doi:10.1093/brain/awq130 Brain 2010: 133; 2136–2147 | 2136

BRAIN
A JOURNAL OF NEUROLOGY

Common genetic variation and susceptibility to partial epilepsies: a genome-wide association study

Dalia Kasperavičiūtė,[1,*] Claudia B. Catarino,[1,2,*] Erin L. Heinzen,[3] Chantal Depondt,[4] Gianpiero L. Cavalleri,[5] Luis O. Caboclo,[1] Sarah K. Tate,[1] Jenny Jamnadas-Khoda,[1] Krishna Chinthapalli,[1] Lisa M. S. Clayton,[1] Kevin V. Shianna,[3] Rodney A. Radtke,[6] Mohamad A. Mikati,[7] William B. Gallentine,[7] Aatif M. Husain,[6] Saud Alhusaini,[5] David Leppert,[8,9] Lefkos T. Middleton,[8,10] Rachel A. Gibson,[8] Michael R. Johnson,[10] Paul M. Matthews,[8,10] David Hosford,[8] Kjell Heuser,[11] Leslie Amos,[8] Marcos Ortega,[12] Dominik Zumsteg,[12] Heinz-Gregor Wieser,[12] Bernhard J. Steinhoff,[13] Günter Krämer,[14] Jörg Hansen,[14] Thomas Dorn,[14] Anne-Mari Kantanen,[15] Leif Gjerstad,[11,16] Terhi Peuralinna,[17] Dena G. Hernandez,[18] Kai J. Eriksson,[19] Reetta K. Kälviäinen,[15,20] Colin P. Doherty,[21] Nicholas W. Wood,[22] Massimo Pandolfo,[4] John S. Duncan,[1,2] Josemir W. Sander,[1,2,23] Norman Delanty,[5] David B. Goldstein[3] and Sanjay M. Sisodiya[1,2]

1 Department of Clinical and Experimental Epilepsy, UCL Institute of Neurology, Queen Square, London, WC1N 3BG, UK
2 National Society for Epilepsy, Chalfont-St-Peter, Bucks, SL9 0RJ, UK
3 Center for Human Genome Variation, School of Medicine, Duke University, Durham, NC 27708, USA
4 Department of Neurology, Hôpital Erasme, Université Libre de Bruxelles, 1070 Brussels, Belgium
5 Molecular and Cellular Therapeutics, The Royal College of Surgeons in Ireland, St. Stephens Green, Dublin, Ireland
6 Department of Medicine (Neurology), Duke University Medical School, Durham, NC 27710, USA
7 Division of Paediatric Neurology, Duke University Medical Centre, Durham, NC 27710, USA
8 Genetics Division, Drug Discovery, GlaxoSmithKline, Research Triangle Park, NC 27709, USA
9 Department of Neurology, University Hospital Basel, Switzerland
10 Department of Clinical Neurosciences, Imperial College, Hammersmith Hospital, London W12 0NN, UK
11 Department of Neurology, Oslo University Hospital, Rikshospitalet, Oslo, Norway
12 Department of Neurology, University Hospital Zurich, 8091 Zurich, Switzerland
13 Kork Epilepsy Centre, Kehl-Kork, Germany
14 Swiss Epilepsy Centre, Bleulerstrasse 60, 8008 Zurich, Switzerland
15 Kuopio Epilepsy Centre, Kuopio University Hospital, Kuopio, Finland
16 Faculty of Medicine, University of Oslo, Oslo, Norway
17 Biomedicum, University of Helsinki and Department of Neurology, Helsinki University Central Hospital, Helsinki, Finland
18 Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health, Bethesda, Maryland, USA
19 Paediatric Neurology Unit, Tampere University Hospital and Paediatric Research Centre, University of Tampere, Tampere, Finland
20 Department of Neurology, Institute of Clinical Medicine, University of Eastern Finland, Kuopio, Finland
21 The Department of Neurology, St James' Hospital Dublin, Ireland
22 Department of Molecular Neuroscience, UCL Institute of Neurology, Queen Square, London, WC1N 3BG UK
23 SEIN, Epilepsy Institutes in the Netherlands Foundation, Achterweg 5, 2103 SW Heemstede, The Netherlands

*These authors contributed equally to this work.

Received February 10, 2010. Revised March 29, 2010. Accepted April 21, 2010. Advance Access publication June 3, 2010

Correspondence to: Sanjay M. Sisodiya,
Department of Clinical and Experimental Epilepsy,
UCL Institute of Neurology,
Queen Square, London,
WC1N 3BG, UK
E-mail: s.sisodiya@ion.ucl.ac.uk

Correspondence may also be addressed to: David B. Goldstein,
Duke University Medical Centre,
450 Research Dr LSRC B Wing,
Box 91009, Durham,
NC 27708, USA
E-mail: d.goldstein@duke.edu

Partial epilepsies have a substantial heritability. However, the actual genetic causes are largely unknown. In contrast to many other common diseases for which genetic association-studies have successfully revealed common variants associated with disease risk, the role of common variation in partial epilepsies has not yet been explored in a well-powered study. We undertook a genome-wide association-study to identify common variants which influence risk for epilepsy shared amongst partial epilepsy syndromes, in 3445 patients and 6935 controls of European ancestry. We did not identify any genome-wide significant association. A few single nucleotide polymorphisms may warrant further investigation. We exclude common genetic variants with effect sizes above a modest 1.3 odds ratio for a single variant as contributors to genetic susceptibility shared across the partial epilepsies. We show that, at best, common genetic variation can only have a modest role in predisposition to the partial epilepsies when considered across syndromes in Europeans. The genetic architecture of the partial epilepsies is likely to be very complex, reflecting genotypic and phenotypic heterogeneity. Larger meta-analyses are required to identify variants of smaller effect sizes (odds ratio <1.3) or syndrome-specific variants. Further, our results suggest research efforts should also be directed towards identifying the multiple rare variants likely to account for at least part of the heritability of the partial epilepsies. Data emerging from genome-wide association-studies will be valuable during the next serious challenge of interpreting all the genetic variation emerging from whole-genome sequencing studies.

Keywords: partial epilepsy; genome-wide association; genetics; common variants
Abbreviation: SNP = single nucleotide polymorphism

Introduction

The epilepsies constitute the commonest serious chronic neurological condition, with a prevalence of 3–16 per 1000 worldwide (Begley *et al.,* 2007). Mendelian epilepsies probably account for only ~1% of cases. The majority—so-called 'sporadic' epilepsies—are considered 'complex': both genetic and environmental factors probably play a role, to different extents, in individual patients. Estimates of partial epilepsy heritability vary between studies, some estimates reaching 70% (Kjeldsen *et al.,* 2001). All published twin and family studies report higher concordance among monozygotic than dizygotic twins and high familial aggregation (e.g. Berkovic *et al.,* 1998; Miller *et al.,* 1998; Hemminki *et al.,* 2006). Nevertheless, even though the importance of genetic factors is clear, the factors themselves remain elusive. Numerous candidate gene studies have failed to identify unambiguous associations (see Discussion section in Tan *et al*., 2004; Cavalleri *et al.,* 2005, 2007). The failure to detect robust associations has been commonly attributed to small sample sizes and hence low study power, and to the choice of candidate genes. Many studies focused on candidates emerging from the genetics of familial epilepsies, such as ion-channel genes, which comprise two-third of genes responsible for Mendelian epilepsies. Interestingly, in single-gene mutant mice exhibiting spontaneous or more readily-evoked seizures, only a quarter of the mutated genes encode ion channels (Frankel, 2009), suggesting a much broader spectrum of pathways and genes for seizure predisposition than currently known.

