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Severe protein aggregate myopathy in a knockout mouse model points to an essential role of cofilin2 in sarcomeric actin exchange and muscle maintenance

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ABSTRACT

Mutations in the human actin depolymerizing factor cofilin2 result in an autosomal dominant form of nemaline myopathy. Here, we report on the targeted ablation of murine cofilin2, which leads to a severe skeletal muscle specific phenotype within the first two weeks after birth. Apart from skeletal muscle, cofilin2 is also expressed in heart and CNS, however the pathology was restricted to skeletal muscle. The two close family members of cofilin2 – ADF and cofilin1 – were co-expressed in muscle, but unable to compensate for the loss of cofilin2. While primary myofibril assembly and muscle development were unaffected in cofilin2 mutant mice, progressive muscle degeneration was observed between postnatal days 3 and 7. Muscle pathology was characterized by sarcoplasmic protein aggregates, fiber size disproportion, mitochondrial abnormalities and internal nuclei. The observed muscle pathology differed from nemaline myopathy, but showed combined features of actin-associated myopathy and myofibrillar myopathy. In cofilin2 mutant mice, the postnatal expression pattern and turnover of sarcomeric α-actin isoforms were altered. Levels of smooth muscle α-actin were increased and remained high in developing muscles, suggesting that cofilin2 plays a crucial role during the exchange of α-actin isoforms during the early postnatal remodeling of the sarcomere.

Introduction

During striated muscle development, the assembly and the precise alignment of actin filaments into sarcomeric structures is a critical part of the formation of myofibrils, which provide the molecular basis for the generation of contractile force. However, the mechanisms of assembly and in particular the maintenance of sarcomeric actin filaments are not well understood. It remains enigmatic how during development the exchange of smooth muscle α-actin *via* cardiac to finally skeletal muscle α-actin is orchestrated, while at the same time sarcomere structure and contractility are perfectly maintained (McHugh et al., 1991; Tondeleir et al., 2009).

In the last two decades a large number of gene mutations affecting sarcomeric proteins have been associated with myopathies. In this context it is puzzling that different mutations in the same gene can cause a broad spectrum of clinical and myopathological phenotypes, while on the other hand a defined clinical condition may be caused by mutations in different genes. For example, mutations in the skeletal muscle α-actin gene (*ACTA1*) give rise to various forms of congenital myopathies and a broad range of distinct myopathological features like actin accumulations, nemaline rods, intranuclear bodies or zebra bodies (Nowak et al., 2012). Conversely, the myopathological presentation of a nemaline myopathy, can be caused by mutations in genes coding for various

