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 with (overall n = 757) and without (overall n = 803) a history of febrile seizures. To dissect out variants related to a history of febrile seizures, we tested cases with hippocampal sclerosis and 3591 control subjects. Meta-analysis revealed a genome-wide significant association for mesial temporal lobe epilepsy with hippocampal sclerosis with (overall n = 757) and without (overall n = 803) a history of febrile seizures. These findings suggest SCN1A involvement in a common epilepsy syndrome, giving 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.
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 prominent features, 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 HumanCNV370-duo, 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 ≥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 × 2 × 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.
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
transcript variant (NM_001202435.1) \(P = 5.26 \times 10^{-8}\), odds ratio for G allele \[\text{OR(G)} = 1.31, 95\% \text{ CI: } 1.19-1.44;\] 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, \(P = 1.19 \times 10^{-7}\) \[\text{OR(A)} = 1.31, 95\% \text{ CI: } 1.19-1.45;\] and rs580041 \(r^2 = 0.806\) with rs11692675, \(P = 5.74 \times 10^{-7}\) \[\text{OR(A)} = 1.29, 95\% \text{ CI: } 1.17-1.43.\)

SCN1A encodes brain-expressed voltage-gated sodium channel type 1, 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 MTLE and febrile seizures (Pittau et al., 2009; O’Dell et al., 2012). Whether febrile seizures cause MTLEHS (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 MTLEHS is heterogeneous (Thom et al., 2010).
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 \( P \)-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, \( P = 5.88 \times 10^{-8} \) [OR(A) = 1.26, 95% CI: 1.07–1.48; Table 2]; this value remains significant at a revised alpha threshold of \( 6.3 \times 10^{-7} \) after Bonferroni correction for multiple comparisons in the replication cohort. No association was detected for MTLEHS – FS.
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. 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 Kasperaviciute 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 (Martín 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 (Martín 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 $1.08 \times 10^{-9}$ and $1.17 \times 10^{-6}$, respectively; Supplementary material). For rs11692675 and rs7587026, respectively, none and 1% of the

<table>
<thead>
<tr>
<th>UK</th>
<th>Germany</th>
<th>Australia</th>
<th>Netherlands</th>
<th>Austria</th>
<th>Switzerland</th>
<th>Ireland</th>
<th>Portugal</th>
<th>USA</th>
<th>Belgium</th>
<th>Finland</th>
<th>Italy</th>
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</tbody>
</table>

Figure 2 Forest plot for association of rs7587026 with MTLEHS + FS. The confidence interval for each study population is given by a horizontal line, and the point estimate is given by a square whose area is inversely proportional to the standard error of the estimate. The combined odds ratio is drawn as a diamond with horizontal limits at the confidence limits and width inversely proportional to its standard error. The study populations are ordered in descending order by the number of MTLEHS + FS cases.

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

<table>
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<tr>
<th>Population</th>
<th>n patients</th>
<th>n controls</th>
<th>Minor allele</th>
<th>Genotype count in patients</th>
<th>Genotype count in controls</th>
<th>Minor allele frequency in patients</th>
<th>Minor allele frequency in controls</th>
<th>P-value in single SNP association (allelic $\chi^2$ test)</th>
<th>P-value in conditional analysis **</th>
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<tr>
<td>rs7587026</td>
<td></td>
<td></td>
<td>A</td>
<td>19/58/81</td>
<td>31/216/337</td>
<td>0.304</td>
<td>0.238</td>
<td>0.017</td>
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<td>158</td>
<td>584</td>
<td>G</td>
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<td>52/100/57</td>
<td>0.365</td>
<td>0.488</td>
<td>0.0015</td>
<td>0.030</td>
</tr>
<tr>
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<td>337</td>
<td>G</td>
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<td>88/166/90</td>
<td>0.382</td>
<td>0.497</td>
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<tr>
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<td>171</td>
<td>6443</td>
<td>A</td>
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<td>0.194</td>
<td>0.33*</td>
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<td>1371/3144/1941</td>
<td>0.433</td>
<td>0.456</td>
<td>0.40</td>
<td>0.83*</td>
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<tr>
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<td>0.488</td>
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<td>0.275</td>
<td>0.194</td>
<td>0.33*</td>
</tr>
<tr>
<td>rs922224 (proxy for rs3812718)</td>
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<td></td>
<td>A</td>
<td>34/81/57</td>
<td>1371/3144/1941</td>
<td>0.433</td>
<td>0.456</td>
<td>0.40</td>
<td>0.83*</td>
</tr>
</tbody>
</table>

*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.

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 MTLEHS + FS as a distinct syndrome, or to the specific development of MTLEHS in the context of remote febrile seizures. If the association does indeed relate to SCNA1 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 MTLE 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 MTLEHS can have devastating consequences. Eventual reliable prediction of significant risk of MTLEHS 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 MTLEHS after febrile seizures.

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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 Tecnicari (BICE TECNICARI 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, Medtronic, 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 Eisai Inc., 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...
Supplementary material

Supplementary material is available at Brain online.

References


Purcell S, Cherny SS, Sham PC. Genetic power calculator: design of linkage and association genetic mapping studies of complex traits. Bioinformatics 2003; 19: 149–50.


