Epilepsy Currents
2021, Vol. 21(2) 126-128

DOI: 10.1177/1535759721990043
journals.sagepub.com/home/epi
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A tRNA Variant Translates Into Seizure Resistance

Expression of the Neuronal tRNA n-Tr20 Regulates Synaptic Transmission and Seizure Susceptibility

Kapur M, Ganguly A, Nagy G, Adamson SI, Chuang JH, Frankel WN, Ackerman SL. *Neuron*. 2020;108(1):193-208. e9. doi:10.1016/j.neuron.2020.07.023; PMID: 32853550; PMCID: PMC7572898

The mammalian genome has hundreds of nuclear-encoded tRNAs, but the contribution of individual tRNA genes to cellular and organismal function remains unknown. Here, we demonstrate that mutations in a neuronally enriched arginine tRNA, *n-Tr20*, increased seizure threshold and altered synaptic transmission. *n-Tr20* expression also modulated seizures caused by an epilepsy-linked mutation in *Gabrg2*, a gene encoding a $GABA_A$ receptor subunit. Loss of *n-Tr20* altered translation initiation by activating the integrated stress response and suppressing mTOR signaling, the latter of which may contribute to altered neurotransmission in mutant mice. Deletion of a highly expressed isoleucine tRNA similarly altered these signaling pathways in the brain, suggesting that regulation of translation initiation is a conserved response to tRNA loss. Our data indicate that loss of a single member of a tRNA family results in multiple cellular phenotypes, highlighting the disease-causing potential of tRNA mutations.

Commentary

Whether you are a basic scientist studying genetic models of epilepsy or clinical epileptologist, you have likely encountered the enigma of phenotypic heterogeneity at some level. Phenotypic heterogeneity is the concept that alterations to a gene or a gene variant can produce different phenotypes, such as a range of epilepsy types and severities, from severe to undetectable, as well as other neurodevelopmental and neuropsychiatric disorders.[1] Basic scientists often encounter this, for example, when examining the same genetic manipulation in different inbred strains of laboratory mice and finding that seizure activity varies considerably between mouse strains with different genetic backgrounds. In humans, family members with the same inherited variants can also show a wide range of phenotypes. Although some genes and variants show strong genotype–phenotype correlation, most can cause a spectrum of phenotypes. The majority of this phenotypic heterogeneity in genetic epilepsies is unexplained, both with regard to the epilepsy phenotype and comorbidities.[2]

A common explanation for this observed phenotypic heterogeneity is the existence of variation in modifier genes, or genes whose expression alters the phenotypic expression of the disease-causing gene. Identification of modifier genes in humans is difficult, especially in epilepsy, as disease-causing variants are individually rare, making identifying genes that modify these already rare alleles exceedingly difficult. Nevertheless, identification of genetic modifiers is important because of its ability to both explain phenotypic heterogeneity, and as a means to targeting these modifiers to treat the disease. The reasoning being that if variation in the modifier gene is strong enough to alter the disease phenotype, then artificially altering the modifier gene or its protein product may also be strong enough to alter the disease.

Identifying genetic modifiers in animal models, while still challenging, is a tractable problem. One approach has been to exploit the aforementioned phenotypic heterogeneity found in different strains of inbred mice. If, for example, you discover that your gene mutation causes severe seizures in C57BL/6 N (B6 N) mice, but mild or no seizures in C57BL/6 J (B6 J), then interbreeding the 2 strains, observing the severity of seizures in the offspring and correlating it to the inherited genomic regions can narrow down genomic regions that influence the seizure phenotype. It is known that B6 J mice are more resistant to electrically and chemically induced seizures than many other inbred strains, and previous studies have identified the distal region of Chromosome 1 as a strong determinant of this trait.[3,4] Although candidate genes in this region have been proposed to underlie the effect, it is unknown whether any single gene exerts a strong influence on susceptibility.

A recent paper in *Neuron* has identified a gene in this region that strongly regulates seizure susceptibility, and it's identity is surprising.[5] A previous study implicated an inwardly rectifying

K^+ channel in this region, party based on the assumption that ion channels are known regulators of neural excitability and seizure activity.[6] While many genes encoding ion channels and synaptic proteins are regulators of seizure activity, this new candidate, *n-Tr20*, doesn't even encode a protein; it's a tRNA. tRNAs are RNA molecules that bring the correct amino acid to the growing polypeptide chain by recognizing the correct codon in the messenger RNA. *n-Tr20* is one of 5 mouse tRNAs that recognize the ACA codon in mRNA and catalyze the addition of an arginine residue to translated proteins.

The authors of this new paper previously found that B6 J mice have a mutation in *n-Tr20* that impairs the processing of the tRNA into its mature form and reduces its function.[7] Because this gene is located on the aforementioned region of Chromosome 1, they decided to test whether it contributed to the known seizure resistance of B6 J mice. Using several genetic models, they show that the mutant *n-Tr20* present in B6 J mice is actually protective against seizures. Correcting the mutation in B6 J mice increased susceptibility to electroconvulsive and pentylenetetrazole-induced seizures, while deleting the gene from the closely related B6 N decreased susceptibility to these challenges. Overexpression of 2 closely related tRNA genes, *n-Tr21* and *n-Tr22*, which also recognize the ACA codon, were able to increase seizure susceptibility in B6 J mice, suggesting that these other tRNAs can functionally substitute for *n-Tr20*, and that depletion of the total $tRNA^{Arg}_{UCU}$ pool underlies the seizure resistance phenotype caused by *n-Tr20* mutation. Finally, overexpressing *n-Tr20* increased the incidence, but not duration, of spike-wave discharge events in mice heterozygous for a patient variant (R43Q) in the $GABA_A$ receptor γ2 subunit (*Gabrg2*), demonstrating that *n-Tr20* can also modify the seizure phenotype of genetic epilepsy mouse model. Overall, these data provide convincing evidence that reduced levels of tRNAs can be protective against seizures.

