

Epilepsy, hippocampal sclerosis and febrile seizures linked by common genetic variation around *SCN1A*

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Epilepsy comprises several syndromes, amongst the most common being mesial temporal lobe epilepsy with hippocampal sclerosis. Seizures in mesial temporal lobe epilepsy with hippocampal sclerosis are typically drug-resistant, and mesial temporal lobe epilepsy with hippocampal sclerosis is frequently associated with important co-morbidities, mandating the search for better understanding and treatment. The cause of mesial temporal lobe epilepsy with hippocampal sclerosis is unknown, but there is an association with childhood febrile seizures. Several rarer epilepsies featuring febrile seizures are caused by mutations in *SCN1A*, which encodes a brain-expressed sodium channel subunit targeted by many anti-epileptic drugs. We undertook a genome-wide association study in 1018 people with mesial temporal lobe epilepsy with hippocampal sclerosis and 7552 control subjects, with validation in an independent sample set comprising 959 people with mesial temporal lobe epilepsy with hippocampal sclerosis and 3591 control subjects. To dissect out variants related to a history of febrile seizures, we tested cases with mesial temporal lobe epilepsy with hippocampal sclerosis with (overall $n = 757$) and without (overall $n = 803$) a history of febrile seizures. Meta-analysis revealed a genome-wide significant association for mesial temporal lobe epilepsy with hippocampal sclerosis with febrile seizures at the sodium channel gene cluster on chromosome 2q24.3 [rs7587026, within an intron of the *SCN1A* gene, $P = 3.36 \times 10^{-9}$, odds ratio (A) = 1.42, 95% confidence interval: 1.26–1.59]. In a cohort of 172 individuals with febrile seizures, who did not develop epilepsy during prospective follow-up to age 13 years, and 6456 controls, no association was found for rs7587026 and febrile seizures. These findings suggest *SCN1A* involvement in a common epilepsy syndrome, give new direction to biological understanding of mesial temporal lobe epilepsy with hippocampal sclerosis with febrile seizures, and open avenues for investigation of prognostic factors and possible prevention of epilepsy in some children with febrile seizures.

Keywords: mesial temporal lobe epilepsy; mesial temporal sclerosis; *SCN1A*; association; complex genetics

Abbreviations: MTLEHS = mesial temporal lobe epilepsy with hippocampal sclerosis; MTLEHS + FS = MTLEHS with febrile seizures; MTLEHS–FS = MTLEHS without febrile seizures

Introduction

Mesial temporal lobe epilepsy with hippocampal sclerosis (MTLEHS) is typically a serious epilepsy syndrome and the most common drug-resistant epilepsy (Berg *et al.*, 2010). It is associated with burdensome co-morbidities, such as memory and psychiatric disorders. MTLEHS is the epilepsy most considered for therapeutic neurosurgery. Although surgery is a proven therapy, only ~50% of patients have sustained postoperative seizure freedom (de Tisi *et al.*, 2011), and surgery can have important adverse consequences. Better treatment options, or even prevention, of MTLEHS are therefore needed, but rational therapy for MTLEHS remains elusive because its causes are obscure (O'Dell *et al.*, 2012).

MTLEHS is associated with a history of febrile seizures in childhood (Pittau *et al.*, 2009; O'Dell *et al.*, 2012). About 3% of children have febrile seizures; why only some go on to develop epilepsy, including MTLEHS, is unknown. There are a number of rare, genetically-determined, epilepsy syndromes in which febrile seizures are a prominent feature, such as Dravet syndrome and 'genetic epilepsy with febrile seizures plus' (GEFS+) (Oliva *et al.*, 2012). MTLEHS has rarely been described in families with GEFS+ (Abou-Khalil *et al.*, 2001) or familial febrile seizures (Mantegazza *et al.*, 2005) associated with *SCN1A* mutations. In familial mesial temporal lobe epilepsy, some family members may have hippocampal sclerosis (Labate *et al.*, 2011). A cluster of families with mesial temporal lobe epilepsy with hippocampal changes has been described in Brazil (Andrade-Valença *et al.*, 2008). Together, this evidence implies genetic susceptibility to MTLEHS, although its heritability is unknown.

We hypothesized that MTLEHS, or MTLEHS with febrile seizures, as common epilepsy syndromes, might be associated with common genetic variation, and tested this 'common disease-common variant' hypothesis in a genetic association study.

Materials and methods

All aspects of the study were approved by the relevant institutional review board. All participants gave written informed consent.

Subjects

Patients were recruited during clinical appointments. MTLEHS was defined as in Wieser (2004). The diagnosis was made and/or reviewed by a consultant epileptologist who was part of this study, with access to history and investigation results. Patients with bilateral hippocampal sclerosis or dual pathology were excluded. One thousand and eighteen patients were included in the discovery stage and 959 patients in the replication. The number of patients by country is shown in Table 1, with further details in Supplementary Table 1. A history of presence or absence of febrile seizures was accepted only if contemporary medical records or a parental account was available; otherwise it was

considered unknown, and not eligible for analysis. Population-based controls ($n = 7552$) were included in the discovery stage, and 3591 in the replication (Table 1 and Supplementary Table 1).

We also studied 542 individuals who had had febrile seizures but by the last follow-up had not had unprovoked seizures. These came from three groups: a German group; an Austrian group and the ALSPAC (Avon Longitudinal Study of Parents and Children) cohort, the latter followed to age 13 years (Supplementary material); MTLEHS after febrile seizures almost always develops by the age of 15 (Neligan *et al.*, 2012). These cases were compared with 7387 control subjects from three relevant populations (Table 1). For the German and Austrian samples, the same controls as in the MTLEHS study were used.

To minimize population stratification, only individuals of white European ancestry were included. In the discovery stage, a combination of self-identified ancestry and EIGENSTRAT principal component methods was used to determine European ancestry. In the replication and febrile seizures analyses, only self-reported white individuals of European ancestry were included. More detailed ancestry data were available from all sources except Austria, allowing exclusion of individuals self-reported as coming from countries other than those where they were recruited.