Despite the heterogeneity of partial epilepsy syndromes, some shared biological features, such as some components of seizures, secondary generalization of partial seizures, shared EEG abnormalities and the fundamental biophysical and neurochemical cellular components of seizures, e.g. action potentials and synaptic transmission processes, suggest there are some common mechanisms for susceptibility to partial seizures in general. Studies indicate that different types of epilepsy can aggregate in families (Ottman *et al.,* 1998; Bianchi *et al.,* 2003; Berkovic *et al.,* 2004), suggesting the existence of shared genetic factors that increase susceptibility to different epilepsies. More recently, microdeletion analyses have shown that apparently the same genetic defect can contribute to different forms of epilepsy (de Kovel *et al.,* 2009), including 'symptomatic' epilepsies (Heinzen *et al.*, 2010).

It is possible, therefore, that shared genetic variants predispose to partial epilepsies, irrespective of syndrome type or any structural cause. Knowledge of such shared variants would significantly increase the understanding of disease biology of partial epilepsies and help identify targets for novel therapeutic interventions effective across partial epilepsies. On the other hand, if well-powered and comprehensive studies cannot detect any common genetic variants for susceptibility for partial epilepsies, our concepts of the genetic architecture of partial epilepsies will evolve, and a different approach may prove more productive in epilepsy genetics research.

Here we report the results from a genome-wide association-study for partial epilepsies in a large cohort of patients of European ancestry. We show that common variants are unlikely to have clinically relevant effects in predisposition to partial epilepsies shared across syndromes.

Methods

Patients

Patients with partial epilepsies were recruited in seven countries (Supplementary material) during clinical appointments. The diagnosis of partial epilepsy was made and/or reviewed by a consultant epileptologist who was part of this study, with access to clinical history and available investigation results. The International League Against Epilepsy (Commission on Classification and Terminology of the International League Against Epilepsy, 1989) definition for partial epilepsy was used. A patient was considered as having epilepsy if he/she had had two or more unprovoked epileptic seizures. Partial epilepsy was defined as epilepsy characterized by seizures the semiology or investigation (ictal EEG) of which disclosed a focal origin of seizures. We did not select patients by syndrome other than partial epilepsy, nor by known structural abnormality, if any. Phenotypic details of the patients for each country's cohort are shown in Table 1, using a scheme adapted from The International League Against Epilepsy revised organization of phenotypes in epilepsies (Berg *et al.*, 2010).

Informed consent was obtained from study participants and the study was approved by the Ethics Committee at each recruitment site according to national standards. Patients of all ethnicities were recruited and genotyped. However, only patients of European ancestry were included in genome-wide association analysis to minimize confounding by population structure (see below).

Controls

We used (Supplementary Fig. 1): (i) 288 controls from Finland and 285 controls from Switzerland without neurological conditions, recruited and genotyped for this study; (ii) 1165 USA controls from the Duke Memory study (Need *et al.*, 2009; Cirulli *et al.*, 2010), who consented to participate in epilepsy genetics research; 84% of participants filled in a questionnaire about their history of neurological conditions and the subjects who reported a history of seizures were excluded from the study; (iii) 5667 population controls from the Wellcome Trust Case Control Consortium (2007) Phase 2, September 2009 data release; (iv) 469 population controls from Finland, all 85-years-old or over at the time of recruitment (Vantaa85+) (Myllykangas *et al.*, 2005; Peuralinna *et al.*, 2008); and (v) 211 Irish neurologically-normal controls from the Study of Irish Amyotrophic Lateral Sclerosis (Cronin *et al.*, 2008).

Genotyping and quality control

DNA was extracted from blood samples using standard procedures. All patients with epilepsy and the Switzerland, Finland and USA controls were genotyped at Duke University. The majority of the in-house genotyped patients (93.4%) and controls (77.4%) were genotyped using Human610-Quadv1genotyping chips (Table 2). Genotype calling and quality control were performed using Beadstudio v3 software as previously described (Fellay *et al.*, 2007) and detailed in the Supplementary material. Finnish control data from the Vantaa85+ study were received in Beadstudio files and processed using the same protocol.

Quality control procedures were applied to the Wellcome Trust Case Control Consortium control dataset in the following order: (i) all individuals listed as 'individual exclusions' in the data release documentation were excluded; (ii) any remaining individuals with >2% missing data were removed; (iii) single nucleotide polymorphisms (SNPs) with >1% missing data were removed; (iv) SNPs with Hardy-Weinberg equilibrium *P*-value below 1×10^{-10} were removed; (v) allele frequencies in 1958 birth cohort and National-Blood cohort subsets were compared using the χ^2 test, and SNPs with *P*-values below 1×10^{-10} were removed; and (vi) principal component analysis was performed on the remaining data using a subset of unlinked SNPs to check for possible plate effects. Such effects were suspected in two plates and these samples were removed (Supplementary material).

The Irish control genotype data were downloaded from the dbGaP database (http://www.ncbi.nlm.nih.gov/gap), dbGaP accession number phs000127.v1.p1. SNPs with call rates below 0.98 and cluster separation values below 0.3, as provided in the data release documentation, were removed. We then checked that none of the individuals had >2% missing data.

Further, we performed gender and relatedness checks for all samples and manually inspected cluster plots for a subset of SNPs as described in the Supplementary material.

Population ancestry and stratification analysis

A combination of self-identified ancestry and EIGENSTRAT principal components methods (Price *et al.*, 2006) were used to identify individuals of European ancestry and to correct further for population stratification. We used a modified EIGENSTRAT method, as previously described (Fellay *et al.*, 2007) and detailed in the Supplementary material.

Principal component analysis also detects correlations in the data that may occur for reasons other than population ancestry. Correlations among individuals may be due to laboratory processing error (batch or plate effects). Correlations among SNPs may be due to large linkage disequilibrium regions or genotype calling differences (e.g. genotyping chip differences or different genotype call algorithms). Therefore, we inspected all EIGENSTRAT axes for these effects and suspect samples were removed. Similarly, we detected 31 SNPs discordant between HumanHap550 and Human610-Quadv1 chips and removed them from the analysis.

Table 1 Phenotypic details of patients for each country's cohort, classified according to the revised organization of phenotypes in epilepsy (Berg *et al.*, 2010), adapted to our study (A), with additional data on MRI findings (B)

