King's Research Portal

DOI:
10.1111/ejn.13420

Document Version
Peer reviewed version

Link to publication record in King's Research Portal

Citation for published version (APA):
Hessel, E. V. S., van Lith, H. A., Wolterink-Donselaar, I. G., de Wit, M., Groot Koerkamp, M. J. A., Holstege, F. C. P., Kas, M. J. H., Fernandes, C., & de Graan, P. N. E. (2016). Mapping of a FEB3 homologous febrile seizure locus on mouse chromosome 2 containing candidate genes Scn1a and Scn3a. *European Journal of Neuroscience*, *44*(11), 2950-2957. https://doi.org/10.1111/ejn.13420

Citing this paper
Please note that where the full-text provided on King's Research Portal is the Author Accepted Manuscript or Post-Print version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version for pagination, volume/issue, and date of publication details. And where the final published version is provided on the Research Portal, if citing you are again advised to check the publisher's website for any subsequent corrections.

General rights
Copyright and moral rights for the publications made accessible in the Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognize and abide by the legal requirements associated with these rights.

•Users may download and print one copy of any publication from the Research Portal for the purpose of private study or research.
•You may not further distribute the material or use it for any profit-making activity or commercial gain
•You may freely distribute the URL identifying the publication in the Research Portal

Take down policy
If you believe that this document breaches copyright please contact librarypure@kcl.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

Download date: 14. Oct. 2023

Mapping of a *FEB3* homologous febrile seizure locus on mouse chromosome 2 containing candidate genes *Scn1a* and *Scn3a* .

Ellen V.S. Hessel.[1], Hein A. van Lith H.A.[2], Inge G. Wolterink-Donselaar[1], Marina de Wit[1], Marian J.A. Groot Koerkamp[3], Frank C.P. Holstege[3], Martien J.H. Kas[1,4] Cathy Fernandes[5] and Pierre N.E. de Graan[1,*]

[1]Brain Center Rudolf Magnus, Department of Translational Neuroscience, University Medical Center Utrecht, Universiteitsweg100, 3584 CG Utrecht, The Netherlands
[2]Division of Animal Welfare & Laboratory Animal Science, Department of Animals in Science & Society, Faculty of VeterinaryMedicine and Brain Center Rudolf Magnus, Utrecht University, Utrecht, The Netherlands
[3]Department of Molecular Cancer Research, University Medical Center Utrecht, Utrecht, The Netherlands
[4]Groningen Institute for Evolutionary Life Sciences, University of Groningen, Groningen, The Netherlands
[5]Social, Genetic & Developmental Psychiatry Centre, Institute of Psychiatry, Psychology and Neuroscience, King's CollegeLondon, London, UK

Keywords: chromosome substitution strain, hyperthermia, quantitatve trait loci, temporal lobe epilepsy

*Corresponding Author

Dr. P.N.E. de Graan
Brain Center Rudolf Magnus, University Medical Center Utrecht, Department of Translational Neuroscience
Universiteitsweg 100, 3584 CG Utrecht, The Netherlands
Phone: +31 887561234
Fax: +3188 7569032
e-mail: p.n.e.degraan@umcutrecht.nl

Running title: A *FEB3* homologous locus in Mice.

Abstract

Febrile seizures (FS) are the most common seizure type in children. Recurrent FS are a risk factor for developing temporal lobe epilepsy later in life and are known to have a strong genetic component. Experimental FS (eFS) can be elicited in mice by warm-air induced hyperthermia. We used this model to screen the chromosome substitution strain (CSS) panel derived from C57BL/6J and A/J for FS susceptibility and identified C57BL/6J-Chr2^{A}/NaJ (CSS2), as the strain with the strongest FS susceptibility phenotype. The aim of this study was to map FS susceptibility loci and select candidate genes on mouse chromosome 2.

We generated an F_2 population by intercrossing the hybrids (F1) that were derived from CSS2 and C57BL/6J mice. All CSS2-F_2 individuals were genotyped and phenotyped for eFS susceptibility, and QTL analysis was performed. Candidate gene selection was based on bioinformatics analyses and differential brain expression between CSS2 and C57BL/6J strains determined by microarray analysis.

Genetic mapping of the eFS susceptibility trait identified two significant loci: FS-QTL2a (LOD-score 3.6) and FS-QTL2b (LOD-score 6.2). FS-QTL2a contained 44 genes expressed in the brain at post natal day 14. Four of these (*Arl6ip6, Cytip, Fmnl2 Ifih1*) contained a non-synonymous SNP comparing CSS2 and C57BL/6J, six genes (*March7, Nr4a2, Gpd2, Grb14, Scn1a, Scn3a*) were differentially expressed between these strains. A region within FS-QTL2a is homologous to the human *FEB3* locus. The fact that we identify mouse FS-QTL2a with high *FEB3* homology is strong support for the validity of the eFS mouse model to study genetics of human FS.

Introduction

Approximately 2-5% of the North-American and European children suffer from febrile seizures (FS) and thereby FS are the most common type of seizures during childhood (Hauser, 1994). FS have a peak occurrence between 6 months and 5 years of age and can be characterized as either simple or complex. Simple FS are usually benign, non-recurring seizures, lasting shorter than fifteen minutes, whereas complex FS are characterized by duration of more than fifteen minutes or recurrence within 24 hours. Complex FS are an important risk factor for the development of mesial temporal lobe epilepsy (mTLE) later in life (French *et al.*, 1993).

Family, twin and association studies indicate a strong genetic component in FS susceptibility. In twins, the concordance rate was higher in monozygotic (36%) than in dizygotic twins (12%) (Kjeldsen *et al.*, 2002). Human linkage studies identified several loci for familial FS (Nakayama, 2009). In addition, mutations in several genes coding for voltage-gated sodium channel subunits (*SCN1A*, *SCN2A* and *SCN1B*) and a GABA(A) receptor subunit (*GABRG2*) were identified in families suffering from generalized epilepsy with FS plus (GEFS+) (Hirose *et al.*, 2003). Common forms of FS are considered to be genetically complex disorders, involving multiple susceptibility genes. So far, association studies have implicated only a limited number of genes for sporadic FS, including *IL1B (Kira et al., 2005)*, *IL10 (Ishizaki et al., 2009)*, *SCN1A (Chou et al., 2003; Feenstra et al., 2014), GABRG2 (Chou et al., 2003)* and *CHRNA4* (Chou *et al.*, 2003). However, the strength of several of these associations has been debated, since it has proven difficult to replicate associations (Nakayama, 2009). Therefore, new genetic strategies are required to identify genes involved in FS susceptibility.

Recently, we employed a forward genetic strategy to search for FS susceptibility genes, combining a mouse model for experimental FS (eFS) with QTL (quantitative trait locus) mapping. Baram and colleagues have previously shown that eFS can be induced

in rat pups by hyperthermia (Baram *et al.*, 1997). We adapted this eFS model to mouse (van Gassen et al., 2008) and performed video/EEG monitoring to validate the mouse model and to subsequently use it as a phenotypic screen to study genetics of eFS susceptibility in inbred strains (Hessel *et al.*, 2009). As a first step in identifying eFS QTLs, we screened a mouse chromosome substitution strain (CSS, also called consomic strain or line) panel derived from C57BL/6J (host strain) and A/J (donor strain) (C57BL/6J-Chr $\#^{A}$/NaJ), a powerful tool to dissect complex genetic traits (Nadeau *et al.*, 2000). We identified six chromosomes carrying putative QTLs for eFS susceptibility and found one of the most significant susceptibility phenotype on mouse chromosome 2 (Hessel *et al.*, 2009). In humans, several genes previously associated with FS are located on chromosome 2 (for review see (Nakayama, 2009)), including *IL1β,* and *SCN1A* and *SCN2A* in the *FEB3* (2q24) locus.

The aim of this study was to map eFS susceptibility QTLs on mouse chromosome 2 and identify candidate genes. We have successfully used this strategy to identify Srp9 as a new FS susceptibility gene on chromosome 1 (Hessel *et al.*, 2014). Therefore, we generated an F_2 population by intercrossing the hybrids (F_1) between the consomic C57BL/6J-Chr 2^{A}/NaJ (CSS2) and C57BL/6J host strains. After pheno- and genotyping the F_2 progeny we performed QTL analysis and identified two highly significant QTLs for eFS susceptibility, one of which contains a sequence with high homology to the human *FEB3* locus, coding for several subunits of the voltage-sensitive sodium channels, including *Scn1a* and *Scn3a*.