Genotyping and quality control

In the discovery stage, all but the Austrian samples and Belgian controls comprised a subset of a previously described data set (Kasperavičiūtė *et al.*, 2010), genotyped on Illumina genome-wide genotyping chips, mostly on Illumina Human610-QuadV1/Human1-2M-DuoCustom. One hundred and fifty-seven Austrian patients and 332 controls were genotyped on Illumina HumanCNV370duo, and 285 Belgian controls were genotyped on Illumina HumanHap300 genotyping chips. Gender and relatedness checks were performed on all samples. The cluster plots of the top-associated single nucleotide polymorphisms were inspected manually. Details are given in Kasperavičiūtė *et al.* (2010) and in the online Supplementary material. For replication analysis, several methods were used for genotyping.

Statistical analysis

In the discovery stage, genome-wide association analysis was performed using PLINK. Only single nucleotide polymorphisms present on both Illumina Human610-QuadV1 and Human1-2M-DuoCustom were analysed. In the discovery stage, we performed logistic regression using an additive model, including all significant EIGENSTRAT axes (assessed using the Tracy-Widom statistic with $P < 0.05$) as covariates. Only single nucleotide polymorphisms with minor allele frequency of $\geq 1\%$ were analysed. Since the replication samples did not have genome-wide data available to calculate EIGENSTRAT axes, we performed stratified analysis using the Cochran-Mantel-Haenszel test for $2 \times 2 \times 8$ stratified case-control subsamples deriving from eight different recruitment countries and self-identified ancestry, using R. The Woolf test was used to assess effect heterogeneity. Meta-analysis of discovery and replication studies was performed using the inverse variance-weighted fixed-effects model as implemented in the GWAMA

Table 1 Number of individuals included in the study, after removal of population outliers and individuals of non-European ancestry

| Population | Patients with MTLEHS | Individuals with a definite history of febrile seizures | Individuals with a definite history of no febrile seizures | Controls |
|-------------------------------|----------------------|---|--|-------------|
| Discovery | | | | |
| Austria | 157 | 45 | 104 | 332 |
| Belgium | 67 | 23 | 20 | 285 |
| USA | 71 | 23 | 45 | 605 |
| Finland | 116 | 18 | 0* | 746 |
| Ireland | 148 | 54 | 90 | 209 |
| UK | 277 | 117 | 101 | 5116 |
| Switzerland | 182 | 61 | 0* | 259 |
| Total discovery | 1018 | 341 | 360 | 7552 |
| Replication | | | | |
| Austria | 57 | 18 | 39 | 254 |
| Germany | 273 | 112 | 161 | 346 |
| Portugal | 102 | 54 | 48 | 190 |
| UK | 80 | 42 | 28 | 857 |
| Netherlands | 164 | 74 | 0* | 601 |
| Italy | 44 | 18 | 26 | 249 |
| Australia | 162 | 83 | 79 | 794 |
| USA | 77 | 15 | 62 | 300 |
| Total replication | 959 | 416 | 443 | 3591 |
| Febrile seizures study | | | | |
| Austria | NA | 158 | NA | 585** |
| Germany | NA | 212 | NA | 346*** |
| UK (ALSPAC) | NA | 172 | NA | 6456 |
| Total febrile seizures | NA | 542 | NA | 7387 |

NA = not applicable.

*Data were not collected according to the criteria used in the study.

**Combined discovery and replication Austrian controls.

***Same as replication German controls.

software (Mägi and Morris, 2010). We considered an association to be genome-wide significant at $P < 5 \times 10^{-8}$.

To fine map the association signal in the discovery stage, we imputed single nucleotide polymorphisms in the 10 Mb region surrounding rs7587026. Imputation was performed using MINIMAC (Howie *et al.*, 2012), and 1000 Genomes Project data (1000 Genomes Project Consortium *et al.*, 2010) as the reference data set. Subsequent association analysis was performed using MACH2DAT (Li *et al.*, 2010) using significant EIGENSTRAT axes as covariates.

Power calculations were performed using Genetic Power Calculator (Purcell *et al.*, 2003).

Expression analysis

We tested association between genotypes of the two top single nucleotide polymorphisms rs7587026 and rs11692675 and *SCN1A* exons and gene expression in the middle temporal cortex (Brodmann areas 20 and 21) from 78 patients with MTLEHS who had undergone surgical resection, compared with 78 neurologically normal individuals from the MRC Sudden Death Brain and Tissue Bank. We specifically chose not to study the hippocampus to avoid confounding due to tissue changes such as cell loss and gliosis. All samples were randomly hybridized to Affymetrix Human Exon 1.0 ST arrays. Differential expression of *SCN1A* transcripts incorporating the 'neonatal' or 'adult' exon 5 form (5N or 5A exon, respectively), and expression of non-coding exons 1a and 1b (GenBank accession numbers DQ993522

and DQ993523, respectively) (Martin *et al.*, 2007) in the 5' region of *SCN1A*, were tested by quantitative RT-PCR as they are not covered by the array. Details are provided in the Supplementary material.

Further, we tested whether the associated single nucleotide polymorphisms have an effect on expression or splicing of any genes in the genome in post-mortem tissue of nine brain regions from 134 control individuals (Supplementary material).

Results

Genome-wide association analyses

We performed a two-stage study. For discovery, we first investigated genome-wide association between all MTLEHS and 531 164 single nucleotide polymorphisms in 1018 MTLEHS cases and 7552 controls from seven populations of European descent (Table 1 and Supplementary Table 1). Using logistic regression analysis and correcting for population stratification, suggestive association emerged for three single nucleotide polymorphisms in a region of strong linkage disequilibrium on chromosome 2q24.3 encompassing *SCN1A* and other sodium channel genes (Supplementary Fig. 1). The most strongly associated single nucleotide polymorphism, rs11692675, is within intron 3 of the *SCN1A* full-length

