A Novel Role of TRAPPC9 in Bone Remodeling and Skeletogenesis

Gabrielle T. Robinson [1,2,3], Ernesto Solorzano-Zepeda [1,2,3], Antoinette C. Holland [1,2], Alexander P. Powell [1,2,3], Trinity A. Kronk[1,2,3] Kenneddy C. Nkachukwu [1,2], Hope C. Ball [1,2], Fayez F. Safadi [1,2,4]

[1]Department of Anatomy and Neurobiology, College of Medicine, Northeast Ohio Medical University (NEOMED), Rootstown, OH, USA,[2] Musculoskeletal Research Focus Area, NEOMED, [3]Basic and Translational Biomedicine Graduate Program, College of Graduate Studies, NEOMED, [4]Rebecca D. Considine Research Institute, Akron Children's Hospital, Akron, OH USA,
grobinson@neomed.edu

INTRODUCTION: On average approximately 17% of the American population suffer from intellectual disability (ID) affects about 4.92 million Americans. Skeletal abnormalities have been described in patients with ID. X-linked forms of ID have been investigated, while the autosomal-recessive forms of ID have yet to be studied. Specifically, Non-syndromic autosomal-recessive intellectual disability (NSARID) develops because of mutations in the trafficking protein particle complex subunit 9 (TRAPPC9) gene. Patients possessing this mutation exhibit obesity, microcephaly, and skeletal abnormalities inclusive of an increase in bone mass, resulting in a denser cranium, limb deformities, and the loss of teeth. The direct linkage by which TRAPPC9 mutations contribute to skeletogenic homeostasis has yet to be characterized and explained. To definitively understand the impact of TRAPPC9 on skeletogenesis, we investigated the physiological role of TRAPPC9 in bone homeostasis. We characterized the skeletal phenotype of TRAPPC9 global knockout (KO) mice utilizing both *in vivo* and *in vitro* approaches.

METHODS: All animal studies were approved by the institutional animal care and use committee (IACUC) of Northeast Ohio Medical University. The functionality of osteoclasts (OCs) was assessed using *in vitro* cellular and biochemical assays of bone marrow-derived hematopoietic stem cells (HSCs) isolated from TRAPPC9 Wild-Type (WT) and TRAPPC9 Knock-Out (KO) mice. HSCs were utilized in the assessment of cell viability, proliferation, differentiation, and function in vitro. OC-related gene expression levels were assessed using qPCR analysis from 8-week-old and 8-month-old TRAPPC9 WT and KO humeri and calvarias. In addition, we also assessed serum C-telopeptide of type I collagen (CTX) levels isolated from TRAPPC9 WT and KO mice, at ages 8 weeks and 8 months of age.

RESULTS SECTION: First, we harvested HSCs from the femurs and tibiae of the anesthetized TRAPPC9 WT (8 weeks old; n =8, 8 months-old; n = 4) and KO (8 weeks old; n = 4, 8 months; n= 6) mice. Using 8-week-old male mice, the osteoclast differentiation of the TRAPPC9 WT mice (Figure 1A) developed normally, while OCs from TRAPPC9 KO mice (Figure 1B) were larger in size with decreased function using resorption assay. Next, we analyzed serum from both ages (8 weeks and 8 months) of TRAPPC9 WT and KO animals. The eight-month-old WT animals displayed an increase in CTX levels compared to the eight-week-old TRAPPC9 WT mice. While the eight-month-old KO mice had decreased serum levels of CTX compared to 8-week-old KO animals. Suggesting a decrease in osteoclast activity in the TRAPPC9 KO mice with age (Figure 1C). RT-qPCR analysis of OC-related markers (TRAP and CTSK) in bone (8 weeks old) showed decreased levels in the TRAPPC9 KO mice compared to WT littermates.

DISCUSSION: Our study is the first to show the role of TRAPPC9 in normal skeletal homeostasis. TRAPPC9 plays a role in osteoclast differentiation and protects against age-related bone loss. Current studies are underway to characterize osteoblast differentiation and function derived inTRAPPC9 KO mice. In summary, here we provide evidence that TRAPPC9 plays a role in osteoclast differentiation and bone resorption.

SIGNIFICANCE/CLINICAL RELEVANCE: Our data provides the first evidence of the potential therapeutic role of modulating TRAPPC9 expression and function in bone.

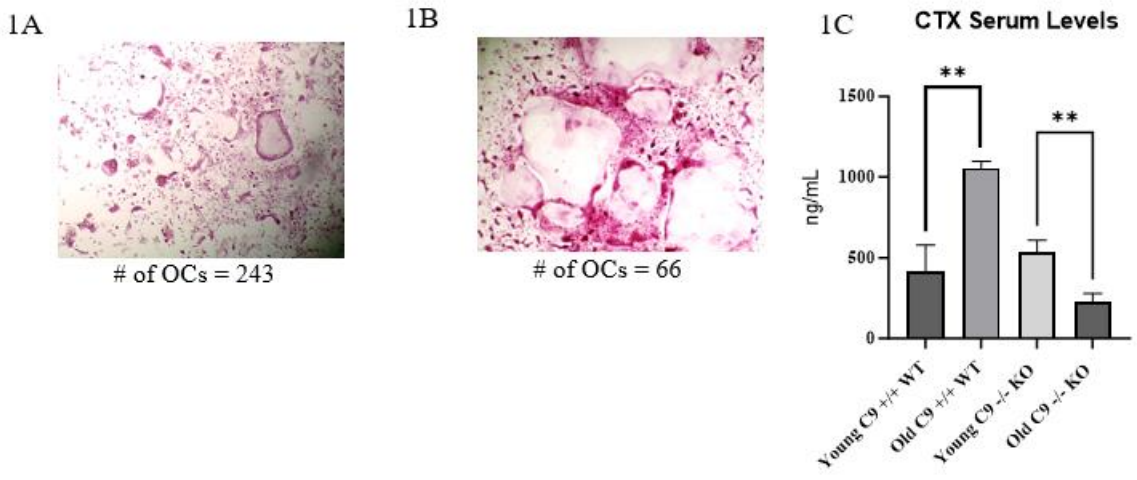

Figure 1A. Osteoclast derived from TRAPPC9 WT mice, stained for TRAP. **Figure 1B.** Osteoclast derived from the TRAPPC9 KO mice, stained for TRAP. TRAPPC9 KO mice exhibit larger OCs which decreased function (not shown). **Figure 1C.** CTX Serum levels in 8-week and 8-month TRAPPC9 WT and KO mice. 8-month-old WT mice had increased levels of CTX, while 8-month-old TRAPPC KO mice had decreased serum levels of CTX.