Genetic complexity of absence seizures in substrains of C3H mice

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Abstract

Absence epilepsy is a common form of idiopathic generalized epilepsy whose etiology is poorly understood due to genetic and phenotypic heterogeneity. The inbred mouse strain C3H/He exhibits spontaneous absence seizures characterized by spike and wave discharges (SWD) on the electroencephalogram concomitant with behavioral arrest. Previous studies using the C3H/HeJ (HeJ) substrain identified a mutation in the *Gria4* gene as a major susceptibility locus. In the present study we found that two closely-related substrains C3H/HeOuJ (OuJ) and C3H/HeSnJ, which have a similar SWD incidence as HeJ, do not contain the *Gria4* mutation. Further analysis of backcross mice segregating OuJ and C57BL/6J alleles shows that, unlike the HeJ substrain, OuJ does not have a major locus for SWD but has suggestive loci at best that would explain only a fraction of the phenotypic variance. These results illustrate how the genetic etiology of a common neurological disorder can differ between substrains with similar phenotypes. We infer that all C3H strains are sensitized to SWD and that additional mutations affecting SWD arose or were fixed independently in the years since the substrains diverged.

Keywords

absence seizure; inbred mouse strains; complex trait; substrain divergence; AMPA receptor

Introduction

Absence epilepsy is a non-convulsive form of idiopathic generalized epilepsy that occurs frequently in childhood and adolescence. An absence seizure is defined by a sudden and brief arrest of normal activity that is accompanied by rhythmic and synchronous spike-wave discharges (SWD) on the electroencephalogram (EEG). Clinical and experimental studies indicate a central role of the thalamocortical circuitry in the genesis of SWD. The molecular pathogenesis, however, remains unclear because the inheritance is usually polygenic or multifactorial (Futatsugi & Riviello, 1998, Huguenard & Mccormick, 2007). Several mutant and inbred rodents strains, exhibiting SWD with behavioral and pharmacological features similar to absence seizure in humans, have long contributed to the field. For example, tottering, stargazer, ducky, and lethargic mutant mice, resulting from mutations in Ca^{2+} channel subunit genes, have been well accepted as absence seizure models despite their ataxic phenotype (Burgess *et al.*, 1997, Cox *et al.*, 1997, Fletcher *et al.*, 1996, Letts *et al.*, 1998, Barclay *et al.*, 2001). More recently the mouse model harboring a $GABA_A$ γ2 missense mutation that segregated in a large family with childhood absence epilepsy and febrile seizures, recapitulated the primary seizure phenotype observed in patients and its absence seizure phenotype was very similar to prior mouse models (Tan *et al.*, 2007). C3H

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is a very commonly used inbred mouse strain with no obvious neurological disease phenotypes, other than retinal degeneration (Fox, 1997). In 2005, EEG recording revealed that the C3H/HeJ (HeJ) substrain exhibited a modest incidence of 7–8-Hz SWD lasting about 2.5 seconds, concomitant with behavioral arrest (Frankel *et al.*, 2005). Segregation tests using a backcross with the C57BL/6J (B6) strain indicated an unusual form of inheritance, with backcross mice showing a much higher incidence of SWD than HeJ itself. Nevertheless, a major HeJ-derived allele for SWD (*spkw1*) was genetically mapped to centromeric Chromosome 9. More recent work identified an intronic proviral insertion in the *Gria4* gene encoding the ionotropic AMPA receptor subunit GRIA4 (also known as GLUR4), very likely to be synonymous with *spkw1* (Beyer *et al.*, 2008). The mutation resulted in the reduction of GRIA4 expression in HeJ mice. The cause-effect relationship between *Gria4* mutation and seizure phenotype was confirmed by use of mice carrying a targeted mutation.

Other substrains of C3H/He, such as C3H/HeOuJ (OuJ) and C3H/HeSnJ (SnJ), have a similar incidence and form of SWD as HeJ. This suggested the hypothesis that susceptibility genes for SWD (e.g. *Gria4*) in HeJ, might be fixed in all C3H/He substrains. In the present study, however, we report that OuJ and SnJ substrains did not have a *Gria4* mutation. The SWD incidence in backcross with OuJ and B6 appeared to show a more continuous distribution reflective of the parental strains, than that seen in crosses with HeJ. In OuJ we did not detect any significant loci associated with SWD, suggesting the genetic component is much more complex. Large-scale quantitative trait locus (QTL) analysis or alternative strategies will be required to further investigate the molecular basis of SWD in OuJ and other substrains of C3H/He.

