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REPORT

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

Wenxi Yu,[1,*] Megan K. Mulligan,[2] Robert W. Williams,[2] and Miriam H. Meisler[1]

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 α2 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 $Gabra2^{B/B}$ was 53 days (n = 15). The median survival of mice with genotype $Gabra2^{B/S}$ or $Gabra2^{S/S}$ was 75 days. The comparable survival of $Gabra2^{B/S}$ and $Gabra2^{S/S}$ mice indicated that the effect of the hypomorphic $Gabra2^{B}$ 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 $Gabra2^{B}$ 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 $Gabra2^{B/B}$.[5] Using Crispr-Cas9 targeting, the single-nucleotide deletion in the $Gabra^{B}$ allele was corrected to generate the $Gabra2^{KI}$ knockin allele, which has the wild-type sequence and expression level.[6]

The two-generation breeding scheme used to determine the effect of the $Gabra^{KI}$ allele is shown in Figure 1. Homozygous C57BL/6J.$Gabra2^{KI/KI}$ mice were crossed with homozygous C57BL6/J.$Emx1^{Cre/Cre}$ mice (JAX 005628) to generate double heterozygotes carrying one copy of *Emx1-Cre* and one copy of the corrected *Gabra2* allele. The double heterozygotes were crossed with homozygous conditional $Scn8a^{R1872W/R1872W}$ mice. Offspring with the genotype $Scn8a^{R1872W/+}$, $Emx1^{Cre/+}$, $Gabra2^{KI/B}$ (heterozygous corrected) were compared with the $Scn8a^{R1872W/+}$, $Emx1^{Cre/+}$, $Gabra2^{B/B}$ (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 $Gabra2^{KI/B}$ mice was 72 days, which was comparable to the long-lived mice in the previous study[5] and significantly longer than the 22-day median survival of the $Gabra2^{BB}$ 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]

[1]Department of Human Genetics, University of Michigan, Ann Arbor, MI, 48109, USA; [2]Department of Genetics, Genomics, and Informatics, University of Tennessee Health Science Center, Memphis, TN, 38103, USA
*Correspondence: wenxiyu@umich.edu
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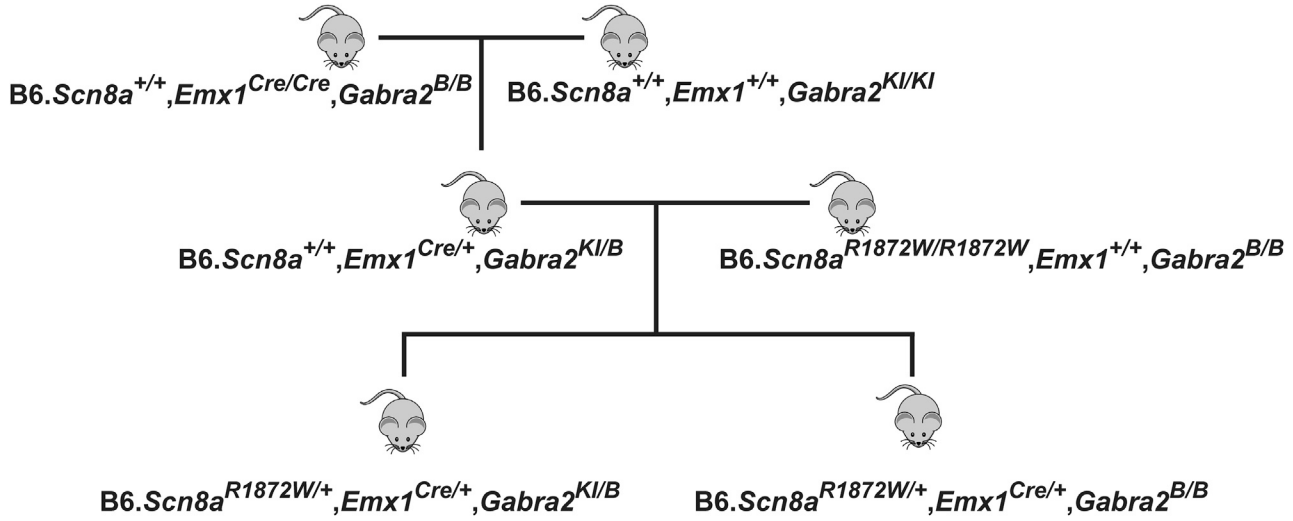

Figure 1. Generation of *Scn8a* mutant mice carrying the corrected allele *Gabra2*^{KI} on the C57BL/6J strain background

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.[6] Since C57BL/6J has been widely used as a wild-type mouse in biomedical research, the *Gabra2*^{B} variant may have had previously unrecognized effects on other neurological phenotypes studied in this strain.

Gabra2 encodes the α2 subunit of the $GABA_A$ receptor, which provides inhibitory input to excitatory neurons. The reduction in inhibitory input due to the hypomorphic *Gabra2*^{B} allele is predicted to result in elevated neuronal excitability, consistent with the early onset of seizures in homozygous *Gabra*$^{B/B}$ mice.[5] A single copy of the wild-type allele is sufficient to rescue early onset and lethality.

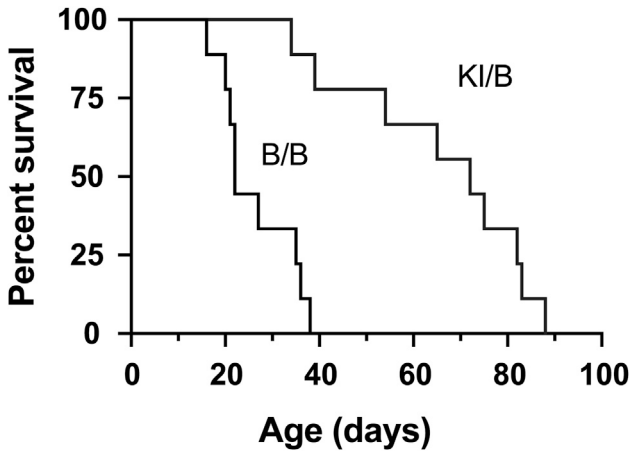

Figure 2. Correction of the hypomorphic *Gabra2* splice site variant in strain C57BL/6J extends the survival of epileptic C57BL6/J *Scn8a*$^{R1872W/+}$, *Emx1*$^{Cre/+}$ mice

Survival was lengthened from a median value of 22 days in *Gabra2*$^{B/B}$ homozygotes (n = 9) to 72 days in *Gabra*$^{B/KI}$ heterozygotes (n = 9). $p < 0.0001$, log-rank (Mantel-Cox) test. B, C57BL/6J allele of *Gabra2*; KI, corrected *Gabra2* allele.

The hypomorphic *Gabra2* variant in C57BL/6J mice also modifies seizure severity in a mouse model of *Scn1a* DEE (Dravet syndrome).[7] 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.[8,9] A genome-wide analysis of 15,212 epileptic individuals found that variants in *GABRA2* were significantly associated with genetic generalized epilepsies.[10] 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 $GABA_A$ receptor activator[11] that is protective in Dravet model mice with genotype *Scn1a* $^{+/-}$, *Gabra2*$^{B/S}$.[12] 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 $GABA_A$ 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.

Acknowledgments

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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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