Table 2 Genotype counts, allele frequencies and association results for rs7587026 and rs11692675 SNPs in the MTL EHS study

| SNP | n patients | n controls | Minor allele | Genotype count in patients | Genotype count in controls | Minor allele frequency in patients | Minor allele frequency in controls | P-value* | Odds ratio (95% CI) |
|--|------------|------------|--------------|----------------------------|----------------------------|------------------------------------|------------------------------------|-----------------------|---------------------|
| Discovery | | | | | | | | | |
| MTLEHS versus controls | | | | | | | | | |
| rs7587026 | 1017 | 7549 | A | 99/440/478 | 536/2895/4118 | 0.314 | 0.263 | 1.19×10^{-7} | 1.31 (1.19–1.45) |
| rs11692675 | 1018 | 7547 | G | 147/477/394 | 794/3352/3401 | 0.379 | 0.327 | 5.26×10^{-8} | 1.31 (1.19–1.44) |
| MTLEHS + FS versus controls | | | | | | | | | |
| rs7587026 | 341 | 7549 | A | 43/161/137 | 536/2895/4118 | 0.362 | 0.263 | 2.64×10^{-8} | 1.59 (1.35–1.87) |
| rs11692675 | 341 | 7547 | G | 61/163/117 | 794/3352/3401 | 0.418 | 0.327 | 1.25×10^{-6} | 1.49 (1.27–1.75) |
| MTLEHS – FS versus controls | | | | | | | | | |
| rs7587026 | 359 | 6544 | A | 30/143/186 | 469/2528/2547 | 0.283 | 0.265 | 0.21 | 1.12 (0.94–1.33) |
| rs11692675 | 360 | 6542 | G | 48/167/145 | 698/2951/2893 | 0.365 | 0.332 | 0.039 | 1.19 (1.01–1.40) |
| MTLEHS + FS versus MTL EHS – FS | | | | | | | | | |
| rs7587026 | 341 | 359 | A | 43/161/137 | 30/143/186 | 0.362 | 0.283 | 1.12×10^{-3} | 1.48 (1.17–1.87) |
| rs11692675 | 341 | 360 | G | 61/163/117 | 48/167/145 | 0.418 | 0.365 | 0.030 | 1.28 (1.03–1.59) |
| Replication | | | | | | | | | |
| MTLEHS versus controls | | | | | | | | | |
| rs7587026 | 933 | 3537 | A | 89/360/484 | 247/1361/1929 | 0.288 | 0.262 | 0.025 | 1.15 (1.02–1.29) |
| rs11692675 | 826 | 3568 | G | 108/364/354 | 394/1615/1559 | 0.351 | 0.337 | 0.19 | 1.08 (0.96–1.21) |
| MTLEHS + FS versus controls | | | | | | | | | |
| rs7587026 | 406 | 3537 | A | 43/163/200 | 247/1361/1929 | 0.307 | 0.262 | 5.88×10^{-3} | 1.26 (1.07–1.48) |
| rs11692675 | 371 | 3568 | G | 56/156/159 | 394/1615/1559 | 0.361 | 0.337 | 0.12 | 1.14 (0.97–1.34) |
| MTLEHS – FS versus controls | | | | | | | | | |
| rs7587026 | 436 | 2972 | A | 42/160/234 | 216/1136/1620 | 0.280 | 0.264 | 0.20 | 1.11 (0.95–1.31) |
| rs11692675 | 357 | 2983 | G | 42/164/151 | 336/1332/1315 | 0.347 | 0.336 | 0.35 | 1.08 (0.92–1.27) |
| MTLEHS + FS versus MTL EHS – FS | | | | | | | | | |
| rs7587026 | 338 | 436 | A | 35/137/166 | 42/160/234 | 0.306 | 0.280 | 0.22 | 1.16 (0.93–1.44) |
| rs11692675 | 298 | 357 | G | 46/125/127 | 42/164/151 | 0.364 | 0.347 | 0.50 | 1.09 (0.87–1.36) |
| Combined (meta-analysis) | | | | | | | | | |
| MTLEHS versus controls | | | | | | | | | |
| rs7587026 | 1950 | 11 086 | A | 188/800/962 | 783/4256/6047 | 0.302 | 0.263 | 3.78×10^{-8} | 1.24 (1.15–1.34) |
| rs11692675 | 1844 | 11 115 | G | 255/841/748 | 1188/4967/4960 | 0.366 | 0.330 | 4.87×10^{-7} | 1.21 (1.12–1.30) |
| MTLEHS + FS versus controls | | | | | | | | | |
| rs7587026 | 747 | 11 086 | A | 86/324/337 | 783/4256/6047 | 0.332 | 0.263 | 3.36×10^{-9} | 1.42 (1.26–1.59) |
| rs11692675 | 712 | 11 115 | G | 117/319/276 | 1188/4967/4960 | 0.388 | 0.330 | 4.78×10^{-6} | 1.30 (1.16–1.46) |
| MTLEHS – FS versus controls | | | | | | | | | |
| rs7587026 | 795 | 9516 | A | 72/303/420 | 685/3664/5167 | 0.281 | 0.265 | 0.067 | 1.12 (0.99–1.25) |
| rs11692675 | 717 | 9525 | G | 90/331/296 | 1034/4283/4208 | 0.356 | 0.333 | 0.033 | 1.13 (1.01–1.27) |
| MTLEHS + FS versus MTL EHS – FS | | | | | | | | | |
| rs7587026 | 679 | 795 | A | 78/298/303 | 72/303/420 | 0.334 | 0.281 | 1.53×10^{-3} | 1.30 (1.10–1.52) |
| rs11692675 | 639 | 717 | G | 107/288/244 | 90/331/296 | 0.393 | 0.356 | 0.039 | 1.18 (1.01–1.38) |

*In discovery stage, *P*-value is logistic regression *P*-value for additive genetic model; in replication stage, Cochran-Mantel-Haenszel test *P*-value.

transcript variant (NM_001202435.1) ($P = 5.26 \times 10^{-8}$, odds ratio for G allele [OR(G)] 1.31, 95% confidence interval (CI) 1.19–1.44; Table 2). Two other single nucleotide polymorphisms within *SCN1A* intron 1, had similarly low *P*-values: rs7587026 ($r^2 = 0.806$ with rs11692675 in CEU population based on 1000 Genomes data set), $P = 1.19 \times 10^{-7}$ [OR(A) = 1.31, 95% CI: 1.19–1.45]; and rs580041 ($r^2 = 0.806$ with rs11692675), $P = 5.74 \times 10^{-7}$ [OR(A) = 1.29, 95% CI: 1.17–1.43].

SCN1A encodes brain-expressed voltage-gated sodium channel type I, alpha subunit. It bears the largest number of known epilepsy-related mutations, some associated with febrile seizures

(Oliva *et al.*, 2012). The common *SCN1A* single nucleotide polymorphism rs3812718, affecting splicing (Heinzen *et al.*, 2007), has also been associated with febrile seizures (Schlachter *et al.*, 2009), though replication has failed (Petrovski *et al.*, 2009). Retrospective studies show association between MTL E and febrile seizures (Pittau *et al.*, 2009; O'Dell *et al.*, 2012). Whether febrile seizures cause MTL EHS (Koyama *et al.*, 2012) or whether pre-existing hippocampal abnormalities predispose to febrile seizures (Cendes, 2004), which may then also be injurious, is unknown. Clinical differences between patients with and without a history of febrile seizures suggest MTL EHS is heterogeneous (Thom *et al.*, 2010).