Materials and Methods

Animals

All mice used in this study were obtained originally from The Jackson Laboratory (Bar Harbor, ME) and housed in the Research Facility at The Jackson Laboratory. All animal procedures were approved by Institutional Animal Care and Use Committee (IACUC).

Cortical EEG recordings

Mice that were between 6 and 10 weeks of age were tested for spontaneous SWD activity as previously described (Beyer *et al.*, 2008). Briefly, mice were anesthetized with tribromoethanol (400mg/kg i.p.) and four Teflon-coated silver wires were implanted on both sides of the skull (1 mm anterior to the bregma, 2 mm posterior to the bregma and 2 mm lateral to the midline). At least 48 hours later, EEG activity of mice was measured twice for two hours on separate days (four hours total) using the Grass EEG Model 12 Neurodata Acquisition System and PolyViewPro software program (Grass-Telefactor, Inc.) or the Stellate Harmonie system (Stellate, Inc.). The mean values of SWD frequency and cumulative length per hour of the two sessions were calculated for individual mouse, with very good correspondence between sessions (r=0.62463; p=0.7282).

Genotyping

Genomic DNA was isolated from tails of the backcross progeny (N_2) or intercross progeny (F_2) that were generated between C3H/HeOuJ and C57BL/6J. Microsatellite markers distinguishing C3H and B6 alleles were amplified with standard PCR techniques and analyzed using agarose gel electrophoresis. N_2 mice were genotyped with the total of 47 markers covering all mouse chromosomes including Chr X and F_2 mice with nine markers based on the initial screening.

Data analysis

Marker regression analysis and interval mapping were performed using MapManagerQTXb20 (http://www.mapmanager.org/). Genome-wide significance thresholds were calculated using the permutation test, for 1,000 permutations. Calculated thresholds were suggestive linkage LRS > 5.0, significant linkage LRS > 8.6, and highly significant linkage LRS > 12.2. Further evaluations of association between genotypes and phenotypes were determined by one-way ANOVA and contingency test using JMP software (SAS Institute, Cary, NC)

Genomic PCR

The intracisternal A-particle (IAP) insertion site found in *Gria4* in C3H/HeJ was amplified using genomic DNAs isolated from excised tail tips of C3H/HeJ, HeOuJ, HeSnJ, C3HeB/FeJ and C57BL/6J with the primers used in the previous study, which were designed in genomic regions located on either side of the mutation sites or within 5′ end of IAP long terminal repeat region (Beyer *et al.*, 2008).

Western blot analysis

Protein lysates were processed using cerebellum of adult mice as previously reported (Beyer *et al.*, 2008). Rabbit polyclonal antibodies for GRIA4 carboxyl terminus (1:200, Chemicon, CA) and for Calbindin D-28-K (1:5000, Swant, Switzerland) were used for primary antibodies. Horseradish peroxidase-conjugated secondary antibody (PerkinElmer, MA) was used. Protein bands were detected using the ECL-plus chemiluminescent reagent (Amersham Biosciences).

Results

The C3H/He substrains: HeJ, OuJ and SnJ exhibit a modest incidence of SWD while another major substrain, C3HeB/FeJ, exhibits a only low incidence (Figure 1) (Frankel *et al.*, 2005). Previous work revealed that the major genetic basis for SWD in HeJ mice is the IAP-insertion mutation in *Gria4* (Beyer *et al.*, 2008). Since the genetic background was expected to be very close among HeJ, OuJ and SnJ substrains, and the features of SWD were observed to be similar, we assumed that SWD in OuJ, SnJ and HeJ would have the same genetic basis. Genomic PCR with primer sets designed to distinguish between the proviral insertion mutation and wild type, and subsequent sequencing, however, showed OuJ and SnJ had the wild-type preintegration site, indicating these two substrains did not have the same allele of *Gria4* as HeJ (Figure 2). Further, while we could not assess whether C3H/HeN mice exhibit SWD, examination of genomic DNA showed the wild–type allele at *Gria4* (data not shown). Western blot analysis further revealed that OuJ and SnJ expressed the GRIA4 protein (Figure 2). These findings suggested that the seizure in both OuJ and SnJ are caused by genetic mutations other than that found in HeJ. This fact prompted us to further investigate the genetic basis of SWD in the C3H/He substrains, focusing on OuJ.

