# Metabolomic Profiling Uncovers a Sex-Specific Bone Phenotype in a Pre-clinical Model of Non-Syndromic-Autosomal Recessive Intellectual Disability

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INTRODUCTION: Intellectual disability (ID) affects approximately 1–3% of the global population, with a U.S. pediatric prevalence of ~1.7–2.2% between 2019 and 2021. While X-linked forms of ID are well characterized, many autosomal-recessive variants remain poorly understood. Between 2019 and 2021, the rise in ID and overall developmental delays was more pronounced in boys than in girls, indicating a sex-dimorphic effect that may stem from genetic, hormonal, or neurodevelopmental factors. Non-syndromic autosomal-recessive ID (NS-ARID), a rare form of ID caused by mutations in the *Trafficking Protein Particle Complex Subunit 9* (*TRAPPC9*) gene, is a prime example. NS-ARID is associated with microcephaly, obesity, and characteristic brain MRI findings, and emerging evidence also highlights skeletal and dental abnormalities. However, the mechanisms by which *TRAPPC9* dysfunction influences skeletogenic homeostasis remain unclear. To address this, we investigated the physiological role of *TRAPPC9* in bone homeostasis in a sex-dependent manner by characterizing the skeletal phenotype of global *TRAPPC9* knockout (KO) mice. TRAPPC9 KO mice have been reported to possess a metabolic disorder, which has been reported in patient populations. Both *in vivo* and *in vitro* analyses were performed in male and female mice at 6–8 weeks (young) and 30–35 weeks (old) of age.

METHODS: All animal studies were approved by the institutional animal care and use committee (IACUC) of Northeast Ohio Medical University. In addition, we assessed the femurs of TRAPPC9 Wild-Type (WT) and TRAPPC9 Knock-Out (KO) mice using micro-CT analysis. Femurs and tibiae from old WT and KO, male and females, were subjected to epiphyseal removal, PBS-washing of the bone marrow, then flash frozen and homogenized for chemical isotope labeling liquid chromatography-mass spectrometry (CIL LC-MS) platform. The samples were assessed for metabolites containing extracted amine/phenol- and carboxyl OC-related gene expression levels were assessed in the males and females using qPCR analysis from 30-40-week-old (old) TRAPPC9 WT and KO humeri and calvariae. The functionality of osteoclasts (OCs) was assessed using *in vitro* cellular and biochemical assays of bone marrow-derived osteoclast precursor cells were isolated from male and female WT and KO mice at young ages.

RESULTS SECTION: At young ages, both male and female mice showed no significant difference in bone mass (BV/TV) between genotypes, while with age, the female KO mice displayed a significant increase in bone volume to tissue volume ratio (BV/TV) (Fig. 1A, and B). However, the KO males decreased BV/TV with age (Fig. 1. C, and D). Using the femurs and tibiae from the old males and female mice, CIL LC-MS was conducted, and the results yielded elevated levels of the metabolite Pantothenic Acid (PA) in KO females compared to WTs, while the males showed no difference (Fig. 1 E and F). PA has been associated with the inhibition of osteoclastogenesis, which then prompted us to assess the levels of Tartrate-resistant acid phosphatase (TRAP) and Osteoclast stimulatory transmembrane protein (OC-STAMP) in both calvariae and humeri, KO females showed decreased expression of TRAP (Fig. 1 G and H), and OC-STAMP (Fig. 1 I and J), Next, using 6-week-old male and female mice, the osteoclast differentiation showed an increase in total OC count in KO compared to WT in both female and male mice (Fig.1 K and L).

DISCUSSION: Our study is the first to show the gender-dependent role of TRAPPC9 in normal skeletal homeostasis. TRAPPC9 plays a role in osteoclast differentiation and regulates age-related bone loss in females, while having no significant effect in males. PA can be the mechanism driving this sex dependent difference, as PA is documented to inhibit osteoclastogenesis and enhance bone mineral density, BV/TV, and Tb. N in ovariectomized mouse models. Current studies are underway to further elucidate the mechanism driving these metabolomic/gender-dependent differences in TRAPPC9 KO males. In summary, here we provide evidence that TRAPPC9 plays a role in gender-dependent skeletal metabolism and homeostasis.

SIGNIFICANCE/CLINICAL RELEVANCE: Our data provide the first study to characterize the sexually dimorphic skeletal phenotype of TRAPPC9 KO mice through bone-targeted metabolomics, offering new insights into the bone-related manifestations of NSARID and potential avenues for therapeutic intervention.

Figure1.

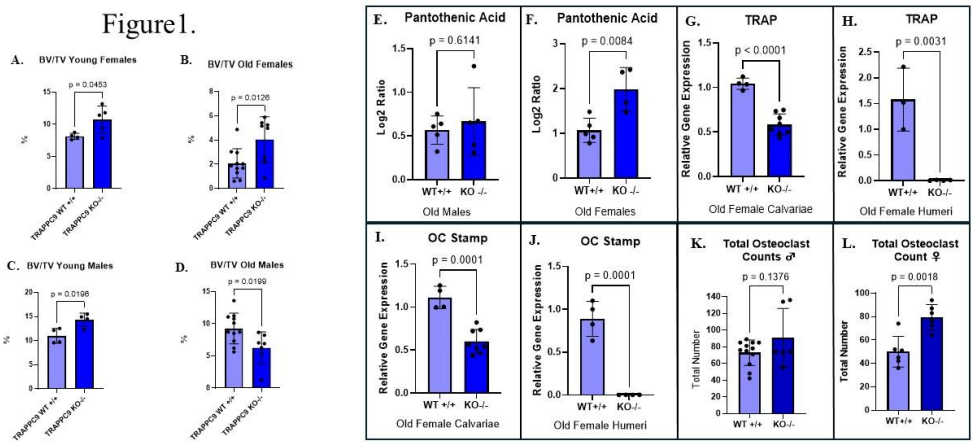


**Figure1. Skeletal and Metabolomic Characterization 1.a.** BV/TV of the young females (6-8-week-old) TRAPPC9 (n=4) and KO (n=5) mice showing no significant differences between genotypes. **1.b.** BV/TV of old (30-40-week-old) KO female mice (n=11) display an increase in BV/TV when compared to WT (n=8) . **1.c**. BV/TV of young male (6-8-week-old) WT (n=4) and KO (n=4), displayed no significance between genotypes. **1.d.** BV/TV of old (30-40-week-old) KO (n=7) male mice displayed a significant decrease in BV/TV when compared to their WT (n=11) counterparts **1e.** Pantothenic Acid (PA) fold change in the old WT and KO males, displaying no significant difference between WTs and KOs. **1f.** PA fold change in the old WT and KO females, displaying a significant increase in KOs compared to their WT counterparts. **1.g-h.** TRAP mRNA expression levels in the humeri and calvariae of old females. TRAP levels were downregulated in the KO females in both humeri and calvariae, when compared to WTs. **1.i-j** OC-STAMP mRNA expression levels in the humeri and calvariae of old females. TRAP levels were downregulated in the KO females in both humeri and calvariae. **1.k-l** Total OC Counts from OC differentation of in male and female cultures displaying an increase in OCs derived from KO mice of both genders. Data presented are the mean +/- SEM.