Phenotype	UK ($n=1185$)	Ireland ($n=607$)	Belgium ($n=418$)	Finland ($n=410$)	USA ($n=393$)	Switzerland ($n=231$)	Norway ($n=201$)	Total ($n=3445$)
A.								
Syndrome or constellation								
Distinctive constellations								
Mesial temporal lobe epilepsy with hippocampal sclerosis	265	148	67	116	71	182	70	919
Epilepsies that can be distinguished first on the basis of the presence or absence of a known structural or metabolic condition (presumed cause) and then on the basis of the primary mode of seizure onset (generalized versus focal)								
Epilepsies attributed to and organized by structural-metabolic causes								
Malformations of cortical development (hemimegalencephaly, heterotopias, etc.)	141	38	21	12	16	12	1	241
Neurocutaneous syndromes (tuberous sclerosis complex, Sturge-Weber, etc.)	1	3	3	0	3	0	0	10
Tumour	42	62	42	3	48	23	2	222
Infection	36	16	9	1	8	0	6	76
Trauma	32	60	22	2	21	0	7	144
Angioma or other vascular malformation	34	26	27	4	9	0	7	107
Perinatal insults	34	12	8	9	2	0	9	74
Stroke	32	27	33	0	9	0	1	102
Other structural-metabolic causes	51	23	18	6	16	5	2	121
Epilepsies of unknown cause	517	192	168	257	190	9	96	1429
B.								
MRI phenotype								
Normal	487	134	94	209	146	8	81	1159
Unilateral hippocampal sclerosis	277	140	65	118	66	182	72	920
Bilateral hippocampal sclerosis	12	6	3	4	5	1	0	31
Malformations of cortical development	138	38	17	12	14	12	1	232
Cerebrovascular disease	27	12	30	0	11	0	1	81
Perinatal injury	34	1	7	8	1	0	9	60
Other acquired injury	59	29	27	3	20	0	8	146
Vascular malformation	34	25	25	4	18	0	7	113
Tumour	42	60	42	3	50	23	2	222
Other	39	23	41	31	6	4	18	162
Incidental MRI findings	26	26	39	11	20	1	2	125
MRI not available, but an X-ray computerized tomography scan is consistent with the presence of a particular abnormality	10	15	10	0	0	0	0	35
MRI data not available[a]	0	98	18	7	36	0	0	159

The organization of phenotypes was adapted to include only the categories for which there were patients in our study. We note that the proposed organization of phenotypes (Berg *et al.*, 2010) is not specific for partial epilepsies, but only patients with partial epilepsies were included in our study. The numbers of patients in some phenotype categories in part A of the table do not necessarily match numbers in the similar categories in the part B, because part A contains the numbers related to causation of epilepsy, which is determined using all available clinical history and investigations, while part B contains data on MRI findings alone.

a Excludes 10 patients from the UK, 15 patients from Ireland and 10 patients from Belgium, for whom an X-ray computerized tomography scan is consistent with the presence of a particular abnormality (cerebrovascular disease, infection or trauma).

Table 2 Subjects of European ancestry included in the analysis

Country	Number of subjects in analysis	Percent of females (number)	Genotyping chip (number of samples)
Patients			
UK	1185	51.1 (605)	Human610-Quadv1 (1018), HumanHap550v3 (167)
Ireland	607	51.1 (310)	Human610-Quadv1 (562), HumanHap300v1 (45)
Belgium	418	53.1 (222)	Human610-Quadv1 (418)
Finland	410	59.0 (242)	Human610-Quadv1 (410)
USA	393	55.2 (217)	Human610-Quadv1 (393)
Norway	201	55.7 (112)	Human610-Quadv1 (201)
Switzerland	231	50.2 (116)	Human610-Quadv1 (231)
All patients	**3445**	**52.9 (1824)**	**Human610-Quadv1 (3233), HumanHap550v3 (167), HumanHap300v1 (45)**
Controls			
UK	5116	49.6 (2535)	Human1-2M-DuoCustom (5116)
Finland	746	72.9 (544)	Human610-Quadv1 (277), Human1M-Duov3 (104), HumanCNV370-Quadv3 (171), HumanCNV370v1 (194)
USA	605	56.9 (344)	Human610-Quadv1 (347), HumanHap550v3 (81), HumanHap550v1 (171), Human1Mv1 (6)
Switzerland	259	56.8 (147)	Human610-Quadv1 (259)
Ireland	209	46.9 (98)	HumanHap550v3 (209)
All controls	**6935**	**52.9 (3668)**	**Human610-Quadv1 (883), Human1-2M-DuoCustom (5116), HumanHap550v3 (81), HumanHap550v1 (380), Human1Mv1 (6), Human1M-Duov3 (104), HumanCNV370-Quadv3 (171), HumanCNV370v1 (194)**

Association analysis

Only SNPs present on both Illumina Human610-Quadv1 and Human1-2M-DuoCustom were included in the analysis. Association analysis was performed using PLINK (Purcell *et al.*, 2007). First, we used the logistic regression additive model, including gender and all significant EIGENSTRAT axes, as assessed using the Tracy-Widom statistic with $P<0.05$, as covariates into the model. Further, we performed a stratified analysis using the Cochran–Mantel–Haenszel test. For this analysis, seven strata were used, each corresponding to the recruitment country. To ensure homogeneity of each stratum, we performed principal component analysis within each stratum separately and removed the outliers.

Only SNPs with minor allele frequency of 1% and above were included in the analysis. We chose this frequency cut-off because we were interested in common variants. Our study was underpowered to detect associations with lower allele frequencies. Genotypes of SNPs with minor allele frequency <1% were less reliably called across the different cohorts.

Power calculations were performed using PGA Power Calculator software (Menashe *et al.*, 2008) assuming a disease prevalence of 0.5%, the additive risk model, and r^2 0.9 between a causal variant and a genotyped marker (Fig. 1).

Gene ontology analysis was performed using the ALIGATOR method (Holmans *et al.*, 2009) to investigate whether there was enrichment for SNPs in genes in any gene ontology categories among the SNPs with low, but not genome-wide significant, *P*-values. We investigated these SNP sets using two thresholds, $P<0.0001$ and $P<0.001$. Only SNPs located within genes were included (based on NCBI SNP build 129 and NCBI sequence build 36.3). One SNP per gene, with the lowest *P*-value, was included in the ALIGATOR analysis using 20 000 simulated replicate gene lists and 5000 simulated replicate studies.

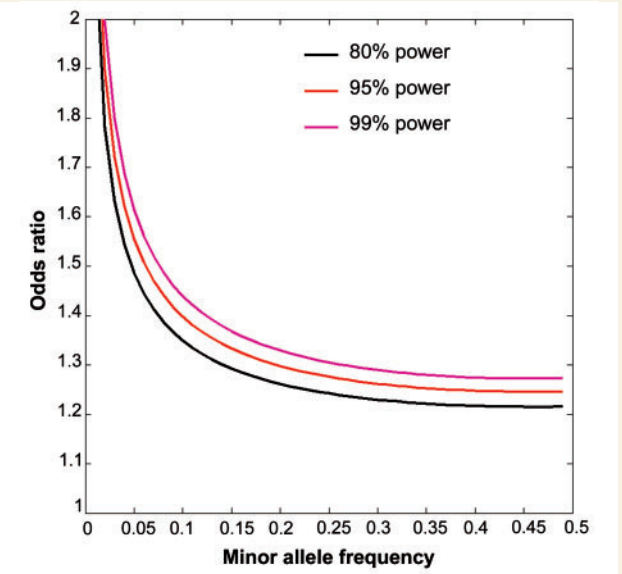

Figure 1 Minimal detectable odds ratio at $P=5\times10^{-8}$ for different power levels in our genome-wide association-study. Power calculations were performed assuming a disease prevalence of 0.5%, the additive risk model and $r^2=0.9$ between a causal variant and a genotyped marker.

Results

Study participants (total 4514, 3941 patients with partial epilepsies and 573 controls) were genotyped in the study (Supplementary Fig. 1). 4383 (97.1%, 3816 patients and 567 controls) passed genotyping quality control filters. After the application of quality

control procedures, the average genotyping call rate was 99.96% for subjects genotyped on Human610-Quadv1 chips and 99.93% for subjects genotyped on HumanHap550v3 chips. Thirty-four known duplicate samples were genotyped. Genotype concordance rate was >99.99% regardless of whether samples were genotyped on the same chip type or on different chips. Twenty subjects (0.4%, 17 patients and three controls) were excluded because sex mismatch was detected between phenotype and genotype data. One sample was removed because the same patient was recruited independently in two cohorts (UK and Ireland). A further 48 subjects (27 patients and 21 controls) were removed because of imputed relatedness to other study participants. The resulting dataset was merged with the quality-controlled control datasets from the Duke Memory study, Wellcome Trust Case Control Consortium, Vantaa85+ and Study of Irish Amyotrophic Lateral Sclerosis, and a further three related controls were removed.