Because of the unexpected nature of the finding that impairing tRNA function lead to seizure resistance, the rest of the paper investigated the underlying mechanisms, using an impressive combination of electrophysiology, gene expression, and measurements of translation efficiency (TE) and cell signaling. As expected, impairment of *n-Tr20* led to increased stalling of translation at ACA codons, and also altered the TE of many genes (330 were decreased while 104 were increased). Analysis of genes with decreased TE upon *n-Tr20* deletion revealed several translation initiation factors, consistent with dysregulation of this process. Strikingly, *n-Tr20* loss decreased the TE of numerous ribosomal protein genes, many of which are known to be regulated by a familiar player in the epilepsy world, the mechanistic target of rapamycin (mTOR) signaling pathway. Further biochemical experiments showed that *n-Tr20* loss decreased several markers of mTOR complex 1 (mTORC1) activity.

Electrophysiology experiments were then used to relate the changes in *n-Tr20*, mTOR signaling, and seizure susceptibility. Although many different conditions and parameters were tested, the strongest and most consistent effect between them was an effect on the frequency of miniature inhibitory postsynaptic currents (mIPSCs). Hippocampal CA1 neurons from B6 J or B6 N mice with impaired *n-Tr20* showed higher mIPSC frequencies than animals with normal *n-Tr20*. Treatment of B6 N mice with the mTOR inhibitor rapamycin also increased mIPSC frequency, and increased seizure threshold in female mice. Thus, the data support a model in which *n-Tr20* dysfunction increases hippocampal inhibitory synaptic transmission, which increases resistance to induced seizures.

Caution is warranted, however, in generalizing the effects of this particular modifier to other mouse strains or genetic models, as the effect of any single gene variant to complex behavioral traits will depend on complex, nonlinear interactions between many variants. Indeed, the initial report on the B6 J *n-Tr20* variant showed that it caused neurodegeneration when combined with a null allele of the *Gtpbp2* gene,[7] demonstrating that the same variant can be both harmful or protective in different disease contexts. Also, B6 J mice do not always show fewer seizures than other strains. A recent paper showed that B6 J mice also have a noncoding variant that reduces the expression of the GABAA receptor subunit-encoding *Gabra2* gene,[8] and this variant may sensitize B6 J mice to seizures caused by loss of the *Scn1a* gene in a mouse model of Dravet Syndrome.[9] Thus, the effect of modifiers on seizures or other phenotypes is highly dependent on the causative insult.

Despite these caveats, this study makes important contributions to our understanding of the biology underlying seizure susceptibility. B6 J mice are the most widely used strain in biomedical research, including epilepsy, and show significant differences in seizure susceptibility and other phenotypes. The discovery that a tRNA gene variant may mediate these differences emphasizes the fact that epilepsy modifier genes may be lurking in unexpected places. And while inhibiting *n-Tr20* may not be the safest therapeutic approach, this paper reinforces the emerging concept that noncoding RNAs and transcriptional regulation play key roles in regulating seizure susceptibility and epileptogenesis. Modulating these processes in clever ways may hold real promise as novel therapeutic approaches.

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References

1. Ellis CA, Petrovski S, Berkovic SF. Epilepsy genetics: clinical impacts and biological insights. *Lancet Neurol*. 2020;19(1):93-100. doi:10.1016/S1474-4422(19)30269-8
2. Helbig I, Tayoun AA. Understanding genotypes and phenotypes in epileptic encephalopathies. *Mol Syndromol*. 2016;7(4):172-181. doi:10.1159/000448530
3. Frankel WN, Taylor L, Beyer B, Tempel BL, White HS. Electroconvulsive thresholds of inbred mouse strains. *Genomics*. 2001;74(3):306-312. doi:10.1006/geno.2001.6564

4. Ferraro TN, Golden GT, Smith GG, et al. Mapping loci for pentylenetetrazol-induced seizure susceptibility in mice. *J Neurosci*. 1999;19(16):6733-6739.
5. Kapur M, Ganguly A, Nagy G, et al. Expression of the neuronal tRNA n-Tr20 regulates synaptic transmission and seizure susceptibility. *Neuron*. 2020;108(1):193-208 e9. doi:10.1016/j.neuron.2020.07.023
6. Ferraro TN, Golden GT, Smith GG, et al. Fine mapping of a seizure susceptibility locus on mouse chromosome 1: nomination of Kcnj10 as a causative gene. *Mamm Genome*. 2004;15(4): 239-251. doi:10.1007/s00335-003-2270-3
7. Ishimura R, Nagy G, Dotu I, et al. RNA function. Ribosome stalling induced by mutation of a CNS-specific tRNA causes neurodegeneration. *Science*. 2014;345(6195):455-459. doi:10.1126/science.1249749
8. Mulligan MK, Abreo T, Neuner SM, et al. Identification of a functional non-coding variant in the GABA A receptor alpha2 subunit of the C57BL/6 J mouse reference genome: major implications for neuroscience research. *Front Genet*. 2019;10:188. doi:10.3389/fgene.2019.00188
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(10). doi:10.1371/journal.pgen.1006398.