

## RESEARCH HIGHLIGHT

## Separation of seizures from comorbidities in the fitful mouse model of *DNM1* epileptic encephalopathy

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The epileptic encephalopathies (EE) are a family of severe brain disorders manifesting early in life and characterized by spasms and/or intractable seizures. The relentless seizure activity impacts both cognitive and behavioral development in the patient <sup>[1]</sup>. Many patients do not respond to typical anti-epileptic therapies and can experience early death. Of late, several novel mutations in newly revealed genes have been associated with the EEs. Through the efforts of such groups as the Epi4K and EuroEPINOMIC-RES consortiums, the Epilepsy Phenome/Genome Project and the Deciphering Developmental Disorders Study, *de novo* variants have surfaced in such genes as *GABRB3*, *ALG13*, *GRINI*, *NEDD4L*, *DNM1* and others <sup>[2-4]</sup>. Whole-exome sequencing of patients has revealed 9 *DNM1* variants <sup>[2-4]</sup>. This research highlight describes our study of the mouse *Dnm1* EE model to illustrate how certain classes of *de novo* variants may give rise to EEs and emphasize the contribution of specific neuronal populations to the comorbid phenotypes. Dynamin-1, the product of the *DNM1* gene, is a large GTPase required for synaptic vesicle recycling in neurons <sup>[5]</sup>. The highly brain specific Dynamin-1 is expressed during synaptogenesis through adulthood and undergoes alternative splicing resulting in the regulated expression of several splice variants.

*Dnm1* patients have differing *de novo* mutations, but similar symptoms with the manifestation of seizures early in

life and global developmental delay <sup>[3,4]</sup>. Seizures, initiating for the most part as infantile spasms, can eventually encompass a range of types such as absence, dyscognitive, myoclonic and/or tonic clonic, for example. The *DNM1* encephalopathy appears to be resistant to standard anti-epileptic drugs, although the ketogenic diet has been reported to help at least one patient <sup>[4]</sup>. Interestingly, while dynamin-1 has five protein domains, each unique human *de novo* variant has appeared in either the GTPase or the middle domain of the protein. *In vitro* studies suggest that while these domains have different functions the mutations have analogous downstream effects on synaptic vesicle endocytosis <sup>[6]</sup>. The GTPase domain is the enzymatic effector and the middle domain is required for protein self-assembly; these findings suggest that without the cycle of assembly/disassembly the GTPase is unable to fission membrane <sup>[7]</sup>.

Comparatively, flies carrying mutations in the *Drosophila melanogaster* dynamin homolog *shibire* display temperature-sensitive paralytic phenotypes. Studies of dominant negative *shibire* mutants have demonstrated that activity-dependent synaptic vesicle depletion underlies the paralysis <sup>[8,9]</sup>. *De novo* human missense *DNM1* mutations appear to be dominant negative as well. The recent publication by Dhindsa et al. investigated the functional effect of human *DNM1* variants on endocytosis. They

presented strong evidence that several of the newly described human EE patient mutations disrupt endocytosis *in vitro* <sup>[6]</sup>.

Loss of dynamin 1 in the mouse causes early lethality and fails to recapitulate either the encephalopathy or developmental delay observed in *DNMI* patients <sup>[10]</sup> or the phenotype of the *Dnm1* EE “fitful” mouse model. The fitful mouse carries a single nucleotide change in *Dnm1* within an alternatively spliced exon. The homozygous mice have a severe seizure phenotype along with ataxia and early lethality and—due to the mutation’s unique location in an alternatively spliced exon and consequently a lower effective gene dosage of the mutation — may best represent a human heterozygous for an identified *DNMI* mutation in the GTPase/middle domains. The heterozygous fitful mice have a milder phenotype with spontaneous seizure onset at 2–3 months of age <sup>[11, 12]</sup>. Previously, we established that the fitful mutation causes a deficiency of dynamin-1 protein self-assembly required for proper function, impaired endocytic recycling, and defective synaptic transmission resulting from inefficient recovery of rapidly reusable vesicles <sup>[12]</sup>. More recently, we have also shown that the synapses of fitful homozygotes have larger synaptic vesicles as compared to wildtype animals suggesting that the mutation disrupts the ability of dynamin-1 to fission membrane properly and efficiently <sup>[6]</sup>.

