3. Results

QTL mapping using maximal electroshock seizure threshold in mice (Ferraro et al., 2001)

MEST in parental mice confirmed previous observations of higher seizure sensitivity in D2 compared to B6 mice (Ferraro et al 1997; Ferraro et al 1999). The distribution of MEST values in B6xD2 F2 intercrossed mice spanned the entire phenotypic range defined by parental strains. Statistical mapping yielded significant evidence for QTLs on chromosomes 1, 2, 5, and 15, which together explained over 60% of the phenotypic variance in the model. The chromosome 1 QTL represents a locus of major effect, accounting for about one-third of the genetic variance. Experiments involving a congenic strain (B6.D2-Mtv7/Ty) enabled more precise mapping of the chromosome 1 QTL and indicate that it lies in the genetic interval between markers *D1Mit145* and *D1Mit17*.

Fine mapping of seizure susceptibility locus on mouse chromosome 1 (Ferraro et al., 2004)

Using reciprocal, interval-specific congenic strains, we confirmed the seizure-related QTL (Szs1) on mouse chromosome 1 and mapped it to a 6.6-Mb segment between *Pbx1* and *D1Mit150*. Haplotype conservation between strains within this segment suggests that Szs1 may be localized more precisely to a 4.1-Mb critical interval between *Fcgr3* and *D1Mit150*. Analysis revealed 12 brain-expressed genes with SNPs that predict a protein amino acid variation. *Kcnj10* revealed a significant effect on seizure sensitivity such that most strains possessing a haplotype containing the B6 variant of *Kcnj10* have higher seizure thresholds than those strains possessing the D2 variant.

BAC transfer confirms effect of QTL for seizure susceptibility in mice (Ferraro et al, 2007, in press)

We created three transgenic lines of mice using a *Kcnj9*- and *Kcnj10*-containing BAC in order to further evaluate Szs1. Quantification of MEST in transgenic mice and wildtype littermates documented a significant "phenotypic rescue" in all three transgenic lines. Results of the study document BAC-mediated rescue of chromosome 1-related seizure susceptibility and narrow the region of DNA harboring the causative gene variation to a 186 Kb interval. In this interval, *Kcnj10* remains the strongest candidate gene so far; however, other genes on this BAC cannot yet be excluded.

Candidate gene *ATP1A2* in human epilepsy (Buono et al., 2000; Lohoff et al., 2005)

Mutation screening of the APT1A2 gene detected a novel 4-base pair insertion polymorphism (6704_6705insTTCC) upstream of exon 2 and novel SNPs in exon 22 (22918 C>T) and intron 22 (23005C>T). We tested the 6704_6705insTTCC in TLE patients and controls and could not find a difference in genotype or allele frequencies. Additional analysis of seven SNPs across the ATP1A2 gene in IGE patients and controls failed to show a significant association with disease. Variations were in strong LD.

Candidate gene *KCNJ10* in human epilepsy (Buono et al., 2004; Lenzen et al., 2005)

Mutations analysis detected and confirmed the missense amino acid polymorphism Arg271Cys (rs1130183). Subsequent association analysis in a heterogeneous clinical sample of epilepsy patients and controls revealed that the Cys-allele was more common among controls ($\chi 2 = 5.65$, df = 1, $P = 0.017$, odds ratio 0.52, 95% CI 0.33–0.82) and reduced in patients (Buono et al 2004).

Replication analysis, using a large homogenous sample of IGE patients (n=563) and controls (n=660) from Germany, confirmed a significant reduction of the Cys271-allele in patients ($\chi 2$ = 4.71, d.f. = 1, P = 0.015, one-sided; $OR_{Cys271+}$ = 0.69; 95% CI: 0.50–0.95). Removal of the 117 IGE patients, who were part of the original study by Buono et al 2004, continued to show a significant result. Consistent with the initial association finding, the independent replication sample of 446 IGE patients revealed a significant lower Cys271 allele frequency (f(Cys271) = 0.067) when compared to the controls ($\chi 2$ = 3.52, df = 1, P = 0.030, one-sided; ORCys271+ = 0.69; 95% CI: 0.50–0.95). The Cys271-frequency was lowest in the JME group (P = 0.011, χ^2 = 5.20, df = 1, one-sided; $OR_{Cys271+}$ = 0.69; 95%-CI: 0.50–0.95) (Lenzen et al 2005).