Material and Methods

Animals

Breeding pairs for C57BL6/J and C57BL/6J-Chr 2^A/NaJ (CSS2) were obtained from The Jackson Laboratory (Bar Harbor, ME, USA). To produce F_1 hybrids, C57BL/6J and CSS2 males and females were reciprocally crossed. F_1 hybrids were then intercrossed producing 143 F_2 progeny (61 mice - male: female = 25 : 36 - originated from CSS2 mothers and 82 - male : female = 52 : 30 - from C57BL/6J mothers).

Animals were kept in a controlled 12 hour light-dark cycle (lights on: 7:00 a.m. to 7:00 p.m.) with a room temperature of 22 ± 1ºC and a humidity of 50-70%. Food (Special Diet Services, Essex, England) and water were available *ad libitum*. All animals were housed in wire topped (Macrolon© Type II cages (Techniplast, Milan, Italy) with sawdust bedding and a paper tissue for nest building. Animals were killed by cervical dislocation or decapitation. All experiments were performed according to the institutional guidelines of the University Medical Center Utrecht (Current allowance number: 2012.I.03.047). The principles outlined in the ARRIVE guidelines and the Basel declaration, including the 3R concept, have been considered when planning the experiments.

Phenotypic screen for eFS susceptibility.

FS susceptibility in inbred mice was measured as described earlier.[12] Prolonged eFS were elicited by exposing 14-day-old mice (P14) to a warm-air stream to induce hyperthermia. Susceptibility to eFS was determined by measuring the latency to tonic-clonic seizures (febrile seizure latency = FSL), a phenotypic parameter which has previously been validated by video/EEG monitoring (Hessel *et al.*, 2009). On P1, litters were culled to 4-6 pups with a balanced male: female ratio, when possible. On P10, a temperature sensitive transponder (IPTT-300, (BioMedic Data Systems) Plexx BV, Elst,

The Netherlands) was injected subcutaneously (separation time from the mother < 2 min) in pups weighing > 5.0 g. At P14, individual pups were exposed to a pre-heated warm-air stream of 50 ± 0.5 ºC until they developed FS. Core body temperature was measured with a wireless temperature reader (WRS-6007, BioMedic Data Systems, Plexx BV) throughout the experiment. Mice were immediately sacrificed after hyperthermia and tails were collected for genotyping. All experiments were performed between 10:00 a.m. – 4:00 p.m. FSL of the complete CSS2-F_2 progeny was determined within a period of two months.

Genotyping and genetic map construction

DNA was isolated from tails as described (Kas *et al.*, 2009). DNA concentration was measured on agarose gel and samples were diluted to 10 ng/µl. To generate a genetic map seventeen DNA markers (fifteen microsatellites and two SNPs) distributed over chromosome 2 were chosen from the mouse genome database based on the presence of allelic differences between the A/J and C57BL/6J inbred strains (Mouse Genome Informatics, http://www.informatics.jax.org). Microsatellite marker genotyping was performed by PCR. Primers (150 nM forward/reverse) flanking these markers (supplementary Table 1) (Sigma-Aldrich, Zwijndrecht, the Netherlands) were used to amplify the DNA (50 µg/reaction) in a Veriti 96 wells thermocycler (PCR system, Applied Bioscience Inc., Foster city, CA, USA) using Super Taq polymerase (SphaeroQ, Gorinchem, The Netherlands: 0.25 units/reaction). The PCR products were separated on 3% agarose gels and visualized by ethidium bromide staining. Two additional TaqMan SNP primers were used. SNP genotyping was performed as described (Kas *et al.*, 2009). The two SNP markers were selected using Genenetwork (http://www.genenetwork.org/): rs27498297 and rs27434812. Primers (Taqman SNP genotyping Assay, Applied Biosystems, USA) were used to amplify the DNA (10

ng/reaction) using TaqMan Universal PCR Master Mix (Applied Biosystems, USA). SNP analyses were done with a sequence detection system (7900HT, Applied Biosystems, Foster City, CA, USA (Kas *et al.*, 2009). Segregation ratios of the genotypes of individual markers were checked by means of the Chi-squared goodness-of-fit-test. None of the markers showed (P < 0.05) segregation distortion (AA:AB:BB ratio 1:2:1). Marker positions (in sex-averaged cM) were taken from the revised genetic map for improved QTL mapping (Cox *et al.*, 2009) by using the 'mouse map converter' (http://cgd.jax.org/mousemapconverter/).

QTL mapping

The Kolmogorov-Smirnov one sample test was used to check Gaussianity of eFS susceptibility in the F_2 population. Because this parameter was not normally distributed, the trait was logarithmically transformed prior to QTL analysis. The location of the (transformed) eFS susceptibility QTLs and the variance explained by each locus were determined by using the parametric interval mapping approach from the MapQTL® software package, version 4.0. Results were expressed as LOD scores. LOD score threshold (confidence level 0.05) was determined using 10,000 random permutations (random shuffling of genotypes with phenotypes), yielding a threshold LOD score value of 2.39 for FSL. Thus, an association was assumed significant when the LOD score was ≥ 2.39. Cosegregation of the phenotype with alleles at a marker locus was evaluated by one-way analysis of variance (ANOVA). All data within genotype groups were normally distributed (Kolmogorov-Smirnov one sample test). In the ANOVA tests, homogeneity of variance was tested (Levene's test). For each marker the data were homoscedastic.

Candidate gene selection

The confidence interval (in bp) for the QTL was determined using the "LOD drop-off" method where minus 1 LOD support interval is constructed by taking the two positions left and right of the maximum value in the LOD profile that have an LOD value of 1 less than this maximum; the two positions are estimated using linear interpolation calculations. Coordinates were obtained with NCBI Build 37 (Blake et al., 2009). Genes (i.e. protein coding genes and non-coding RNA genes) present in the 1-LOD support interval were selected on http://www.informatics.jax.org/. Brain expressed genes were selected based on microarray analysis of P14 mouse brain samples (see below). Genes on HSA2 (human chromosome 2) previously implicated in human FS or epilepsy were studied using OMIM http://www.ncbi.nlm.nih.gov/sites/entrez?db=OMIM, CarpeDB http://www.carpedb.ua.edu/ or Egad http://www.epigad.org/egad/list. (non-synonymous) SNP differences between A/J and C57BL/6J were studied with Mouse Genome Database (http://www.informatics.jax.org/strains_SNPs.shtml). The selected non-synonymous SNP differences between A/J and C57BL/6J were analysed with *Polyphen 2* and *SIFT* programs to determined whether they are missense or nonsense mutations. Other QTLs and eQTLs mapped in this region were studied with the Mouse Genome Database (http://www.informatics.jax.org/)

Microarray gene expression analysis

Total RNA was isolated from P14 C57BL/6J (n = 4) and CSS2 (n = 4) brains. These were compared in a common reference experiment design using 8 dual channel microarrays with each sample hybridized against an identical common reference total RNA sample consisting of pooled total RNA from the four C57BL/6J samples. Within each group of four microarrays for each genotype, sample versus common reference hybridisations were performed in balanced dye-swap. The microarrays used were

mouse whole genome GE 4x44K v2 (Agilent, Belgium) representing 39429 M. musculus 60-mer probes in a 4x44K layout. cDNA synthesis, cRNA amplification, labeling, quantification, quality control and fragmentation were performed with an automated system (Caliper Life Sciences NV/SA, Belgium), starting with 3 ug total RNA from each sample, all as previously described in detail (van Wageningen *et al.*, 2010). Microarray hybridization and washing was with a HS4800PRO system with QuadChambers (Tecan, Benelux) using 1000 ng, 1-2% Cy5/Cy3 labeled cRNA per channel as described (van Wageningen et al., 2010). Slides were scanned on an Agilent G2565BA scanner at 100% laser power, 30% PMT. After automated data extraction using Imagene 8.0 (BioDiscovery), Loess normalization was performed on mean spot-intensities (Yang *et al.*, 2002). Gene-specific dye bias was corrected by a within-set estimate. Data were further analysed by MAANOVA (Wu *et al.*, 2003) modeling sample, array and dye effects in a fixed effect analysis. The *P*-values were determined by a permutation F2-test, in which residuals were shuffled 10000 times globally. Gene probes with $P < 0.05$ after false discovery rate determination (FDR by Benjamini-Hochberg) were considered significantly changed. In cases of multiple probes per gene, the values from the most 3' probe were used. Prediction of brain expression was based on A-values. For genes with A-values < 7.0 brain expression could not reliably be determined (these genes are marked * in table 2).