After the population structure analysis, 3445 patients with partial epilepsies and 6935 controls of European ancestry were included for genome-wide association-analysis (Table 2). 528 745 SNPs were included in the analysis. We note, however, that for the SNPs which were on Human610-Quadv1 and Human1-2M-DuoCustom only, but not on other types of chips, the sample size was smaller. Therefore the minimal sample size was 3233 patients and 5999 controls, if a SNP was not present on any other type of the chip.

First, we performed association analysis using logistic regression, including sex and 15 EIGENSTRAT axes as covariates in an additive genetic model. Inspection of quantile–quantile plots showed a slight departure from 'normal' expectation (Fig. 2A) with a genomic inflation factor λ 1.05. This could indicate the existence of many causal alleles with small effect sizes. However, we were concerned there could be residual population stratification. Unequal sample sizes from different European subpopulations can bias principal component-based population structure analysis, overemphasizing variation within the largest cohorts. Also, because of the differences in ratio of patients and controls from the different populations, the most significant principal component axes correlated with case–control status. Therefore principal component-based correction could have over-compensated. To check the robustness of the association results, we performed a stratified association analysis using the Cochran–Mantel–Haenszel test. The quantile–quantile plot indicated a slight excess of low *P*-values, with a genomic inflation factor λ 1.02 indicating adequate correction for population structure (Fig. 2B).

Manhattan plots of the genome-wide association-results are shown in Fig. 3. Results for SNPs with *P*-values below 5×10^{-5} in both analyses are shown in Table 3. All SNPs with *P*-values below 1×10^{-4} in either Cochran–Mantel–Haenszel or logistic regression tests are shown in the Supplementary material. The *P*-values for all SNPs are available from http://www.ion.ucl.ac.uk/departments/epilepsy/themes/genetics/PEvsCTRL.

None of the *P*-values in our study reach the now widely-accepted 5×10^{-8} threshold for genome-wide significance in association studies (McCarthy *et al.*, 2008), nor the 9.46×10^{-8} threshold required to achieve significance after applying Bonferroni correction for 528 745 tests in our study specifically.

The top SNP, rs346291, ($P = 3.34 \times 10^{-7}$) is located on chromosome 6 within a predicted pseudogene and is located 95 and 116 kb from the closest known genes, *SH3BGRL2* and *ELOVL4*, respectively. There is little linkage disequilibrium in the region, and the second most-associated SNP, rs9341799, is in only moderate linkage disequilibrium with rs346291 ($r^2 = 0.34$ in our dataset) (Fig. 4). To test the independence of association signals for these two SNPs, we performed logistic regression analysis for rs9341799 conditioned on the genotype of rs346291, i.e. incorporating this genotype as a covariate in the model. We detected a

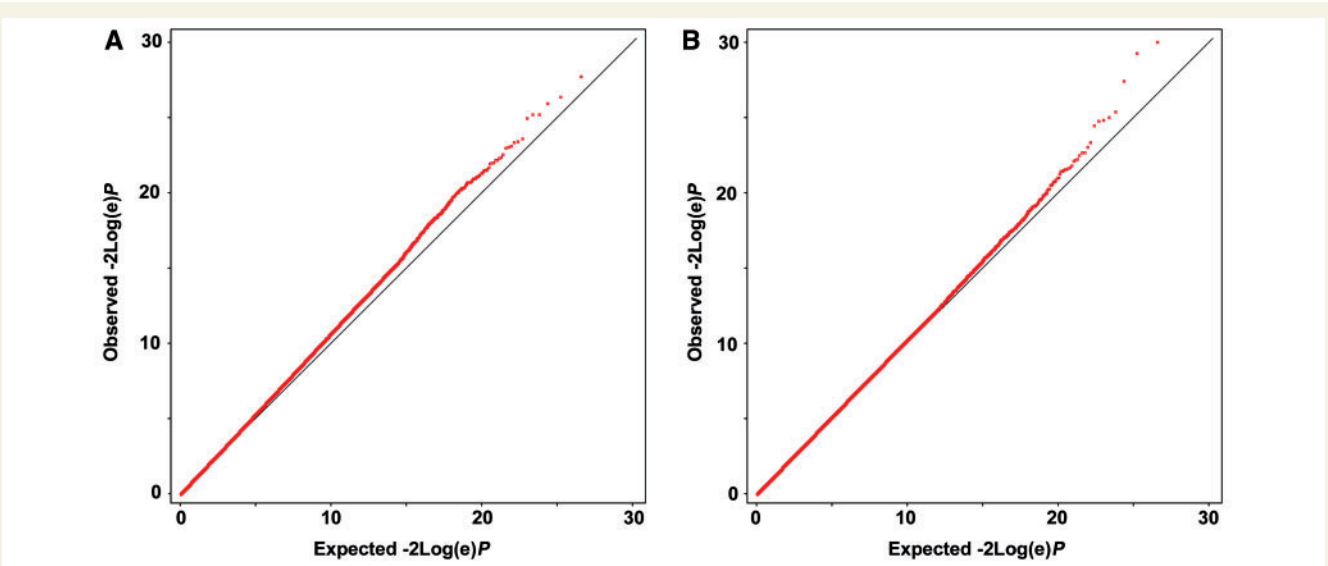

Figure 2 Quantile–quantile plots of *P*-values (red dots) of genome-wide association-analysis in partial epilepsies based on *P*-values calculated using logistic regression and including significant EIGENSTRAT axes as covariates (**A**) and using the Cochran–Mantel–Haenszel test (**B**). Figure generated in WGAviewer (Ge *et al.*, 2008).

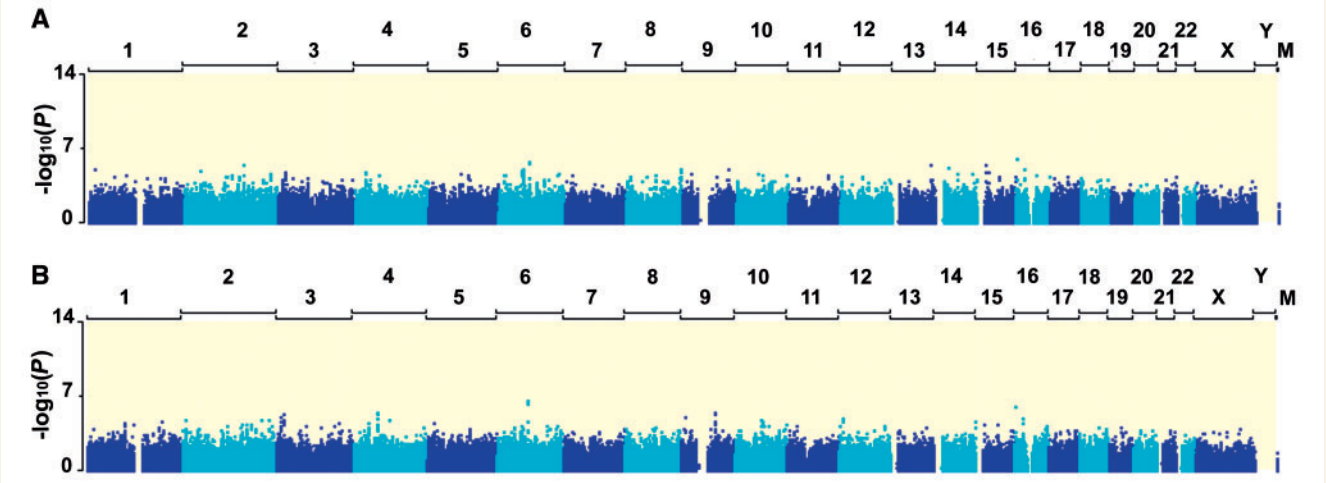

Figure 3 Manhattan plots for genome-wide association-analysis results. $-\log_{10}$ *P*-values of the logistic regression test (**A**) and the Cochran–Mantel–Haenszel test (**B**) for quality-control-positive SNPs are plotted against SNP positions on each chromosome. Chromosomes are shown in alternating colours for clarity.

residual association ($P = 0.0102$), indicating that these two signals are not completely dependent on each other.

