Matrix Biology 29 (2010) 357–368

Contents lists available at ScienceDirect

Matrix Biology

journal homepage: www.elsevier.com/locate/matbio

Fibrillin-2 is dispensable for peripheral nerve development, myelination and regeneration

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ARTICLE INFO

Article history:
Received 16 October 2009
Received in revised form 15 February 2010
Accepted 18 February 2010

Keywords:
Fibrillin
Extracellular matrix
Peripheral nerve

ABSTRACT

The extracellular matrix of peripheral nerve is formed from a diverse set of macromolecules, including glycoproteins, collagens and proteoglycans. Recent studies using knockout animal models have demonstrated that individual components of the extracellular matrix play a vital role in peripheral nerve development and regeneration. In this study we identified fibrillin-1 and fibrillin-2, large modular structural glycoproteins, as components of the extracellular matrix of peripheral nerve. Previously it was found that fibrillin-2 null mice display joint contractures, suggesting a possible defect of the peripheral nervous system in these animals. Close examination of the peripheral nerves of fibrillin-2 deficient animals described here revealed some structural abnormalities in the perineurium, while general structure of the nerve and molecular composition of nerve extracellular matrix remained unchanged. We also found that in spite of the obvious motor function impairment, fibrillin-2 null mice failed to display changes of nerve conduction properties or nerve regeneration capacity. Based on the data obtained we can conclude that peripheral neuropathy should be excluded as the cause of the impairment of locomotory function and joint contractures observed in fibrillin-2 deficient animals.

1. Introduction

Fibrillin-1 and -2 are large multidomain glycoproteins and the structural components of extracellular microfibrils that impart physical properties to a large variety of tissues, alone or together with elastin as elastic fibers, and that modulate cell performance during organ growth and tissue remodeling by sequestering TGFβ and BMP complexes and interacting with integrins and cells surface proteoglycans (Ramirez and Dietz, 2009). In spite of being part of the same extracellular macro-aggregates, structural mutations in fibrillin-1 and fibrillin-2 are causally associated with distinct human conditions (Marfan syndrome (MFS) and congenital contractual arachnodactyly (CCA), respectively) that are in part replicated in the discrete phenotypes of mice lacking fibrillin-1 (*Fbn1*) or fibrillin-2 (*Fbn2*) gene expression (Ramirez and Dietz, 2009). Like CCA patients, *Fbn2*$^{-/-}$ mice display transient contractures of small joints, persistent contractures of large joints and reduced bone mass, in addition to a bone-patterning defect, bilateral syndactyly, which was genetically linked with impaired BMP signaling and is observed in neither CCA patients nor *Fbn1*$^{-/-}$ mice (Arteaga-Solis et al., 2001; Carta et al., 2006; Viljoen, 1994). Whereas syndactyly is in line with the broad profile of *Fbn2* activity in embryonic tissues, bone and joint manifestations are consistent with histological evidence that fetal and early postnatal expression of the protein is predominantly restricted to tendon/ligament, perichondrium, bone and peripheral nerves (Boregowda et al., 2008; Charbonneau et al., 2003; Quondamatteo et al., 2002; Ritty et al., 2003; Zhang et al., 1995).

The present study was designed to investigate the formal possibility that joint contractures in *Fbn2*$^{-/-}$ mice (and by extrapolation in CCA patients) may be accounted for by neurological abnormalities that affect peripheral nerve development and/or myelination. To this end, *Fbn2*$^{-/-}$ mice were subjected to a series of behavioral and electrophysiological tests, as well as extensive immunohistological analyses. Although architectural abnormalities were identified in the mutant perineurium, these experiments revealed no major defects in peripheral nerves, neuromuscular junctions (NMJ) or nerve conduction properties that could explain the large joint contractures and severe locomotory impairment of *Fbn2*$^{-/-}$ mice. Hence, mechanism(s) other than peripheral neuropathy is (are) responsible for these manifestations in CCA and mouse models of the disease.

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doi:10.1016/j.matbio.2010.02.006

2. Results and discussion

Like CCA patients, newborn *Fbn2*$^{-/-}$ mice display small joint contractures that disappear within the first few days of postnatal life and large joint contractures that persist throughout adulthood (Arteaga-Solis et al., 2001). While being lifted by the tail, *Fbn2*$^{-/-}$ mice retracted the hindlimbs toward their body whereas wild type (*WT*) and *Fbn2*$^{+/-}$ littermates extended their limbs downward (Fig. 1A and B). We have also occasionally seen forelimb flexion in tail-suspended *Fbn2*$^{-/-}$ mice. To assess possible functional deficits in forelimbs of *Fbn2*$^{-/-}$ animals, a wire hanging test was performed. We found that *Fbn2*$^{-/-}$ mice exhibited a significant deficit in the ability to hang on the wire relative to *WT* or *Fbn2*$^{+/-}$ littermates (Fig. 1C; $p<0.0001$). These data suggest that a possible neurological defect, likely but not exclusively affecting the peripheral nervous system, is involved in the phenotype of fibrillin-2 deficient animals. To further explore this possibility, mice were subjected to a Rotarod test that measures balance and motor coordination. Consistent with motor function impairment, *Fbn2*$^{-/-}$ mice performed significantly worse than *WT* or *Fbn2*$^{+/-}$ littermates (Fig. 1D; $p<0.0001$). By contrast, a Hot plate analgesia test did not reveal appreciable differences between mice of the three different genotypes, indicating that the nociceptive sensory function was not altered in *Fbn2*$^{-/-}$ animals (Fig. 1E). To analyze whether Fbn2-deficient mice have a defect in proprioceptive sensory organs, cross-sections of soleus muscle of adult *Fbn2*$^{-/-}$ and *WT* mice were dual stained using antibodies to slow-tonic myosin heavy chain, a marker of muscle spindles, and to collagen type IV, which encapsulates muscle spindles. Muscle spindles could readily be detected in the muscles of both *WT* and *Fbn2*$^{-/-}$ animals, and no apparent differences in the amount or distribution of the spindles were found (Fig. 2A–D). Consistent with that, no differences were also detected when sections of cervical (Fig. 2E,F) or lumbar (data not shown) dorsal root ganglia of *WT* and *Fbn2*$^{-/-}$ mice were stained using antibody to proprioceptive neuronal marker parvalbumin. Collectively, these data suggest the absence of both nociceptive and proprioceptive sensory defects in fibrillin-2 deficient animals.