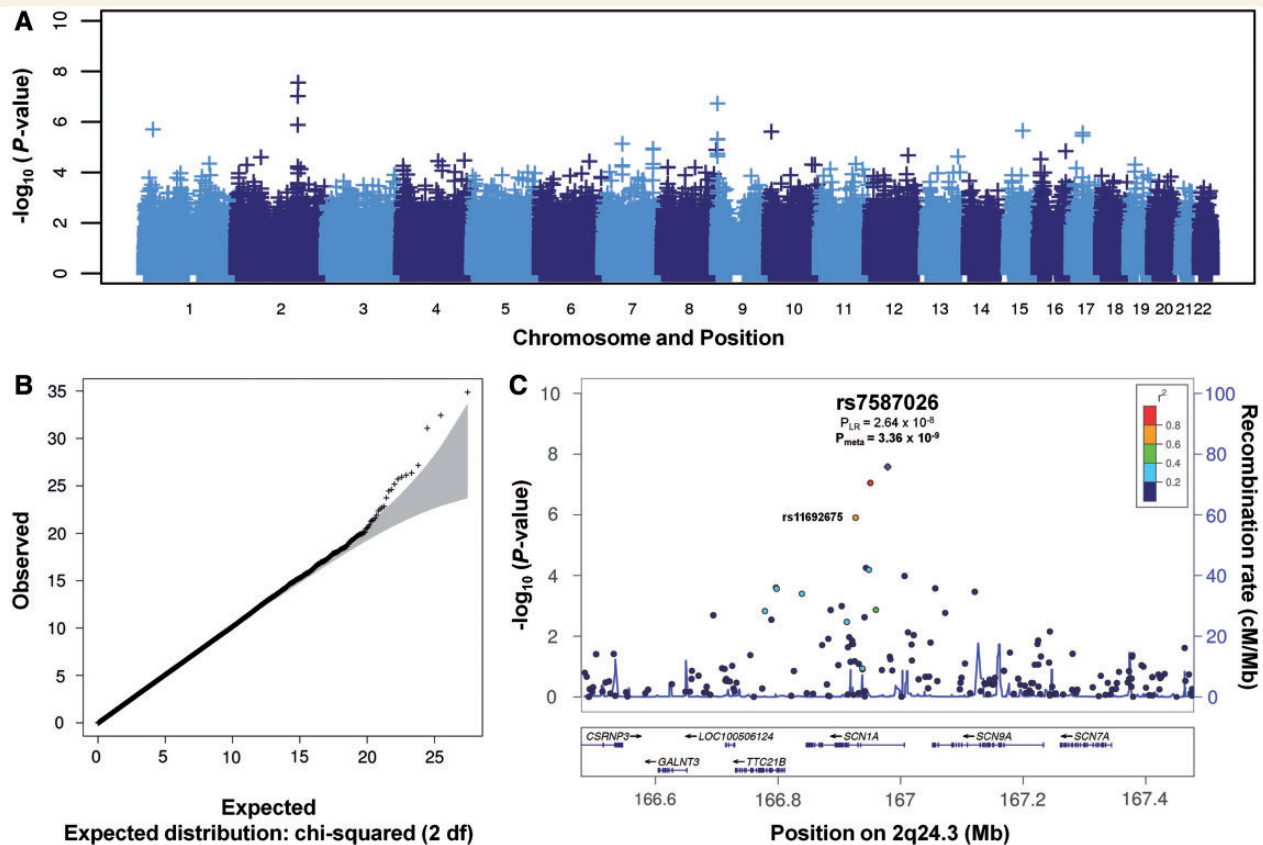


Figure 1 The results of genome-wide association analysis in MTLEHS + FS in discovery stage. (A) Manhattan plot, $-\log_{10}(P\text{-values})$ of the logistic regression test are plotted against single nucleotide polymorphism positions on each chromosome. (B) Quantile-quantile plot, the grey shaded area represents the 95% confidence interval of expected $-\log_{10}(P\text{-values})$. Black dots represent the observed $P\text{-values}$; $\lambda = 1.022$. (C) Regional association results for the chromosome 2q24.3 locus. The left y-axis represents $-\log_{10}(P\text{-values})$ for association with MTLEHS, the right y-axis represents the recombination rate, and the x-axis represents base-pair positions along the chromosome (human genome Build 37). The top single nucleotide polymorphism, rs7587026, is shown in purple, the rest of the single nucleotide polymorphisms are coloured according to their linkage disequilibrium r^2 value with rs7587026.

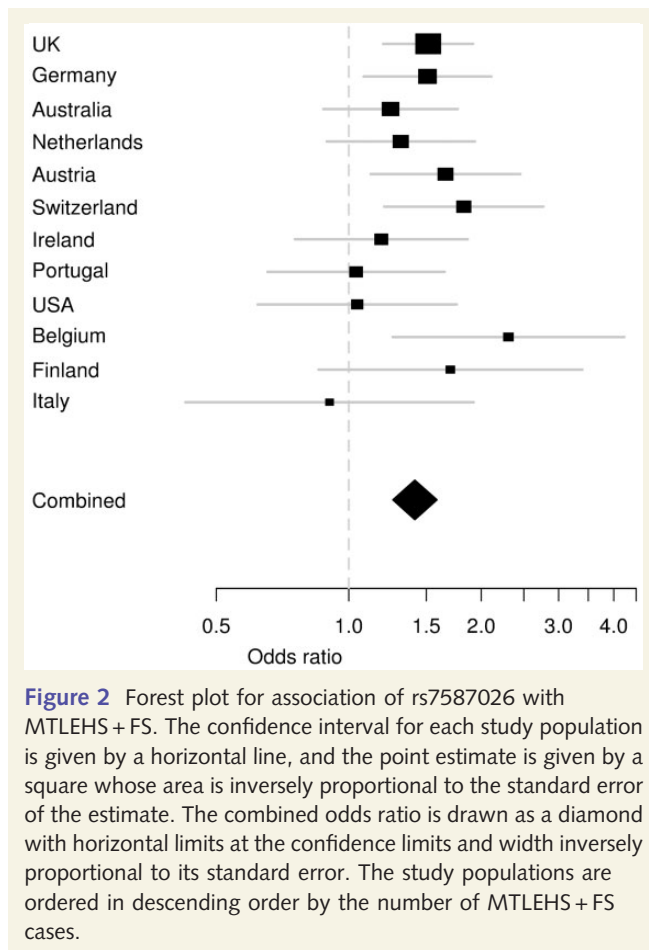
This evidence motivated our pre-analysis collection of febrile seizure data, and our previous study of febrile seizures (Petrovski *et al.*, 2009). We performed analysis of patients in the discovery cohort with a known history of presence of childhood febrile seizures (MTLEHS + FS, $n = 341$) (Table 2 and Fig. 1). The strongest association was for rs7587026, $P = 2.64 \times 10^{-8}$ [OR(A) = 1.59, 95% CI: 1.35–1.87] and rs580041, $P = 8.91 \times 10^{-7}$ [OR(A) = 1.56, 95% CI: 1.33–1.84], whereas the signal for rs11692675 was slightly weaker, $P = 1.25 \times 10^{-6}$ [OR(G) = 1.49, 95% CI: 1.27–1.75]. No association was seen in patients with MTLEHS without febrile seizures (MTLEHS – FS), despite similar sample size.