We crossed OuJ to B6 or FeJ to determine the mode of inheritance. Of five OuJ x B6 F_1 hybrid mice tested, SWD were observed at low incidence in two animals and no SWD in others, suggesting that inheritance was mostly recessive (Figure 3). We then crossed the F_1 hybrid mice to OuJ mice and assessed SWD in the resultant backcross generation. Almost all progeny showed some SWD, the frequency of which spanned the entire range defined by parental strains, with only one mouse exhibiting much higher frequency (56 SWD/hr) (Figure 3); this is in contrast with previous results from HeJ x B6 where a large number of backcross mice had SWD at a much higher rate than HeJ itself (Frankel *et al.*, 2005). About one third of the current backcross showed modest frequency similar to OuJ (10–30 SWD/hr) and the rest showed lower frequency (<10 SWD/hr). Those findings further suggested that

the inheritance was recessive but non-Mendelian in this OuJ and B6 cross. On the other hand, in F_1 hybrids between OuJ and FeJ substrains of C3H/He, all mice exhibited a similar frequency to OuJ, and the backcross progeny to OuJ also showed a modest frequency of SWD except for one mouse with a low incidence (Figure 3). We infer that the major genes underlying SWD in OuJ are dominant or semidominant when on the C3H background - showing further that background appears to influence the mode of inheritance of the seizure phenotype of OuJ.

To explore the genetic basis of SWD in OuJ mice, we first conducted parametric analysis using 56 OuJ and B6 N_2 backcross mice. The number of SWD per hour was determined and transformed into natural log to obtain a more normal distribution and to equalize the variance as much as possible; a genome scan was then performed. The preliminary marker regression analysis using 42 polymorphic microsatellite markers gave potential evidence that Chr 5, 12, 14 and 16 are associated with the phenotype (Table 1). The susceptibility alleles on Chr 14 and 16 would be derived from OuJ, but those on Chr 5 and 12 would be transgressive susceptibility alleles derived from the B6 strain (Figure 4). Subsequently, additional markers for potential OuJ susceptibility loci were genotyped and tested. As a result, suggestive associations between phenotype and genotype were detected at the centromere-proximal markers on Chr 16 (*D16Mit165*; Table 2 and Figure 4). We then performed interval mapping, and modest peaks were detected only within Chr 5 and Chr 16, surpassing the suggestive threshold as estimated by using a permutation test (Figure 5). Neither main effect loci nor pairwise interactions were found to be significantly associated with the phenotype, nor was there a significant effect of gender. Moreover, no association was seen in the *Gria4* region of Chr 9, suggesting that *Gria4* variants are not involved in SWD of OuJ mice (*D9Mit67*, $p=0.759$; *D9Mit18*, $p=0.768$).

As an alternative, we applied a non-parametric approach to detecting SWD loci, hypothesizing that an obligate interaction between several loci might be required for high seizure incidence. We noted that five N_2 progeny showed a much higher incidence of SWD (>20 SWD) compared with the remaining animals (Figure 3). An obligate interaction between three recessive alleles would result in approximately 1/8 of all the backcross mice having a high seizure incidence – not significantly different from 1/10 observed. An allele frequency test between markers and phenotype was therefore conducted between these two groups. While none of markers on Chr 5, 12, 14 and 16, previously suggested by parametric analysis, showed a significant difference between groups, the non-parametric approach showed that all individuals in the high-incidence group had OuJ homozygous alleles at *D4Mit54* and *D18Mit120* (Fisher's exact test: $p<0.0257$ and $p<0.0445$, respectively; Table 3).

Although the evidence was weak for OuJ-derived SWD susceptibility loci obtained from either parametric or non-parametric analysis, we thought that it was still possible that major loci could be located on Chr 16 or 14 (the two chromosomes with the most promising OuJ-derived susceptibility loci), but perhaps their effects were masked by other complexities in the backcross. An intercross would provide a more diffuse population structure than the corresponding backcross, for example, breaking-up certain types of interactions that might interfere with the ability to detect major effects. Such complexities might include transgressive alleles – even minor ones - that are linked to Chr 16 or Chr 14, or epistasis between main effect loci, and recessive OuJ-derived modifiers in the background, which would be less prolific in an intercross than in a backcross.

