More Genes, Better Outcome?

Gabra2 Is a Genetic Modifier of Scn8a Encephalopathy in the Mouse

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Objective: SCN8A encephalopathy is a developmental epileptic encephalopathy typically caused by de novo gain-of-function mutations in Nav 1.6. Severely affected individuals exhibit refractory seizures, developmental delay, cognitive disabilities, movement disorders, and elevated risk of sudden death. Patients with the identical SCN8A variant can differ in clinical course, suggesting a role for modifier genes in determining disease severity. The identification of genetic modifiers contributes to understanding disease pathogenesis and suggesting therapeutic interventions. Methods: We generated F1 and F2 crosses between inbred mouse strains and mice carrying the human pathogenic variants SCN8A-R1872W and SCN8A-N1768D. Quantitative trait locus (QTL) analysis of seizure-related phenotypes was used for chromosomal mapping of modifier loci. Results: In an F2 cross between strain SJL/J and C57BL/6J mice carrying the patient mutation R1872W, we identified a major QTL on chromosome 5 containing the Gabra2 gene. Strain C57BL/6J carries a splice site mutation that reduces expression of Gabra2, encoding the α2 subunit of the aminobutyric acid type A receptor. The protective wild-type allele of Gabra2 from strain SJL/J delays the age at seizure onset and extends life span of the Scn8a mutant mice. Additional Scn8a modifiers were observed in the F2 cross and in an F1 cross with strain C3HeB/FeJ. Significance: These studies demonstrate that the SJL/J strain carries multiple modifiers with protective effects against seizures induced by gain-of-function mutations in Scn8a. Homozygosity for the hypomorphic variant of Gabra2 in strain C57BL/6J is associated with early seizure onset and short life span. GABRA2 is a potential therapeutic target for SCN8A encephalopathy.

Commentary

Next-generation sequencing has facilitated discoveries of causal molecular etiology across a broad spectrum of epilepsies. Over 100 genes are now firmly linked to Mendelian epilepsies and many more are associated with sporadic epilepsies. Still, we face an enduring challenge of explaining and predicting diverse phenotypic evolution even in defined monogenic epilepsy types. Qualitative properties of a causal variant may explain some phenotypic features as exemplified by channelopathies. In the *SCN8A*-related disorders; the gain-of-function variants result in epilepsy of varied severity and intellectual disability (ID) while autism spectrum disorder and neurodevelopmental disorders are the predominant manifestation of loss-of-function variants.[1] Yet, many features, such as epilepsy penetrance, age of onset, propensity to remission or early mortality, presence and severity of intellectual disability or other comorbidities are less likely to be explained by the causal variant properties. Results from the Center for Mendelian Genomics parallel discoveries of several other initiatives aimed at understanding molecular causality and phenotypic heterogeneity of Mendelian disorders. Evaluation of over 61 000 samples has delivered evidence for multilocus variation that is seen in an estimated 4.9% of clinical exomes and together with genetic modifiers modulates phenotypic diversity.[2] Similarly, an oligogenic inheritance structure was proposed in a recent study of cases with developmental epileptic encephalopathy (DEE) that showed an enrichment in damaging ultra rare variation in known DEE genes but also in genes without a prior association with DEE, such as *NF1*, *AP5B1*, *DNMT3L*, and *ARGFEF1*.[3]

How shall one distill the evidence from the large-scale studies to a clinically relevant understanding of phenotypic heterogeneity and disease course in a specific epilepsy type? While human genomic studies are delivering novel epilepsy and candidate modifier genes, model systems are becoming ever more important in understanding the genetic interactions and phenotypic outcomes as evidenced by the genetically engineered di-genic mouse models and the modifier loci identified by classical forward genetic approaches in inbred mouse strains.[4] Current work by Yu et al. adopts similar approaches to isolate a modifier locus and a candidate gene *Gabra2* and thus contributes towards our understanding of phenotypic heterogeneity in *SCN8A*-related epilepsy.[5]