To refine the association signal, we performed regional imputation in the discovery data set of a 10 Mb region surrounding rs7587026 using the 1000 Genomes reference panel. Two single nucleotide polymorphisms had slightly lower $P\text{-values}$ in the MTLEHS + FS analysis than the original single nucleotide polymorphisms [rs16851603 ($P = 2.23 \times 10^{-8}$) and rs3919196 ($P = 2.26 \times 10^{-8}$)], but neither were significantly stronger than the original associations, and these signals reflected regional linkage disequilibrium structure (Supplementary Fig. 2). No known functional variants in *SCN1A*, nor in other genes in the region,

were in high linkage disequilibrium with rs7587026. The association signal is localized within one linkage disequilibrium block that also spans the promoter and 5' UTR region of *SCN1A* (Supplementary Fig. 2).

Replication and combined analyses

We selected the two top single nucleotide polymorphisms, rs7587026 and rs11692675, for replication in an independent sample of 959 patients with MTLEHS, of whom 416 had MTLEHS + FS, and 3591 population-matched controls of European descent from eight populations (Table 1 and Supplementary Table 1). We did not study rs580041 because of its perfect linkage disequilibrium with rs7587026 in white Europeans ($r^2 = 1$). We detected an association between rs7587026 and MTLEHS + FS, $P = 5.88 \times 10^{-3}$ [OR(A) = 1.26, 95% CI: 1.07–1.48; Table 2]; this value remains significant at a revised alpha threshold of 6.3×10^{-3} after Bonferroni correction for multiple comparisons in the replication cohort. No association was detected for MTLEHS – FS.



Meta-analysis of the discovery and replication samples confirmed the association of the 2q24.3 locus with MTLEHS+FS at genome-wide significant level for rs7587026 [$P_{meta} = 3.36 \times 10^{-9}$, OR(A) = 1.42, 95% CI: 1.26–1.59]; the signal for rs11692675 did not reach genome-wide significance [$P_{meta} = 4.78 \times 10^{-6}$, OR(G) = 1.30, 95% CI: 1.16–1.46]. No significant heterogeneity in effect sizes was detected among different populations (Fig. 2, Woolf's test for heterogeneity $P = 0.45$, see Supplementary Tables 7–10 for allele frequencies in all populations).

Febrile seizure analysis

To explore whether the observed association with rs7587026 is specific for MTLEHS+FS, or is specific for febrile seizures in general, we examined a total of 7387 controls and three data sets of patients (totalling 542) who had had febrile seizures but had not developed epilepsy by the time of the latest follow-up. It has been shown that almost all children who go on to develop any type of epilepsy after febrile seizures have done so by the age of 15 years (Neligan *et al.*, 2012). Therefore, to address the specificity of the association for MTLEHS+FS rather than febrile seizures alone, the ideal febrile seizures cohort would have been followed to age 15 at least. Of the three data sets available to us, only one met this criterion closely. The ALSPAC prospective cohort, which has the most comprehensive phenotypic data of the three data sets,

followed children to age 13: there was no association of rs7587026 with febrile seizures in 171 individuals who did not go on to develop epilepsy (Table 3) in comparison with 6443 controls from the same cohort. The two other cohorts of children with febrile seizures, from Austria [samples partially overlapping with those reported in Schlachter *et al.* (2009)] and Germany, were ascertained at a young age (to six years of age only) and had no follow-up to establish whether the children had febrile seizures only, or febrile seizures in the context of subsequent epilepsy including MTLEHS, and are therefore not best suited to address the question, but were examined as few cohorts overall are available. Bearing this key caveat in mind, in these two data sets there was an observed association of febrile seizures with rs7587026 (Table 3). The previously reported association in the Austrian population with an *SCN1A* functional splice-site single nucleotide polymorphism, rs3812718, was also seen in our Austrian sample [which is not unexpected as there is partial overlap of cases with those in the original report (Schlachter *et al.*, 2009)] and was present in the German sample. The observed association of rs7587026 with febrile seizures disappeared in both Austrian and German data sets when analysis was conditioned on rs3812718 ($P > 0.19$; Table 3). Moreover, although the association of febrile seizures with rs3812718 may be thought to be of interest for pure febrile seizures alone, we note there is no association of rs3812718 with febrile seizures in the best characterized cohort, from ALSPAC (Table 3), nor in a published sample (Petrovski *et al.*, 2009).

Thus, although other single nucleotide polymorphisms in or near *SCN1A* may predispose to pure febrile seizures, the signal we observed in MTLEHS+FS is very unlikely to be due to the history of febrile seizures alone. Moreover, no significant association was detected in a group of patients with other partial epilepsies with a history of febrile seizures [data set from Kasperavičiūtė *et al.* (2010); rs7587026, $P = 0.24$, OR(A) = 1.15, 95% CI: 0.91–1.45]. The sample for this analysis was smaller (177 patients; 7552 controls), but had 81% power to detect association of OR ≥ 1.42 (as seen in MTLEHS+FS group combined analysis) under 0.05 significance level. Collectively, we found no evidence that the MTLEHS+FS association was due to febrile seizures, or that it holds for all partial epilepsies with febrile seizures.