To examine whether the suggestive associations on Chr 16 and Chr 14 could be detected in intercross mice, 40 F_2 progeny of OuJ and B6 were generated and genotyped before examining phenotype. To perform the hypothesis test efficiently, EEG was ultimately

recorded from 22 F_2 mice that were homozygous for one or the other parental allele of Chr 16. As shown in Figure 3, one third (seven) of F_2 mice exhibited the similar frequency of SWD (>10 SWDs/hr) to OuJ parental strain while one third showed fewer than five times per hour. Linear regression analysis was applied to Chr 16 markers to explore possible correlation between genotype and phenotype in the F_2 population. Similarly, we tested for Chr 14. Consistent with the backcross results there was no evidence for major effects on either chromosome and only a persistent trend for OuJ-derived susceptibility loci (Table 4).

With similar rationale, the F_2 population was analyzed non-parametrically for Chr 4 and Chr 18. Four of the 22 F_2 mice showed a relatively high seizure incidence compared to the remainder (>20 SWD). Although it is difficult to reconcile the N_2 and F_2 incidences with a similar epistatic model, all F_2 progeny were nevertheless genotyped for *D4Mit54* and *D18Mit120* and an allele frequency test was again performed. In this analysis, a suggestive trend was noted on Chr 4, where all high-incidence mice had OuJ homozygous or heterozygous allele. Together with the non-parametric results from N_2 progeny, this implies a potential involvement of Chr 4 in seizure incidence. However, considering the two datasets together, the overall evidence for trait loci remains suggestive at best.

Discussion

In 2005, it was reported that C3H/He substrains are prone to absence seizures (Frankel *et al.*, 2005). Subsequently, a retroelement insertion mutation in the *Gria4* gene was discovered as the major cause of SWD in the HeJ substrain (Beyer *et al.*, 2008). While HeJ, OuJ and SnJ are genetically very similar, the genetic basis for SWD in these other He substrains was not directly assessed. The present study revealed that OuJ and SnJ substrains do not have the *Gria4* mutation associated with their seizure phenotype, and genetic analysis provided only suggestive evidence for susceptibility loci of any kind. Although our study was not powered to detect minor locus effects to a high degree of confidence, it was larger than that used to initially implicate *Gria4* as a candidate gene and to detect an epistatic interaction in HeJ mice. Therefore, we would have detected a major effect locus if it did exist. From the results, however, we conclude that the etiology of SWD in OuJ is most likely to be genetically complex, with multiple susceptibility loci interacting to yield a modest level of SWD.

Indeed, we found no evidence that *Gria4* variants were involved in the OuJ phenotype. OuJ and SnJ substrains expressed normal amount levels of GRIA4 protein, and marker-phenotype assessment in N_2 progeny derived from OuJ and B6 mice did not indicate any correlations – even suggestive ones - between SWD phenotype and Chr 9 near *Gria4*. Lastly, unlike the previous study involving HeJ in which it was likely that unmasking of recessive SWD resistance loci in HeJ led to many *Gria4*-deficient backcross progeny having a much higher SWD incidence than either parent, in the present study the phenotype of most OuJ backcross progeny was well within the range of the parents (Beyer *et al.*, 2008, Frankel *et al.*, 2005). This suggests that the innate effect of *Gria4* deficiency, in the absence of resistance loci, is quite profound. In contrast, in the equivalent backcross for OuJ, we saw only suggestive correlations at best on Chr 5, 12, 14 and 16, and Chr 4 for high SWD incidence. Acknowledging the limitation of our study to confidently detect trait loci of small effects, these results suggest that there are multiple variants which together contrive to cause SWD in OuJ. We may infer, but we do not know formally, that additional substrains of C3H/He such as SnJ and HeN have the same etiology as OuJ.

