4. Discussion

While the genetic contribution to common forms of epilepsy has been widely documented (Ottman 2005), the identification of the responsible genes has been difficult (Mulley et al 2005). This might be due to the complex mode of inheritance, clinical and genetic heterogeneity and the fact that multiple genes, each only contributing a small fraction to the overall risk, are necessary in order to develop epilepsy. Based on the complexity and experimental limitations of human epilepsy, we developed a multigenic mouse model to identify common genetic factors for seizure susceptibility. This mouse model was based on the observation that inbred mice strains differ significantly in their endogenous seizure threshold (Hall 1947; Schlesinger et al 1968). Previous QTL mapping experiments by Ferraro et al. (Ferraro et al 1997; Ferraro et al 1999; Ferraro et al 1998b), using kainic acid and pentylenetetrazol to induce seizures, have well documented this paradigm and identified a seizure susceptibility locus on distal mouse chromosome 1 (Szs1). We performed another QTL mapping study using electroshock as seizure induction method. This was an important addition to the paradigm because it would exclude the possibility that pharmacokinetic effects might be responsible for observed effects. Our results confirm a locus on chromosome 1, most notably overlapping with the two previous scans. We also detected loci of significant effects on chromosomes 2, 5, and 15, consistent with the multigenic hypothesis of seizure susceptibility (Ferraro et al 2001). Interestingly, QTL mapping efforts of alcohol withdrawal, using seizures as an outcome measure, confirmed as well a locus on distal mouse chromosome 1 (Buck and Finn 2001; Buck et al 1997).

Based on these results, we moved forward and used a candidate gene approach to further investigate the genetic factors underlying *Szs1*. Because the interval of interest was fairly large, possibly harbouring hundreds of genes, we prioritized our candidate gene analysis to only brain expressed genes. In addition, we focused on biological relevance and hypothesized that coding variations between the two inbred strains used for mapping would differentiate phenotype. We identified 12 brain-expressed genes with SNPs that predict a protein amino acid variation (Ferraro et al 2004). Of these, the most compelling seizure susceptibility candidate is *Kcnj10*, which differed for aminoacid 262 (Serine D2, Threonine B6). The *Kcnj10* gene encodes an inward-rectifying potassium ion channel. Ion channels in general and

inward recitifiers in particular, have been recently suggested to be involved in epilepsy (Chioza et al 2002; Jansen et al 2005; Schroder et al 2000). They might represent a new target for therapeutic intervention (Wickenden 2002). Other biological high ranking candidate genes are the sodium-potassium pump genes *Atp1a2* and *Atp1a4*; however, there were no coding region differences between the two strains. Since we did not screen for intronic or regulatory differences between the two strains, it is still possible that these candidate genes contribute to the Szs1 effect. In order to further narrow down the genomic region of Szs1 and to identify a gene or genes that contribute to seizure threshold, we designed a gene transfer experiment. Results documented a "phenotypic rescue" of a seizure phenotype through *in vivo* transfer of a BAC that harbors six linked genes, of which *Kcnj9*, *Kcnj10 and Atp1a2* are primary candidates for major seizure susceptibility QTLs on distal chromosome 1 (Ferraro 2007). The rescue strategy involved random insertion and must be interpreted with caution. The integration of the BAC clone could interrupt the sequence of other gene(s) or regulatory elements; however we generated three separate transgenic lines, all demonstrating phenotypic rescue, maximizing the likelihood that the effect is due to a gene or genes on the BAC clone. The non-synonymous (Thr262Ser) SNP in *Kcnj10* remains the variation that is most likely to define the seizure related QTL on distal chromosome 1.

The mouse model provided us with high ranking candidate genes, which we planing to investigate in human epilepsy. Although there was no evidence for coding variation in the mouse model for *ATP1A2*, this gene remained a high ranking candidate gene based on it's involvement in familial hemiplegic migraine and evidence of co-segregation of seizures in affected families (De Fusco et al 2003; Kaunisto et al 2004; Segall et al 2004; Spadaro et al 2004; Swoboda et al 2004; Vanmolkot et al 2003). We screened the *ATP1A2* gene for variations and performed a case control association study. We failed to detect an association with either TLE or IGE. Although our result did not support the involvement of variation in the *ATP1A2* gene with common forms of epilepsy, there are several potential factors why we might have missed an association. Besides population stratification concerns and clinical heterogeneity, the major concern was our small sample size (TLE: n=56, IGE: n=152), limiting our power to detect alleles of small effect. Additional large scale studies are necessary to rule out *ATP1A2* as seizure susceptibility gene.

Results from our murin model suggested strongly that *KCNJ10* might contribute to the seizure susceptibility locus on mouse chromosome 1 and further might play also a role in human epilepsy. Mutation screening of human *KCNJ10* revealed a non-synonymous SNP, Arg271Cys, which predicts an amino acid variation at a relative position nine residues removed from the variation discovered in the mouse. We performed an association study using a heterogeneous sample of epilepsy patients (n = 407) and controls (n = 284). Results suggest a seizure resistant effect of the Cys-allele (*P* = 0.017, OR = 0.52, 95% CI: 0.33–0.82) (Buono et al 2004). Since this study used both TLE and IGE patients, which were ascertained from Germany and the USA, the finding might be false positive due to population stratification. In an attempt to address this issue, we genotyped this missense variation in an independent sample of German IGE patients and controls. Results confirmed a moderate seizure protective effect of the Cys-allele (OR=0.69). While *In vitro* electrophysiological studies using Xenopus oocytes failed to document a significant functional differences when *Kcnj10* variants were expressed (Shang et al 2005), interpretation of these results is limited by the experimental design. Influences of this variation might be subtle and might require participation of other molecular elements not present in oocytes. Although animal models, in vitro and in vivo experiments can be useful in discovering new pathways and disease mechanisms, they lack by default the complexity of the human body, thus limiting the interpretation and “translation” of results to human physiology.

Future studies of human epilepsy will require large, multi-center samples, with standardized protocols for clinical characterization. In addition, the use of endo-phenotypes would further homogenize the clinical sample and improve power to detect small effects. While gene-gene and gene-environment interaction remain challenges for future studies, it can be expected that that most molecular defects will affect common pathways of cortical synchronization (Ferraro and Buono 2006; Noebels 2003). Identification of these pathways might provide important insights into disease mechanism and might have significant therapeutic implications.