SCN1A expression in the human brain

The observed association could act by modulating *SCN1A* gene expression. The associated region harbours several alternative untranslated *SCN1A* exons (Martin *et al.*, 2007; Nakayama *et al.*, 2010). We did not detect association between rs7587026 and any protein-coding exon except one (see below) or total *SCN1A* expression, or with expression of untranslated 5' exons 1a and 1b (Martin *et al.*, 2007) (data not shown) in 78 patients and 78 control subjects.

The presence or absence of transcripts incorporating the 'neonatal' *SCN1A* exon 5 ('5N') was significantly different according to genotype of the two top single nucleotide polymorphisms (rs11692675 and rs7587026, P -values 1.08×10^{-9} and 1.17×10^{-6} , respectively; Supplementary material). For rs11692675 and rs7587026, respectively, none and 1% of the

Table 3 Genotype counts, allele frequencies and association results for rs7587026, rs3812718 and rs922224 in febrile seizures stage

| Population | n patients | n controls | Minor allele | Genotype count in patients | Genotype count in controls | Minor allele frequency in patients | Minor allele frequency in controls | P-value in single SNP association (allelic χ^2 test) | P-value in conditional analysis** |
|---------------------------------------|------------|------------|--------------|----------------------------|----------------------------|------------------------------------|------------------------------------|---|-----------------------------------|
| rs7587026 | | | | | | | | | |
| Austria | 158 | 584 | A | 19/58/81 | 31/216/337 | 0.304 | 0.238 | 0.017 | 0.19 |
| Germany | 194 | 337 | A | 15/92/87 | 20/116/201 | 0.314 | 0.231 | 0.003 | 0.43 |
| UK (ALSPAC) | 171 | 6443 | A | 23/59/89 | 498/2550/3395 | 0.307 | 0.275 | 0.194 | 0.33* |
| rs3812718 | | | | | | | | | |
| Austria | 133 | 209 | G | 16/65/52 | 52/100/57 | 0.365 | 0.488 | 0.0015 | 0.030 |
| Germany | 212 | 344 | G | 32/98/82 | 88/166/90 | 0.382 | 0.497 | 0.00018 | 0.0012 |
| rs922224 (proxy for rs3812718) | | | | | | | | | |
| UK (ALSPAC) | 172 | 6456 | G | 34/81/57 | 1371/3144/1941 | 0.433 | 0.456 | 0.40 | 0.83* |

*Conditional analysis performed despite a non-significant single SNP association.

**In conditional analyses, rs7587026 was conditioned for rs3812718 (or its proxy, rs922224, for the ALSPAC cohort), while rs3812718 and rs922224 were conditioned for rs7587026.

individuals with the GG and AA genotype showed *SCN1A* transcripts in the neonatal form, compared with 83% and 81% with the genotype AA or CC. This alternative splicing event is influenced by rs3812718 (Heinzen *et al.*, 2007). The association of alternative splicing with rs922224 ($r^2 = 1$ with rs3812718) was stronger, $P = 2.33 \times 10^{-31}$. The level of expression of *SCN1A* exon 5N was also significantly different according to genotype ($P = 1.62 \times 10^{-11}$ for rs11692675, 2.70×10^{-6} for rs7587026, 7.40×10^{-34} for rs922224). In conditional analyses including all three single nucleotide polymorphisms, only rs922224 remained significant ($P = 1.08 \times 10^{-25}$). Finally, expression quantitative trait loci analyses for subsets of patients according to a known history of presence ($n = 46$) or absence ($n = 27$) of febrile seizures in childhood for rs11692675 or rs7587026 showed significant differences in the level of expression of 5N exon according to genotype in both MTLEHS+FS and MTLEHS–FS. Including both rs11692675 or rs11692675 and rs922224 in the regression models, only rs922224 remained significant in both MTLEHS+FS and MTLEHS–FS groups (Supplementary material).

We cannot exclude the possibility that rs7587026 (or another single nucleotide polymorphism in the high linkage disequilibrium region) may act as an additional splicing controller to rs3812718, but our data are consistent with rs7587026 having no solo effect on 5N splicing. We also did not detect any correlation using a significance level of $P < 5 \times 10^{-5}$ between rs7587026 and expression/splicing of any other genes across the genome (Supplementary material).

Discussion

We show that common variation in and near *SCN1A* may increase susceptibility to MTLEHS+FS. Our previously published larger genome-wide association study for a broader range of focal epilepsies did not identify any single-single nucleotide polymorphism association (Kasperaviciute *et al.*, 2010), but the findings here demonstrate that associated variants may exist for more

narrowly-defined syndromes. Because the biology of most of the epilepsies is poorly understood, there are few *a priori* data upon which to base selection of the range of phenotypes to include in studies of possible genetic causation. Our findings suggest that focussing on clinically recognized syndromes or constellations (Berg *et al.*, 2010) may prove fruitful by reducing heterogeneity before genomic analyses.

Our association seems to be specific for MTLEHS+FS, with no association for MTLEHS–FS, febrile seizures alone or non-MTLEHS partial epilepsies with febrile seizures. Our findings suggest that there is genetic susceptibility to MTLEHS, and that it, or hippocampal sclerosis, may not necessarily be only acquired. The results support the concept of heterogeneity in MTLEHS, beyond that already documented clinico-pathologically (Tassi *et al.*, 2009; Thom *et al.*, 2010; Blümcke *et al.*, 2012). However, further work will be needed to confirm the specificity of our findings, as we did not formally establish a significant difference in odds ratios between MTLEHS+FS and MTLEHS–FS. It would also be interesting to explore, in a suitably-powered study, whether there is any association with MTLE without hippocampal sclerosis.

The notably weaker association in the replication stage could be due to several factors, the most important of which is the 'winner's curse' (Ioannidis *et al.*, 2009); there may be a large number of weak but real associations in the data, some of which achieve genome-wide significance in a particular study through random stochastic chance, but will not do so in another study. The association in our discovery cohort was replicated in the second independent sample, but it is nevertheless important that other studies are undertaken to further replicate our findings. Other limitations of our study are the lack of genome-wide data in the replication sample, preventing direct population stratification assessment, though self-identification closely corresponds to genetically-determined ancestry (Lao *et al.*, 2008; Wang *et al.*, 2010), a phenomenon we confirmed in the discovery stage, and the small size of some of the replication groups, reducing replication power, and magnifying effects of undetected population admixture.