C3H/He is a general purpose inbred strain used in a wide variety of research areas. The HeJ substrain arose from progenitor C3H/He strain in 1947 and SnJ had been separated from C3H/He earlier than HeJ. HeJ and OuJ were separated in 1952 (Figure 6). Those substrains

share the same genetic background and are well known to have a high incidence of mammary tumors caused by an exogenous mouse mammary tumor virus and also severe visual impairment in young adulthood caused by a retinal degeneration mutation (Fox, 1997., Bowes *et al.*, 1993). On the other hand, several mutations which have become differentially fixed in these substrains have been reported. Perhaps the most famous is one that occurred in HeJ in 1960's at the lipopolysaccharide response locus (in the toll-like receptor 4 gene, *Tlr4^{lps}*) making HeJ mice endotoxin resistant while the other C3H strains are endotoxin sensitive (Poltorak *et al.*, 1998). It seems likely that the progenitor strain already had a predisposition to SWD because at least four substrains, including C3HeB/FeJ, which was separated in 1948, exhibit at least some SWD. It is presumed that, during approximately 60 years (and some 200 generations) of divergence, the major-effect *Gria4* mutation arose in HeJ and during the same period independent, but more minor-effect mutations arose in OuJ, resulting in the modest aggravation of its susceptibility for SWD. It is also possible that the minor mutations might occur before segregating of OuJ and are present in HeJ mice, their effects on phenotype being dwarfed by that of *Gria4* deficiency, or that FeJ acquired resistance alleles that reduced SWD frequency. Regardless of the specifics, this study illustrates the general principle that even substrains with the same progenitor and with the same or very similar phenotypes, may have a different genetic basis.

In recent years, genetic study of human families has provided strong evidence of a genetic etiology in idiopathic generalized epilepsy (IGE), common forms of which are childhood absence epilepsy (CAE), juvenile absence epilepsy (JAE) and juvenile myoclonic epilepsy. Several potential loci and genes associated with IGE, such as *CLCN2* (chloride channel 2) and *CACNA1H* (T-type voltage-dependent calcium channel α1H subunit), as reported in linkage analyses and genetic association studies (Chen *et al.*, 2003, Crunelli & Leresche, 2002, Haug *et al.*, 2003, Steinlein, 2004, Wallace *et al.*, 2001). Replication studies, however, are often unsuccessful because of strong phenotypic and genotypic heterogeneity. Genetic animal models are therefore powerful complementary tools for studying complex disorders such as epilepsy, to understand the molecular and physiological bases, and to guide the development of novel antiepileptic drugs (Frankel, 1999, Futatsugi & Riviello, 1998, Smith *et al.*, 2007). C3H substrains seem a particularly attractive mouse model for absence epilepsy because they do not have any other neurological symptoms or gross morphological changes in the brain (their retinal degeneration is a defect of the peripheral CNS). On the other hand, in behavioral tests C3H should be used with care given their visual impairment in addition to their absence seizure episodes (Schmidt & Lolley, 1973).

Despite the suggestive nature of the SWD loci we detected, it is noteworthy that mouse Chr 16 has conserved synteny with human Chr 21q22 where the *GRIK1* gene (encoding the ionotropic glutamate receptor GluR5) is located. It has been reported that a polymorphism of *GRIK1* was associated with JAE in family-based association analysis (Sander *et al.*, 1997). Chr 14 also has conserved synteny with human Chr 14q23 where suggestive evidence for IGE has been found previously (Sander *et al.*, 2000).