The other top associated SNPs lie within interesting candidate genes and may warrant further investigation. The third most-associated SNP, rs2601828 ($P = 1.21 \times 10^{-6}$) is an intronic SNP in the *ADCY9* gene, encoding adenylate cyclase 9, which catalyses the formation of cyclic AMP from ATP and is involved in neuronal signalling. The *PRKCB* gene, which encodes protein kinase C, also involved in neuronal signalling, is another interesting candidate. However, we note that these associations did not reach genome-wide significance in our study and require replication in an independent study.

Further, we performed gene ontology analysis to see whether any gene ontology categories are overrepresented among genes with SNPs with low *P* values (Holmans *et al.*, 2009). Ion-channel and receptor coding genes showed significant enrichment (Tables 4 and 5).

Discussion

The contribution of genetic factors to sporadic partial epilepsies is undoubted, but the identity of these factors remains almost entirely unknown. We show that, for modest (or greater) effect sizes, common SNPs appear not to contribute to causation shared across the partial epilepsies. Previous failed attempts to identify associated variants (including our own) have usually been attributed to small sample sizes coupled with a candidate gene approach, which necessarily relies on the limited existing biological understanding of epilepsy. However, despite the less-biased genome-wide approach and a large cohort, with over 80% power to detect common variants with odds ratio above 1.25–1.3, and close to 100% power to detect common variants with odds ratio above 1.35–1.4 (Fig. 1), we still did not detect genome-wide significant associations. The genotyping platform used has a high genomic coverage—the markers, spaced evenly throughout the genome, tag 87% of common variants (with minor allele frequency above 0.05) with $r^2 > 0.8$, and 95% with r^2 of at least 0.55 in European populations (Illumina technical note, based on HapMap release 24 data, www.illumina.com). Although it is possible that we failed to detect real associations with common variants with high effect sizes, if these variants were not represented or poorly tagged, it is highly unlikely that we missed multiple associations, such as are predicted by the common variant—common disease model, and found in many other complex diseases (e.g. Wellcome Trust Case Control Consortium, 2007). Therefore, it is unlikely that there is any shared common genetic causation for the partial epilepsies that acts across syndromes in European populations.

We only investigated genetic factors shared across partial epilepsies, disregarding the type of partial epilepsy. The differing risks of epilepsy in first-degree relatives of those with idiopathic or cryptogenic partial epilepsy compared to those with symptomatic partial epilepsy have led to the suggestion that genetic influences are primarily restricted to the idiopathic and cryptogenic subgroups (Ottman *et al.*, 1996; Bianchi *et al.*, 2003). Heritabilities vary even among 'idiopathic' partial epilepsy syndromes; for example, inherited factors seem to play only a minor role in benign epilepsy with centrotemporal spikes (Vadlamudi *et al.*, 2006). Notably, the distinction between 'idiopathic', 'cryptogenic' and 'symptomatic' is not always obvious. For example, in some older studies, MRI quality (if MRI had indeed been undertaken) might have been such that some 'symptomatic' were misclassified as 'idiopathic' or 'cryptogenic'. In addition, the recent organization of the epilepsies recommended by the International League Against Epilepsy discourages the use of these terms (Berg *et al.*, 2010). Since the majority of patients in our study were recruited in tertiary clinical centres, our cohort might have included more patients with more severe, lesion-associated epilepsies that may have lower heritabilities, in comparison to the general population of patients with epilepsy, from which heritability estimates are predominantly derived (Kjeldsen *et al.*, 2001).

Table 3 SNPs with $P < 5 \times 10^{-5}$ both in Cochran–Mantel–Haenszel and logistic regression tests

SNP	Chr	Position (NCBI build 36)	Type	Closest gene	P (CMH)	P (LR)	Odds ratio (95% CI, CMH)	Minor allele	MAF in patients	MAF in controls	Genotype counts in patients	Genotype counts in controls
rs346291	6	80 564 836	Within pseudogene	*AL132875.2*	3.34×10^{-7}	2.51×10^{-6}	0.83 (0.77–0.89)	A	0.335	0.366	384/1538/1523	950/3180/2802
rs9341799	6	80 564 519	Within pseudogene	*AL132875.2*	4.82×10^{-7}	2.08×10^{-6}	1.20 (1.12–1.28)	G	0.405	0.373	569/1617/1215	943/3005/2617
rs2601828	16	4 103 871	Intronic	*ADCY9*	1.21×10^{-6}	1.04×10^{-6}	1.21 (1.12–1.31)	A	0.253	0.222	200/1342/1903	349/2380/4206
rs1490157	3	21 719 246	Intronic	*ZNF385D*	5.30×10^{-6}	2.36×10^{-5}	0.83 (0.76–0.90)	G	0.229	0.261	163/1229/2004	444/2538/3572
rs1989647	16	23 959 420	Intronic	*PRKCB*	1.28×10^{-5}	8.89×10^{-6}	1.18 (1.09–1.26)	A	0.351	0.312	423/1536/1438	654/2791/3122
rs1320292	3	21 701 712	Intronic	*ZNF385D*	1.61×10^{-5}	1.82×10^{-5}	0.83 (0.76–0.90)	A	0.208	0.240	140/1127/2116	361/2434/3772
rs951997	2	223 567 016	Intronic	*MOGAT1*	2.00×10^{-5}	4.53×10^{-5}	1.16 (1.08–1.24)	A	0.476	0.443	796/1690/959	1354/3441/2138
rs1942006	10	67 653 901	Intergenic	*CTNNA3*	2.12×10^{-5}	4.07×10^{-5}	1.17 (1.09–1.26)	A	0.300	0.274	306/1451/1687	538/2726/3666
rs1387822	3	21 686 466	Intronic	*ZNF385D*	2.92×10^{-5}	2.51×10^{-5}	0.86 (0.79–0.92)	G	0.298	0.326	294/1462/1688	725/3070/3137
rs1396626	1	96 025 546	Within known processed transcript	*AL683887.1*	3.36×10^{-5}	3.32×10^{-5}	1.17 (1.08–1.25)	A	0.318	0.288	351/1487/1607	585/2823/3522
rs16834756	2	154 745 009	Intronic	*GALNT13*	4.89×10^{-5}	3.68×10^{-6}	0.67 (0.55–0.81)	G	0.030	0.046	6/190/3205	9/582/5973

CMH = Cochran–Mantel–Haenszel; LR = logistic regression; Chr = chromosome; CI = confidence intervals; MAF = minor allele frequency.

