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Dynamin 1 isoform roles in a mouse model of severe childhood epileptic encephalopathy

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ABSTRACT

Dynamin 1 is a large neuron-specific GTPase involved in the endocytosis and recycling of pre-synaptic membranes and synaptic vesicles. Mutations in the gene encoding dynamin 1 (*DNM1*) underlie two epileptic encephalopathy syndromes, Lennox-Gastaut Syndrome and Infantile Spasms. Mice homozygous for the *Dnm1* "fitful" mutation, a non-synonymous coding variant in an alternatively spliced exon of *Dnm1* (exon 10a; isoform designation: Dnm1a^{Ftfl}) have an epileptic encephalopathy-like disorder including lethal early onset seizures, locomotor and neurosensory deficits. Although fitful heterozygotes have milder recurrent seizures later in life, suggesting an additive or semi-dominant mechanism, the molecular etiology must also consider the fact that Dnm1a^{Ftfl} exerts a dominant negative effect on endocytosis in vitro. Another complication is that the fitful mutation induces alterations in the relative abundance of Dnm1 splice variants; mutants have a downregulation of Dnm1a and an upregulation of Dnm1b, changes which may contribute to the epileptic pathology. To examine whether Dnm1a loss of function, Dnm1a^{Ftfl} dominance or compensation by Dnm1b is the most critical for severe seizures, we studied alternate isoform-specific mutant mice. Mice lacking *Dnm1* exon 10a or *Dnm1* exon 10b have neither spontaneous seizures nor other overt abnormalities, suggesting that in normal conditions the major role of each isoform is redundant. However, in the presence of Dnm1a^{Ftfl} only exon 10a deleted mice experience severe seizures. These results reveal functional differences between Dnm1a and Dnm1b isoforms in the presence of a challenge, i.e. toxic *Dnm1*^{Ftfl}, while reinforcing its effect explicitly in this model of severe pediatric epilepsy.

1. Introduction

Dnm1 encodes a large neuron-specific GTPase involved in the endocytosis and recycling of pre-synaptic membranes and synaptic vesicles. This gene undergoes alternative splicing resulting in the regulated expression of several splice variants. The first alternatively spliced site is exon 10, resulting in two mutually-exclusive variants, termed Dnm1a and Dnm1b. The fitful mutation (*Dnm1a*^{Ftfl}) confers a single amino acid substitution to the respective *Dnm1a* isoform leaving the alternative *Dnm1b* isoform intact (Boumil et al., 2010). Mutant Dnm1a^{Ftfl} protein does not assemble properly into the homo-oligomeric complexes necessary for proper function (Boumil et al., 2010). Complete knockout of *Dnm1* (all isoforms) in the mouse demonstrated that a sub-population of neurons is more sensitive to the loss of *Dnm1* and resulted in endocytic defects (Ferguson et al., 2007, Hayashi et al., 2008). Homozygous *Dnm1* knockout mice display early lethality, but neither the homozygous nor heterozygous knockout mice have seizures. By comparison, the fitful mutation, while present only in the Dnm1a isoform, is associated with recurrent seizures in both heterozygous and homozygous genotypes, with the latter being severe and accompanied by behavioral comorbidities similar to human epileptic encephalopathy (EE) patients that carry *de novo DNM1* variants (Boumil et al., 2010, Asinof et al., 2015, Dhindsa et al., 2015).

Expression and alternative splicing of *Dnm1* is regulated during early postnatal development and synaptogenesis (Gray et al., 2003, Ferguson et al., 2007). There is a developmental shift in the expression of the Dnm1a and Dnm1b isoforms (Boumil et al., 2010). *Dnm1*b is expressed at the highest levels during embryonic and early postnatal development and decreases during the time of synaptogenesis. *Dnm1a* expression increases at this stage (Boumil et al., 2010). The Dnm1a-Dnm1b alternative exon encodes a portion of the "middle" domain of dynamin 1, previously shown to be required for multi-molecular assembly (Okamoto et al., 1999, Smirnova et al., 1999, Ramachandran et al., 2007). A paralogous middle domain exon is also present in *Dnm2*, but not in *Dnm3* (reviewed in (Marks et al., 2001)), although the developmental

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expression profile of this domain is not known (Sontag et al., 1994). Interestingly, the Dnm2 alternative isoforms have Golgi- specific differential functions (Cao et al., 1998, Liu et al., 2008). In fitful animals exon 10 is spliced abnormally; *Dnm1*b expression persists well into the late postnatal period (Boumil et al., 2010). It is tempting to speculate that altered dynamin 1 composition during synaptic maturation may contribute to the disease phenotype – perhaps reflecting changing dynamin 1 subunit assembly requirements for endocytosis from early to late postnatal development. Indeed, the modes of endocytosis mature from basal endocytosis to stimulation-induced endocytosis as the requirement for presynaptic synaptic vesicle recycling becomes more demanding (Bonanomi et al., 2008, Clayton and Cousin, 2009). Inappropriate expression of *Dnm1b* in mature neurons that should express the adult *Dnm1a* isoform may affect the kinetics of endocytosis and exacerbate the fitful phenotype.

Dnm1b is expressed earlier than *Dnm1a* and, interestingly, homozygous fitful mice survive until two to three weeks of age suggesting that *Dnm1b* is necessary and perhaps sufficient for providing normal *Dnm1* function during this stage (Boumil et al., 2010). *Dnm1* null mice only survive through the first week of life (Ferguson et al., 2007). In both wild-type and fitful mutant mice, *Dnm1a* expression increases and becomes the more dominantly expressed isoform by two weeks of age. This is concurrent with the observed decline of fitful mutant mice, suggesting that *Dnm1a* is necessary for maturation and the expression of *Dnm1b* can no longer compensate.