Statistical analyses

All statistical analyses were carried out according to Field (Field, 2009) using a IBM© SPSS® Statistics for MS Windows (version 20.0) computer program (IBM Nederland N.V., Amsterdam, The Netherlands), and paying attention to the assumptions that underlie the various statistical procedures. Two-sided, exact (i.e. for the nonparametric tests) probabilities were estimated throughout. The data were summarized by means

and standard error of the means (SEM). The Kolmogorov-Smirnov one-sample test was used to check Gaussianity of the data.

The phenotypic characteristics of C57BL/6J and CSS2 mice were normally distributed and subjected to a two-way ANOVA with strain and gender as main factors. Homoscedasticity was tested using the Levene's test, which is a powerful and robust test based on the *F* statistic. If the ANOVA showed significant effects the group means were further compared with the unpaired Student's *t* test. The unpaired Student's *t* tests were performed using pooled (for equal variances) or separate (for unequal variances) variance estimates. Temperature increase was analysed with a univariate repeated measures two-way ANOVA and Bonferroni posthoc test was performed if allowed. The choice of a univariate instead of a multivariate statistic in the repeated measures ANOVA is based on the criteria given by Algina and Keselman (Algina & Keselman, 1997). In case of univariate repeated measures ANOVA violations to sphericity were addressed with a Huynh-Feldt correction to degrees of freedom. The equality of variances was tested with the Levene's test. For the unpaired Student's *t* test with separate variance estimates, SPSS® uses the Welch-Satterthwaite correction. Performing many ANOVA's in the F_2 population (see QTL analyses) increases the risk of a Type I error. To avoid this, the level of statistical significance for these ANOVA's was adjusted by using the so-called Dunn—Šidák method ($\alpha = 1 - [1 - 0.05]^{1/\gamma}$; γ = total number of DNA markers [17, $\alpha \approx 0.003$]). In all other cases (i.e. the Kolmogorov-Smirnov one sample test, Levene's test, statistical testing of host and consomic strain, Chi-squared goodness-of-fit-test) the probability of a Type I error < 0.05 was taken as the criterion of significance.

Results:

Comparison of FS susceptibility in C57BL/6J and CSS2

The difference in FSL between CSS2 (445.1 ± 15.1 s) and C57BL/6J (597.4 ± 36.2 s) mice (Fig.1 panel A) was confirmed in a separate group of male and female CSS2 (n = 9,) and C57BL/6J (n = 9) (two-way ANOVA: strain effect, $F_{strain(1,14)}$ = 11.867, P = 0.0001; gender effect, $F_{gender(1,14)}$ = 0.298, P = 0.594; interaction effect, $F_{interaction(1,14)}$ = 0.008, P = 0.929). The time-course of body temperature increase during exposure to hyperthermia did not differ between C57BL/6J and CSS2 mice (Fig. 1, panel B); time $F_{(1,14)}$ = 1704.63, P = *0.0001*; time×group $F_{(1,14)}$ = 1.363, P = 0.264). There was neither a difference in P14 body weight between the C57BL/6J (7.36 ± 0.24 g) and CSS2 (7.07 ± 0.13 g) (two-way ANOVA: strain effect, $F_{strain(1,14)}$ = 3.091, P = 0.101; gender effect, $F_{gender(1,14)}$ = 0.439, P = 0.518; interaction effect, $F_{interaction(1,14)}$ = 1.137, P = 0.304) nor a difference in core-body temperature at the start of the hyperthermia induction between the C57BL/6J (T_{start} = 36.0 ± 0.1°C) and CSS2 (T_{start} = 35.9 ± 0.2 °C) (two-way ANOVA: strain effect, $F_{strain(1,14)}$ = 0.202, P = 0.660; gender effect, $F_{gender(1,14)}$ = 1.153, P = 0.301; interaction effect, $F_{interaction(1,14)}$ = 2.502, P = 0.136)

QTL analysis CSS2

To map the QTLs for eFS susceptibility on mouse chromosome 2, we bred a CSS2-F_2 generation (n = 143) by intercrossing the hybrids (F_1) between the CSS2 and C57BL/6J inbred strains. As expected, phenotyping these genetically unique F_2 mice revealed a (left) shift toward a shorter FSL compared to C57BL/6J mice (Fig. 2A). Two QTLs were mapped on chromosome 2 (Fig. 2B). The most proximal FS-QTL2a had a LOD score of 3.6, accounted for 13.8% of the F_2 phenotypic variance and the effect was recessive with respect to the A/J grandparent's allele (Table 1). The 1-LOD support interval of FS-

QTL2a was 52,581,428 – 66,278,629 bp. FS-QTL2b had a LOD score of 6.2, accounted for 19.8% of the F_2 phenotypic variance and the effect is additive for the A/J grandparent's allele (Table 1). The 1 -LOD support interval for QTL2b was 114,875,854 – 133,817,501 bp (Fig. 2B). Detailed analysis of FS-QTL2b will be described elsewhere (Hessel et al., submitted).

FS-QTL2a

The 1-LOD support interval of FS-QTL2a (52,581,428 – 66,278,629 bp) contains 53 coding genes (and 47 predicted genes (GM)). Microarray analysis of brain tissue of C57BL/6J P14 mouse pups confirmed that 44 of these genes (and 8 GM genes) were brain expressed (Table 2). DNA microarray data has been submitted to GEO (accession nr GSE41320). During the review process the microarray data are available through the following link:
http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=hbwzjwcusywugtu&acc=GSE41320). In other mice studies no other QTLs related to epilepsy or other relevant (neurological) disorders were mapped in this QTL2A region.

Candidate gene selection was based on differential expression and SNPs between CSS2 and C57BL/6J. Six brain expressed genes in FS-QTL2a showed differential expression between C57BL/6J and CSS2 at P14 (*March7, Nr4a2, Gpd2, Grb14, Scn1a, Scn3a,* (Table 2, genes in Bold)). Four brain expressed genes in the QTL contained a non-synonymous SNP that may affect protein function *(Arl6ip6, Cytip, Fmnl2* and *Ifih1* (Table 2, genes underlined)). All these non-synonymous SNPs resulted in a missense mutation by changing the amino acid of the protein and did not cause a nonsense mutation (premature stop codon). Thus, based on expression difference and/or the presence of non-synonymous SNPs these 10 genes were designated candidate genes within FS-QTL2a. Further analysis of FS-QTL2a revealed that *Scn1a* and *Scn3a*, genes

previously associated with epilepsy, are genes encoded in the human *FEB3* locus. FS-QTL2a contains a sequence which is highly homologous to the human *FEB3* locus (2q23-q24 (Fig. 3), coding for several subunits of voltage gated sodium channels, some of which have previously been associated with human febrile or developmental seizures (Fig 3).

Discussion

In a forward genetic screen for eFS susceptibility using the C57BL/6J-Chr #^{A}/NaJ CSS panel, we recently identified mouse chromosome 2 as the chromosome with the strongest eFS susceptibility phenotype) (Hessel *et al.*, 2009). In the present study, we mapped two QTLs for eFS susceptibility on chromosome 2, FS-QTL2a and FS-QTL2b. Further analysis of FS-QTL2b will be described elsewhere (Hessel et al., submitted). The 1-LOD support interval of FS-QTL2a was 52,581,428 – 66,278,629 bp. This locus codes for 44 genes, which were confirmed to be expressed in brain tissue of P14 pups. Candidate gene selection identified 10 candidate genes, six based on differential brain expression (*March7, Nr4a2, Gpd2, Grb14, Scn1a* and *Scn3a)* and 4 genes containing a non-synonymous SNP that may affect protein function (*Arl6ip6, Cytip, Fmnl2* and *Ifih1*) (see Table 2). The CSS2 strain, which is most susceptible to FS, showed reduced expression of *March7, Nr4a2, Gpd2, Scn1a* and *Scn3* compared to the background strain C57BL/6J. *March7,* which showed the most significant reduction in expression, is a member of the MARCH family of membrane-bound E3 ubiquitin ligases (EC 6.3.2.19), which add ubiquitin to substrate proteins, thereby signaling their vesicular transport between membrane compartments (Bartee *et al.*, 2004). *Grb14* (growth factor receptor-bound protein 14), which is the only candidate gene which showed increased expression in CSS2, encodes a growth factor receptor-binding protein that interacts with insulin

receptors and insulin-like growth-factor receptors, probably inhibiting receptor tyrosine kinase signaling (Daly *et al.*, 1996).

