INTRODUCTION:
Neurofibromatosis 1 (Morbus von Recklinghausen, NF1) is a common genetic disease with an incidence of 1:3000. It is caused by mutations in the NF1 tumor suppressor gene that encodes for the protein neurofibromin (NF1). The GTPase activity is the best considered function of NF1. Neurofibromin functions as a down-regulator of Ras proto-oncogene signaling by accelerating the switch of active Ras-GTP into inactive Ras-GDP. Approximately half of all patients with NF1 deficiency revealed a loss of heterozygosity (LOH) of the NF1 gene (Sakamoto et al. 2007). As animals with a homozygous knock-out of NF1 do not survive, studies of LOH are only possible with conditional knock-out mouse models (Kolanczyk et al. 2007).

The aim of the current study is to analyze fracture healing and its disturbance in NF1 with LOH in mesenchymal cells.

METHODS:
Male 8-10 weeks old C57BL/6N mice (WT, N=32) and NF1 knock-out mice with C57BL/6N background (KO, N=4) were included in this study. A unilateral femoral, transverse, mid-diaphyseal closed fracture was created in the left femur and stabilized with an intramedullary nail. Studies of tibial fracture healing in NF1 knock-out mice revealed no diagnostic criterion. Bone healing in NF1 patients is problematic and can lead to pseudarthrosis that is challenging to treat.

Studies of tibial fracture healing in NF1 knock-out mice revealed no disturbed healing in the mid-diaphysis (Schindeler et al. 2008). However, studies of human tibial pseudarthrosis in patients with NF1 deficiency revealed a loss of heterozygosity (LOH) of the NF1 gene (Sakamoto et al. 2007). As animals with a homozygous knock-out of NF1 do not survive, studies of LOH are only possible with conditional knock-out mouse models (Kolanczyk et al. 2007).

RESULTS:
\[ \text{FIG. 1. Total Callus Volume (TV, left) and Bone Volume (BV, right) over the healing course in the WT mice and at day 21 in the KO mice (*p < 0.05).} \]

DISCUSSION:
The WT mice displayed regular, secondary bone healing with bony bridged callus after day 21. The fracture healing process in the NF1 KO was disturbed, as demonstrated by a significantly lower mineralization of the callus tissue, a lower callus volume and a disturbed endochondral ossification. The Ras-MAP-Kinase pathway is a highly conserved module that is involved in various cellular functions including migration, proliferation, differentiation and survival. Various studies suggest that activation of the Ras-MAP-Kinase pathway inhibits osteogenic differentiation and promotes proliferation. The results of this study provide further evidence for the regulatory role of neurofibromin in fracture healing (Kuorilehto et al. 2006). The delayed healing in the KO with broad osteoid seams might have been caused by a decreased differentiation and increased proliferation of osteoblasts due to the activation of the Ras-MAPK-pathway. Further experiments will be carried out to investigate the role of neurofibromin in fracture healing and to study the therapeutic potential of a MAP-Kinase inhibitor to stimulate fracture healing in Neurofibromatosis 1.

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