The major goal of our studies is to identify and characterize modifier genes that influence epilepsy susceptibility and severity. We will determine the molecular basis of the \textit{Scn2aQ54} gene on Chromosome 11 and delayed onset and improved survival on the 129S6/SvEvTac strain background. Based on these observations, we hypothesize that multiple genetic modifiers act to influence penetrance and expressivity of the primary epilepsy mutation. Conversely, the epilepsy phenotype of \textit{Scn1a}+/− mice is more severe on the C57BL/6J background, while they have delayed onset and improved survival on the 129S6/SvEvTac strain background. Based on these observations, we hypothesize that multiple genetic modifiers act to influence penetrance and expressivity of the primary epilepsy mutation. Previously we mapped two modifier loci that are responsible for the strain difference in epilepsy modifier genes will provide insight into the basic biology of epileptogenesis and may identify novel therapeutic targets for the treatment of human patients.

\textbf{Active Grants}

1) “Combined Approach to Genetic Modifiers of Inherited Epilepsy”

Project Period: 05/25/2006-02/28/2016

Funding Agency: National Institute of Neurological Disorders and Stroke

Goals: To identify modifier genes influencing the clinical course of epilepsy in mouse models.

Abstract:
Mutations in voltage-gated sodium channels have been associated with several types of human epilepsy, including Genetic (Generalized) Epilepsy with Febrile Seizure Plus (GEFS+) and Dravet Syndrome. Within these genetic epilepsies, there is variable penetrance and expressivity of the clinical phenotype, suggesting a role for genetic modifiers. We have developed mouse models with mutations in voltage-gated sodium channels and seizure-related phenotypes with different underlying mechanisms. \textit{Scn2aQ54} transgenic mice have a gain-of-function mutation that results in spontaneous, adult-onset partial motor seizures and models features of GEFS+. Heterozygous \textit{Scn1a}+/− null mice are a model of Dravet Syndrome, a severe, infant-onset epilepsy with progressive worsening accompanied by psychomotor regression. A common feature of these mouse models is that epilepsy severity varies depending on the genetic strain background, suggesting that genetic modifiers influence the phenotype. \textit{Scn2aQ54} on the resistant C57BL/6J background have delayed onset, decreased severity and improved survival compared to the susceptible (C57BL/6J × SJL/J)F1 background. Conversely, the epilepsy phenotype of \textit{Scn1a}+/− mice is more severe on the C57BL/6J background, while they have delayed onset and improved survival on the 129S6/SvEvTac strain background. Based on these observations, we hypothesize that multiple genetic modifiers act to influence penetrance and expressivity of the primary epilepsy mutation. Previously we mapped two modifier loci that are responsible for the strain difference in \textit{Scn2aQ54} mice: \textit{Moe1} (modifier of epilepsy 1) on Chromosome 11 and \textit{Moe2} on Chromosome 19. During the previous funding period we performed fine mapping and candidate gene analysis of the \textit{Moe1} region, identified the \textit{Moe2} modifier gene, and identified additional modifier loci that influence the \textit{Scn2aQ54} phenotype. We propose to continue our analysis of epilepsy modifiers using the \textit{Scn2aQ54} and \textit{Scn1a}+/− mouse models. First, we will identify the responsible genes at \textit{Scn2aQ54} modifier loci. Next, we will determine the molecular basis of the \textit{Moe2} modifier effect. Finally, we will map genetic modifier loci in the \textit{Scn1a}+/− mouse model and perform transcriptome analysis via RNA-seq for accelerated identification of candidate genes. The major goal of our studies is to identify and characterize modifier genes that influence epilepsy susceptibility and severity. These genes are likely to contribute to common epilepsy syndromes with more complex genetics. Identification of epilepsy modifier genes will provide insight into the basic biology of epileptogenesis and may identify novel therapeutic targets for the treatment of human patients.

2) “Genetic Mapping of Modifier Loci in a Mouse Model of Dravet Syndrome”

Project Period: 12/15/2012-12/14/2017

Funding Agency: National Human Genome Research Institute / Center for Inherited Disease Research

Goals: To map modifier genes influencing the epilepsy severity in a mouse model of Dravet syndrome.

This is a peer reviewed NIH grant application that requested the Center for Inherited Disease Research (CIDR) to perform whole genome genotyping of progeny from a large mouse mapping cross. It was competitively reviewed and received approval for whole genome genotyping of 300 DNA samples.