Only three (*Scn1a, Scn3a and Nr4a2*) of the ten candidate genes have previously been associated with epilepsy. *Scn1a* and *Scn3a,* coding for voltage dependent sodium channels, are located in a conserved region which is homologous to the human *FEB3* locus (Fig. 3, 2q23-24) (Peiffer *et al.*, 1999) in which a *SCN1A (Mantegazza et al., 2005)* and a *SCN9A* gene mutation (Singh *et al.*, 2009) were identified. *SCN1A* mutations (loss- or gain-of-function) have been detected in a variety of genetic epilepsy syndromes including several with FS as a prominent phenotype, such as GEFS+ and Dravet Syndrome (Mulley *et al.*, 2005). Recent publications reviewing the identified gene mutations for SCN1A and their functions in animal and cell models emphasize the role of SCN1A in the susceptibility to different forms of epilepsy and febrile seizures (Brunklaus & Zuberi, 2014; Tang *et al.*, 2014; Schutte *et al.*, 2016). *SCN3A* mutations have not directly been associated with FS, but duplications in the *SCN* locus, which includes *SCN3A* but not *SCN1A,* have been associated with epilepsy in patients also suffering from FS (Vecchi *et al.*, 2011). One *SCN3A* mutation has been associated with pediatric epilepsy (Holland *et al.*, 2008). Recently, four novel *SCN3A* missense variants were identified in pediatric patients with focal epilepsy of unknown cause (Vanoye *et al.*, 2014). The third gene previously linked to epilepsy is *Nr4a2.* Distribution of *NURR1* (the human homologous of the *Nr4a2* gene) positive cells was found to be altered in the cortex of temporal lobe epilepsy patients (Rossini *et al.*, 2011). The other candidate genes in FS-QTL2a (*March7*, *Arl6ip6*, *Cytip, Fmnl2, Gpd2, Grp14* and *Ifih1*) have not previously been associated with epilepsy.

Based on our mouse differential expression data and above mentioned human studies *Scn1a* and *Scn3a* are strong candidate genes within FS-QTL2a. No non-synonymous SNPs were found between C57BL/6J and CSS2, indicating that *Scn1a* and/or *Scn3a*

expression levels, rather than protein (mal)function may be important in regulating FS susceptibility. For all ten candidate genes a potential causal relationship with FS susceptibility needs to be further investigated by performing, as recently described for the new FS susceptibility gene Srp9 (Hessel *et al.*, 2014), for instance protein expression and functional interference studies *in vivo* and *in vitro.* It is important to realize that only candidate genes showing expression or haplotype/genotype differences between parental strains of the CSS can be detected with our approach. Moreover, using this CSS approach, we cannot rule out that cis or trans effects influence the results of our QTL mapping (Nadeau *et al.*, 2000).

Interestingly, previous seizure susceptibility mapping studies in mice, using chemically or electrically-induced seizure models, show that C57BL/6J mice, which are most susceptible to FS, are relatively resistant to seizures and seizure-induced cell death (Ferraro *et al.*, 1995). This indicates that genes involved in FS susceptibility are distinct from those involved in chemical- or electrically-induced seizures. This notion is further substantiated by the fact that the only two other studies using the C57BL/6J-Chr $\#^{A}$/NaJ panel did not identify any seizure QTLs on chromosome 2, screening for susceptibility for sleep-related epilepsy (Strohl *et al.*, 2007) or chemoconvulsant pilocarpine (Winawer *et al.*, 2007). Mapping a mouse eFS QTL with homology to the human *FEB3* locus is strong support for the validity of our forward genetic strategy in mouse to detect human FS genes. The mouse phenotypic screen is based on measuring the latency to eFS elicited by warm-air induced hyperthermia. Thus, in contrast to FS in children, eFS are elicited by hyperthermia without an underlying infection. In children vulnerable to FS, hyperthermia alone, for instance induced by a hot bath, may also cause FS (Fukuda *et al.*, 1997). Thus, our data suggest that temperature sensitivity, rather than sensitivity to infection, is a key factor in the genetic predisposition to FS susceptibility. A recent study using a Dutch Cohort suffering from Dravet Syndrome with pathogenic SCN1A

mutations studied the events that trigger seizures in Dravet patients (Verbeek *et al.*, 2015). These authors identified elevated body temperature as an important seizure precipitant. The seizures could be caused by fever, a warm bath, ambient warmth, or physical exercise (Verbeek *et al.*, 2015).

In humans complex FS are a risk factor for the development of TLE later in life (French *et al.*, 1993). Complex FS are associated with 30-70% of the adult TLE cases (French *et al.*, 1993). Also, complex FS in rodents increase the risk to develop spontaneous recurrent seizures. Hyperthermia-induced FS elicit spontaneous recurrent seizures in 35-50% of the animals (Dube *et al.*, 2006; Koyama *et al.*, 2012). One interpretation of these data is that complex FS and TLE share a common genetic predisposition. Indeed, association and linkage studies have shown that the *SCN1A* mutations are associated with TLE and FS susceptibility (Table 3) (Van Poppel *et al.*, 2012; Kasperaviciute *et al.*, 2013). If FS and TLE indeed share a common genetic predisposition, candidate genes for FS susceptibility identified in the present study (such as Scn1a and Scn3a) may also be relevant for TLE.

Acknowledgements

This study was sponsored by the Epilepsy fund of The Netherlands (grant 06-09 & 11-09) and by the EPOCH Foundation (Focal Epilepsies of Childhood). We thank Prof. Dr. D. Collier (Institute of Psychiatry, King's College London) for the SNP markers.

Disclosures

None of the authors has any conflict of interest to disclose. The authors confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

References

Algina, J. & Keselman, H.J. (1997) Detecting repeated measures effects with univariate and multivariate statistics. . **2**, 208-218.

Baram, T.Z., Gerth, A. & Schultz, L. (1997) Febrile seizures: an appropriate-aged model suitable for long-term studies. *Brain research. Developmental brain research*, **98**, 265-270.

Bartee, E., Mansouri, M., Hovey Nerenberg, B.T., Gouveia, K. & Fruh, K. (2004) Downregulation of major histocompatibility complex class I by human ubiquitin ligases related to viral immune evasion proteins. *Journal of virology*, **78**, 1109-1120.

Brunklaus, A. & Zuberi, S.M. (2014) Dravet syndrome--from epileptic encephalopathy to channelopathy. *Epilepsia*, **55**, 979-984.

Chou, I.C., Peng, C.T., Huang, C.C., Tsai, J.J., Tsai, F.J. & Tsai, C.H. (2003) Association analysis of gamma 2 subunit of gamma- aminobutyric acid type A receptor polymorphisms with febrile seizures. *Pediatric research*, **54**, 26-29.

Cox, A., Ackert-Bicknell, C.L., Dumont, B.L., Ding, Y., Bell, J.T., Brockmann, G.A., Wergedal, J.E., Bult, C., Paigen, B., Flint, J., Tsaih, S.W., Churchill, G.A. & Broman, K.W. (2009) A new standard genetic map for the laboratory mouse. *Genetics*, **182**, 1335-1344.

Daly, R.J., Sanderson, G.M., Janes, P.W. & Sutherland, R.L. (1996) Cloning and characterization of GRB14, a novel member of the GRB7 gene family. *The Journal of biological chemistry*, **271**, 12502-12510.

Dube, C., Richichi, C., Bender, R.A., Chung, G., Litt, B. & Baram, T.Z. (2006) Temporal lobe epilepsy after experimental prolonged febrile seizures: prospective analysis. *Brain : a journal of neurology*, **129**, 911-922.

Feenstra, B., Pasternak, B., Geller, F., Carstensen, L., Wang, T., Huang, F., Eitson, J.L., Hollegaard, M.V., Svanstrom, H., Vestergaard, M., Hougaard, D.M., Schoggins, J.W., Jan, L.Y., Melbye, M. & Hviid, A. (2014) Common variants associated with general and MMR vaccine-related febrile seizures. *Nature genetics*, **46**, 1274-1282.

