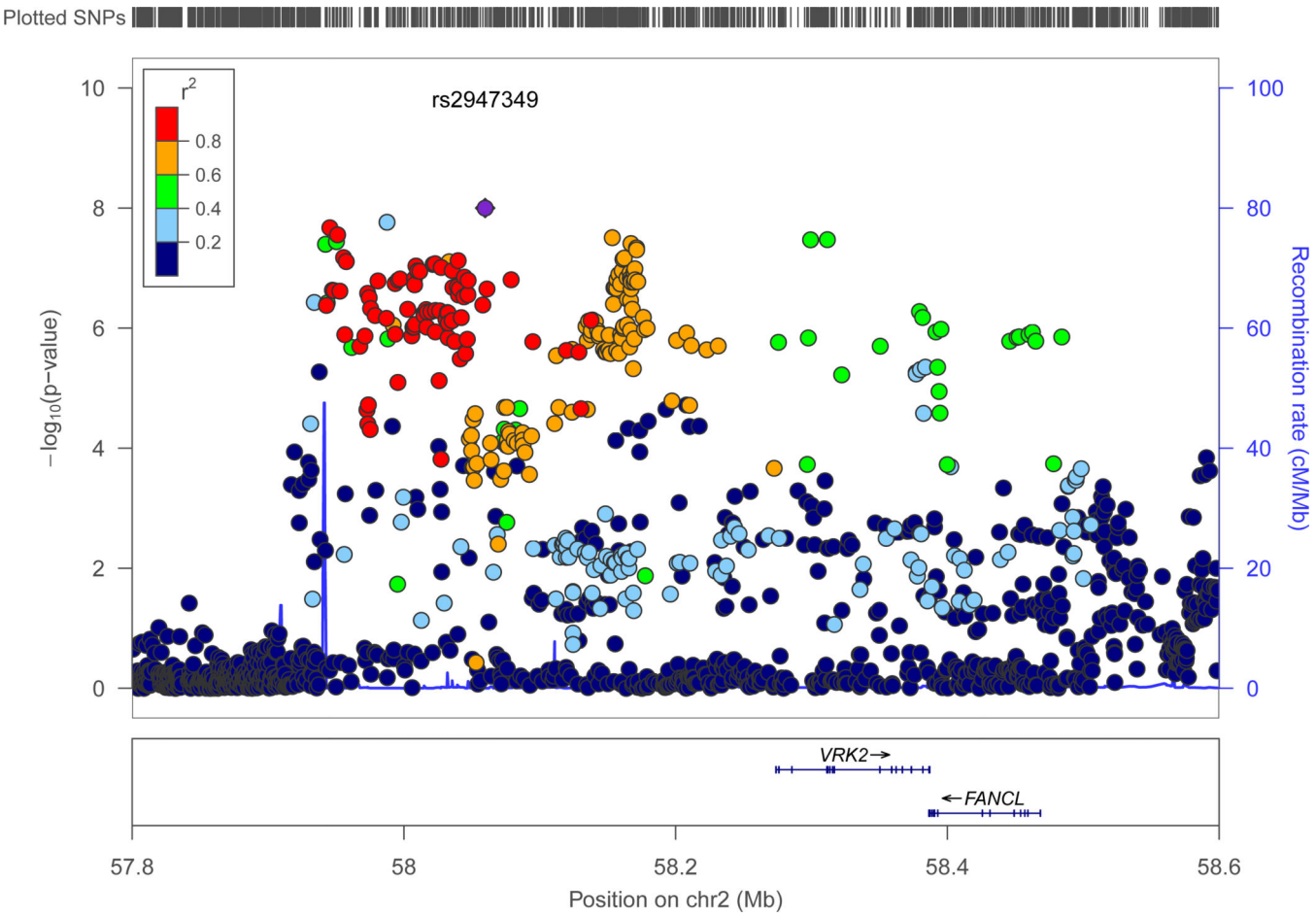

Figure 4. Genomic context of 2p16.1 signal from GGE analysis

Plot created using LocusZoom[60]. Linkage disequilibrium data are taken from 1000 Genomes Project, HG19, March 2012.