In conclusion, C3H strains appear to harbor a variety of susceptibility and modifier genes involved in absence seizures. Their further study has the potential to contribute to understanding the etiology of absence epilepsy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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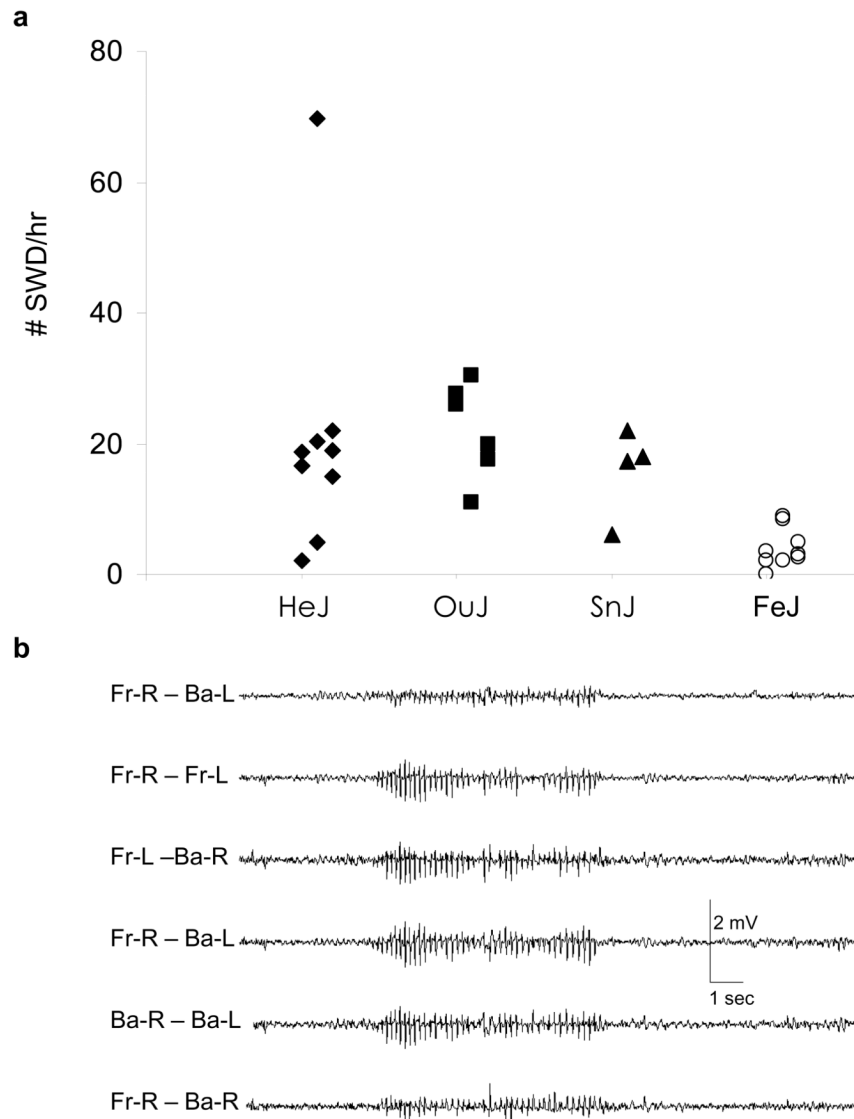**Figure 1. Spike and wave discharge (SWD) in C3H substrains**
(a) Distribution of SWD per hour in C3H/HeJ (HeJ), C3H/HeOuJ (OuJ), C3H/HeSnJ (SnJ) and C3HeB/FeJ (FeJ). HeJ and FeJ data were replotted from Beyer et al., 2008. (HeJ: 20.9 ± 6.51 SWD/hr (n=9), OuJ: 22.1 ± 2.96 SWD/hr (n=6), SnJ: 15.9 ± 3.43 SWD/hr (n=4), FeJ: 3.96 ± 1.00 SWD/hr (n=9). Mean ± S.E.M.)
(b) Representatives of EEG trace recorded with the OuJ mouse. EEG was obtained from electrodes placed over the right front (Fr-R), left front (Fr-L), right back (Ba-R), and left back (Ba-L) surface of the cerebral cortex. SWD were characterized by a high amplitude, synchronous, bilateral and generalized signal.
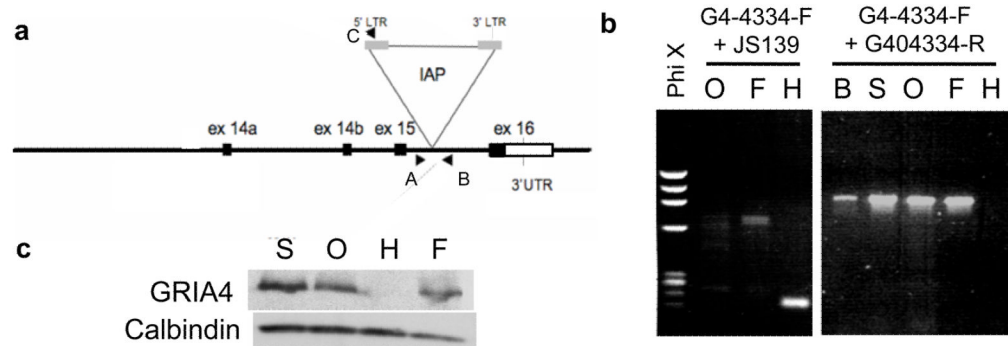
Figure 2. The IAP insertion mutation in *Gria4* in HeJ is not present in other C3H substrains

(a) Schematic map of the 3′ end of the *Gria4* gene (modified from Beyer et al, 2008). The IAP proviral insertion was located within intron 15 in the C3H/HeJ substrain. Arrow heads represent the location of primers used in genomic PCR; A: G4-4334-F, B:G4-4334-R, C:JS139.

