Age and Sex Dependent Variations in Knee Anatomy During Skeletal Maturation in Children and Adolescents

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Abstract

Knee joint is the most common site of injury in children and adolescents and accounts for up to 60% of all sports-related injuries in this population. Ruptures of the anterior cruciate ligament (ACL) are among the most frequent knee injuries in pediatric population with up to 10 times greater prevalence in females than males. These injuries are often immediately disabling and are associated with significant short- and long-term clinical complications such as increased risk of posttraumatic osteoarthritis (OA), despite our best current treatment methods. Several unique anatomical features of the knee joint, including tibial slope and femoral notch size, have shown to affect joint and ACL loading and risk of injury. Despite the well-established role of these anatomical features on ACL injury risk, it remains unclear how these anatomical features change during skeletal growth and maturation. We are proposing to systematically investigate how age and sex affect multiple prominent anatomical features of the knee joint in children and adolescents. This will be done retrospectively by analyzing the Magnetic Resonance (MR) images of the knees of patients who underwent a knee MRI at Boston Children’s Hospital. The 3D MR image stacks will be used to measure a comprehensive set of anatomical indices of the knee joint based on previously established techniques. The associations between age and each of the quantified anatomical indices will be assessed. The age-dependent changes in knee anatomy will be compared between boys and girls as well as between those who had an ACL injury and those with healthy uninjured knees. Findings will help to better understand how prominent anatomical features of the knee joint, in particular those identified as risk factors for ACL injury, are being developed and/or changed during skeletal growth and maturation. Findings will also improve our knowledge of how sex may affect the age dependent changes in knee anatomy. The outcomes of this work will improve our knowledge on how and when anatomy becomes different between subjects at low risk of ACL injury and “at risk” population. Such improved knowledge can be used to develop novel risk screening models to more effectively identify children at high risk of ACL injury based on their anatomical profile. Individuals with high-risk bony anatomy can be subjected to neuromuscular and biomechanical interventions early on to minimize their risk of sustaining and ACL tear.
A. Specific Aims

Injuries to the anterior cruciate ligament (ACL) is among the most common sports injuries in children and adolescents, in particular among girls.\textsuperscript{30} Multiple anatomical features of the tibial plateau (i.e. medial and lateral slopes, and medial concavity) and femoral condyle (i.e. femoral notch size) have been proposed as potential risk factors for ACL injury.\textsuperscript{6, 21, 22, 43, 56, 57, 68} Despite well-established role of these anatomical features on ACL injury risk, it remains unclear how these anatomical features are being developed and/or changed during the course of skeletal growth and maturation. Our preliminary results show that several prominent anatomical features of the knee joint change by age in a cohort of 268 healthy uninjured boys and girls (3-18 years; 51\% girls). We have also shown that the age dependent changes in knee anatomy are different in boys compared to girls. We are now poised to investigate how age and sex affect knee anatomy during the course of skeletal growth and maturation in boys and girls who sustained an ACL injury and how those trends are different with our earlier observations in healthy uninjured knees.

**Aim 1:** Determine how age and sex affect knee anatomy during skeletal growth and maturation in children and adolescents with torn ACL. Multiple anatomical features of femoral condyles and tibial plateau will be measured from MR images of a cohort of 240 subjects (Age: 7-18 years old; ~50\% girls) who have sustained an ACL injury. The effect of age on anatomy will be assessed for both boys and girls. Quantified anatomical indices will also be compared between boys and girls across a range of age groups corresponding to different stages of puberty and skeletal maturation.

**H 1.1:** There are age-dependent variations in quantified anatomical indices for both boys and girls who sustain an ACL injury.

**H 1.2:** The age-dependent changes in quantified anatomical indices are different between boys and girls, in particular at later stages of skeletal maturation (11-18 years).

**Aim 2:** Determine how observed age and sex dependent variations in anatomy are different between individuals with ACL injury (cases) and those with no injury (controls). Quantified anatomical indices in cases will be compared with the same measurements previously done in an age- and sex-matched cohort of healthy uninjured controls. The comparisons will be done across a range of age groups corresponding to different stages of puberty and skeletal maturation. Separate analysis will be done for boys and girls.

**H 2.1:** There are significant differences in how age affects the anatomy between cases and controls, with cases developing a more high-risk anatomical profile.
B. Background

The Knee is the largest and the most complex joint in human body and also the most common site of injury in children and adolescents.\textsuperscript{1, 51} Dynamic knee stability is affected by both passive (ligamentous) and active (neuromuscular) joint restraints. Among the contributors to knee joint stability, the anterior cruciate ligament (ACL) has been long considered as the primary passive restraint to anterior translation of the tibia with respect to the femur.\textsuperscript{8, 31} Moreover, the ACL contributes to knee rotational stability in both frontal and transverse planes due to its specific orientation.\textsuperscript{36, 49}