The authors evaluated a known knock-in mouse model of *SCN8A*-related epileptic encephalopathy (EE), carrying a recurrent human variant *SCN8A*-R1872W. In the model, the R1872W is expressed in the murine forebrain when activated by a cross to Emxl[Cre] mice expressing Cre recombinase in forebrain excitatory neurons beginning at the age of E9.5. Typically, Scn8a[R1872W], *Emxl*[Cre] mice develop seizures between 1-2 months of age with a median survival of 46 days.[6] The first interesting finding was the discovery that shifting Scn8a[R1872W], Emxl[Cre] mice from a SJL/J background to a C57BL/B6 background by repeated backcrosses reduced age at seizure onset and decreased survival with a direct correlation between these two indices ($r^2 = 0.7$, n = 60). By the 8th generation, both the age of seizure onset and the length of survival were tightly clustered at 20-30 and 20-40 days, respectively. In the F2 Scn8a[R1872W], Emxl[Cre] generation, seizures occurred in most animals between 30-60 days. However, the range was quite broad (20–180 days) while the proportion of transcript containing the mutant R1872W sequence was stable at $26 \pm 2\%$, thus speaking for a uniform Cre mediated activation of the conditional allele. Interestingly, longevity interval—seizure onset to death—was similar between mice with early-vs late-onset seizures. This finding recalls a spontaneous mouse mutant Scn8a[medJ] carrying a splice variant resulting in a partial exon skipping with resultant progressive dystonia and ataxia but a normal life span on C3H background but a much more severe phenotype and early lethality on C57BL/6 background. The phenotypic severity correlated with the amount of correctly spliced Scn8a transcript that was 10% in C3H but 6% in C57BL/6 due to a co-existent pathogenic variant in an auxiliary spliceosomal protein encoded by *Scnm1* gene located on mouse chromosome 3.[7]

In keeping with their prior investigations, the authors set out to better understand genetic contributors to survival in the Scn8a[R1872W], Emxl[Cre] dual background (C57BL/B6 and SJL/J) model and performed quantitative trait locus (QTL) mapping using a microarray interrogating 10 819 biallelic SNPs. They found a statistically significant lod score ($P < 0.05$) for one locus on chromosome 5 and hits for 2 additional loci on chromosome 1 at a less stringent $P < 0.1$. An additional peak was seen at chromosomes 19, albeit it did not reach significance. SNP haplotype in 5 mice surviving less than 30 days showed that they were homozygous for an allele containing a single base pair deletion in the splice acceptor site of exon 5 in the *Gabra2* gene (rs29547790, aka B allele) and a homozygous B allele leads to a 75% reduction of a *Gabra2* transcript levels.[8] Results from the Scn8a[R1872W] mouse parallel earlier findings in *Scn1a*+/– model of Dravet syndrome. A mixed 129xC57BL/6J background resulted in hyperthermia-induced as well as spontaneous seizures and 80% lethality by three months of age. The *Gabra2* gene was identified as the modifier allele in this model as well.[9]

In the case of the Scn8a[R1872W] model, the modulatory effect of the *Gabra2* B allele was evident by the significantly shorter survival of Scn8a[R1872W], Emxl[Cre] Gabra2[B/B] as opposed to animals that were either B allele heterozygous (*Gabra2*[B/S]) or wild type (S allele) homozygotes (*Gabra2*[S/S]). Moreover, Scn8a[R1872W], Emxl[Cre] *Gabra2*[B/B] animals manifested an earlier median age at seizure onset (53 days) than their *Gabra2*[B/S] (70 days) or *Gabra2*[S/S] (79 days) counterparts. While, it was tempting to assume that *Gabra2*[B/B] haplotype was singularly essential in influencing age of seizure onset and survival, 2 Scn8a[R1872W], Emxl[Cre] *Gabra2*[B/B] mice manifested age at seizure onset beyond 50 days albeit without an effect on their survival. These results indicated a possible role of additional genetic modifiers other than *Gabra2* in the manifestation of their epilepsy and the investigators aimed to elucidate this by analyzing the C3HeB/FeJ Scn8a[N1768D24] mouse model with 50% lethality at 6 months. Interestingly, the C3HeB/FeJ strain does not carry the *Gabra2* splice variant and crosses with the SJL/J strain resulted in a rescue of survival beyond 6 months. No such effect was seen when C3HeB/FeJ Scn8a[N1768D24] mice were crossed with FVB/NJ and DBA/2J mice, thus indicating SJL/J strain carries specific modifiers protective in seizure-related lethality.