Based on the above evidence, the profiles of fibrillin-1 and fibrillin-2 immunostaining were compared in the hindlimbs obtained from

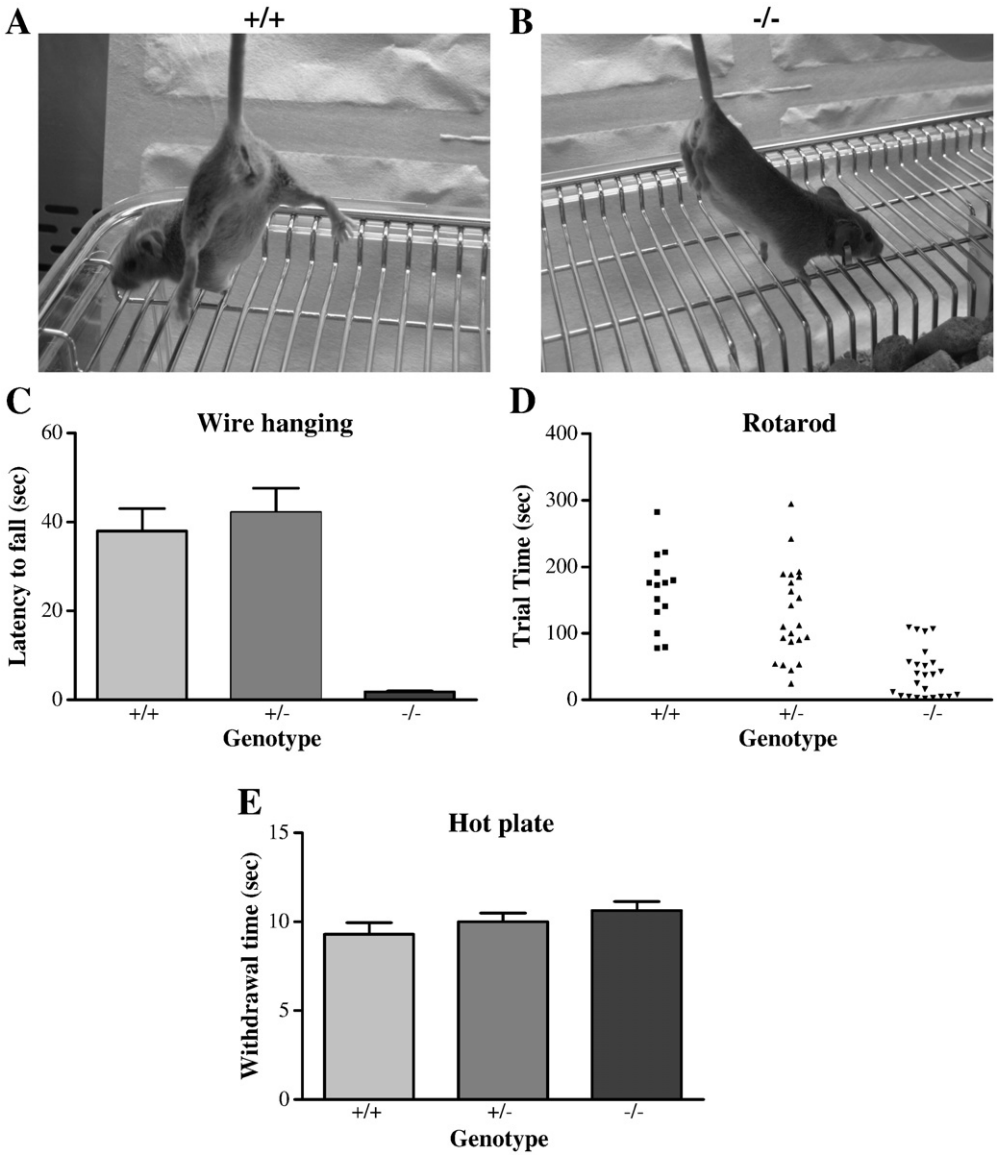

Fig. 1. Ablation of fibrillin-2 causes motor dysfunction. When suspended, *Fbn2*$^{-/-}$ mice retracted their hindlimbs toward the body (B). This was never observed with *WT* mice (A). C: Performance in wire hanging test. Latency to fall was recorded. D: Analysis of motor function by Rotarod test. The duration time for which the mice could stay on accelerating rotarod was measured. Means of the two final trials for each of the tested animals are shown. E: Hot plate test. Mice were placed on the hot plate and latency to first paw lift and lick was recorded. In C and E, each bar represents the mean ± S.E. of each genotype group.