(b) 250 bp of the IAP junction fragment in HeJ is amplified with the genomic primer G4-4334-F and the IAP primer JS139, but this fragment was not present in lanes O and F (left panel). The genomic primer G4-4334-F and G4-4334-R amplified an 800 bp fragments from all the strains except for HeJ (right panel). The fragment length for HeJ was expected to be 5 kb, too large to be amplified under the PCR conditions of the present study. The absence of the IAP insertion mutation in OuJ and SnJ was also confirmed by genomic sequence (data not shown). (H: C3H/HeJ, O: C3H/HeOuJ, S: C3H/HeSnJ, F: C3H HeB/FeJ, B: C57B6/6J)

(c) Expression of the GRIA4 protein. Western blot analysis of cerebellum was performed using anti-GRIA4 and anti-calbindin for loading control. OuJ, FeJ and SnJ mice expressed GRIA4 as well as FeJ but no GRIA4 expression was detected in HeJ.
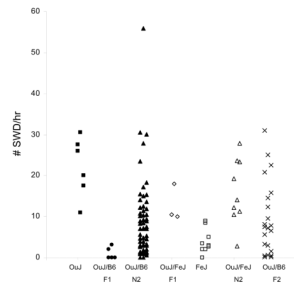
Figure 3.
Spike and wave discharge incidence in OuJ mice, F_1 hybrids, intercross F_2, and backcross N_2 progeny with B6 or FeJ mice. OuJ and FeJ data was replotted from Figure 1.
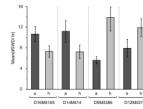
Figure 4. SWD effects for marker alleles with suggestive evidence or association in backcross mice

The top of each bar represents mean ± S.E.M. The mean SWD/hr of homozygous alleles was larger than that of heterozygous alleles at *D16Mit165* or *D14Mit14*. In contrast, the mean SWD/hr of homozygous alleles was smaller than that of heterozygous alleles at *D5Mit386* or *D12Mit37*; a: C3H/HeOuJ homozygous alleles, h: heterozygous alleles. The number of animal (a, h) is: (33, 23) for *D16Mit165*, (29, 27) for *D14Mit14*, (31, 25) for *D5Mit386* and (37, 19) for *D12Mit37*.
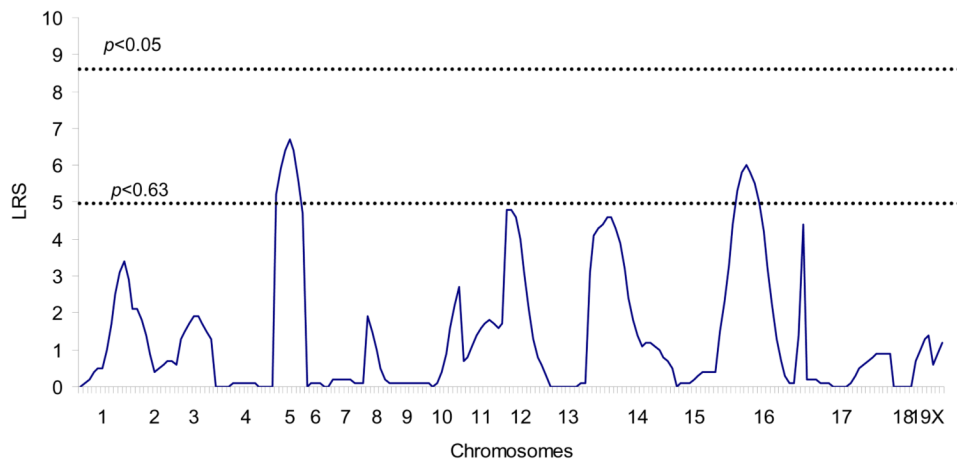
Figure 5.
Genetic map indicating interval likelihood ratio test statistics (LRS) scores for linkage to loci associated with SWD in OuJ and B6 backcross progeny (n=56). LRS scores were determined with MapManager QTX20 using ln (#SWD) as the trait measure. Significance thresholds were estimated by permutation test.