Ferraro, T.N., Golden, G.T., Smith, G.G. & Berrettini, W.H. (1995) Differential susceptibility to seizures induced by systemic kainic acid treatment in mature DBA/2J and C57BL/6J mice. *Epilepsia*, **36**, 301-307.

Field, A. (2009) *Discovering Statistics Using SPSS.* London: SAGE Publications Ltd;.

French, J.A., Williamson, P.D., Thadani, V.M., Darcey, T.M., Mattson, R.H., Spencer, S.S. & Spencer, D.D. (1993) Characteristics of medial temporal lobe epilepsy: I. Results of history and physical examination. *Annals of neurology*, **34**, 774-780.

Fukuda, M., Morimoto, T., Nagao, H. & Kida, K. (1997) Clinical study of epilepsy with severe febrile seizures and seizures induced by hot water bath. *Brain & development*, **19**, 212-216.

Hauser, W.A. (1994) The prevalence and incidence of convulsive disorders in children. *Epilepsia*, **35 Suppl 2**, S1-6.

Hessel, E.V., de Wit, M., Wolterink-Donselaar, I.G., Karst, H., de Graaff, E., van Lith, H.A., de Bruijn, E., de Sonnaville, S., Verbeek, N.E., Lindhout, D., de Kovel, C.G., Koeleman, B.P., van Kempen, M., Brilstra, E., Cuppen, E., Loos, M., Spijker, S.S., Kan, A.A., Baars, S.E., van Rijen, P.C., Gosselaar, P.H., Groot Koerkamp, M.J., Holstege, F.C., van Duijn, C., Vergeer, J., Moll, H.A., Tauboll, E., Heuser, K., Ramakers, G.M., Pasterkamp, R.J., van Nieuwenhuizen, O., Hoogenraad, C.C., Kas, M.J. & de Graan, P.N. (2014) Identification of Srp9 as a febrile seizure susceptibility gene. *Annals of clinical and translational neurology*, **1**, 239-250.

Hessel, E.V., van Gassen, K.L., Wolterink-Donselaar, I.G., Stienen, P.J., Fernandes, C., Brakkee, J.H., Kas, M.J. & de Graan, P.N. (2009) Phenotyping mouse chromosome substitution strains reveal multiple QTLs for febrile seizure susceptibility. *Genes, brain, and behavior*, **8**, 248-255.

Hirose, S., Mohney, R.P., Okada, M., Kaneko, S. & Mitsudome, A. (2003) The genetics of febrile seizures and related epilepsy syndromes. *Brain & development*, **25**, 304-312.

Holland, K.D., Kearney, J.A., Glauser, T.A., Buck, G., Keddache, M., Blankston, J.R., Glaaser, I.W., Kass, R.S. & Meisler, M.H. (2008) Mutation of sodium channel SCN3A in a patient with cryptogenic pediatric partial epilepsy. *Neuroscience letters*, **433**, 65-70.

Ishizaki, Y., Kira, R., Fukuda, M., Torisu, H., Sakai, Y., Sanefuji, M., Yukaya, N. & Hara, T. (2009) Interleukin-10 is associated with resistance to febrile seizures: genetic association and experimental animal studies. *Epilepsia*, **50**, 761-767.

Kas, M.J., de Mooij-van Malsen, J.G., de Krom, M., van Gassen, K.L., van Lith, H.A., Olivier, B., Oppelaar, H., Hendriks, J., de Wit, M., Groot Koerkamp, M.J., Holstege, F.C., van Oost, B.A. & de Graan, P.N. (2009) High-resolution genetic mapping of mammalian motor activity levels in mice. *Genes, brain, and behavior*, **8**, 13-22.

Kasperaviciute, D., Catarino, C.B., Matarin, M., Leu, C., Novy, J., Tostevin, A., Leal, B., Hessel, E.V., Hallmann, K., Hildebrand, M.S., Dahl, H.H., Ryten, M., Trabzuni, D., Ramasamy, A., Alhusaini, S., Doherty, C.P., Dorn, T., Hansen, J., Kramer, G., Steinhoff, B.J., Zumsteg, D., Duncan, S., Kalviainen, R.K., Eriksson, K.J., Kantanen, A.M., Pandolfo, M., Gruber-Sedlmayr, U., Schlachter, K., Reinthaler, E.M., Stogmann, E., Zimprich, F., Theatre, E., Smith, C., O'Brien, T.J., Meng Tan, K., Petrovski, S., Robbiano, A., Paravidino, R., Zara, F., Striano, P., Sperling, M.R., Buono, R.J., Hakonarson, H., Chaves, J., Costa, P.P., Silva, B.M., da Silva, A.M., de Graan, P.N., Koeleman, B.P., Becker, A., Schoch, S., von Lehe, M., Reif, P.S., Rosenow, F., Becker, F., Weber, Y., Lerche, H., Rossler, K., Buchfelder, M., Hamer, H.M., Kobow, K., Coras, R., Blumcke, I., Scheffer, I.E., Berkovic, S.F., Weale, M.E., Delanty, N., Depondt, C., Cavalleri, G.L., Kunz, W.S. & Sisodiya, S.M. (2013) Epilepsy, hippocampal sclerosis and febrile seizures linked by common genetic variation around SCN1A. *Brain : a journal of neurology*, **136**, 3140-3150.

Kira, R., Torisu, H., Takemoto, M., Nomura, A., Sakai, Y., Sanefuji, M., Sakamoto, K., Matsumoto, S., Gondo, K. & Hara, T. (2005) Genetic susceptibility to simple febrile seizures: interleukin-1beta promoter polymorphisms are associated with sporadic cases. *Neuroscience letters*, **384**, 239-244.

Kjeldsen, M.J., Kyvik, K.O., Friis, M.L. & Christensen, K. (2002) Genetic and environmental factors in febrile seizures: a Danish population-based twin study. *Epilepsy research*, **51**, 167-177.

Koyama, R., Tao, K., Sasaki, T., Ichikawa, J., Miyamoto, D., Muramatsu, R., Matsuki, N. & Ikegaya, Y. (2012) GABAergic excitation after febrile seizures induces ectopic granule cells and adult epilepsy. *Nature medicine*, **18**, 1271-1278.

Mantegazza, M., Gambardella, A., Rusconi, R., Schiavon, E., Annesi, F., Cassulini, R.R., Labate, A., Carrideo, S., Chifari, R., Canevini, M.P., Canger, R., Franceschetti, S., Annesi, G., Wanke, E. & Quattrone, A. (2005) Identification of an Nav1.1 sodium channel (SCN1A) loss-of-function mutation associated with familial simple febrile seizures. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 18177-18182.

Mulley, J.C., Scheffer, I.E., Petrou, S., Dibbens, L.M., Berkovic, S.F. & Harkin, L.A. (2005) SCN1A mutations and epilepsy. *Human mutation*, **25**, 535-542.

Nadeau, J.H., Singer, J.B., Matin, A. & Lander, E.S. (2000) Analysing complex genetic traits with chromosome substitution strains. *Nature genetics*, **24**, 221-225.

Nakayama, J. (2009) Progress in searching for the febrile seizure susceptibility genes. *Brain & development*, **31**, 359-365.

Peiffer, A., Thompson, J., Charlier, C., Otterud, B., Varvil, T., Pappas, C., Barnitz, C., Gruenthal, K., Kuhn, R. & Leppert, M. (1999) A locus for febrile seizures (FEB3) maps to chromosome 2q23-24. *Annals of neurology*, **46**, 671-678.

Rossini, L., Moroni, R.F., Tassi, L., Watakabe, A., Yamamori, T., Spreafico, R. & Garbelli, R. (2011) Altered layer-specific gene expression in cortical samples from patients with temporal lobe epilepsy. *Epilepsia*, **52**, 1928-1937.

Schutte, S.S., Schutte, R.J., Barragan, E.V. & O'Dowd, D.K. (2016) Model systems for studying cellular mechanisms of SCN1A-related epilepsy. *Journal of neurophysiology*, **115**, 1755-1766.

Singh, N.A., Pappas, C., Dahle, E.J., Claes, L.R., Pruess, T.H., De Jonghe, P., Thompson, J., Dixon, M., Gurnett, C., Peiffer, A., White, H.S., Filloux, F. & Leppert, M.F. (2009) A role of SCN9A in human epilepsies, as a cause of febrile seizures and as a potential modifier of Dravet syndrome. *PLoS genetics*, **5**, e1000649.