In order to determine whether the *Dnm1* middle domain isoform usage is critical for normal development or for the seizure phenotype in fitful mice we generated and characterized mice that lack either *Dnm1a* or *Dnm1b*. Although neither deletion alone has any obvious effect on development, in the presence of the $Dnm1a^{Ftfl}$ mutation normal *Dnm1a* isoform is required to prevent lethal seizures. We conclude that subtle functional differences between the middle domain isoforms have evolved in vertebrates to specialize in differential requirements for developmental and adult brain functions.

2. Results

2.1. Generation of dynamin 1 isoform specific knock-out mice

It was previously demonstrated that the developmentally regulated shift in dynamin-1 isoform expression is disrupted in fitful mice carrying an isoform specific mutation (Boumil et al., 2010). Therefore, we postulated that Dnm1a and Dnm1b isoforms may have divergent functions. In order to isolate and distinguish possible altered functional requirements for the *Dnm1a* and *Dnm1b* isoforms, we generated two independent lines of knockout mice, by homologous recombination in ES cells using targeting vectors containing loxP recombination sites flanking either exon 10b or exon 10a and a *Tkneo* selection cassette flanked by FRT recombination sites (Fig.1). The targeted isoform exon was deleted and the remaining isoform was left intact. Independent mouse lines were backcrossed to C57BL/6J mice for at least ten generations to establish congenic strains. The established *Dnm1a* and *Dnm1b* isoform knockout mice were viable, had a Mendelian distribution of expected genotypes and no obvious health alterations. The mice were initially observed for overt behavior and seizure phenotypes. Neither the *Dnm1a* nor the *Dnm1b* knockout mice displayed any spontaneous seizure phenotype either as young mice or as aged adults.

2.2. Expression levels of dynamin 1 isoforms

Analysis of RNA and protein levels demonstrated a complete loss of isoform expression in the respective deletion strain (Fig. 2). While a lack of specific isoform expression was detected, the overall level of dynamin 1 was maintained indicating upregulation of the remaining isoform.

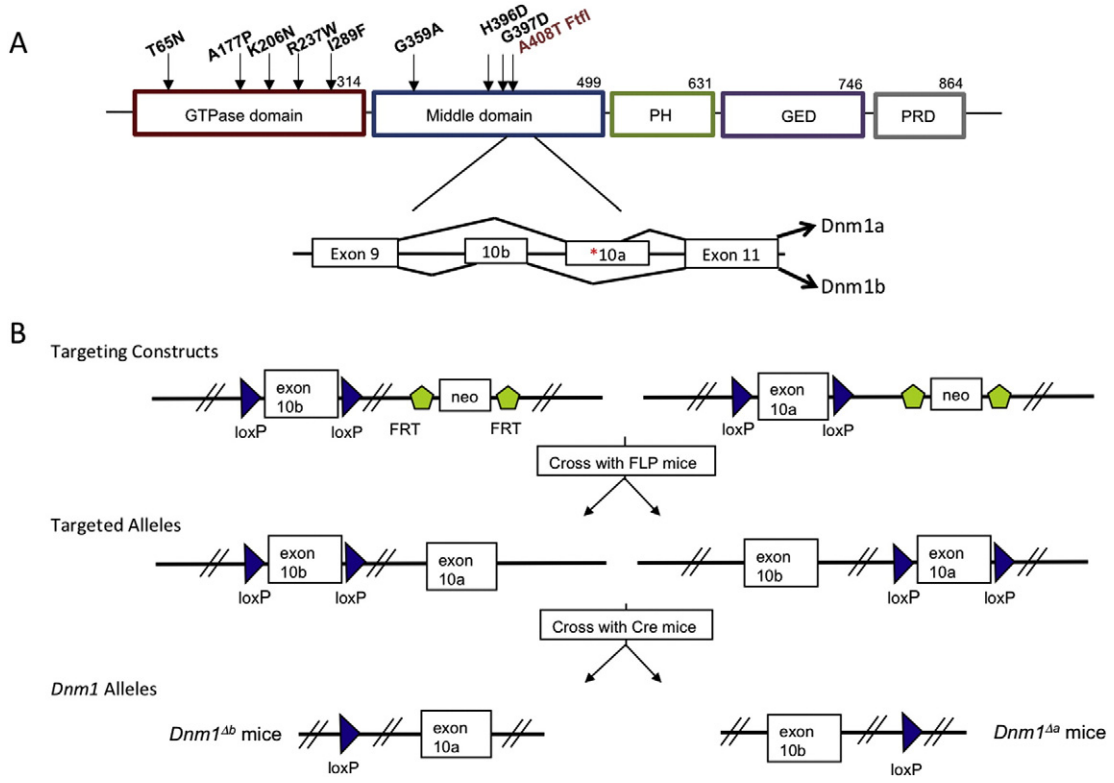

Fig. 1. Dynamin 1 isoform specific mice. (A) Schematic of DNM1 with enlargement of the alternatively spliced exon structure found in the middle domain. Above the protein domains are the known disease-causing human variants (black) and the fitful mutation (red). The fitful mutation is located in exon 10a. (B) Targeting strategy. loxP sites were created flanking either exon 10b or exon 10a with an adjacent FRT flanked neomycin selection cassette. Correctly targeted recombined mice were crossed with FLP mice to remove the neomycin cassette resulting in "floxed" mice. The floxed mice were crossed with germline cre recombinase mice to recombine the loxP sites and remove the targeted exon resulting in isoform specific deletion mice.