The ACL has been the focus of many biomechanical/anatomical studies and is among the most frequently studied structures of the human musculoskeletal system over the past decades. ACL injuries are one of the most common and devastating knee injuries, with a prevalence estimated to be 1 in 3,000 in the United States (greater than 120,000 cases annually).\textsuperscript{32} Despite trivial injury incidences in the general population, ACL injury frequently affects young, active individuals, and females are at a reported 2-10 fold greater risk than males playing the same sport activity.\textsuperscript{3, 4, 19, 37, 44, 46, 50, 60} High risk of injury in females along with the high rate of sports participation among females, over the last three decades, has led to a rapid rise in ACL injuries in adolescent females. These injuries are often immediately disabling and may result in often result in joint effusion, altered movement, muscle weakness, reduced functional performance, and the loss of entire season or more of sports participation among young athletes.\textsuperscript{49} ACL injuries are also associated with other concomitant articular injuries and result in an increased risk of posttraumatic osteoarthritis (OA) 10 to 15 years post-injury (as high as 78%).\textsuperscript{9, 39, 41, 66}

In addition to pain, instability and associated long-term sequelae, ACL injury affects a patient’s quality of life economically as well as socially.\textsuperscript{38, 41} Using a conservative cost estimate of $17,000 - $25,000 per patient for surgery and rehabilitation, the estimated cost for treatment for ACL injured patients in the United States is over $1.7 billion annually. This estimate does not consider the resources necessary for non-surgical treatment, or to treat the long-term complication of post-traumatic OA associated with both the ACL-injured and ACL-reconstructed knee.\textsuperscript{18}

Although ACL reconstruction has become the current gold standard for restoring the gross stability of a symptomatic ACL-deficient knee, significant problems persist. In
the short term, conventional ACL reconstruction fails in restoring the normal joint kinematics and kinetics.\textsuperscript{20, 24} This alteration in joint mechanics has been mainly associated with non-anatomic ligament insertion (location and geometry) and alignment, loss of tissue neuro-sensory function (proprioception), graft tissue degeneration, and neuromuscular deficit.\textsuperscript{27, 29, 55} Many studies have shown significantly greater translational and rotational laxity of the reconstructed knees relative to the contralateral uninjured sides regardless of the graft type.\textsuperscript{5, 16, 47, 65} Additionally, reconstruction requires tissue harvest from the knee (autograft) which is associated with additional morbidity. Alternatively, using allografts is associated with high risk of biologic incorporation failure and disease transmission in addition to financial and tissue availability complications. Moreover, ACL reconstruction is associated with relatively high rates of graft failure and re-injury, especially in young patients.\textsuperscript{48, 67} Most importantly, patients remain at a high risk for development of early onset posttraumatic OA (up to 78\%).\textsuperscript{9, 26, 39, 45, 53, 58, 64, 66} A meta-analysis of 33 clinical follow-ups reported that ACL reconstruction was unable to slow the premature onset of OA following ACL tear.\textsuperscript{40} On average, patients with OA following ACL injury are 15 to 20 years younger than those with primary OA when they seek medical help for their symptoms.\textsuperscript{39, 54} This represents a critical health issue as it places a growing number of young active individuals at high risk for knee OA before the age of 40, which may in turn impose a significant burden in the society, financially as well as socially.

Considering the high prevalence of ACL injuries in addition to unsatisfactory outcomes of ACL surgery, proper risk screening and injury prevention strategies can be considered as an appealing option to avoid short- and long-term complications associated with these injuries.\textsuperscript{30} This has motivated many studies to identify the variables associated with increased risk of ACL injury in an effort to develop more effective risk assessment and prevention strategies so that those at increased risk can be identified and targeted for intervention.\textsuperscript{57} Among studied risk factors, knee anatomy has shown to significantly affect the joint biomechanics as well as ACL loading and risk of injury.\textsuperscript{21, 42, 43, 56}

The knee joint comprises of many unique and complex morphological features. The role of these anatomical features on ACL loading and injury risk has been the focus of many research efforts over the past two decades.\textsuperscript{2, 6, 7, 17, 21, 22, 34, 38, 43, 52, 56, 61-63, 68} Multiple anatomical indices including the tibial plateau morphology (posterior slope and concavity),\textsuperscript{6, 7, 17, 21, 22, 38, 43} femoral inter-condylar notch size and bony ridge thickness,\textsuperscript{2, 34},
and tibial spine size\textsuperscript{61} have been associated with increased ACL loading and risk of injury. Despite well-established role of these anatomical features on ACL injury risk, it remains unclear how these anatomical features are being developed and/or changed during the course of skeletal growth and maturation.

C. Innovation, Significance and Clinical Impact

This project is novel and is among the first studies to systematically investigate the age and sex dependent changes in multiple anatomical features of the knee joint during skeletal growth and maturation among pediatric population at high risk of ACL injury. The proposed work will take advantage of a comprehensive and unique set of clinical and imaging data at Boston Children’s Hospital. There are certain advantages to this work that underline the novelty and clinical significance of the proposed study including: A) access to 3D MR imaging data which will enable more accurate anatomy measurements compared to measurement from 2D X-Rays, B) large sample size over a wide age range covering all skeletal maturity and puberty stages from preschoolers to late adolescents, and C) the ability to conduct direct comparisons between healthy uninjured knees and those with injured ACL.