The study by Yu et al. is an elegant investigation marrying classical forward genetics with contemporary methods of genetic engineering. It delivers an important glimpse into the complex biology that may underlie phenotypic heterogeneity not explained by the qualitative properties of pathogenic variation in *SCN8A*-related epilepsy phenotype. It is a valuable addition to the library of di-genic mouse models and it opens avenues for further investigation related to age at epilepsy onset and mechanisms responsible for intellectual disability and premature mortality. Onset of model epilepsy was assessed in a daily 8-hour observation of convulsive seizures. Continuous prolonged behavioral and EEG monitoring will be invaluable in defining the onset of behavioral, developmental, and electrophysiological abnormalities that might occur even prior to the onset of overt behavioral seizures. Additional insight might then be guiding for early therapeutic interventions. This model is also likely to motivate re-evaluation of existing exome data in patients affected by *SCN8A*-related neurodevelopmental disorders for co-existing variation in the *GABRA1* gene as it may open treatment opportunities. As shown in the *Scn1a*+/– *Gabra2*[B/B] mouse model, administration of a GABAA receptor allosteric modulator clobazam was protective against hyperthermia induced seizures. In the *SCN8A*-related EE due to GOF mutations, sodium channel blockers seem to be preferentially effective in seizure control. It will be interesting to explore Scn8a[R1872W], Emxl[Cre] *Gabra2*[B/B] for therapies aimed at these molecular targets for future translation into clinic. The model also begs for further dissection of candidate modifier loci seen at chromosomes 1 and 19 housing 2 candidate modifier epilepsy genes, *Kcnv2* and *Smarca2*.[10] Understanding their possible role in the *Scn8a* phenotype may guide reciprocal searches for variation in these genes in patients and offer additional therapeutic targets.

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References

1. Brunklaus A, Du J, Steckler F, et al. Biological concepts in human sodium channel epilepsies and their relevance in clinical practice. *Epilepsia*. 2020;61:387-399.
2. Posey JE, O'Donnell-Luria AH, Chong JX, et al. Insights into genetics, human biology and disease gleaned from family based genomic studies. *Genet Med*. 2019;21:798-812.
3. Takata A, Nakashima M, Saitsu H, et al. Comprehensive analysis of coding variants highlights genetic complexity in developmental and epileptic encephalopathy. *Nat Commun*. 2019;10:2506.
4. Kearney JA. Genetic modifiers of neurological disease. *Curr Opin Genet Dev*. 2011;21:349-353.
5. Yu W, Hill SF, Xenakis JG, Pardo-Manuel de Villena F, Wagnon JL, Meisler MH. Gabra2 is a genetic modifier of Scn8a encephalopathy in the mouse. *Epilepsia*. 2020;61:2847-2856.
6. Bunton-Stasyshyn RKA, Wagnon JL, Wengert ER, Barker BS, Faulkner A, Wagley PK, et al. Prominent role of forebrain excitatory neurons in SCN8A encephalopathy. *Brain* 2019;142: 362-375.
7. Sprunger L, Escayg A, Tallaksen-Greene S, Albin RL, Meisler MH. Dystonia associated with mutation of the neuronal sodium channel Scn8a and identification of the modifier locus Scnm1 on mouse chromosome 3. *Hum Mol Genet* 1999;8:471-479.
8. Mulligan MK, Abreo T, Neuner SM, et al. identification of a functional non-coding variant in the GABAA receptor α2 subunit of the C57BL/6J mouse reference genome: Major implications for neuroscience research. *Front Genet*. 2019;10:188.
9. Hawkins NA, Zachwieja NJ, Miller AR, Anderson LL, Kearney JA. Fine mapping of a dravet syndrome modifier locus on mouse chromosome 5 and candidate gene analysis by RNA-Seq. *PLoS Genet*. 2016;12:e1006398.
10. Bergren SK, Rutter ED, Kearney JA. Fine mapping of an epilepsy modifier gene on mouse Chromosome 19. *Mamm Genome*. 2009; 20:359-366.