Abstract:
Mutations in voltage-gated sodium channels are responsible for several types of human epilepsy. More than 800 mutations have been reported in patients with several epilepsy syndromes, including genetic epilepsy with febrile seizures plus (GEFS+) and Dravet syndrome. Variable penetrance and expressivity in family members with the same sodium channel mutation is a common feature of these genetic epilepsies, suggesting a potential contribution of genetic modifiers. We have developed a mouse model Dravet syndrome, a severe infant-onset epileptic encephalopathy with progressive worsening and psychomotor regression. Mice with targeted deletion of \textit{Scn1a} provide a model of Dravet syndrome, in which approximately half of patient mutations are protein truncation alleles. Heterozygous \textit{Scn1a}+/− null mice develop spontaneous generalized seizures and sporadic death beginning in the third week of life. The epilepsy phenotype of \textit{Scn1a}+/− mice is more severe on the C57BL/6J (B6) strain background, while they have delayed onset and improved survival on the 129S6/SvEvTac (129) background, suggesting that genetic modifiers influence the phenotype. Based on these observations, we hypothesize that genetic modifiers act to influence expressivity of the primary \textit{Scn1a} mutation. To address our hypothesis, we will perform genetic mapping to identify modifier loci that influence epilepsy severity in the \textit{Scn1a}+/− mouse model of Dravet syndrome. The focus of this CIDR Access Request is to perform the genome-wide genotyping that is necessary for genetic mapping of modifier loci that influence phenotype severity. Identification of epilepsy modifier loci will provide insight into the etiology of epilepsy and may identify novel therapeutic targets for the improved treatment of human patients.
3) “Optogenetic Approach to Dravet Syndrome”
Project Period: 04/01/2012 – 03/31/2014       Role:  PI (5% effort)
Funding Agency:  Dravet.org
Goals: To determine the contribution of interneurons to the pathophysiology of Dravet Syndrome.
Abstract:
Dravet syndrome (DS) is a severe, infant-onset epileptic encephalopathy with progressive worsening and psychomotor regression. Over 70% of DS patients have mutations in the voltage-gated sodium channel gene SCN1A. To date, more than 600 SCN1A mutations have been reported in DS patients. Approximately half of SCN1A mutations in DS result in protein truncation, suggesting that SCN1A is haploinsufficient. Mice with targeted deletion of Scn1a are a model of DS. Electrophysiological analysis of Scn1a+/− heterozygous null mice demonstrated decreased activity of inhibitory interneurons. Based on these results, it has been hypothesized that DS is an “interneuronopathy”, with hyperexcitability resulting from reduced activity of GABAergic interneurons. We propose to use novel optogenetic tools to further explore the contribution of GABAergic interneurons to DS. Several lines of transgenic mice have been developed expressing Channelrhodopsin-2 (ChR2) under the control of promoters targeting specific neuronal subtypes. To address the interneuronopathy hypothesis, we will cross Scn1a+/− mice with mice expressing ChR2 specifically in inhibitory interneurons. Stimulation of the ChR2 cation channel with blue light will result in depolarization and enhanced activity of interneurons that are under-active in the Scn1a+/− mice. We will determine whether increased activity of inhibitory interneurons can rescue the DS phenotype. In Aim 1, we will cross Scn1a+/− heterozygotes with mice expressing ChR2 under the control of the vesicular GABA transporter promoter and test the ability of ChR2-mediated activation of inhibitory interneurons to terminate or prevent seizures in awake, behaving Scn1a+/− mice. In Aim 2, we will evaluate the effect of ChR2-mediated activation of interneurons on network excitability in hippocampal slice recordings of Scn1a+/− mice. Together these experiments will further delineate the contribution of inhibitory interneurons to DS pathophysiology and may suggest a novel therapeutic strategy.

4) “Humanized Mouse Model of a Dravet Syndrome Missense Mutation”
Funding Agency:  Epilepsy Foundation, Targeted Research Initiative for Severe Symptomatic Epilepsies
Project Period: 07/01/2012 – 06/30/2013       Role:  PI (10% effort)
Goals: To determine the molecular basis of Dravet syndrome arising from an Scn1a missense mutation.
Abstract:
Mutations in voltage-gated sodium channels are responsible for several types of human epilepsy with varying degrees of clinical severity, including Genetic Epilepsy with Febrile Seizures Plus (GEFS+) and Dravet syndrome. SCN1A missense mutations with variable clinical severity exhibit shared biophysical defects in heterologous expression systems. Thus, there is not an obvious genotype-phenotype correlation between Nav1.1 dysfunction in vitro and severity of the clinical phenotype. We hypothesize that variable clinical severity results from unique functional consequences of missense mutations in different neuron types. To address this hypothesis, we will utilize humanized mice carrying the Dravet syndrome SCN1A-R1648C missense mutation in the context of the human Nav1.1 channel. In vitro characterization of this mutation revealed gain-of-function biophysical defects of inactivation and persistent current. We will determine the in vivo functional consequences of the mutation on clinical and neuronal phenotypes. In Aim 1, we will perform neurological phenotyping including assessment of spontaneous and induced seizures. In Aim 2, we will determine the functional consequences of the mutation by electrophysiological recording of acutely isolated neurons. These studies will advance our understanding of the molecular basis of Dravet syndrome. This will provide insight into the basic biology of epileptogenesis and suggest novel therapeutic strategies for improved treatment of patients.