As for many genome-wide association studies, we could not narrow the association to a single gene or functional variant. There are other genes designated 'SCNxA' in the vicinity: *SCN3A*, *SCN2A*, *SCN9A* and *SCN7A* (this last does not show any sodium channel activity in exogenous expression systems) (Meisler *et al.*, 2010). Among these genes, *SCN2A* has the most published evidence to support its role in the epilepsies. We cannot exclude the possibility that the association is driven by deleterious variants in these or other nearby genes. *SCN1A*, however, emerges as the most plausible candidate, due both to its proximity to the associated region and its role in other epilepsies with febrile seizures. Notably, our association is with a syndrome involving hippocampal damage, whereas typically no hippocampal damage is observed in patients with Dravet syndrome caused by deleterious changes affecting *SCN1A* (Catarino *et al.*, 2011), suggesting that *SCN1A* might influence epileptogenesis through various mechanisms.

The location of the associated variants within *SCN1A* and overlapping its promoter regions (Long *et al.*, 2008), was suggestive of possible roles in *SCN1A* expression modulation. In fact, we did not detect a definitive effect on expression of *SCN1A* or its exons in temporal neocortex. However, this analysis may have been confounded by many factors: effects may be brain-region or cell-population specific, as in *SCN1A*-related Dravet syndrome, where consequences are only found in interneurons (Ogiwara *et al.*, 2007); our whole-tissue expression analysis would not detect such subtle signals. Moreover, noting the febrile seizures association, the effects may be temporally or spatially restricted, acting only in childhood or/and in the stress of febrile seizures (Koyama *et al.*, 2012). Further studies will be needed to explore possible functional effects.

The detected association could act in different ways, predisposing to MTLAHS + FS as a distinct syndrome, or to the specific development of MTLAHS in the context of remote febrile seizures. If the association does indeed relate to *SCN1A* and function of the encoded protein, new lines of investigation may prove possible in the context of the existing deep knowledge of *SCN1A*, experimental models of MTLAHS and *in vitro* study of mechanisms of hippocampal dysfunction in epilepsy, as well as intriguing reports of the role of *SCN1A* in many epilepsies, such as the suggestion that mutations in *SCN1A* in Dravet Syndrome may protect against hippocampal sclerosis (Auvin *et al.*, 2008; Catarino *et al.*, 2011). Stratifying by febrile seizures type could also prove illuminating, as prolonged, lateralized or repeated febrile seizures within a short interval may have different effects to 'uncomplicated' febrile seizures. Our retrospective febrile seizures data were insufficiently resolved to permit such analysis. This is an important avenue for further investigation, because no predictors exist for the development of epilepsy in the 3% of all the children who have febrile seizures, and because established MTLAHS can have devastating consequences. Eventual reliable prediction of significant risk of MTLAHS after febrile seizures could lead to novel preventative measures in at-risk individuals: here, we note that *SCN1A* encodes an important anti-epileptic drug target and that it is possible to pharmacologically prevent the development of epilepsy after febrile seizures in an animal model (Koyama *et al.*, 2012). Our findings suggest that further work on *SCN1A* variation may

contribute to understanding the risk of developing MTLAHS after febrile seizures.

Acknowledgements

We thank all patients, their families and physicians for participating in this study. We thank Drs. Goldstein, Heinzen and Radtke for use of data from patients at Duke University School of Medicine, and for their comments. We thank Drs. D. Lowenstein and A-E. Lehesjoki, and the ILAE Consortium on Complex Epilepsies (including Carolien de Kovel, Thomas Sander, Dennis Dlugos, Warren Lo, Tom Ferraro, Mike Johnson, Tony Marson, Douglas Speed, Patrick Kwan, Larry Baum, Stacey Cherny, Zhi Wei, Larry Brody, Pak Sham, David Balding and Aarno Palotie) for their support of the work, and for their comments. We thank Prof J. Hardy for his support. We thank the members of the Dutch Collaborative Epilepsy Surgery Program for their cooperation. We acknowledge the Italian League against Epilepsy (LICE) collaborative group for its support. We gratefully acknowledge Professor John Hopper (School of Population Health, The University of Melbourne) for providing the control samples for the Melbourne cohort and Dr. Marian Todaro for processing the DNA samples for the Melbourne cohort; Dr. Liisa Myllykangas (Folkhalsan Institute of Genetics and Department of Pathology, University of Helsinki) and Dr. Pentti Tienari (Molecular Neurology Programme, Biomedicum, University of Helsinki and Department of Neurology, Helsinki University Central Hospital) for providing us the Vantaa85+ Study GWAS genotypes; Professor Edouard Louis (MD, PhD), Head of the Gastroenterology Unit, University Hospital Centre (CHU) of Liège and Professor Michel Georges (DVM, PhD), Head of the Animal Genomics Unit, Faculty of Veterinary Medicine and Groupe Interdisciplinaire de Génoprotéomique Appliquée (GIGA-R) for providing us the Belgian control cohort; Drs. P. C. van Rijen and P. H. Gosselaar, Department of Neurosurgery, Rudolf Magnus Institute of Neuroscience, University Medical Centre Utrecht, The Netherlands; Christian Hengsbach for expert assistance with recruitment for the Tübingen MTLAHS cohort; Susanne Beyer and Ulrike Strube for the technical assistance in SNP genotyping for the Bonn MTLAHS cohort; and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses. We would like to thank AROS Applied Biotechnology AS company laboratories and Affymetrix for their valuable input. We are grateful to the Banner Sun Health Research Institute Brain and Body Donation Program of Sun City, Arizona for the provision of human biospecimens contributing to gene expression analysis of nine brain regions from 134 control individuals. The Brain and Body Donation Program is supported by the National Institute of Neurological Disorders and Stroke (U24 NS072026 National Brain and Tissue Resource for Parkinson's Disease and Related Disorders), the National Institute on Aging (P30 AG19610 Arizona Alzheimer's Disease Core Centre), the Arizona Department of Health Services (contract 211002, Arizona Alzheimer's Research Centre), the Arizona Biomedical Research Commission (contracts 4001, 0011, 05-901 and 1001 to the

Arizona Parkinson's Disease Consortium) and the Michael J. Fox Foundation for Parkinson's Research.