Upon completion, we will have a better understanding of how prominent anatomical features of the knee joint, in particular those identified as risk factors for ACL injury, are changing during skeletal growth and maturation in children and adolescents with injured ACL. Findings will also improve our knowledge of how sex may affect the observed age-dependent changes in knee anatomy. This work will build upon our earlier efforts and improve our knowledge on how and when anatomy becomes different between subjects at low risk of ACL injury and “at risk” population. Such improved knowledge can be used to develop novel risk screening models to more effectively identify children at high risk of ACL injury based on their anatomical profile. Individuals with high-risk bony anatomy can be subjected to neuromuscular and biomechanical interventions early on to minimize their risk of sustaining an ACL tear. Findings will also lay the foundation for future studies to determine intrinsic (i.e. genetic background) and extrinsic (i.e. physical activity and life style) factors affecting the bony anatomy, in particular at early stages of skeletal maturation, when the bones have the highest potential for remodeling and adaptation.
D. Preliminary Studies

Our preliminary analyses show significant age and sex dependent variations in multiple anatomical features of the healthy uninjured knees during skeletal growth and maturation. Following IRB approval, electronic medical records and imaging records of all the patients who have visited the Orthopedics or Sports Medicine clinics at the Boston Children's Hospital (from 2011 till now) were reviewed retrospectively. A total of 268 sets of MR images, from 268 unique patients (Age: 3-18 years old; 51% girls), were randomly selected from boys and girls with complete set of high-quality knee MRIs who had no previous knee injuries, growth-related and congenital skeletal disorders, bony deformity, fractures of femoral condyles and tibial plateau, and substantial cartilage damage. The subjects were selected in a way to ensure equal distribution across each age (~10 boys and 10 girls for each age). Due to lack of sufficient eligible MRIs available for ages 3 to 6, all the available eligible MRIs for that age range were analyzed. The distribution of the analyzed subjects is presented in Figure 1.

![Age and Sex Distribution](image)

**Figure 1**: The age and sex distribution of the eligible subjects used in our preliminary studies.

3D MR image stacks of the knee joint were used to measure multiple anatomical features of the ACL, femoral condyle and tibial plateau following previously established techniques (Section E.2). The list of measured anatomical indices are presented in Table 1.
Table 1: Quantified anatomical indices of the knee joint

<table>
<thead>
<tr>
<th>Segment</th>
<th>Anatomical Index</th>
<th>Measurement Technique</th>
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<tbody>
<tr>
<td>ACL</td>
<td>Length</td>
<td>Cohen et al. 2009&lt;sup&gt;10&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Cross-sectional Area</td>
<td>Lipps et al. 2012&lt;sup&gt;38&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Cross-Sectional Area : Length Ratio</td>
<td>Dividing ACL cross-sectional area by the ACL length</td>
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<td></td>
<td>Sagittal Elevation Angle</td>
<td>Jordan et al. 2007&lt;sup&gt;28&lt;/sup&gt;</td>
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<td></td>
<td>Coronal Elevation Angle</td>
<td>Jordan et al. 2007&lt;sup&gt;28&lt;/sup&gt;</td>
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<tr>
<td>Femoral Condyle</td>
<td>Bi-condylar Width</td>
<td>Whitney et al. 2014&lt;sup&gt;68&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Notch Width</td>
<td>Whitney et al. 2014&lt;sup&gt;68&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Notch Width Index</td>
<td>Dividing the notch width over bi-condylar width</td>
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<td></td>
<td>Sagittal Femoral Condyle Curvature (medial)</td>
<td>Howell et al. 2010&lt;sup&gt;25&lt;/sup&gt;</td>
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<td></td>
<td>Sagittal Femoral Condyle Curvature (lateral)</td>
<td>Howell et al. 2010&lt;sup&gt;25&lt;/sup&gt;</td>
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<td>Tibial Plateau</td>
<td>Width</td>
<td>Lee DC et al. 2012&lt;sup&gt;35&lt;/sup&gt;</td>
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<td></td>
<td>Tibial Spine Height (medial)</td>
<td>Surnick et al. 2014&lt;sup&gt;61&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Tibial Spine Height (lateral)</td>
<td>Surnick et al. 2014&lt;sup&gt;61&lt;/sup&gt;</td>
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<td></td>
<td>Coronal Slope</td>
<td>Hashemi et al. 2008&lt;sup&gt;21&lt;/sup&gt;</td>
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<td>Posterior Slope (lateral)</td>
<td>Hashemi et al. 2008&lt;sup&gt;21&lt;/sup&gt;</td>
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<td></td>
<td>Posterior Slope (medial)</td>
<td>Hashemi et al. 2008&lt;sup&gt;21&lt;/sup&gt;</td>
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<td></td>
<td>Maximum Depth of the Medial Tibial Plateau</td>
<td>Hashemi et al. 2008&lt;sup&gt;21&lt;/sup&gt;</td>
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<td></td>
<td>Curvature mismatch between the medial femoral condyle and medial tibial plateau in sagittal plane</td>
<td>Medial femoral condyle curvature – medial tibial plateau curvature</td>
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Linear regression analysis was used to investigate the relationships between age and quantified anatomical indices in healthy uninjured knees. Separate analysis was done for each sex. For each model, both anatomy (dependent variable) and age (independent variable) were used as continuous variables. Second order polynomial and logarithmic models were used where regression diagnostics indicated that the linear models are not appropriate. The distribution of the measurements over the age range along with the regression line were plotted for each anatomical index to visualize the changes in anatomy by age and the differences between boys and girls. Measurements were also grouped to 8 subgroups (4 age groups for each sex) based on following age categories:

- Preschool aged children (3–6 years old)
- Pre-pubertal school-aged children (7–10 years old)
- Early adolescents (11–14 years old)
- Late adolescents (15–18 years old)

A two-way analysis of variance (ANOVA) was used to investigate the effects of age and sex on quantified anatomy for the abovementioned 8 subgroups. For these analyses, the anatomic parameter (dependent variable) was defined as continuous with age group and sex (independent variables) being defined as categorical and dichotomous variables, respectively. The p values from the ANOVA analysis were used to test whether age and/or sex significantly affect the anatomy or not. Further the interaction term between sex and age group was also added to the model to further investigate whether the age dependent changes in knee anatomy are affected by sex. This was tested by looking at the p value corresponding to the interaction term in the model. Using the same ANOVA model, the quantified anatomy at each age group was compared between boys and girls (4 pairwise comparisons). The p values for all 4 pairwise comparisons were then adjusted based on Hochberg correction method to account for potential increases in type I error due to multiple comparisons. Following is a summary of the findings in healthy uninjured knees:

**D.1. ACL Anatomy:** As expected, ACL length was significantly increased by age in both boys and girls (p < .0001; Figure 2). Both boys and girls had similar linear increases in their ACL length prior to becoming adolescent. While ACL length remained unchanged in adolescent girls, adolescent boys had a continuous increase in the length of their ACL,
which happened at a slower rate compared to the pre-adolescence stages. As shown in Figure 2, both age and sex significantly affected the length of the ACL ($p < .0001$) with different patterns in boys compared to girls ($p = .003$). Finally, late adolescent boys had a significantly longer ACL compared to age-matched girls ($p < .0001$).

![ACL Length graph](image)

**Figure 2:** Age and sex dependent changes in ACL length during skeletal growth and maturation (the graph on the top right corner shows Mean ± SEM).

Similarly, ACL cross-sectional area was significantly increased by age in both boys and girls ($p < .0001$; Figure 3). While boys and girls had similar age related changes in their ACL area at early stages, boys had a significantly greater rate of increase in ACL cross-sectional area as they became adolescent ($p < .0001$). Adolescent boys had a significantly larger ACL cross-sectional area to age-matched girls ($p < .001$). As shown in Figure 3, both age and sex significantly affected the cross-sectional area of the ACL ($p < .0001$) with different patterns in boys compared to girls ($p = .041$).
Figure 3: Age and sex dependent changes in ACL cross-sectional area during skeletal growth and maturation (the graph on the top right corner shows Mean ± SEM).

With regards to the ACL area:length ratio, boys had a sharp increase in their ACL area:length ratio while transitioning from pre-pubertal school-aged to early and then late adolescents (Figure 4). Girls also showed a significant increase in their ACL area:length ratio but only when they become adolescents. These variations in changes in ACL area:length ratio between boys and girls resulted in significantly greater ratios in early and late adolescent boys compared to the adolescent girls of the same age group (p < .05; Figure 4).
Figure 4: Age and sex dependent changes in ACL cross-sectional area:length ratio during skeletal growth and maturation (the graph on the top right corner shows Mean ± SEM).

Interestingly, there were significant increases in ACL sagittal and coronal elevation angles by age in both boys and girls (P < .0001; Figure 5 and 6). For both sexes, the ACL became more vertical, in both sagittal and coronal planes, by age with similar patterns in boys and girls (p > .370). In general, girls had a more vertical ACL than boys across the whole tested age range (p < .05). However, pairwise comparison only showed a significant difference in ACL coronal elevation angle between boys and girls at 7-10 years old age group (p = .012; Figure 6).
Figure 5: Age and sex dependent changes in ACL sagittal elevation angle during skeletal growth and maturation (the graph on the top right corner shows Mean ± SEM).

Figure 6: Age and sex dependent changes in ACL coronal elevation angle during skeletal growth and maturation (the graph on the top right corner shows Mean ± SEM).
In summary, current findings showed significant differences in ACL size and orientation by age in both girls and boys. Data also showed significant differences in most of the quantified anatomical features of the ACL between boys and girls, in particular at later stages of skeletal maturation. Most importantly, results demonstrated that the age dependent changes in ACL size (length, area and area:length ratio) are significantly different between boys and girls. The smaller ACL has been shown to be more susceptible to injury during high-risk loading conditions.\textsuperscript{11, 12} The smaller ACL in girls, particularly smaller cross-sectional area and area:length ratio, can be a contributing factor to the previously reported higher risk of ACL injury among young adolescent females compared to age-matched males.\textsuperscript{3, 4, 19, 37, 44, 46, 50, 60}

**D.2. Femoral Condyles Anatomy:** As expected, bi-condylar femoral width was significantly increased by age in both boys and girls (p < .0001; Figure 7). Both boys and girls had similar linear increases in their bi-condylar width prior to becoming adolescent. While bi-condylar width remained unchanged in adolescent girls, adolescent boys had a continuous increase in the width of their femoral condyles, which happened at a slower rate compared to the pre-adolescence stages. As shown in Figure 7, both age and sex significantly affected the bi-condylar width (p < .0001) with different patterns in boys compared to girls (p = .014). Finally, at all age groups, boys had a significantly wider femoral condyles compared to age-matched girls (p < .0001).