Given these considerations, it is possible that syndrome-specific common genetic causes do exist, and that a genome-wide association-study in a more homogeneous and narrowly-defined group of patients might detect them. Gathering patients with a single syndrome in cohorts big enough for adequately-powered genome-wide association-studies will be challenging, especially if population structure is also considered.

There may yet be susceptibility loci shared across the partial epilepsies, but with comparatively small (<1.35) effect sizes, a phenomenon observed for both normal traits [e.g. height (Soranzo *et al.,* 2009), pulmonary function (Hancock *et al.,* 2010)] and other common diseases [e.g. obesity (Willer *et al.,* 2009), diabetes (Barrett *et al.,* 2009)]. Discovery of such small-effect variants is unlikely to have immediate clinical application, but could be illuminating for disease biology.

For detection of common causative variants specific to partial epilepsy syndromes, or those of small effect size shared across the partial epilepsies, the assembly of much larger cohorts will be necessary as has been successfully achieved for other conditions, such as diabetes (Barrett *et al.,* 2009) and multiple sclerosis (De Jager *et al.,* 2009). Such an effort is underway for meta-analysis of genetic data in epilepsy, and we urge all interested groups with data to join in with this important effort (please contact Prof. S. Berkovic, Chair, Genetics Commission, International League Against Epilepsy: s.berkovic@unimelb.edu.au). The top hits in our study, as well as the gene ontology analysis results, hint at possible insights into mechanisms in the partial epilepsies and may warrant further investigation—but this will also require larger cohorts. It is now difficult to justify smaller, under-powered, genome-wide association-studies of susceptibility to partial epilepsies in pan-syndromic cohorts of European ancestry.

We only investigated patients of European ancestry. The types and proportions of acquired epilepsies with known causes differ across populations. For example, the higher rates of epilepsy in some developing countries are thought mainly to be due to neurocysticercosis (García *et al.*, 2003). It is also possible that due to genetic differences between populations, genetic factors influencing susceptibility to sporadic partial epilepsies also differ. But it is still likely that large cohorts will be needed to discover such variants or to exclude their role in partial epilepsies in populations of other ethnicities.

More broadly, these results suggest the need to re-evaluate strategies for detection of causal genetic variants in the partial epilepsies: the genetic and mechanistic architecture of the partial epilepsies must be more complex or heterogeneous than considered here. In contrast to the common variant-common disease approach, recent discoveries of putatively-causal structural abnormalities (e.g. de Kovel *et al.,* 2009, Heinzen *et al.,* 2010) show that rare variants with large effect sizes can cause a broad spectrum of epilepsies, and can even manifest with different neuropsychiatric conditions. Therefore, it seems likely that rare, or even individual, variants might constitute a substantial proportion of genetic causes in partial epilepsies, accounting for the 'missing heritability' (Manolio *et al.,* 2009). Similar findings have emerged in other neuropsychiatric conditions (Merikangas *et al.,* 2009).

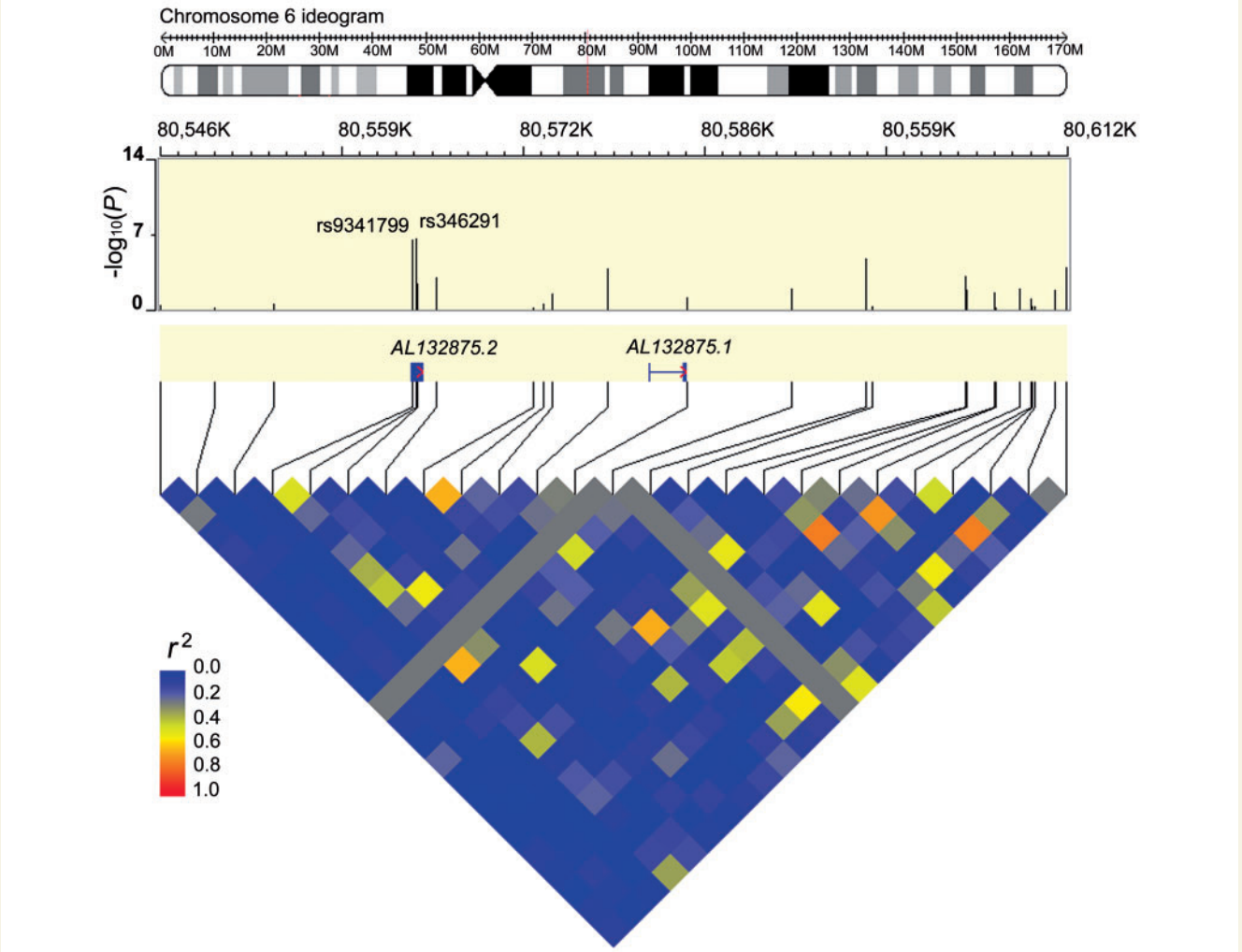

Figure 4 Overview of chromosome 6 region with top associated SNPs. *Top*: $-\log_{10}$ *P*-values for Cochran–Mantel–Haenszel test, each bar represents a SNP; *bottom*: linkage disequilibrium structure for this region in HapMap CEU samples, colouring according to r^2. Figure generated in WGAviewer (Ge *et al.*, 2008).

