IL-3/IL-3 Receptor Contributes to Skeletal Sexual-Dimorphism in a Pre-Clinical Model for Non-Syndromic Autosomal Recessive Intellectual Disability

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INTRODUCTION: Intellectual disability (ID) affects roughly 1–3% of the global population, with a diagnosed prevalence of approximately 1.7–2.2% during 2019–2021 in children in the US. X-linked causes of ID have been extensively characterized, while many autosomal-recessive forms remain understudied. Among the increases in ID and overall developmental delays during 2019-2012, boys were disproportionately affected compared to girls, underscoring a sex-dimorphic effect that may reflect underlying genetic, hormonal, or neurodevelopmental differences. One being, 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. NS-ARID is associated with microcephaly, obesity, and characteristic brain MRI abnormalities. Emerging reports also note skeletal and dental features in some individuals and animal models, but the mechanisms linking TRAPPC9 dysfunction to skeletogenic homeostasis are unclear. Upon validation of the TRAPPC9 Knock-Out (KO) animal model for ND-ARID, it was stated that a sexual dimorphic effect was observed. Primarily with the TRAPPC9 KO Females displayed less interest in interacting with behavioral analysis, which correlates with learning and memory. To definitively understand the impact of TRAPPC9 on skeletogenesis, we investigated the physiological role of TRAPPC9 in bone homeostasis in a gender-dependent manner. We characterized the skeletal phenotype of TRAPPC9 global knockout (KO) mice utilizing both *in vivo* and *in vitro* approaches in both male and female mice at 6-8 weeks of age (Young) and 30-35 weeks of age (Old).

Figure1.

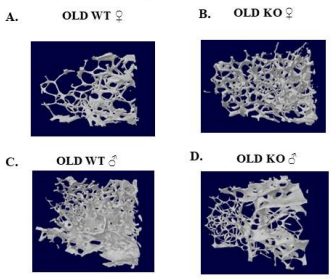

Figure1. Skeletal Characterization of TRAPPC9 KO animals1.a. Micro-computed tomography (μCT) Analysis –3-D rendering of the trabeculae of old TRAPPC9 WT female mice (n=8). **1b**. 3D rendering of the KO female (n=11). The KO female mice have an increase in trabeculae compared to WT. **1c.** 3D rendering of old WT male (n=11). **1.d.** 3D rendering of old KO male (n=7). The old KO male mice displayed a decrease in trabeculae compared to WT.

METHODS: All animal studies were approved by the institutional animal care and use committee (IACUC) of Northeast Ohio Medical University. First, we assessed the femurs of TRAPPC9 Wild-Type (WT) and TRAPPC9 Knock-Out (KO) mice using micro-CT analyses. Sera analysis was conducted using the O-link multiplex system using sera isolated from young and old, WT and TRAPPC9 KO male and female mice. The functionality of osteoclasts (OCs) was assessed using *in vitro* cellular and biochemical assays of bone marrow-OC precursors (OCPs) isolated from male and female WT and KO mice at young ages. OCPs were utilized for the assessment of OC differentiation and function analyses. Interleukin 3 (IL-3) and its alpha receptor (CD123) gene expression levels were assessed using qPCR analysis from 8-12-week-old (young) and -30-40-week-old (old) TRAPPC9 WT and KO humeri.

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). However, the KO males showed decreased BV/TV with age (Fig.1 A, B, C and D). Olink Multiplex system yielded no significant changes between young WT (n=3) and KO (n=3) males and females. While at old ages. In addition, our O-link data showed that sera from female TRAPPC9 KO mice displayed a marked increase in IL-3 levels compared to WT, while males displayed no significant differences (data not shown). Next, IL-3's expression in the humeri of WT and KO mice at young and old ages, in both sexes (Y ♀ WT n=6, Y ♀ KO n=5), (O ♀ WT n=6, O ♀ KO n=6). IL-3 mRNA levels were significantly increased in both young and old KO females (Fig.2. A, and B). While at young ages the KO males displayed a decrease in IL-3, but at older ages IL-3 levels were upregulated in the TRAPPC9 KO males (Y ♂ WT n=9, Y ♂ KO n=7), (O ♂ WT n=6, O ♂ KO n=6) (Fig.2. C and D). We further assessed the expression of IL-3 receptor (CD123α) and observed that CD123 mRNA expression was upregulated in bone from females at young sexes (Y ♀ WT n=5, Y ♀ KO n=4), (O ♀ WT n=6, and O♀ KO n=3) and old ages compared to WT (Fig. 2. E and F). In male mice, CD123 levels were decreased in TRAPPC9 KO compared to their WT littermates (Y ♂ WT n=6, Y ♂ KO n=5), (O ♂ WT n=8, O ♂ KO n=5) (Fig. 1. G and H).

Figure 2.

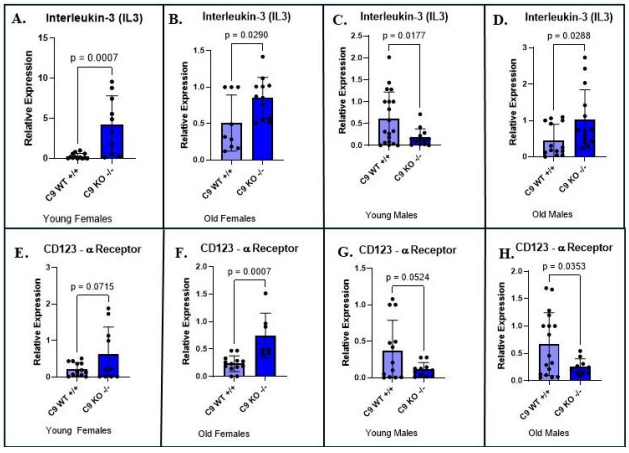

Figure 2. Interleukin-3 Expression 1.A.–B. IL-3 mRNA expression levels in the humeri of young and old females. IL-3 levels were upregulated in KO females at young and old ages. **1.C-D.** IL-3 mRNA expression levels in the humeri of young and old males. IL-3 levels were downregulated in the young KO males, while the old KO males were increased. **1E.-F.** CD123 mRNA expression levels in the humeri of young and old females. IL-3 levels were upregulated in KO females at young and old ages. **1.G.-H.**CD123 levels in the humeri of young and old males. CD123 levels were downregulated in KO males at young and old ages. (N=6-8) Data presented are the mean +/- SEM.

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. We also identified IL-3 as a possible modulator of these results. As IL-3 has been associated with the promotion of bone formation, while also promoting early stage OC- differentiation but inhibiting late stage differentiation. These data led us to conclude that the skeletal phenotype observed in the female KO mice could be mediated by OCs via IL-3/IL-3 signaling. In summary, here we provide evidence that TRAPPC9 plays a role in gender-dependent skeletal homeostasis.

SIGNIFICANCE/CLINICAL RELEVANCE: Our data provide the first characterization of the skeletal dimorphic phenotype of TRAPPC9 KO mice and will aid in developing new therapeutic approaches for the treatment of NSARID.