Similar to observed changes in bi-condylar width, the width of the femoral notch was also significantly increased by age in both boys and girls (p < .0001; Figure 8). At almost all age groups, except 7-10 years old group, boys had a significantly wider femoral notch compared to age-matched girls (p < .015). Both boys and girls had a similar increasing trend in the width of their femoral notch at early stages prior to becoming adolescent (Figure 8). However, boys and girls showed different age dependent patterns at later stages (p = .044). While boys had a continuous growth in the width of their femoral notch even after becoming adolescent, notch width remained unchanged in adolescent girls.
**Figure 7:** Age and sex dependent changes in bi-condylar femoral width during skeletal growth and maturation (the graph on the top right corner shows Mean ± SEM).

**Figure 8:** Age and sex dependent changes in femoral notch width during skeletal growth and maturation (the graph on the top right corner shows Mean ± SEM).
In contrast to observed age and sex dependent variations in width of the femoral notch, notch width index remained unchanged in both boys and girls through the course of skeletal growth and maturation (p = .584; Figure 9). Similar magnitudes (p = .691) and age dependent changes in notch width index (p = .361).

Notch Width Index

![Graph showing notch width index](image)

**Figure 9:** Age and sex dependent changes in notch width index during skeletal growth and maturation (the graph on the top right corner shows Mean ± SEM).

We further tested the relationships between notch width and ACL cross-sectional area using linear regression analysis (Figure 10 A and B). We also repeated the same analyses to investigate if notch width index is a better predictor of ACL cross-sectional area (Figure 10 C and D). Results showed that notch width is a significant predictor of ACL cross-sectional area in both boys and girls and is responsible for 24% – 26% variations in ACL cross-sectional area. However, weak associations were observed between notch width index and ACL cross-sectional area which was only significant in
girls. Data showed that notch width index can only explain up to 4% variations in ACL cross-sectional area. These findings suggest that notch width is a more relevant and stronger predictor of ACL size in pediatric population.

![Graphs showing associations between ACL cross-sectional area and notch width (A and B) and notch width index (C and D) in boys and girls.](image)

**Figure 10:** Associations between ACL cross-sectional area and notch width (A and B) and notch width index (C and D) in boys and girls.

Interestingly, there were significant decreases in medial and lateral femoral condyles curvature by age in both boys and girls (p < .0001; Figure 11 and 12). Boys and girls had similar linear decreases in the curvature of their medial femoral condyle prior to becoming adolescent. However, medial femoral condyle curvature remained unchanged in adolescent girls while it continuously decreased in adolescent boys (Figure 11). This
resulted in significantly higher medial femoral condyle curvature in late adolescent girls compared to age-match boys (p = .001).

**Figure 11**: Age and sex dependent changes in medial femoral condyle curvature during skeletal growth and maturation (the graph on the top right corner shows Mean ± SEM).

Similar patterns were observed in lateral femoral condyle. At almost all age-groups, except at 11-14 years old group, girls had a significantly more curved lateral femoral condyle compared to boys (p < .035; Figure 12). For both the condyles, age and sex significantly affected the sagittal curvature of the femoral condyle (p < .0001) with different patterns seen in girls compared to boys (p = .031).
Figure 12: Age and sex dependent changes in lateral femoral condyle curvature during skeletal growth and maturation (the graph on the top right corner shows Mean ± SEM).

In summary, current findings showed significant differences in femoral condyles size and curvature by age in both girls and boys. Data also showed significant differences in size and curvature of the femoral condyles between boys and girls, in particular at later stages of skeletal maturation. Most importantly, results demonstrated that the age dependent changes in femoral condyles size and curvature are significantly different between boys and girls. Narrow femoral notch have been associated with increased ACL loading and greater risk of ACL injuries.\(^2, 34, 52, 56, 68\) It has been theorized that a small notch is an indication of a small ACL, which is more susceptible to injuries.\(^11, 12\) This is supported by our current findings showing strong associations between the width of the femoral notch and ACL cross-sectional area (Figure 10). Another described mechanism is that a narrow notch produces mechanical impingement of the ACL, which results in localized shear forces that could act to tear the ligament during high-risk movements or where repetitive contact between the ligament and bone reduces the structural properties of the ligament.
over time.\textsuperscript{56} The smaller femoral notch in girls can be a contributing factor to previously reported higher risk of ACL injury among young adolescent females compared to age-matched males.\textsuperscript{3, 4, 19, 37, 44, 46, 50, 60}

**D.3. Tibial Plateau Anatomy:** As expected, width of the tibial plateau significantly increased by age in both boys and girls (p < .0001; Figure 13). Boys and girls had a similar increasing trend in the width of their tibial plateau at early stages prior to becoming adolescent (Figure 13). However, boys and girls showed different age dependent patterns at later stages (p < .0001). While adolescent boys had a continuous growth in the width of their tibial plateau, tibial plateau width remained unchanged in adolescent girls. At all age groups boys had a significantly wider tibial plateau compared to age-matched girls (p < .002).