Table 1

Post QC case/control numbers, as structured for analysis.

Index GWAS	**Ethnicity**[1]	**Epilepsy cases**	**GGE**	**Focal**	**Population controls**[3]
EPIGEN-Dublin	Irish	638	-	520	2232
EPIGEN-Brussels	Belgian	505	48	406	1675
EPIGEN-Duke	AA[2]& EA	760	102	551	504
EPIGEN-London	British + other	1007	93	773	2494
ILM Collaboration	European-descent	1703	212	1263	2699
GenEpa	Finnish	422	-	422	1963
EPICURE	NW- European	1440	1440	-	2454
Philadelphia_550_AA[2]	African American	324	81	222	2746
Philadelphia_550_CAU	European American	819	440	378	5736
Philadelphia_Omni_AA	African American	106	-	-	97
Philadelphia_Omni_CAU	European American	485	190	288	682
Hong Kong	Asian-Han	487	-	487	2875
TOTAL		**8696**	**2606**	**5310**	**26157**

[1]Broad ethnicity of the cohort. AA: African American. EA: European American. Other: indicates mixed, as would be expected in a cosmopolitan population. European-descent: European-caucasian. NW-European: North-West European.

[2]EPIGEN-Duke individuals of AA ancestry were merged with Philadelphia_550_AAcohort.

[3]See Supplementary Table 2 for further details on control cohorts.

Table 2

Summary of loci at $p<5{\cdot}0\times10^{-7}$.

rs number	chr	pos (bp)	A1/A2	MAF	cand. gene	annotation	pheno	OR (CI)	LMM p	condp
rs6732655	2q24.3	166895066	T*/A	0.22(A)	*SCN1A*	intronic	allepi	0·89 (0·86-0·93)	$8{\cdot}71\times10^{-10}$	$4{\cdot}95\times10^{-7}$
rs28498976	4p15.1	31151357	A/G*	0.46(A)	*PCDH7*	intergenic	allepi	0·90 (0·87-0·94)	$5{\cdot}44\times10^{-9}$	$2{\cdot}29\times10^{-4}$
rs111577701	3q26.2	167861408	T/C*	0.09(T)	*GOLIM4*	intergenic	allepi	1.16 (1.09-1.24)	$4{\cdot}42\times10^{-7}$	-
rs535066	4p12	46240287	T/G*	0.40(G)	*GABRA2*	intergenic	allepi	1.10 (1.05-1.16)	$1{\cdot}71\times10^{-7}$	-
rs2947349	2p16.1	58059803	A*/C	0.26(C)	*VRK2/FANCL*	intergenic	GGE	1·23 (1·16-1·31	$9{\cdot}99\times10^{-9}$	1×10^{-4}
rs1939012	11q22.2	102595135	C/T*	0.40(T)	*MMP8*	intronic	GGE	1.12 (1.07-1.17)	$2{\cdot}37\times10^{-8}$	-
rs1044352	4p15.1	31147874	T*/G	0.50(T)	*PCDH7*	synonymous	GGE	0.88 (0.82-0.93)	$1{\cdot}87\times10^{-7}$	-
rs55670112	5q22.3	114268470	A/C*	0.47(C)	none	intergenic	GGE	1.18 (1.1-1.26)	$6{\cdot}34\times10^{-8}$	-
rs12987787	2q24.3	166858391	C/T*	0.21(C)	*SCN1A*	intronic	focal	1.12 (1.01-1.14)	$1{\cdot}45\times10^{-7}$	-

Chr: cytogenetic band; pos (bp): base pair position, build 37 (hg19); A1/A2: allele 1, allele 2

MAF: minor allele frequency, from all 1000Genomes populations; cand. gene: most plausible candidate gene attributable to the signal; annotation: type of SNP; pheno: phenotype; OR (CI): odds ratio, corresponds to allele 2, computed from logistic regression; 95% confidence interval in brackets; LMM p: p value from linear mixed model meta analysis; cond: p value when conditioning on this specific SNP., to determine independent signals from same locus.

* indicates ancestral/chimp allele