Funding

Supported by the Wellcome Trust (grant 084730 to S.M.S., N.D., C.D.); the UK Medical Research Council (MRC grants G0400126 to S.M.S., G1100616 to C.S., G0901254 to J.H. and M.E.W., and Training Fellowship G0802462 to M.R.); the King Faisal Specialist Hospital and Research Centre (KFSH & RC grant to D.T.); the University College London Hospitals Charities and the Clinical Research and Development Committee (UCLH/CRDC grant F136 to S.M.S.); the National Institute for Health Research (NIHR grant 08-08-SCC to S.M.S.); the National Society for Epilepsy; the National Institute for Health Research University College London Hospitals Biomedical Research Centre; Marie Curie International Re-integration Grant (FP7-PEOPLE-2009-RG grant No 256545 to M.M. and S.M.S.); the European Commission (FP7 project EpiPGX, grant 279062 to H.L., S.M.S and W.S.K.; FP6 project Epicure, grant LSHM-CT-2006-037315 to H.L., P.S.R. and F.R.); the Robert Bosch Foundation, Stuttgart, and the University of Tübingen (IZEPHA project 18-0-0, grant to H.L.); the German Federal Ministry of Education and Research, National Genome Research Network (NGFNplus: EMINet grant 01GS08123 to H.L., S.S., A.J.B.); BONFOR (S.S., A.J.B.); the German Federal Ministry of Education and Research (independent research groups in neuroscience); the German Research Foundation (EUROCORES program, EuroEPINOMICS-RES grant RO3396/2-1 to P.S.R. and F.R. and EuroEPINOMICS-RES grant DFG BL421/3-1 to I.B. and K.K.); the Austrian Science Fund (project FWF I643, grant to F. Zimprich); the National Health and Medical Research Council (NHMRC grant 628952 to S.F.B.); the Royal Melbourne Hospital Neuroscience Foundation (grant to T.J.O'B.); the Foundation of Science and Technology, Lisbon (FCT grant PIC/IC/83297/2007 to B.M.S.); the National Institutes of Health, USA (NIH grants R01-NS-49306-01 to R.J.B. and R01-NS-064154-01 to R.J.B. and H.H.); the collection of the Irish patient cohort was supported by the Irish Higher Education Authority Programme for Research in Third Level Institutions (PRTL13) through a Science Foundation Ireland Research Frontiers Programme award (08/RFP/GEN1538) and a Medical Research Charities Group of Ireland/Health Research Board award (2009/001) from Brainwave—the Irish Epilepsy Association. 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 Fonds National de la Recherche Scientifique, grant n. FC 63574/3.4.620.06 F, and the Fonds Erasme, Université Libre de Bruxelles. The collection of the Belgian control cohort was supported by the Walloon Region and the French-Speaking Community of Belgium, the Belgian Science Policy and the University of Liège. J.N. is supported by the Swiss National Science Foundation-Fellowships for prospective researchers and the SICPA Foundation, Prilly, Switzerland. Computing facilities used at King's College London were supported by the National

Institute for Health Research (NIHR) Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London. Funding support for the ALSPAC was provided by The UK Medical Research Council (grant refs: 74882) the Wellcome Trust (grant refs: 076467) and the University of Bristol.

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 the project was provided by the Wellcome Trust under award 076113 and 085475) and the Irish Amyotrophic Lateral Sclerosis Study (funding support was provided by Muscular Dystrophy Association, USA, Irish Institute of Clinical Neurosciences Travel Award, and National Institutes of Health, USA; additional genotyping was provided by the National Institute of Neurological Disorders and Stroke (NINDS); the dataset used for the analyses described in this manuscript were obtained from the NINDS Database found at <http://www.ncbi.nlm.nih.gov/gap> through dbGaP accession number phs000127.v1.p1).

Conflict of interest

Y.W. has served on scientific advisory boards for UCB Pharma and has received funding for travel and speaker honoraria from UCB, Desitin Pharmaceuticals, GmbH, and Eisai Inc.; H.L. has served on scientific advisory boards for Eisai Inc., GlaxoSmithKline, Pfizer Inc, UCB, and Valeant Pharmaceuticals International, has received funding for travel from GlaxoSmithKline, Medtronic, Pfizer and UCB and speaker honoraria from Desitin Pharmaceuticals, GmbH, Eisai Inc., GlaxoSmithKline, Pfizer, and UCB, and has received research support from Sanofi-Aventis, UCB, DFG, BMBF, and the EU; J.C. has received funding from the Tecnifar group (BICE Tecnifar Grant 2009); F.R. has received within the last two years honoraria as scientific advisor from GSK, Eisai, UCB and Pfizer, and has received speaker honoraria from UCB, GSK, Eisai, Desitin and Medtronic and educational grants from Nihon-Kohden, UCB, Medtronics, Cyberonics and Cerbomed (F.R. has, however, no conflicts of interest regarding this study); P.S.R. has received travel grants from UCB; G.L.C. has received research funding from UCB and speaker honoraria from Eisai; S.M.S. has received research funding or personal/institutional honoraria from UCB Pharma, GlaxoSmithKline and Eisai Inc; H.M.H. has served on the scientific advisory board of Eisai, Pfizer, GlaxoSmithKline and UCB Pharma, has served on the speakers' bureau of Desitin, Eisai, GlaxoSmithKline and UCB Pharma and received research funding from Desitin, Janssen-Cilag, GlaxoSmithKline and UCB Pharma; I.B. received speaker honoraria from Desitin Pharmaceuticals, GmbH, Eisai Inc., and UCB, and has received research support from Boehringer-Ingelheim; R.K.K. has served on scientific advisory boards for UCB Pharma, Eisai, Lundbeck, GlaxoSmithKline, and Fennomedical and has received funding for travel and speaker honoraria from UCB Pharma, Eisai, GlaxoSmithKline, Medtronic, Pfizer, Orion, Fennomedical and institutional research funding from UCB Pharma and GlaxoSmithKline; A-M.K. has received funding for travel from UCB Pharma, Eisai, Abbott and Biogen; S.F.B. is an inventor on a patent for SCN1A testing held by

Bionomics Inc and licensed to various diagnostic companies. All other authors declare that they have no conflicts of interest.

Supplementary material

Supplementary material is available at *Brain* online.

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