![Tibial Plateau Width](image)

**Figure 13:** Age and sex dependent changes in width of the tibial plateau during skeletal growth and maturation (the graph on the top right corner shows Mean ± SEM).
Both boys and girls showed significant increases in the height of their medial and lateral tibial spine during skeletal growth and maturation ($p < .0001$; Figures 14 and 15). Boys showed a continuous and linear increases in tibial spine height at early stages followed by a slow growth in height after becoming adolescent (Figures 14 and 15). Similar trend was observed in girls but the rate at which the tibial spine grew dramatically decreased after girls entered the pre-pubertal school-age stage (Figures 14 and 15). These trends were observed for both medial and lateral tibial spines. At almost all age groups, except the 7-10 years old group, boys had a significantly bigger tibial spine height compared to age-match girls ($p < .045$).

**Figure 14:** Age and sex dependent changes in medial tibial spine height during skeletal growth and maturation (the graph on the top right corner shows Mean ± SEM).
Figure 15: Age and sex dependent changes in lateral tibial spine height during skeletal growth and maturation (the graph on the top right corner shows Mean ± SEM).

With regards to the slope of the tibial plateau in the coronal plane, boys had a consistent and linear increase in their coronal tibial slope at early stages, which then remained unchanged when they became adolescent (Figure 16). In contrast, the coronal tibial slope in girls did not change by age. At almost all age groups, except the 15-18 years old group, girls had a steeper coronal tibial slope compared to age-matched boys which were only statistically significant at pre-adolescence stages (p < .015; Figure 16).

Similar to the observed trends in coronal tibial slope, the posterior slope of the lateral tibial plateau remained relatively unchanged in girls during the course of skeletal growth and maturation, whereas it significantly decreased in boys by age (p < .011; Figure 17). At almost all age groups, except the 3-6 years old group, girls had a significantly steeper posterior tibial slope across the lateral compartment compared to age-matched boys (p < .015).
Figure 16: Age and sex dependent changes in coronal tibial slope during skeletal growth and maturation (the graph on the top right corner shows Mean ± SEM).

Figure 17: Age and sex dependent changes in lateral tibial slope during skeletal growth and maturation (the graph on the top right corner shows Mean ± SEM).
With regards to the posterior tibial slope in the medial compartment, both boys and girl showed similar age dependent changes ($p = .788$; Figure 18). In both sexes, the posterior slope of the medial tibial plateau remained almost unchanged during the early stages of skeletal growth and maturation and slowly decreased over time after becoming adolescent (Figure 18). Despite similar patterns between boys and girls, girls consistently had a steeper medial tibial slope compare to age-matched boys which were statistically significant at 11-14 and 15-18 years old age groups ($p < .05$).

**Figure 18**: Age and sex dependent changes in medial tibial slope during skeletal growth and maturation (the graph on the top right corner shows Mean ± SEM).

Both girls and boys had steadily increases in the maximum depth of their medial tibial plateau during skeletal growth and maturation ($p < .0001$; Figure 19). While they both had similar rates at pre-adolescence stages, adolescent boys showed a higher rate of increases in medial tibial depth (Figure 19). In general, boys had a deeper medial tibial
plateau compared to age-matched girls. These difference were statistically significant across all age groups except the 3-6 years old group (p < .030).

**Figure 19:** Age and sex dependent changes in maximum depth of the medial tibial plateau during skeletal growth and maturation (the graph on the top right corner shows Mean ± SEM).

We also calculated the mismatch between the sagittal curvature of the medial femoral condyle and sagittal curvature of the medial tibial plateau. This was calculated by subtracting the curvature of the medial tibial plateau from the curvature of the medial femoral condyle. Interestingly, there were significant decreases in curvature mismatch between the medial femoral condyle and medial tibial plateau by age in both boys and girls (p < .0001; Figure 20). Both boys and girls had similar linear decreases in the curvature mismatch between their femoral condyle and tibial plateau prior to becoming adolescent. However, the curvature mismatch remained unchanged in adolescent girls.
whereas it continued to drop in adolescent boys (p < .0001; Figure 20). In general, boys had a smaller curvature mismatch compared to age-matched girls. These difference were statistically significant across all age groups except the 3-6 years old group (p < .050).

**Figure 20**: Age and sex dependent changes in curvature mismatch between medial femoral condyle and medial tibial plateau during skeletal growth and maturation (the graph on the top right corner shows Mean ± SEM).