Table 4 Results of gene ontology analysis for partial epilepsies associated SNPs with $P<0.0001$ (Cochran–Mantel–Haenszel test), gene ontology categories with enrichment $P<0.05$

Gene ontology number	Type	Total number genes in category	Number of genes on list	Expected number of genes on list	*P*-value	Function
GO:0005230	FUNCTION	69	3	0.3	0.0032	Extracellular ligand-gated ion channel activity
GO:0005234	FUNCTION	19	2	0.2	0.016	Extracellular-glutamate-gated ion channel activity
GO:0004970	FUNCTION	18	2	0.2	0.016	Ionotropic glutamate receptor activity
GO:0015276	FUNCTION	112	3	0.58	0.019	Ligand-gated ion channel activity
GO:0022834	FUNCTION	112	3	0.58	0.019	Ligand-gated channel activity
GO:0005231	FUNCTION	46	2	0.23	0.021	Excitatory extracellular ligand-gated ion channel activity
GO:0004888	FUNCTION	832	6	2.28	0.022	Transmembrane receptor activity
GO:0044248	PROCESS	839	4	1.22	0.032	Cellular catabolic process
GO:0046982	FUNCTION	125	2	0.29	0.032	Protein heterodimerization activity
GO:0045211	CELLULAR	115	3	0.72	0.035	Postsynaptic membrane
GO:0022836	FUNCTION	277	4	1.25	0.035	Gated channel activity
GO:0016788	FUNCTION	547	4	1.27	0.036	Hydrolase activity, acting on ester bonds
GO:0000122	PROCESS	148	2	0.31	0.039	Negative regulation of transcription from RNA polymerase II promoter
GO:0008066	FUNCTION	29	2	0.32	0.040	Glutamate receptor activity
GO:0005529	FUNCTION	145	2	0.32	0.041	Sugar binding
GO:0009056	PROCESS	952	4	1.41	0.049	Catabolic process

Table 5 Results of gene ontology analysis for partial epilepsies associated SNPs with $P < 0.001$ (Cochran–Mantel–Haenszel test), gene ontology categories with enrichment P-values < 0.01

Gene ontology number	Type	Total number genes in category	Number of genes on list	Expected number of genes on list	P-value	Function
GO:0005272	FUNCTION	30	5	0.71	0.00005	Sodium channel activity
GO:0001518	CELLULAR	12	3	0.2	0.0007	Voltage-gated sodium channel complex
GO:0034706	CELLULAR	12	3	0.2	0.0007	Sodium channel complex
GO:0005248	FUNCTION	15	3	0.22	0.0008	Voltage-gated sodium channel activity
GO:0022836	FUNCTION	277	15	7.17	0.0043	Gated channel activity
GO:0030324	PROCESS	58	4	0.72	0.0051	Lung development
GO:0005882	CELLULAR	85	3	0.38	0.0058	Intermediate filament
GO:0045111	CELLULAR	86	3	0.39	0.0061	Intermediate filament cytoskeleton
GO:0046873	FUNCTION	282	14	6.88	0.0062	Metal ion transmembrane transporter activity
GO:0006368	PROCESS	35	2	0.13	0.0068	RNA elongation from RNA polymerase II promoter
GO:0030323	PROCESS	60	4	0.81	0.0075	Respiratory tube development
GO:0006354	PROCESS	38	2	0.14	0.0079	RNA elongation
GO:0005216	FUNCTION	341	16	8.38	0.0084	Ion channel activity
GO:0022838	FUNCTION	349	16	8.41	0.0087	Substrate specific channel activity
GO:0015267	FUNCTION	355	16	8.42	0.0088	Channel activity
GO:0022803	FUNCTION	355	16	8.42	0.0088	Passive transmembrane transporter activity
GO:0006213	PROCESS	8	2	0.23	0.0091	Pyrimidine nucleoside metabolic process
GO:0048286	PROCESS	10	2	0.16	0.0094	Alveolus development
GO:0008266	FUNCTION	5	2	0.2	0.0097	Poly(U) binding

Our study provides further support for a rare variant(s)—common disease model (Iyengar and Elston, 2007). We did detect a slight excess of SNPs with borderline significant P-values, and our most associated SNPs suggest multiple independent signals clustered in the same genomic region. The strongest signals in our study are generated by two SNPs in only moderate linkage disequilibrium with each other ($r^2 = 0.34$) and the association is not completely explained by either SNP alone. Such independent signals of common variants are the typical features of 'synthetic' associations (Dickson *et al.*, 2010), actually caused by one or several rare variants with larger effect sizes. Although the causal variants can be located at relatively long distances from the 'associated' common alleles, the detected common variant associations are the best available pointers to the genomic regions where some of the 'missing heritability' of partial epilepsies might lie.

The results of our study, combined with emerging knowledge from studies of rare structural variants, strongly promote a shift to the next stage of epilepsy genetics—not only large meta-analyses, but also the search for rare variants with larger effects using exome or whole-genome sequencing. The raw data from genome-wide association-studies, such as ours, can provide a powerful tool with which upcoming sequencing findings could be more successfully interpreted.

Acknowledgements

We thank the patients who kindly participated, and the physicians who recruited them. We thank Eylert Brodtkorb, Bernt Engelsen, Morten Lossius, Karl Otto Nakken, Erik Taubøl and Erik Sætre for their contribution to the Norwegian part of GenEpA sudy.
We thank Dr Liisa Myllykangas (Folkhalsan Institute of Genetics and Department of Pathology, University of Helsinki), Dr Raimo Sulkava (Department of Public Health and General Practice Division of Geriatrics, University of Kuopio) and Dr Pentti Tienari (Molecular Neurology Programme, Biomedicum, University of Helsinki and Department of Neurology, Helsinki University Central Hospital) for providing the Vantaa85+ study genotypes.
This study makes use of data generated by the Wellcome Trust Case-Control Consortium. A full list of the investigators who contributed to the generation of the data is available from www.wtccc.org.uk. Funding for that project was provided by the Wellcome Trust under award 076113.

Funding

This work was supported by grants from the Medical Research Council (G0400126); The Wellcome Trust (084730); UCLH CRDC (F136); the National Institute for Health Research (08-08-SCC); the National Society for Epilepsy. This work was partly undertaken at UCLH/UCL, which received a proportion of funding from the Department of Health's NIHR Biomedical Research Centres funding scheme; The collection of the Irish patient cohort was supported by the Irish Higher Education Authority Programme for Research in Third Level Institutions (PRTLI3); phenotyping by a Science Foundation Ireland Research Frontiers Programme award (08/RFP/GEN1538); GlaxoSmithKline funded the recruitment and phenotypic data collection of the GenEpA Consortium samples used in this study and contributed to the

genotyping costs associated with their study; the collection of the Belgian patients was supported by the Funds National de la Recherche Scientifique, grant no. FC 63574/3.4.620.06 F and the Fondation Erasme, Université Libre de Bruxelles; Funding support for Study of Irish Amyotrophic Lateral Sclerosis was provided by Muscular Dystrophy Association, USA; Irish Institute of Clinical Neurosciences Travel Award; and National Institutes of Health, USA and the genotyping of samples was provided by the National Institute of Neurological Disorders and Stroke (NINDS). The dataset used for the analyses described in this manuscript was obtained from the NINDS Database found at http://www.ncbi.nlm.nih.gov/gap through dbGaP accession number phs000127.v1.p1.

Supplementary material

Supplementary material is available at *Brain* online.

References

Barrett JC, Clayton DG, Concannon P, Akolkar B, Cooper JD, Erlich HA, et al. Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. Nat Genet 2009; 41: 703–7.

Begley CE, Baker GA, Beghi E, Butler J, Chisholm D, Langfitt JT, et al. Cross-country measures for monitoring epilepsy care. Epilepsia 2007; 48: 990–1001.

Berg AT, Berkovic SF, Brodie MJ, Buchhalter J, Cross JH, van Emde Boas W, et al. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005–2009. Epilepsia 2010; 51: 676–685.