Strohl, K.P., Gallaugher, L., Lynn, A., Friedman, L., Hill, A., Singer, J.B., Lander, E.S. & Nadeau, J. (2007) Sleep-related epilepsy in the A/J mouse. *Sleep*, **30**, 169-176.

Tang, L., Lu, X., Tao, Y., Zheng, J., Zhao, P., Li, K. & Li, L. (2014) SCN1A rs3812718 polymorphism and susceptibility to epilepsy with febrile seizures: a meta-analysis. *Gene*, **533**, 26-31.

van Gassen, K.L., Hessel, E.V., Ramakers, G.M., Notenboom, R.G., Wolterink-Donselaar, I.G., Brakkee, J.H., Godschalk, T.C., Qiao, X., Spruijt, B.M., van Nieuwenhuizen, O. & de Graan, P.N. (2008) Characterization of febrile seizures and febrile seizure susceptibility in mouse inbred strains. *Genes, brain, and behavior*, **7**, 578-586.

Van Poppel, K., Patay, Z., Roberts, D., Clarke, D.F., McGregor, A., Perkins, F.F. & Wheless, J.W. (2012) Mesial temporal sclerosis in a cohort of children with SCN1A gene mutation. *Journal of child neurology*, **27**, 893-897.

van Wageningen, S., Kemmeren, P., Lijnzaad, P., Margaritis, T., Benschop, J.J., de Castro, I.J., van Leenen, D., Groot Koerkamp, M.J., Ko, C.W., Miles, A.J., Brabers, N., Brok, M.O., Lenstra, T.L., Fiedler, D., Fokkens, L., Aldecoa, R., Apweiler, E., Taliadouros, V., Sameith, K., van de Pasch, L.A., van Hooff, S.R., Bakker, L.V., Krogan, N.J., Snel, B. & Holstege, F.C. (2010) Functional overlap and regulatory links shape genetic interactions between signaling pathways. *Cell*, **143**, 991-1004.

Vanoye, C.G., Gurnett, C.A., Holland, K.D., George, A.L., Jr. & Kearney, J.A. (2014) Novel SCN3A variants associated with focal epilepsy in children. *Neurobiology of disease*, **62**, 313-322.

Vecchi, M., Cassina, M., Casarin, A., Rigon, C., Drigo, P., De Palma, L. & Clementi, M. (2011) Infantile epilepsy associated with mosaic 2q24 duplication including SCN2A and SCN3A. *Seizure*, **20**, 813-816.

Verbeek, N.E., Wassenaar, M., van Campen, J.S., Sonsma, A., Gunning, B., Knoers, N., Lindhout, D., Jansen, F.E., Leijten, F., Brilstra, E.H. & Kasteleijn-Nolst Trenite, D. (2015) Seizure precipitants in Dravet syndrome: What events and activities are specifically provocative compared with other epilepsies? *Epilepsy & behavior : E&B*, **47**, 39-44.

Winawer, M.R., Kuperman, R., Niethammer, M., Sherman, S., Rabinowitz, D., Guell, I.P., Ponder, C.A. & Palmer, A.A. (2007) Use of chromosome substitution strains to identify seizure susceptibility loci in mice. *Mammalian genome : official journal of the International Mammalian Genome Society*, **18**, 23-31.

Wu, H.M., Kerr, K., Cui, X. & Churchill, G.A. (2003) MAANOVA: A Software Package for the Analysis of Spotted cDNA Microarray Experiments. *The Analysis of Gene Expression Data: Methods and Software, New York: Springer*, 313-341.

Yang, Y.H., Dudoit, S., Luu, P., Lin, D.M., Peng, V., Ngai, J. & Speed, T.P. (2002) Normalization for cDNA microarray data: a robust composite method addressing single and multiple slide systematic variation. *Nucleic acids research*, **30**, e15.

Table 1. Co-segregation analysis of the CSS2-F2 progeny

Febrile seizure latencies (FSL, s) are expressed as means (±SEM). Numbers in parentheses indicate number of mice. Some DNA samples failed to give a conclusive genotype, hence the number of mice typed varied slightly with each locus. Numbers in bold indicate significant difference ($P < 0.003$). Alleles: A = A/J-allel, B = C57BL/6J-allel.

Marker (cM)	AA	AB	BB	*F*	*P*
D2Mit80 (14.7)	572.2 ± 20.8 (38)	560.9 ± 14.6 (72)	546.9 ± 17.8 (31)	0.378	0.686
D2Mit370 (24.5)	600.9 ± 23.5 (36)	546.3 ± 13.4 (66)	546.5 ± 15.4 (41)	3.008	0.053
D2Mit458 (29.3)	618.0 ± 24.6 (31)	558.7 ± 14.6 (66)	533.0 ± 14.5 (46)	5.012	0.008
D2Mit156 (31.7)	634.1 ± 23.4 (37)	542.6 ± 12.9 (64)	533.0 ± 15.3 (41)	**9.735**	**0.000**
D2Mit380 (40.9)	612.5 ± 25.6 (33)	567.8 ± 13.9 (63)	526.4 ± 14.9 (44)	5.171	0.007
D2Mit94 (47.9)	615.1 ± 24.6 (36)	563.5 ± 12.4 (70)	511.7 ± 16.9 (35)	**7.08**	**0.001**
D2Mit66 (49.5)	616.2 ± 24.6 (36)	559.6 ± 12.7 (67)	506.3 ± 14.6 (33)	**8.147**	**0.000**
D2Mit184 (53.1)	612.3 ± 23.1 (38)	562.2 ± 12.9 (75)	501.7 ± 14.2 (30)	**7.778**	**0.001**
D2Mit336 (57.7)	643.3 ± 26.0 (30)	552.6 ± 11.9 (72)	518.9 ± 18.2 (38)	**10.59**	**0.000**
rs27498297 (59.1)	644.0 ± 26.0 (31)	557.2 ± 12.2 (73)	506.8 ± 15.1 (38)	**13.1**	**0.000**
D2Mit277 (60.7)	649.3 ± 26.3 (30)	557.9 ± 12.3 (72)	507.2 ± 14.0 (41)	**14.5**	**0.000**
rs27434812 (62.0)	645.5 ± 27.6 (28)	563.0 ± 12.5 (73)	506.3 ± 14.4 (40)	**12.79**	**0.000**
D2Mit401 (70.4)	637.3 ± 25.4 (31)	552.7 ± 12.2 (75)	519.1 ± 17.3 (36)	**9.567**	**0.000**
D2Mit282 (73.6)	624.3 ± 23.1 (36)	548.3 ± 12.8 (70)	528.5 ± 17.8 (36)	**7.224**	**0.001**
D2MIT493 (76.1)	615.9 ± 22.2 (39)	544.6 ± 13.2 (66)	540.6 ± 18.6 (35)	5.395	0.006
D2MIT51 (84.3)	609.6 ± 22.9 (39)	548.0 ± 13.0 (67)	538.6 ± 17.9 (36)	4.354	0.015
D2MIT113 (96.2)	600.0 ± 22.1 (40)	546.3 ± 13.4 (65)	550.4 ± 18.7 (37)	2.771	0.066

Table 2. Differential expression between C57BL/6J and CSS2 of brain expressed genes in FS-QTL2A. mRNA profiling of C57BL/6J (n = 4) and CSS2 (n = 4) mouse brains confirmed that 44 genes were brain expressed at P14 [and eight predicted (GM)] in FS-QTL2A. Six genes (in bold) were differentially expressed between strains (ratio CSS2/C57BL/6J, P-value for multiple testing $P < 0.05$). Four genes (underlined) contain non-synonymous SNPs (www.genenetwork.org). Candidate gene selection identified 10 candidate genes, six based on differential brain expression [March7 (membrane associated ring finger 7), Nr4a2 (nuclear receptor subfamily 4, group A, member 2), Gpd2 (glycerol phosphate dehydrogenase 2, mitochondrial), Grp14, (growth factor receptor bound protein 14) Scn1a (sodium channel, voltage-gated, type I, alpha), and Scn3a (sodium channel, voltage-gated, type III, alpha)] and four genes containing a non-synonymous SNP that may affect protein function [Arl6ip6 (ADP-ribosylation factor-like six interacting protein 6), Cytip (cytohesin 1 interacting protein), Fmnl2 (formin-like 2) and Ifih1 (interferon induced with helicase C domain 1)]. *A-value < 7: brain expression cannot be confirmed in our P14 microarray.