In summary, current findings showed significant differences in multiple prominent anatomical features of the tibial plateau by age in both girls and boys. Data also showed significant differences in size, slope and depth of the tibial plateau between boys and girls, in particular at later stages of skeletal maturation. Most importantly, results demonstrated that the age dependent changes in all the quantified anatomical feature of the tibial plateau, except the medial tibial slope, are significantly different between boys and girls.
Steeper slope of the posterior lateral tibial plateau have been associated with increased ACL loading and greater risk of ACL injuries.\textsuperscript{6, 7, 17, 21, 22, 38, 43} During weight bearing tasks, the posterior tibial slope results in generation of an anterior shear force component from the axial compression force across the knee joint.\textsuperscript{17} Accordingly, increased posterior tibial slope would lead to increased anterior tibial shear force,\textsuperscript{42} anterior tibial acceleration and translation,\textsuperscript{43} and ACL strain\textsuperscript{38, 43} during weight-bearing activities. A more shallow medial tibial plateau (smaller medial tibial depth) has also shown to significantly affect the ACL injury risk.\textsuperscript{21, 22} A shallow plateau provides less resistance to anterior tibial translation, which may in turn result in increased ACL loading and risk of injury.\textsuperscript{21, 22} Recent reports have highlighted the links between small tibial spine and increased risk of ACL injury.\textsuperscript{61} Tibial spine is among the main bony stabilizers of the tibiofemoral joint, in particular against axial rotation and medial - lateral translation. A smaller tibial spine provides less resistance to femoral external rotation and lateral translation, which may in turn result in increased ACL loading and risk of injury.\textsuperscript{61} The steeper lateral tibial slope, shallower medial tibial plateau and smaller tibial spine in girls can be contributing factors to the previously reported higher risk of ACL injury among young adolescent females compared to age-matched males.\textsuperscript{3, 4, 19, 37, 44, 46, 50, 60}

Our preliminary studies show significant changes in almost all anatomical features of the knee joint by age in children and adolescents. Findings also indicate that, in most cases, the way age affect the anatomy during skeletal growth and maturation is different in boys compared to girls. Results also highlights that girls develop a more high-risk anatomical profile than boys, which is consistent with the observed greater risk of ACL injury in girls than boys.\textsuperscript{3, 4, 19, 37, 44, 46, 50, 60} These preliminary results support the clinical relevance of our specific aims and hypotheses and further highlight our abilities and experience to conduct the proposed study.
E. Approach

E.1. Study Population: Following IRB approval, electronic medical records and imaging records of all the patients who have visited the Orthopedics or Sports Medicine clinics at the Boston Children’s Hospital (from 2011 till now) will be reviewed retrospectively. A total of 240 sets of MR images, from 240 unique patients, will be randomly selected based on the following criteria:

- **Boys and girls (7-18 years old)**
- **Confirmed ACL tear**
- **Complete set of high-quality preoperative MRI**
- **No growth-related and congenital skeletal disorders**
- **No bony deformities**
- **No fractures of femoral condyles and tibial plateau**
- **No cartilage damage across the knee joint**

MR images will be selected in a way to ensure equal distribution across each age (~10 boys and 10 girls for each age). Children younger than 7 will be excluded due to very low prevalence of ACL injuries in this age group.

E.2. Anatomical Index Measurement: The 3D MR image stacks will be used to measure following anatomical indices using previously established measurement protocols:

I. Femoral Condyles: The central slice in the medial-lateral direction of each compartment in sagittal plane will be used to measure the curvature of the medial and lateral femoral condyles. The radii of the femoral condyles will be determined with use of a circle-fitting technique in which the femoral condyle is assumed to have a single radius of curvature from 10° to 160°. A similar approach will be used to measure the medial femoral condyle’s curvature in the coronal plane. The measurement will be done in the central slice, in an anterior-posterior direction, in the coronal plane. The curvature will be defined as the inverse of the radius of the biggest circle that can be fitted to each femoral condyle.
Bicondylar femoral width and width of the intercondylar femoral notch will be measured from 3D MR image stacks in coronal-oblique plane (oblique to ACL). The femoral notch width will be measured at the middle of the ACL femoral attachment. Bicondylar femoral width will also be measured as the maximum distance between the outer surfaces of the medial and femoral condyles in the same coronal oblique plane. Measurements will be done in parallel to a reference line tangent to the posterior subchondral aspects of both femoral condyles. Notch width index will be calculated as notch width divided by bicondylar width.

II. Tibial Plateau: MR images from the identified sagittal plane slices at the middle of each condyle will be used to measure the posterior slopes of the medial and lateral tibial plateaus using the well-established technique described by Hashemi et al.21, 22 Briefly, the posterior slope of the tibial plateau (medial and lateral) will be measured as the angle between a line that connects the peak points on the anterior and posterior rims of the plateau and a line perpendicular to tibial longitudinal axis.21, 22 This method has been shown to be able to quantify the tibial slope with a sensitivity of 1 degree.21 The sagittal curvature radius of medial tibial plateau will also be measured in the same sagittal slice, used to quantify medial tibial slope, using the same circle-fitting approach described above. Sagittal curvature radius of the tibial plateau will only be measured in the medial side considering the very flat surface of the lateral tibial plateau in sagittal plane.6, 21 The maximum depth of the medial tibial plateau will also be measured as the perpendicular distance between a line connecting the anterior and posterior rims of the medial tibial plateau and the deepest point of the medial plateau in the same slice that the medial slope was measured.21 The maximum height of both medial and lateral tibial spine will be measured in sagittal MR slices passing through the middle, in medial-lateral direction, of each spine. The height will be defined at the perpendicular distance between the highest point of the spine in sagittal view and the line tangent to the tibial plateau.

