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Leigh has no Brakes: Impaired Inhibition in a Mouse Model of Leigh Syndrome Leads to Enhanced Seizure Sensitivity

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Elevated Susceptibility to Exogenous Seizure Triggers and Impaired Interneuron Excitability in a Mouse Model of Leigh Syndrome Epilepsy

Manning A, Han V, Stephens A, Wang R, Bush N, Bard M, Ramirez JM, and Kalume F. *Neurobiol Dis*. 2023;187:106288

Mutations in the nicotinamide adenine dinucleotide dehydrogenase (ubiquinone reductase) iron-sulfur protein 4 (*NDUFS4*) gene, which encodes for a key structural subunit of the OXFOS complex I, lead to the most common form of mitochondrial disease in children known as Leigh syndrome (LS). As in other mitochondrial diseases, epileptic seizures constitute one of the most significant clinical features of LS. These seizures are often very difficult to treat and are a sign of poor disease prognosis. Mice with whole-body *Ndufs4* KO are a well-validated model of LS; they exhibit epilepsy and several other clinical features of LS. We have previously shown that mice with *Ndufs4* KO in only GABAergic interneurons (Gad2-*Ndufs4*-KO) reproduce the severe epilepsy phenotype observed in the global KO mice. This observation indicated that these mice represent an excellent model of LS epilepsy isolated from other clinical manifestations of the disease. To further characterize this epilepsy phenotype, we investigated seizure susceptibility to selected exogenous seizure triggers in Gad2-*Ndufs4*-KO mice. Then, using electrophysiology, imaging, and immunohistochemistry, we studied the cellular, physiological, and neuroanatomical consequences of *Ndufs4* KO in GABAergic interneurons. Homozygous KO of *Ndufs4* in GABAergic interneurons leads to a prominent susceptibility to exogenous seizure triggers, impaired interneuron excitability, and interneuron loss. Finally, we found that the hippocampus and cortex participate in the generation of seizure activity in Gad2- *Ndufs4*-KO mice. These findings further define the LS epilepsy phenotype and provide important insights into the cellular mechanisms underlying epilepsy in LS and other mitochondrial diseases.

Commentary

Leigh syndrome (LS) is one of the most common forms of mitochondrial disease in children.[1] LS is due to one of several mutations in genes involved in mitochondrial function, especially Complex I of the oxidative phosphorylation component of the respiratory chain. One particular gene found to be involved in patients with LS is *Ndusf4*, which encodes a subunit of Complex I that provides structural stability to the complex.[2] Mitochondrial diseases including LS commonly feature seizures that are often the first sign of disease and are particularly difficult to control with medications. These diseases and their related animal models, also feature many other phenotypes that confound study of the seizures and epilepsy. Patients who have intractable seizures are at a high risk of dying from sudden unexpected death in epilepsy (SUDEP).[3] LS can be associated with early, sudden, and unexpected death.[4]

Manning et al[5] set out to characterize cellular, physiological, and neuroanatomical changes in a mouse model of LS that they developed in which the *Ndusf4* gene is knocked out only inhibitory neurons (Gad2-Ndufs4-KO; Gad2 mutant). This mouse prominently features increased susceptibility to seizures as well as spontaneous seizures, but does not manifest most other disease-associated abnormalities, making it a nice model in which to examine mechanisms for the seizures independent of the other signs and symptoms. Notably, they previously determined that these mice had an increased death rate that they believe was associated with seizures and suggestive of SUDEP events.[6]

In this study,[5] to determine whether these mice had increased seizure susceptibility they employed two experimental models. First, they challenged animals with an ordinarily subconvulsive dose of the chemo-convulsant, pentylenetetrazol (PTZ). They found that this lower dose of PTZ was sufficient to induce generalized convulsive seizures in the Gad2 mutant, but only induced lesser myoclonic seizures in wildtype mice. Second, they exposed the animals to moderate exercise to create a metabolic challenge. This was sufficient to induce seizures in the Gad2 mutant mice, but not in wildtypes. This is a somewhat unusual seizure-induction method. While exercised-induced seizures are rare in patients,[7] exercise does cause

measurable changes in electroencephalogram that could be conducive to seizures.[8]