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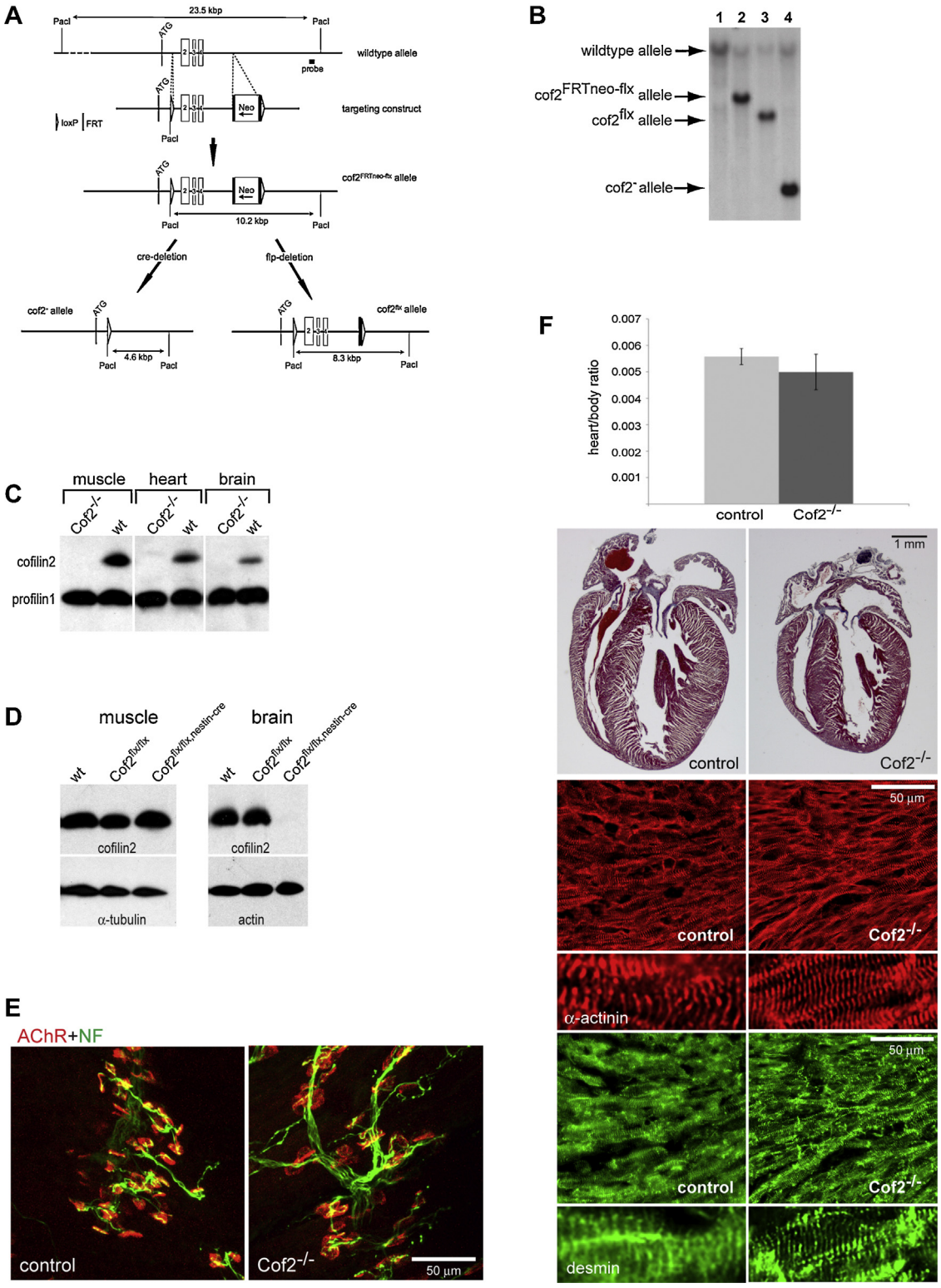

Fig. 1. Generation of a conditional knockout mouse model for cofilin2. (A) Targeting of the cofilin2 gene. Schematic outline of the cofilin2 wildtype allele (*cof2*^{wt}), the targeting construct and the targeted allele (*cof2*$^{FRTneo\text{-}flx}$ allele), which carries loxP sites (open triangles) in the first intron and downstream of the cofilin2 gene, respectively. The FRT (black boxes)-flanked neomycin resistance cassette was inserted upstream of the second loxP site. Flp mediated removal of the neomycin resistance cassette leads to the conditional cofilin2 allele *cof2*^{flx} (bottom right). Cre mediated deletion of exons 2–4 including the neomycin resistance cassette results in the knockout allele *cof2*$^{-}$ (bottom left). The probe for Southern blot hybridization is indicated. The location of the diagnostic PacI restriction sites and the size of the respective restriction fragments are shown. (B) Southern blot analysis of mouse genomic DNA from wildtype (*cof2*^{wt}) and different transgenic animals after PacI digestion and probing with a fragment from the 3′UTR as indicated in (A). *Cof2*$^{wt/wt}$ DNA (lane 1), *cof2*$^{wt/FRTneo\text{-}flx}$ DNA (lane 2), *cof2*$^{wt/flx}$ DNA (lane 3), and *cof2*$^{wt/-}$ DNA (lane 4) were loaded, the sizes of the different alleles are indicated in (A). (C) Western blot analysis of wildtype (wt) and cofilin2 knockout (*cof2*$^{-/-}$) tissue lysates from muscle, heart and brain at postnatal day 7 were incubated with the cofilin2 specific antibody FHU1. The same membrane was probed for profilin1 to normalize for loading. (D) Conditional deletion of cofilin2 in brain using nestin-cre. Western blot of muscle and brain lysates from adult wildtype (wt), cofilin2 conditional (*cof2*$^{flx/flx}$), and cofilin2 conditionally deleted (*cof2*$^{flx/flx,nestin\text{-}cre}$) animals were probed for cofilin2 (FHU1). α-Tubulin and actin were used for normalization, respectively. (E) Morphology of neuromuscular junctions. Sections of the diaphragm from control and *cof2*$^{-/-}$ mice at P7 were stained for acetylcholine receptor (AChR, red) and neurofilament (NF, green). (F) Heart analysis of *cof2*$^{-/-}$ mice. Heart weight to body weight index for

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