Our latest study sought to dissect the neural underpinnings of the fitful mouse’s complex phenotype. Using the cre-loxP system, we were able to delete wildtype dynamin-1 from either inhibitory or excitatory neurons, leaving the mutated version alone in those populations (making them “hemizygous” for the mutant gene). When all inhibitory neurons in the forebrain were made hemizygous for *Dnm1*<sup>F<sup>fl</sup></sup> (using either the pan-inhibitory *Gad2*-cre or the forebrain interneuron-specific *Dlx5/6*-cre driver lines), the mice succumbed to seizures at the same age as homozygous fitful mice without the other physiological or behavioral abnormalities characteristic of the model. Using a similar approach, we assayed several subpopulations of inhibitory neurons. We discovered that mice hemizygous for *Dnm1*<sup>F<sup>fl</sup></sup> in parvalbumin-positive neurons (fast-spiking interneurons) also had shortened lifespans and latencies to lethal seizures comparable to fitful mice. Similar manipulations in other inhibitory subpopulations (e.g. somatostatin-positive interneurons and corticostatin-positive interneurons) also produced lethal seizure phenotypes, but at far later time points <sup>[11]</sup>.

On the other hand, we found that mice hemizygous for *Dnm1*<sup>F<sup>fl</sup></sup> in excitatory neurons did not have seizures, but importantly did have abnormalities in locomotor, exploratory, and repetitive behaviors. The fact that dominant

mutations in *Dnm1* can lead to behavioral abnormalities without seizures implies that the behavioral comorbidities of EEs are not connected to the progression of a seizure disorder. Further, this suggests that treating seizures would do little to control the other neurodevelopmental symptoms of many EEs. Given the broad pharmacological profiles of many antiepileptic drugs, and the diversity of neural populations at play, it is possible that these treatments might even exacerbate other symptoms. To this point, treating mice hemizygous for *Dnm1*<sup>F<sup>fl</sup></sup> in excitatory neurons with the glutamatergic antagonist drug MK801 (dizocilpine), which has anticonvulsant properties <sup>[13]</sup>, resulted in increased hyperactivity <sup>[11]</sup>. This study highlights the need to further investigate the contributions of specific neuronal populations to complex neurological disorders such as EEs using mouse models. This will help to examine the effects of prospective drugs/treatments for each aspect of the disorder.

Dynamin-1 is just one of many genes involved at the presynapse in vesicle dynamics. Interestingly, other vesicle trafficking genes such as *SYN1*, *STXBPI*, *LRRK2* and the very recently published *NAPB* are human epilepsy susceptibility genes <sup>[14–16]</sup>. Genes encoding the auxiliary protein *DNAJC* (a chaperone co-factor) and the syntaxin *SNX33* (a SNARE) are in a duplicated chromosomal region implicated in idiopathic generalized and focal epilepsy <sup>[17]</sup>. Most of these genes produce protein products that interact with dynamin-1 (according to coimmunoprecipitation studies). This demonstrates an increasing body of evidence for dysfunctional presynaptic vesicle recycling as a mechanism underlying epileptic disorders such as the EEs. Undoubtedly, further exome-sequencing studies will identify mutations in these genes and others encoding presynaptic proteins. Future work should determine whether the cellular populations important to the fitful seizure pathology (e.g. parvalbumin expressing interneurons) are similarly critical to the deleterious effects of EE mutations in these other presynaptic genes.

Given the low efficacy of standard anti-epileptic drugs in the context of these mutations, other forms of treatment are required. Gene therapy may be a viable option for patients given the potential for incompatible responses of different neuronal populations to target drug therapies. *DNMI* patients have diverse pathogenic mutations therefore increasing wildtype dynamin-1 may be an effective approach to restore endocytosis in the presence of dysfunctional protein regardless of individual mutations. This approach might also be applicable to mutations in other presynaptic genes, if their deleterious effects manifest through converging mechanisms (such as depletion of synaptic vesicles in rapidly-firing inhibitory cells).

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