Since the mutation was only expressed in GABAergic neurons, they examined whether it had any effect on GABAergic neuron function. For this, they performed whole-cell current clamp recordings from CA1 hippocampal interneurons and found that ~60% of the studied neurons had a biphasic response with increased firing following lower depolarization currents and reduced action potential firing ranging up to abject failure to produce action potentials at higher depolarization current injections. These neurons also displayed impaired action potential dynamics as evidenced by reduced capacitance, reduced rheobase, reduced action potential height, increased action potential width, increased input resistance, and increased afterhyperpolarization. These changes are generally consistent with a hypoactivity phenotype. The other ~40% of neurons did not demonstrate an appreciable reduction in firing rate and showed lesser changes in action potential dynamics compared to neurons from control mice.

To determine whether the mutation affected the interneuron number, they performed immunohistochemistry on the brains of mutants. They found a reduction in GABAergic neurons in the caudal portion of the hippocampal CA1 region and the central and posteromedial cortical amygdala nuclei. No cell loss was noted in the dentate gyrus, rostral CA1, or CA2/CA3.

Finally, to determine which areas were activated in response to seizures, they euthanized a subset of animals shortly after thermally—induced seizures and measured immunoreactivity of the immediate early gene, *cFos*. With this method, they identified increased activity in the dentate gyrus of the hippocampus and frontal cortex.

Epilepsies are generally due to excitation–inhibition imbalance with either excessive excitation or insufficient inhibition; with the latter often leading to the former. Here they identify a specific role of the *Ndufs4* mutation in reducing excitability in inhibitory interneurons. It will be important to identify which interneuron subtype (eg, somatostatin, parvalbumin, etc) is primarily involved as interneuron types are not uniformly affected by mitochondrial abnormalities. It is intriguing that they found two different populations of interneurons, one demonstrating impaired firing due to the *Ndufs4* mutation and one that was relatively intact. It will be interesting to understand more rigorously what specific single-cellular mechanisms may be at play to orchestrate the differences in phenotypes. Single nuclear RNA sequencing might be a good way to examine this.

They chose to probe for increased seizure susceptibility using PTZ, a GABAergic antagonist. Given that these mice already harbored the mutation in GABAergic neurons and demonstrated impaired GABAergic neuron function, they considered this model a "second hit" leading to heightened excitability and seizure proclivity. This is comparable to the human phenotype in which seizures are provoked by some additional extrinsic stimulus such as increased metabolic demand. The authors postulated that the concept of seizures also increasing metabolic demand may explain why neuronal activity is more affected in older animals who have experienced more lifetime seizures and cumulative metabolic derangement.

This study employed a relatively crude means to identify brain regions activated by seizures; however, this is a reasonable way to screen for affected areas. Perhaps it would be beneficial to follow up with a technique such as fiber photometry to assess calcium activity or multi-unit electrophysiology to verify increased activity in these regions and perhaps determine which specific cells are involved. Coupling these methods with a technique to express calcium indicators or opsins only in activated cells, such as targeted recombination in active populations would be a powerful way to examine activated circuits.[9]

Mitochondria are energetic powerhouses, presenting innumerable potential nodes at which to alter cellular energetics and by extrapolation, excitability. It follows that mitochondrial dysfunction is at the heart of a wide range of neurological diseases.[10] Despite this, mitochondrial signaling is relatively unexplored in the development of anti-seizure medications. When patients are refractory to medications, several alternative therapies are considered including surgical management, device implantation (eg, vagal, deep brain/thalamic, or responsive neural stimulators), or dietary therapies. A mainstay of dietary therapies is the ketogenic diet.[11] The ketogenic diet exerts its action at least in part through altering mitochondrial energetics. Many studies have been undertaken to try to identify potentially druggable targets to produce a pharmacological ketogenic diet of sorts. The mutation examined in this study may represent such a druggable target.

It will be interesting to see whether these findings could be translatable to other mitochondrial disorders. Could manipulating *Ndufs4* such that its function was enhanced in other mitochondrial disorders correct various phenotypes, especially seizures, in these other diseases? Similarly, could this be generalizable to other intractable epilepsies? Novel avenues for drug development are desperately needed for the millions of patients worldwide with intractable epilepsy.[12]

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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