All the measurements will be done using Osirix Viewer (http://www.osirix-viewer.com). Measurements will be done by a well-trained reader blind to subjects’ age and sex. To test the measurements reliability, the original reader and another independent reader will repeat the measurements on a random subset of subjects. Variance component analysis will be used to estimate the variability within- and between-examiners,
and between-subjects. Intraclass correlation coefficients (ICCs) will also be calculated for each measurement to assess interobserver and intraobserver reliability.\textsuperscript{15}

\textbf{E.3. Analysis Plan:} Similar analytical methods, used in our preliminary studies, will be used to address the Aim 1 of the proposed work here. Briefly, linear regression analysis will be used to investigate the relationships between age and quantified anatomical indices in ACL injured knees. Separate analysis will be done for each sex considering our previous observations of significant sex difference in knee anatomy (Preliminary Results). For each model, both anatomy (dependent variable) and age (independent variable) will be used as continuous variables. Second order polynomial and logarithmic models will be used where regression diagnostics indicated that the linear models are not appropriate. The distribution of the measurements over the age range along with the regression line will be plotted for each anatomical index to visualize the changes in anatomy by age and the differences between boys and girls. Measurements will also be grouped to 6 subgroups (3 age groups for each sex) based on following age categories:\textsuperscript{33}

- \textit{Pre-pubertal school-aged children} (7–10 years old)
- \textit{Early adolescents} (11–14 years old)
- \textit{Late adolescents} (15–18 years old)

A two-way ANOVA will be used to investigate the effects of age and sex on quantified anatomy for the abovementioned 6 subgroups. For these analyses, the anatomic parameter (dependent variable) will be defined as continuous with age group and sex (independent variables) being defined as categorical and dichotomous variables, respectively. The p values from the the ANOVA analysis will be used to test whether age and/or sex significantly affect the anatomy or not. Further the interaction term between sex and age group will also be added to the model to further investigate whether the age dependent changes in knee anatomy are affected by sex. This will be tested by looking at the p value corresponding to the interaction term in the model. Using the same ANOVA model, the quantified anatomy at each age group will be compared between boys and girls at each age group (3 pairwise comparisons). The p values for all 3 pairwise comparisons will be adjusted based on Hochberg correction method to account for potential increases in type I error due to multiple comparisons.
For Aim 2, we will pool the measurements obtained from Aims 1 with those done in our preliminary studies on healthy uninjured knees into two separate groups, one for each sex. Similar to the approach being used in Aim 1, measurements will be grouped into 6 subgroups (3 age groups for each sex and 2 injury states). A two-way ANOVA will be used to investigate the effects of age group and injury state (injured / uninjured) on quantified anatomy. For these analyses, anatomy (dependent variable) will be defined as continuous variable with age group and injury state (independent variables) defined as categorical and dichotomous variables, respectively. Further the interaction term between injury state and age group will also be added to the model to further investigate whether the age dependent changes are different between uninjured and injured knees. This will be tested by looking at the p value corresponding to the interaction term in the model. Using the same ANOVA model, the quantified anatomy at each age group will be compared between injured and uninjured knees for each sex group (3 pairwise comparisons for each sex). The p values for all 6 pairwise comparisons will be adjusted based on Hochberg correction method to account for potential increases in type I error due to multiple comparisons.

F. Expected Outcomes

Similar to our preliminary observations, we anticipate that there will be strong associations between the age and quantified anatomical indices of the knee joint in both boys and girls with injured ACL (Hypotheses 1.1). We also expect to see substantial sex differences in how age affects knee anatomy in knees with injured ACL (Hypotheses 1.2). We further anticipate to see girls developing a more high-risk anatomical profile compared with boys, in particular at later stages of skeletal maturation. We also expect seeing substantial differences in how anatomy changes by age between uninjured knees and those with injured ACL (Hypothesis 2.1). We expect to see that ACL injured knees develop a more high-risk anatomical profile compared to the healthy uninjured knees of the same sex group.
G. Ethical Considerations of the Proposed Research

This is a retrospective study of the pre-existing imaging and clinical data from the patients who have been seen at Boston Children's Hospital. No identifiable patient’s information will be stored except the Medical Record Numbers (MRN), which will be used to link the imaging data and the medical records (needed to assess the general health status, weight, height, age, sex, ...) and to assess subjects’ eligibility. After linking the imaging data and health record data for each subject, the MRNs will be coded to randomly generated ID numbers. The links between MRNs and specimen IDs will be kept on a single spreadsheet. MR images will be de-identified prior to measurement process. During the study, all the acquired de-identified data along with the MRN to specimen ID conversion spreadsheet will be kept on an encrypted and password protected computer located in PI's office at Boston Children's Hospital. No one else will have access to these data other than the PI.

There are no potential risks to patients’ health and privacy as: 1) all the data will be de-identified prior to analysis and measurements and no identifiable information, except patients’ MRN, will be used or stored for this study, and 2) the imaging data to be used in this study have already being obtained as part of patients’ routine clinical care. There is no direct benefit to the patients whose data will be used for this retrospective analysis. However, the findings of this study may result in an improved knowledge of the sources responsible for such a significant discrepancy in ACL injury risk between young men and women. This may in turn lead to development of novel risk screening models to more effectively identify children at high risk of ACL injury based on their anatomical profile. Individuals with high-risk bony anatomy can be subjected to neuromuscular and biomechanical interventions early on to minimize their risk of sustaining and ACL tear.
H. Bibliography