Figure 6. Schematic figure of the genealogical tree for C3H/He substrains
The C3H/He progenitor strain arose in 1942 from original the C3H inbred strain which had been derived from a cross of a Bagg albino female with a DBA male. C3H/HeJ (HeJ) was introduced to The Jackson Laboratory in 1947 and C3HeB/Fe was developed in 1948 by transfer of fertilized ova of HeJ to C57BL/6. HeJ and C3H/HeOuJ (OuJ) substrains were separated in 1952. C3H/HeSnJ (SnJ) arose from C3H/He prior to the separation of HeJ. HeJ, OuJ and SnJ show an average of 20–30 SWD/hr while C3HeB/FeJ shows 4–5 SWD/hr. The present generation of each substrain was from JAX® Mice database (http://jaxmice.jax.org, Jan 2008). Black arrows represent events of mutations identified in HeJ in the previous study (Frankel *et al.*, 2005). Sparkled and white arrows represent hypothetical events of mutations in OuJ and SnJ, respectively.

Table 1

SWD susceptibility loci detected by marker regression analysis [a]

Chr	Locus	Position (cM)	LRS	Genetic variance explained (%)	p-value	Additive effect [b]	Susceptibility allele
5	D5Mit386	9.8	5.2	9	0.022	1.14	B6
5	D5Mit95	57.9	3.8	7	0.049	0.98	B6
12	D12Mit37	1.1	4.8	8	0.028	1.15	B6
14	D14Mit14	16.4	4.3	7	0.039	−1.03	OuJ
16	D16Mit152	45.9	4.6	8	0.032	−1.09	OuJ

[a] 56 N2 progeny were genotyped with 42 polymorphic markers. Marker regression analysis was performed using MapManager QTXb20. (Markers associated at p<0.05 are shown.)

[b] Additive effect was calculated by MapManager program.

Table 2

Likelihood ratio test statistics (LRS) scores of additional markers on Chr 16 and 14

Chr	Locus [a]	Position (cM)	LRS	Genetic variance explained (%)	p-value
16	D16Mit165	10.9	6.0	10	0.014
	D16Mit4	25.1	0.1	0	0.786
	D16Mit30	30.6	4.4	8	0.036
14	D14Mit10	3.3	3.1	5	0.076
	D14Mit102	37.2	1.1	2	0.287

[a]Five markers were further tested by marker regression analysis with 56 N2 progeny.

Table 3

Non-parametric analysis with high (>20 SWD/hr) and low (<20 SWD/hr) seizure groups

		N₂ backcross			F₂ intercross	
	Genotype	**High**	**Low**	*Genotype*	**High**	**Low**
D4Mit54	A/A	5 (8.9%)	23 (41.1%)	A/A	2 (9.1%)	4 (18.2%)
	A/B	0 (0.0%)	28 (50.0%)	A/B	2 (9.1%)	10 (45.5%)
				B/B	0 (0.0%)	4 (18.2%)
		Fisher's test [a]	*p=0.0257*			
D18Mit120	A/A	5 (8.9%)	26 (46.4%)	A/A	1 (4.5%)	2 (9.1%)
	A/B	0 (0.0%)	25 (44.6%)	A/B	2 (9.1%)	12 (54.6%)
				B/B	1(4.5%)	4 (18.2%)
		Fisher's test [a]	*p=0.0445*			

A: OuJ allele, B: B6 allele

[a] Contingency analysis using JMP software.

Table 4

Linear regression analysis of the frequency of SWD on Chr 16 and 14 in F_2 mice

| | F | χ^2 | p-value | SWD/hr (mean ±S.E.M.) | | |
				A/A (n)	A/B (n)	B/B (n)
D16Mit9	1.56	0.07	0.23	11.4±3.2 (8)	9.9±3.0 (9)	4.8±4.0 (4)
D16Mit152	1.78	0.08	0.20	12.0±2.8 (10)	-	7.0±2.5 (12)
D14Mit14	0.05	0.002	0.82	10.9±4.7 (4)	9.4±3.0 (8)	8.3±3.3 (10)
D14Mit102	0.74	0.03	0.40	11.4±3.3 (8)	8.4±2.8 (11)	6.8±5.3 (3)

A: OuJ allele, B: B6 allele