

A Phe377del Mutation in ANK Leads to Impaired Osteoblastogenesis and Osteoclastogenesis in a Mouse Model for Craniometaphyseal Dysplasia (CMD)

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Craniometaphyseal dysplasia is a rare genetic disorder with hyperostosis of craniofacial bones and widened metaphyses in long bones. Patients often suffer from neurological symptoms due to obstruction of cranial foramina. No proven treatment is available and the pathophysiology is largely unknown. A Phe377 (TTC₁₁₃₀₋₁₁₃₂) deletion in exon 9 of the pyrophosphate (PPi) transporter ANK leads to CMD-like features in a *Ank*^{KI/KI} mouse model. Here, we investigated the effects of CMD-mutant ANK on mineralization and bone mass at a cellular level. *Ank*^{KI/KI} osteoblast cultures showed decreased mineral deposition. Expression of regulator genes for bone mineralization *Mmp13*, *Ocn*, *Osx*, *Phex* was reduced in *Ank*^{KI/KI} osteoblasts while *Fgf23* was highly expressed in calvarial and femoral bones, which corresponds with findings of hypomineralized cortical bone in *Ank*^{KI/KI} mice. Since ANK is a known PPi transporter, we examined other regulators of Pi/PPi homeostasis *Enpp1* and *Tnap*. Significantly increased ENPP1 activity in *Ank*^{KI/KI} osteoblasts may compensate for dysfunctional mutant ANK. Similar to *Ank*^{KI/KI} bone marrow-derived macrophage cultures, peripheral blood cultures from CMD patients exhibited reduced osteoclastogenesis. Cell-autonomous effects in *Ank*^{KI/KI} osteoclasts result in disrupted actin ring formation and cell fusion. In addition, *Ank*^{KI/KI} osteoblasts fail to adequately support osteoclastogenesis. Increased bone mass can partially be rescued by bone marrow transplants supporting our hypothesis that reduced osteoclastogenesis contributes at least in part to hyperostosis. We conclude that the Phe377del mutation in ANK causes impaired osteoblastogenesis and osteoclastogenesis resulting in hypomineralization and a high bone mass phenotype.