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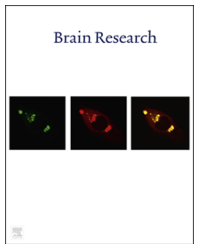

Research Report

Transcriptional upregulation of myelin components in spontaneous myelin basic protein-deficient mice

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ABSTRACT

Myelin is essential for efficient signal transduction in the nervous system comprising of multiple proteins. The intricacies of the regulation of the formation of myelin, and its components, are not fully understood. Here, we describe the characterization of a novel myelin basic protein (Mbp) mutant mouse, *mbp*^{jive}, which spontaneously occurred in our mouse colony. These mice displayed the onset of a shaking gait before 3 weeks of age and seizure onset before 2 months of age. Due to a progressive increase of seizure intensity, *mbp*^{jive} mice experienced premature lethality at around 3 months of age. *Mbp* mRNA transcript or protein was undetectable and, accordingly, genetic analysis demonstrated a homozygous loss of exons 3 to 6 of *Mbp*. Peripheral nerve conductance was mostly unimpaired. Additionally, we observed grave structural changes in white matter predominant structures were detected by T1, T2 and diffusion weighted magnetic resonance imaging. We additionally observed that Mbp-deficiency results in an upregulation of *Qkl*, *Mag* and *Cnp*, suggestive of a regulatory feedback mechanism whereby compensatory increases in *Qkl* have downstream effects on *Mag* and *Cnp*. Further research will clarify the role and specifications of this myelin feedback loop, as well as determine its potential role in therapeutic strategies for demyelinating disorders.

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1. Introduction

The myelin sheath is an insulating layer covering the axon of neurons, which is essential for efficient signal transduction in the nervous system. This white matter sheath is produced by either oligodendrocytes in the central nervous system or by Schwann cells in the peripheral nervous system. Recent work has shown that the intricacies of myelin formation allow for complex networks in the mammalian brain (Fields, 2014; Tomassy et al., 2014).

Myelin is constituted by a complex mixture of proteins, lipids and small molecules (such as cholesterol), with a different composition in the central versus peripheral nervous system. In the central nervous system, proteolipid protein (PLP) and myelin basic protein (MBP) are the most abundant proteins in myelin. MBP has been described as the 'executive molecule of myelin', as it is the only component of myelin that appears essential for myelin function (reviewed in Boggs, 2006).

Defects in myelin have been associated with a variety of complex neurological disorders such as schizophrenia and bipolar disorder (Yu et al., 2014), but also alcoholism (Levey et al., 2014). In schizophrenia a loss of white matter is described as one of the earliest measurable effects and genetic polymorphisms in *MBP* are associated with the disease (reviewed in Roussos and Haroutunian, 2014). A number of spontaneous mutant rodents have been discovered with mutations affecting myelin components. The effect of such mutations vary widely: from lethal in the Long Evans shaker rat (Carre et al., 2002) and the shiverer mouse (Chernoff, 1981), because of large scale hypomyelination, to mice that do not display a dysmyelinating phenotype despite lacking PLP. Here we discovered and characterized a novel spontaneous mbp-deficient mouse strain and provide evidence for a compensation feedback loop from Mbp to Mag (myelin-associated glycoprotein) and Cnp (cyclic nucleotide phosphodiesterase).

2. Results

2.1. Discovery of a spontaneous mutant mouse strain with neurological manifestations

During routine breeding a severe shaking phenotype was observed in young C57BL/6 mice of common descent, denoted "*jive*" mice after their jive dance-like gait. Further breeding of the parents of such mice resulted in ~25% of the mice manifesting this phenotype, suggestive of a spontaneous recessive mutation. The *jive* mice, retrospectively identified as "mbp^{jive}", developed the shaking phenotype before weaning at 3 weeks of age (Fig. 1A). This phenotype was followed by silent seizures around 50 days of age, progressing into tonic seizures and premature death around 75 days of age (Fig. 1A). The shaking phenotype occurred upon locomotion (Supplemental video 1) and the seizures upon external stimuli such as cage opening and researcher interference in the cage (Supplemental video 1). Mbp^{jive} mice also display decreased absolute body weight at young ages (Fig. 1B). We assessed the mice at approximately 50 days of age with the 6-min open field test. The gait of wildtype (Fig. 1C) and mbp^{jive} mice (Fig. 1D) was distinctly different, with less locomotion of the mbp^{jive} mice (Fig. 1E) despite similar mobility (Fig. 1F) and time spent in the different areas of the open field (Supplemental Fig. 1).

Supplementary material related to this article can be found online at doi:10.1016/j.brainres.2015.02.021.

2.2. Spontaneous loss-of-function mutation caused by deletion of exons 3–6 of Mbp

Due to the similarities of the *jive* phenotype to other mouse strains with spontaneous (Roach et al., 1985) or designed (Akowitz et al., 1987) deficiencies in *Mbp*, we investigated the potential for the *jive* phenotype to be caused by a *Mbp* mutation. Relative gene expression measurements did not detect any *Mbp* transcript in *jive* mice when testing 2 different qPCR assays spanning alternative exons (Fig. 2A and B). This loss of *mbp* mRNA resulted in complete Mbp protein deficiency, as demonstrated by Western blot analysis of brain tissue of adult wildtype and mbp^{jive} mice (Fig. 2C). For the loading control tubulin and full blot images, please see Supplemental Fig. 2A and B. Genotyping for previously described Mbp mutations ruled out known mutations, so we assessed each exon of the *Mbp* gene for their presence in the mbp^{jive} genome (strategy depicted in Fig. 2D). The result of this exon testing was the discovery that exons 3 to 6 of the *Mbp* gene were homozygously deleted (Fig. 2E). As we were unable to bridge the deleted junction with extensive efforts, we suspect that this genetic variation may include a translocation or inversion. Retrospective genotyping of all previous *jive* mice and wildtype siblings demonstrated 100% concordance of the *Mbp* exon 3–6 deletion with the *jive* phenotype, confirming the identification of a novel *Mbp* mutant mouse strain.

2.3. Mbp^{jive} mice maintain normal peripheral neurological responses despite altered structure of the central nervous system

To fully characterize the effects of the *jive* phenotype in mbp^{jive} mice, we performed nerve conductance measurements over sciatic nerve and the dorsal caudal nerve to assess integrity and functionality of the peripheral nervous system. Measurements of sensory nerve action potential (SNAP) only showed a slight decrease of the latencies at 8 weeks (Fig. 3A), while the shaking phenotype was present from 3 weeks of age on. SNAP amplitude, compound muscle action potential (CMAP) latency and CMAP amplitude measurements (Fig. 3B–D) all showed no differences between the shaking mice and wildtype age-matched controls at three different time points following the onset of shaking.

To assess the effect of the mbp^{jive} mutation on the central nervous system T2-weighted imaging and T2 relaxometry was performed (Fig. 4A). Here, we discovered an increase in the T2-weighted contrast and a significant increase in the T2 relaxation times for the corpus callosum in mbp^{jive} mice (Fig. 4B), but not in the more prominent grey matter structures such as the cortex and hippocampus (Fig. 4C and D). Contrast changes were also apparent on T1-weighted images in the corpus callosum and cerebellum. Finally, diffusion tensor imaging further