Gene Symbol	*P*-value	Ratio	Gene Symbol	*P*-value	Ratio
*4930555B11Rik**	0.99	0.98	*Gm13619**	0.48	1.08
*5330411J11Rik**	1.00	0.99	*Gm13620*	0.85	0.95
A930012O16Rik	0.95	0.96	***Gpd2***	**0.04**	**0.88**
Acvr1c	1.00	1.00	***Grb14***	**0.00**	**1.25**
Arl6ip6	0.99	1.02	*Ifih1*	0.94	0.96
Baz2b	1.00	1.02	*Itgb6*	0.99	1.04
BB557941	0.11	0.89	*Kcnh7*	1.00	1.00
Cacnb4	0.28	0.88	*Kcnj3*	0.95	0.97
Ccdc148	1.00	0.99	*Ldha-ps*		
Cd302	0.67	1.07	*Ly75**	0.97	1.03
Cobll1	0.87	1.06	***March7***	**0.00**	**0.83**
Csrnp3	0.99	0.98	***Nr4a2***	**0.01**	**0.87**
*Cytip**	0.96	0.97	*Pkp4*	0.85	1.05
*Dapl1**	0.99	1.02	*Pla2r1*	0.83	1.05
Dpp4	1.00	0.98	*Prpf40a*	0.19	0.91
Ermn	0.99	0.95	*Psmd14*	1.00	0.99
Fap	0.64	0.92	*Rbms1*	0.96	0.95
Fign	0.18	0.88	*Rprm*	0.99	0.98
Fmnl2	1.00	1.01	***Scn1a***	**0.03**	**0.88**
Galnt13	1.00	0.99	*Scn2a1*	1.00	1.00
Galnt3	1.00	0.99	***Scn3a***	**0.04**	**0.83**
*Galnt5**	1.00	1.01	*Scn9a*	0.99	1.03
Gca	0.82	1.08	*Slc38a11*	0.93	1.04
*Gcg**	0.99	0.98	*Slc4a10*	0.83	1.06
*Gm13531**	1.00	1.02	*Stam2*	0.99	1.02
Gm13545	0.98	0.97	*Tanc1**	1.00	1.02
Gm13546	1.00	1.00	*Tank*	0.99	0.97
Gm13549	1.00	1.00	*Tbr1*	0.99	1.02
Gm13573	0.61	0.92	*Ttc21b*	0.99	0.97
Gm13580	0.13	0.87	*Upp2**	0.87	1.05
Gm13582	1.00	1.01	*Wdsub1*	0.99	1.02
Gm13592	0.72	1.10			

Table 3. Linkage and association studies in FS patients investigating genes in the FEB3 locus (http://www.epigad.org/egad/list). SCN3A is associated with pediatric epilepsies (Holland et al., 2008; Vanoye et al., 2014). 1 Schlachter et al. (2009); 2 Le Gal et al. (2011); 3 Peiffer et al. (1999); 4 Mantegazza et al. (2005); 5 Zhang et al. (2010); 6 Chou et al. (2003); 7 Nakayama & Arinami (2006); 8 Berkovic et al. (2004) 9 Sugawara et al. (2001); 10Singh et al. (2009). All SNPs in BOLD are missense SNPs.

Gene symbol	FS association studies			FS linkage studies
	SNP	Positive	Negative	
SCN1A	**IVS5N+5G>A**	< 0.0001[1]	> 0.15[2]	FEB3A/ GEFS+[3,4]
	rs3812718		> 0.02[5]	
	rs2298771		0.81[6]	
SCN2a	**R19K**		0.18[7]; 0.19	GEFS+[8]
	R188W		1[9]	
SCN3a	no			no
SCN9a	no			FEB3B[3,10]

Figure legends

Fig 1. Febrile seizure susceptibility in C57BL/6J and CSS2 mice. (A) CSS2 mice (n = 9, 445.1 15.1 s) have a shorter FSL (s) then C57BL/6J (n = 9, 597.4 36.2 s) Two-way ANOVA: strain effect, $F_{strain}(1,14) = 11.867$, $P = 0.0001$; gender effect, $F_{gender}(1,14) = 0.298$, $P = 0.594$; interaction effect, $F_{interaction}$ $(1,14) = 0.008$, $P = 0.929$. (B) The time-course of body temperature increase during exposure to hyperthermia did not differ between C57BL/6J and CSS2 mice, time F1,14 = 1704.63, P = 0.0001; time 9 group F1,14 = 1.363, P = 0.264. The time-course of body temperature increase during exposure to hyperthermia did not differ between C57BL/6J and CSS2 mice (time F1,14 = 1704.63, P = 0.0001; time 9 group F1,14 = 1.363, P = 0.264). Error Bars represents Standard Error of the Mean. The asterisk (*) denotes significant difference ($P < 0.05$).

Fig. 2. QTL mapping on chromosome 2. (A) Frequency histogram of FS latencies of CSS2-F2 progeny (n = 143) shows that, as expected, the majority of these genetically unique CSS2-F2 mice have a shorter FSL than C57BL/6J (black bar is C57BL/6J average). (B) Identification of 2 FSL QTLs: QTL2a LOD = 3.6, (1-LOD support interval: 52 581 428–66 278 629 bp) and QT2b LOD = 6.2, (1-LOD support interval: 114 875 854–133 817 501 bp). 1-LOD support interval indicated with the dotted line (Cox et al., 2009).

Fig. 3. Homology between mouse QTL2A (left box) and a human chromosome 2 region (right box) containing the FEB3 locus (2q24) (Peiffer et al., 1999; Mantegazza et al., 2005; Singh et al., 2009). Figure based on data derived from www.ensembl.org.

Figure 1.

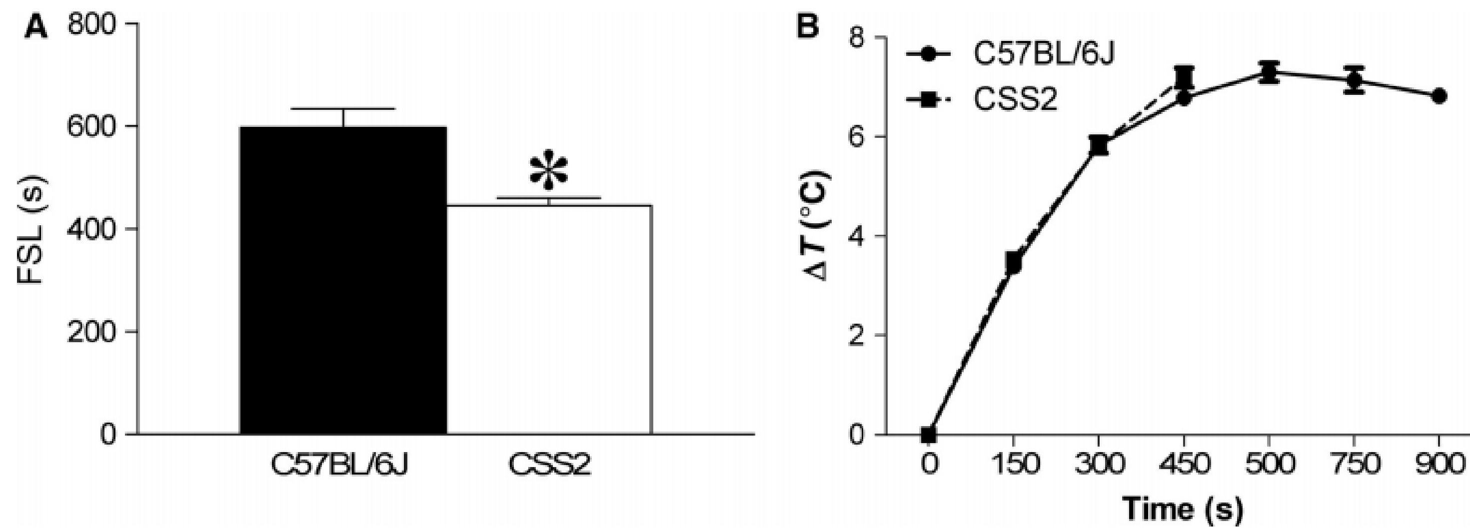
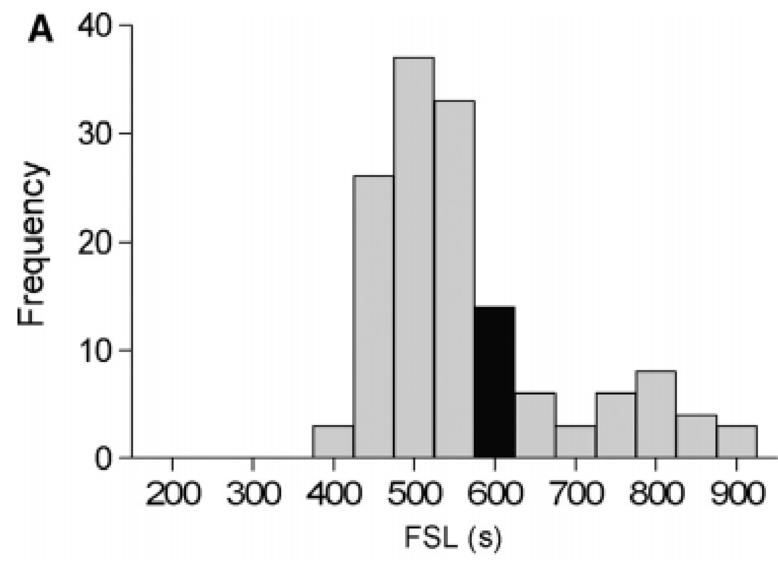


