Contribution of Gonads & Sex Chromosomes to Early Knee & ACL Structure & Function in Four Core Genotypes Mouse Model

Taylor Turner[1], Claire Bruns[2], Cade Chezem[1] Adam Carter[1], Simran Shergill[1], Roufael Hanna[1], Benjamin Loflin[1], Stephen H. Schlecht[1,3]
[1]Department of Orthopaedic Surgery, Indiana University School of Medicine, Indianapolis, IN, [2]Wood College of Osteopathic Medicine, Marian University, Indianapolis, IN, [3]Indiana Center for Musculoskeletal Health, Indiana University School of Medicine, Indianapolis, IN

Disclosures: None applicable

INTRODUCTION: ACL injuries are a major public health issue, particularly among young females. Anterior cruciate ligament (ACL) injuries are one of the most common orthopaedic injuries. Adolescent females are at least 2 times more likely to suffer a first-time noncontact ACL failure than males when accounting for activity type and level of competition.[1] Reasons for this sex disparity are multifactorial with structural and functional ACL differences being primary contributors. Females, on average, have smaller ACLs with a less dense concentration of larger diameter collagen fibrils.[2,3] This in part contributes to a functional difference wherein female ACLs are less stiff and strong compared to those of males, contributing to an increase in knee laxity.[4] This combination of traits can result in increased risk of collagen matrix damage accrual and subsequent ACL failure.[5] Females, on average, also have increased posterior tibial slopes and a narrower intercondylar femoral notch.[6] A steep posterior tibial slope can increase the propensity for internal tibial rotation upon ground contact, increasing ACL strain, while a narrower femoral notch is believed to increase the likelihood of ACL impingement when moving from knee flexion to extension. Sex hormones are implicated, albeit inconsistently, as underlying some female-biased ACL phenotypic differences, however, the extent and age at which they have a significant effect remain unclear. Moreover, investigations have not considered X and Y chromosome differences that likely also contribute to these phenotypic differences, either independently or via sex hormone interaction. To address these previous limitations, we employed the Four Core Genotypes (FCG) transgenic mouse model that consists of females and males having normal gonads and sex hormone levels but mixed complements of X and Y chromosomes (Fig 1).[7] Here, we characterize the effects of sex hormones and chromosomes on ACL structure and function, as well as knee morphology in young adolescent (6 week) mice, which will at a later date be compared to late adolescent (12 week) and adult (18 week) mice . We hypothesize that female sex hormones have a significant influence on ACL function, but not ACL size or knee morphology, which is more influenced by sex chromosomes at this young age.

METHODS: This study was approved by Institutional Animal Care and Use Committee. Transgenic male mice that had the testis-determining gene (*Sry*) deleted from the Y chromosome and reinserted onto an autosome, were bred with wild-type B6 female mouse to produce progeny with different complements of sex chromosomes (XX_{Ovary}, XY_{Ovary}, XX_{Testis}, XY_{Testis}). Mice were provided a standard rodent diet and water ad libitum. At 6 weeks of age (n=77), mice were genotyped, weighed, and euthanized. For knee morphology measures, left hindlimbs were removed and imaged using micro-computed tomography at an 8µm voxel size. Pertinent traits measured from the resulting image stacks included 1) bicondylar femoral width (BFW), 2) femoral notch height (NH), 3) anterior (ANW), central (CNW) and posterior (PNW) femoral notch widths, and 3) posterior medial (PMTS) and lateral (PLTS) tibial slopes.[8] From the femoral notch and bicondylar width/height measures, femoral notch shape indices (CNW/NH) and notch width indices (CNW/BFW) were derived. All measures were done using established methods, repeated 3 separate times per trait, evaluated for interobserver reliability, and averaged. Right legs were evaluated for ACL size, strength, and stiffness using ex-vivo mechanics. For ACL structural measures, right hindlimbs were removed and the knee was microdissected under a stereoscope to remove all joint tissues except the ACL. Biplanar images were then taken of the ACL, and elliptical cross-sectional area (ACL CSA) was quantified.[8] For functional measures, the dissected knees were placed in custom fixture to hold the knee at 30° of flexion, affixed to a universal testing system. While hydrated, each ACL was axially tensioned to failure.[8] Custom MATLAB code was then used to extract stiffness and maximum load to failure data from the resulting load-displacement curves. All data was standardized by body weight using linear regression. Adjusted data were then analyzed by 2-way ANOVA using GraphPad, with gonads and sex chromosomes as the two variables, followed by Sidak's multiple comparisons test for XX_O vs XX_T, XY_T vs XY_O, XX_O vs XY_O, XX_T vs XY_T, and XX_O vs XY_T.

RESULTS: Results are provided in Figure 2. There is a strong sex hormone effect on body weight, with gonadally-intact females being significantly smaller than their male counterparts, irrespective of sex chromosome complement. Similarly, ACL max load was dependent on sex hormones with XX_O mice being less strong than XX_T. There were no significant sex hormone or chromosome effects on ACL stiffness. Interestingly, ACL CSA showed both a sex hormone and chromosome effect, with XX_O mice having larger ACLs than XX_T and XY_T also having larger ACLs than XX_T. No significant effects were found among the femoral or tibial morphology measures.

DISCUSSION: Outcomes from this study suggest that at 6 weeks of age, soft tissues are largely influenced by sex hormones. Body weight and ACL strength were significantly higher in gonadal males compared to females. In contrast, ACL size was larger in gonadal females compared to males. This is interesting considering tissue size typically significantly correlates with tissue strength. That gonadal females have larger yet weaker ACLs than males suggest there may be significant differences in ACL composition between gonadal females and males at this young age. No sex hormone and chromosome effects were observed across knee morphology measures suggesting that at this translational growth phase these discriminating sex factors have little influence on the sexual dimorphism among knee traits that are widely clinically associated with ACL injury risk and mirrored in the mouse knee.[9]

SIGNIFICANCE/CLINICAL RELEVANCE: Determining the influence of sex hormones and chromosomes on knee development will provide a better understanding of how sexually dimorphic risk factors for ACL, and other knee injuries, mature throughout adolescence into young adulthood. Elucidating their regulatory role on these critical traits *in vivo*, particularly those influencing ACL mechanics, will potentially facilitate sex-specific strategies based partially on participant hormone profiles to mitigate excessive collagen matrix damage accrual and subsequent ACL failure.

REFERENCES: [1]Beynnon et al., *Am J Sports Med*, 2014; [2]Hashemi et al., *J Bone Joint Surg*, 2008; [3]Chandrashekar et al., *J Biomech*, 2006; [4]Lipps et al., *Am J Sports Med*, 2012; [5]Loflin et al., *Am J Sports Med*, 2023, [6]Barnum et al., *Am J Sports Med*, 2021; [6]Arnold, *Neurosci Biobehav Rev*, 2020; [7]Ochocki et al., *J Exp Orthop*, 2022; Liu et al., *J Orthop Res*, 2023.

ACKNOWLEDGEMENTS: Funding support from Indiana University School of Medicine. Knee imaging was facilitated through the Indiana Center for Musculoskeletal Health.