Correction of the hypomorphic Gabra2 splice site variant in mouse strain C57BL/6J modifies the severity of Scn8a encephalopathy

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Summary

De novo gain-of-function mutations of SCN8A are a significant cause of developmental and epileptic encephalopathy (DEE) (MIM: 614558). The severely affected individuals exhibit refractory seizures, developmental delay, and cognitive disabilities, often accompanied by impaired movement. Individuals with the identical SCN8A variant often differ in clinical course, suggesting a role for modifier genes in disease severity. In a previous study we demonstrated genetic linkage between a hypomorphic mutation in the Gabra2 gene and seizure severity in a mouse model of the human SCN8A pathogenic variant p.Arg1872Trp. Homozygosity for the hypomorphic Gabra2 mutation was associated with early seizure onset and shortened lifespan. We have now confirmed Gabra2 as the modifier gene using a knock-in allele that corrects the splice site variant in strain C57BL/6J. Correction of the Gabra2 variant restores transcript abundance, increases the age of seizure onset, and extends survival of the Scn8a mutant mice. Gabra2 encodes the γ2 subunit of the GABA_A receptor that provides inhibitory input to dendrites and the the axon initial segment of excitatory neurons. Quantitative variation in human GABA_A receptor expression could contribute to variation in the severity of genetic epilepsies and suggests a potential therapeutic intervention.

De novo gain-of-function mutations in SCN8A, encoding the voltage-gated sodium channel Na_v1.6, have been identified in more than 400 individuals with developmental and epileptic encephalopathy (DEE). The pathogenic variant p.Arg1872Trp results in delayed channel inactivation and has been identified in several individuals with DEE.1-3 We generated a conditional knockin mouse model carrying the Scn8a-R1872W variant.4 When combined with global expression of Cre recombinase, this allele is activated and generates early-onset, lethal, convulsive seizures. Activation of the conditional allele by Emx1-Cre, with selective expression in forebrain excitatory neurons, generates the complete phenotype of early-onset convulsive seizures and juvenile lethality.4

To identify genetic modifiers of the epilepsy phenotype, mice carrying Scn8a-R1872W and Emx1-Cre on the C57BL/6J strain background were previously crossed with wild-type mice from strain SJL/J.5 In the F2 generation, variation in the age of seizure onset co-segregated with a region of chromosome 5 containing the Gabra2 gene. The median survival of F2 mice with genotype Gabra2B/B was 53 days (n = 15). The median survival of mice with genotype Gabra2B/S or Gabra2S/S was 75 days. The comparable survival of Gabra2B/B and Gabra2S/S mice indicated that the effect of the hypomorphic Gabra2 allele is recessively inherited.

To directly test the role of the splice site mutation, we have now used a corrected knockin line of C57BL/6J carrying a wild-type Gabra2 allele.6 The mutant Gabra2B allele is characterized by deletion of a single nucleotide, a thymidine residue at the −3 position of the splice acceptor site of exon 5.6 This splice variant reduces the abundance of the Gabra2 transcript to 25% of wild-type level. We predicted that the quantitative difference in GABRA2 protein expression was responsible for the shorter survival of mice with genotype Gabra2B/B.5 Using Crispr-Cas9 targeting, the single-nucleotide deletion in the GabrB allele was corrected to generate the Gabra2KI knockin allele, which has the wild-type sequence and expression level.6

The two-generation breeding scheme used to determine the effect of the Gabra2KI allele is shown in Figure 1. Homozygous C57BL/6J, Gabra2K/KI mice were crossed with homozygous C57BL/6J, Emx1Cre/Cre mice (JAX 005628) to generate double heterozygotes carrying one copy of Emx1Cre and one copy of the corrected Gabra2 allele. The double heterozygotes were crossed with homozygous conditional Scn8aR1872W/R1872W mice. Offspring with the genotype Scn8aR1872W/+ , Emx1Cre/+ , Gabra2KI/+ (heterozygous corrected) were compared with the Scn8aR1872W/+ , Emx1Cre/+ , Gabra2R/R (uncorrected) offspring. We observed a 3-fold increase in lifespan in mice inheriting one copy of the corrected splice site (Figure 2). The median lifespan of the Gabra2K/KI mice was 72 days, which was comparable to the long-lived mice in the previous study and significantly longer than the 22-day median survival of the Gabra2B/B mice (p < 0.0001, log-rank [Mantel-Cox] test). These data demonstrate that Gabra2 was the major modifier locus segregating in the C57BL/6J X SJL/J cross.5

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It is important to note that the Gabra2 splice site variant is a private variant in the C57BL/6J strain and is not found in other B6 sublines such as C57BL/6N or in any other of the common inbred strains. Since C57BL/6J has been widely used as a wild-type mouse in biomedical research, the Gabra2 variant may have had previously unrecognized effects on other neurological phenotypes studied in this strain.

Gabra2 encodes the α2 subunit of the GABAA receptor, which provides inhibitory input to excitatory neurons. The reduction in inhibitory input due to the hypomorphic Gabra2B allele is predicted to result in elevated neuronal excitability, consistent with the early onset of seizures in homozygous Gabra2B/B mice. A single copy of the wild-type allele is sufficient to rescue early onset and lethality.

The hypomorphic Gabra2 variant in C57BL/6J mice also modifies seizure severity in a mouse model of Scn1a DEE (Dravet syndrome). Loss-of-function variants of human GABRA2 have been identified in multiple individuals with epileptic encephalopathies, and the variants with greater reduction in function were associated with greater clinical severity. A genome-wide analysis of 15,212 epileptic individuals found that variants in GABRA2 were significantly associated with genetic generalized epilepsies. In addition, loss-of-function variants of GABRA2 are underrepresented in the Genome Aggregation Database (gnomAD) (pLI = 1.0, observed/expected = 0.05), suggesting that there has been selection against haploinsufficiency. The commonly used anticonvulsant clobazam is a GABAA receptor activator that is protective in Dravet model mice with genotype Scn1a+/−, Gabra2B/S. Together these findings suggest that reduced GABRA2 activity could be a contributing modifier in human sodium-channel-related epilepsy and that pharmacological augmentation of α2 subunit-containing GABAA receptor function may be a relevant therapy for these disorders.

Data and code availability
The published article includes all datasets generated or analyzed during this study.

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Declaration of interests

The authors declare no competing interests.

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Web resources

Basel declaration, https://www.basel-declaration.org/
OMIM, https://www.omim.org

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