Figure 2



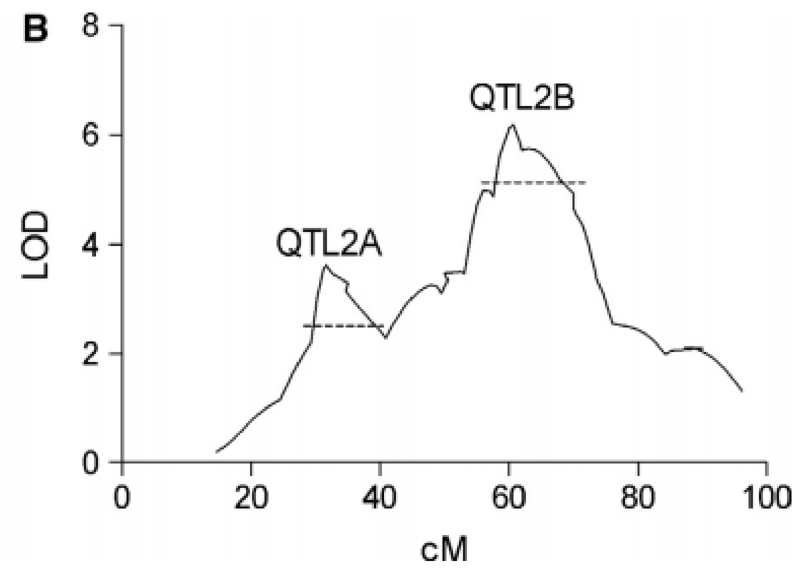
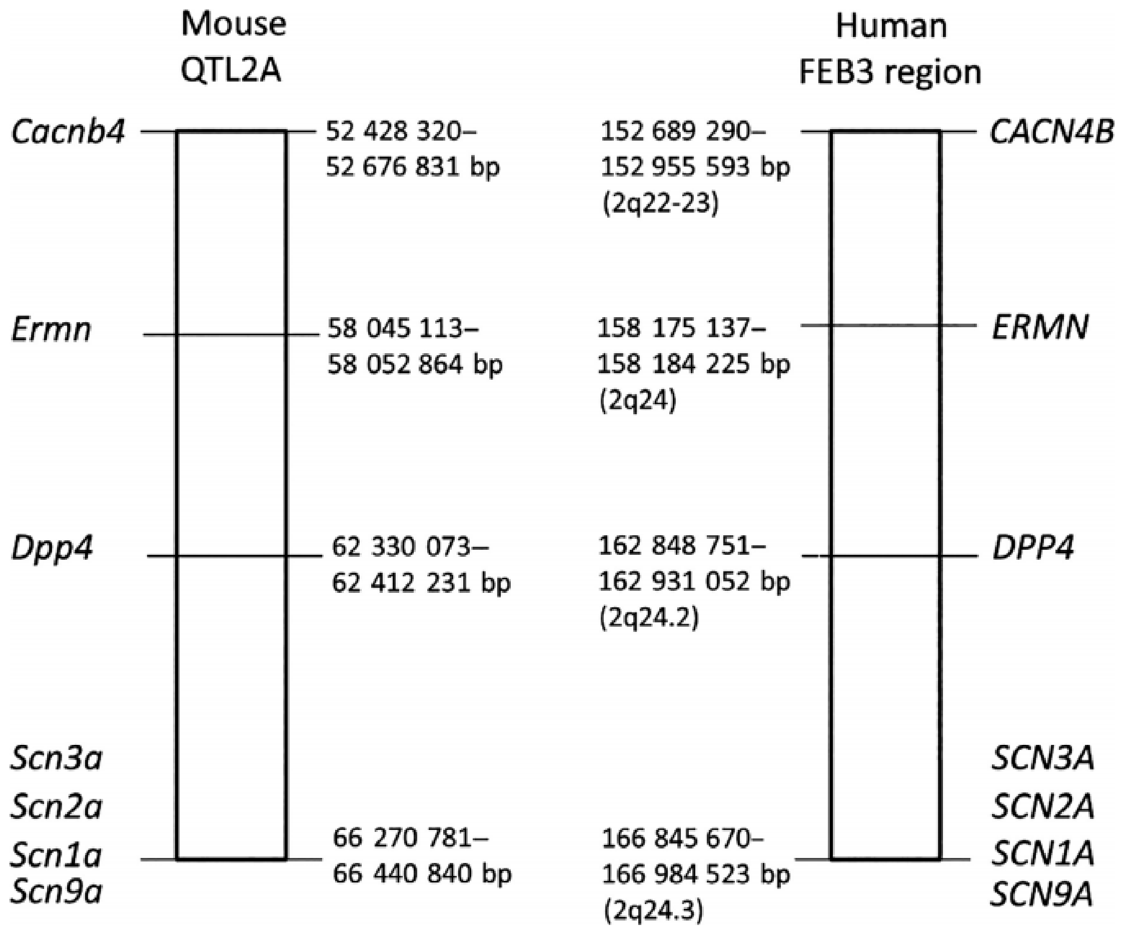


Figure 3



Supplemental Table 1 microsatelite markers chromosome 2

Primers / markers	Forward	Reverse	Size marker (bp)
D2Mit80	TAGCCTACAGAGTGGACAGCC	CTAGGCTTTATGTAGCCTCTTTGC	12
D2Mit370	ATCAAAGGAAGACAGGTGAATG	TTTCTTCCCTGTCTATGTGATAAGG	17
D2MIT458	GTAGTTGAGGAAGACAATTGACACA	AGTGCTGTCCTCTGGGCTTA	24
D2Mit156	ACTGGGGAGACTAAATGGGG	ACTCTTCCATGCAACCGATT	14
D2Mit380	CCTCAGGTCTGAAATGAGGTG	AATGATGTGCATGTGCGC	14
D2Mit94	GGCTTCGACCCTGGTTTTAG	TGAAAGTTCAGATGACCACAGG	34
D2Mit66	GTTGCACAGGCAATCAACC	ATCTATCACTGGGGCTGTGC	22
D2Mit184	TGCCCTAAAACTTTAATTTACTTAACA	TCTTACAATATCACTAGCAAGTGAACA	21
D2Mit336	CTGGATTCCACAGGCACCTA	ACAAACAATATTTTTAAAAGGGTGTG	20
D2Mit277	TATTTCCCTGTCACTCCTCCC	TGTCTTTATGCTCAGACATACACA	129
D2Mit401	TTTCTCCTTAGTAACCTCTGCCC	ATTTACTTCAGCCACATTTGCA	10
D2Mit282	GCAACCTCAAACATACTCCATG	CTCTTCACAGATTCCCCCTG	14
D2Mit493	GTCTCTACCTGAGTTTCCATCACA	TCCCGAGTTGTCCCTCTATG	12
D2Mit51	GTGAGGGGTCAATGCCAC	GGCTCAGTTGTAAGCACAAGG	14
D2MIT113	CTCACGTGAGGGTCATGAGA	CTTCTCTACCTTCCTCAGAAGCC	20