Berkovic SF, Howell RA, Hay DA, Hopper JL. Epilepsies in twins: genetics of the major epilepsy syndromes. Ann Neurol 1998; 43: 435–45.

Berkovic SF, Serratosa JM, Phillips HA, Xiong L, Andermann E, Díaz-Otero F, et al. Familial partial epilepsy with variable foci: clinical features and linkage to chromosome 22q12. Epilepsia 2004; 45: 1054–60.

Bianchi A, Viaggi S, Chiossi E. Family study of epilepsy in first degree relatives: data from the Italian Episcreen Study. Seizure 2003; 12: 203–10.

Cavalleri GL, Lynch JM, Depondt C, Burley MW, Wood NW, Sisodiya SM, et al. Failure to replicate previously reported genetic associations with sporadic temporal lobe epilepsy: where to from here? Brain 2005; 128: 1832–40.

Cavalleri GL, Weale ME, Shianna KV, Singh R, Lynch JM, Grinton B, et al. Multicentre search for genetic susceptibility loci in sporadic epilepsy syndrome and seizure types: a case-control study. Lancet Neurol 2007; 6: 970–80.

Cirulli ET, Kasperaviciute D, Attix DK, Need AC, Ge D, Gibson G, et al. Common genetic variation and performance on standardized cognitive tests. Eur J Hum Genet 2010; Epub ahead of print.

Commission on Classification and Terminology of the International League Against Epilepsy. Proposal for revised classification of epilepsies and epileptic syndromes. Epilepsia 1989; 30: 389–99.

Cronin S, Berger S, Ding J, Schymick JC, Washecka N, Hernandez DG, et al. A genome-wide association study of sporadic ALS in a homogenous Irish population. Hum Mol Genet 2008; 17: 768–74.

De Jager PL, Jia X, Wang J, de Bakker PI, Ottoboni L, Aggarwal NT, et al. Meta-analysis of genome scans and replication identify CD6, IRF8 and TNFRSF1A as new multiple sclerosis susceptibility loci. Nat Genet 2009; 41: 776–82.

de Kovel CG, Trucks H, Helbig I, Mefford HC, Baker C, Leu C, et al. Recurrent microdeletions at 15q11.2 and 16p13.11 predispose to idiopathic generalized epilepsies. Brain 2010; 133: 23–32.

Dickson SP, Wang K, Krantz I, Hakonarson H, Goldstein DB. Rare variants create synthetic genome-wide associations. PloS Biol 2010; 8: e1000294.

Fellay J, Shianna KV, Ge D, Colombo S, Ledergerber B, Weale M, et al. A whole-genome association study of major determinants for host control of HIV-1. Science 2007; 317: 944–7.

Frankel WN. Genetics of complex neurological disease: challenges and opportunities for modeling epilepsy in mice and rats. Trends Genet 2009; 25: 361–7.

García HH, Gonzalez AE, Evans CA, Gilman RH. Cysticercosis Working Group in Peru. Taenia solium cysticercosis. Lancet 2003; 362: 547–56.

Ge D, Zhang K, Need AC, Martin O, Fellay J, Urban TJ, et al. WGAViewer: software for genomic annotation of whole genome association studies. Genome Res 2008; 18: 640–3.

Hancock DB, Eijgelsheim M, Wilk JB, Gharib SA, Loehr LR, Marciante KD, et al. Meta-analyses of genome-wide association studies identify multiple loci associated with pulmonary function. Nat Genet 2010; 42: 45–52.

Heinzen EL, Radtke RA, Urban TJ, Cavalleri GL, Depondt C, Need AC, et al. Rare deletions at 16p13.11 predispose to a diverse spectrum of sporadic epilepsy syndromes. Am J Hum Genet 2010; 86: 707–18.

Hemminki K, Li X, Johansson SE, Sundquist K, Sundquist J. Familial risks for epilepsy among siblings based on hospitalizations in Sweden. Neuroepidemiology 2006; 27: 67–73.

Holmans P, Green EK, Pahwa JS, Ferreira MA, Purcell SM, Sklar P, et al. Gene ontology analysis of GWA study data sets provides insights into the biology of bipolar disorder. Am J Hum Genet 2009; 85: 13–24.

Iyengar SK, Elston RC. The genetic basis of complex traits: rare variants or ''common gene, common disease''? Methods Mol Biol 2007; 376: 71–84.

Kjeldsen MJ, Kyvik KO, Christensen K, Friis ML. Genetic and environmental factors in epilepsy: a population-based study of 11900 Danish twin pairs. Epilepsy Res 2001; 44: 167–78.

Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, et al. Finding the missing heritability of complex diseases. Nature 2009; 461: 747–53.

McCarthy MI, Abecasis GR, Cardon LR, Goldstein DB, Little J, Ioannidis JP, et al. Genome-wide association studies for complex traits: consensus, uncertainty and challenges. Nat Rev Genet 2008; 9: 356–69.

Menashe I, Rosenberg PS, Chen BE. PGA: power calculator for case-control genetic association analyses. BMC Genet 2008; 9: 36.

Merikangas AK, Corvin AP, Gallagher L. Copy-number variants in neurodevelopmental disorders: promises and challenges. Trends Genet 2009; 25: 536–44.

Miller LL, Pellock JM, DeLorenzo RJ, Meyer JM, Corey LA. Univariate genetic analyses of epilepsy and seizures in a population-based twin study: the Virginia Twin Registry. Genet Epidemiol 1998; 15: 33–49.

Myllykangas L, Wavrant-De Vrièze F, Polvikoski T, Notkola IL, Sulkava R, Niinistö L, et al. Chromosome 21 BACE2 haplotype associates with Alzheimer's disease: a two-stage study. J Neurol Sci 2005; 236: 17–24.

Need AC, Attix DK, McEvoy JM, Cirulli ET, Linney KL, Hunt P, et al. A genome-wide study of common SNPs and CNVs in cognitive performance in the CANTAB. Hum Mol Genet 2009; 18: 4650–61.

Ottman R, Annegers JF, Risch N, Hauser WA, Susser M. Relations of genetic and environmental factors in the etiology of epilepsy. Ann Neurol 1996; 39: 442–9.

Ottman R, Lee JH, Hauser WA, Risch N. Are generalized and localization-related epilepsies genetically distinct? Arch Neurol 1998; 55: 339–44.

Peuralinna T, Oinas M, Polvikoski T, Paetau A, Sulkava R, Niinistö L, et al. Neurofibrillary tau pathology modulated by genetic variation of alpha-synuclein. Ann Neurol 2008; 64: 348–52.

Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet 2006; 38: 904–9.

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007; 81: 559–75.

Soranzo N, Rivadeneira F, Chinappen-Horsley U, Malkina I, Richards JB, Hammond N, et al. Meta-analysis of genome-wide scans for human adult stature identifies novel loci and associations with measures of skeletal frame size. PLoS Genet 2009; 5: e1000445.

Tan NC, Mulley JC, Berkovic SF. Genetic association studies in epilepsy: ''the truth is out there''. Epilepsia 2004; 45: 1429–42.

Vadlamudi L, Kjeldsen MJ, Corey LA, Solaas MH, Friis ML, Pellock JM. Analyzing the etiology of benign rolandic epilepsy: a multicenter twin collaboration. Epilepsia 2006; 47: 550–5.

Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007; 447: 661–78.

Willer CJ, Speliotes EK, Loos RJ, Li S, Lindgren CM, Heid IM, et al. Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. Nat Genet 2009; 41: 25–34.