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Structural and Functional Maturation of Distal Femoral Cartilage and Bone during Postnatal Development and Growth in Humans and Mice

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SYNOPSIS

Introduction—The size and shape of joints markedly affect their biomechanical properties, but the macroscopic 3-dimensional (3-D) mechanism and extent of cartilage and joint maturation during normal growth are largely unknown.

Aims—The purposes of this study were to qualitatively illustrate the development of the cartilage-bone interface in the knee during postnatal growth in humans and C57BL/6 wild-type mice, and to quantitatively define the 3-D shape using statistical shape modeling as well as to assess growth strain rates in the mouse distal femur.

Methods—Clinical computed tomography (CT) scans of asymptomatic knees (0.3–0.6mm in-plane resolution, 0.63mm slice thickness) were obtained from six patients between 4 to 12 years old. Micro-CT scans of mouse knees (9µm isotropic resolution) were from twenty-one mice between 12 to 120 days postnatal. Human and mouse images were compared qualitatively with 2-D images and 3-D reconstructions. Mouse femora shape parameters were determined with statistical shape modeling, and strain rates and directions during growth were mapped.

Results—The attainment of cartilage-bone interface shape of the distal femur and proximal tibia were qualitatively similar in humans and mice, with marked differences in growth plate

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morphology. Mouse distal femur shape was described by 11 independent parameters that accounted for >90% of total shape variation during growth. Each shape parameter described changes in specific anatomical regions of the distal femur and varied with age. Shape parameters and strains in the medial and lateral condyles, as well as intercondylar notch, varied greatly between postnatal days 16 to 30. Directions of growth strain across ages corresponded well with the appearance of anatomical landmarks within the distal femur.

Conclusion—Accurate quantification of the cartilage-bone interface geometry is imperative for furthering the understanding of the macroscopic mechanisms of cartilage maturation and overall joint development.

Keywords

distal femur; postnatal development; growth strain; joint shape; statistical shape modeling

INTRODUCTION

The load-bearing and compositional properties of articular cartilage vary with both the size and shape of the diarthrodial joint. In skeletally mature animals and humans, the biomechanical and biochemical characteristics of articular cartilage vary both by site (ie. patellofemoral groove and femoral condyles) and joint (ie. knee versus ankle),^{1–7} complementing the local geometry to respond to various loading demands of the body. During postnatal development (the processes of differentiation and growth), joint size increases, and cartilage thickness and chondrocyte density decrease.^{8–10} Concomitantly, articular cartilage load-bearing material properties also improve. In rabbit and bovine knee cartilage, increases during development in the tensile and compressive moduli of articular cartilage have been associated with increases in collagen content and cross-linking.^{6,7,11,12}

The macroscopic mechanisms that dictate cartilage material maturation and joint shaping remain to be determined. At the articular surface, appositional growth^{13,14} occurs with chondrocyte proliferation in the superficial zone^{15,16} and replacement of collagen fibers.¹⁷ Articular cartilage maturation is also affected by changes at the cartilage-bone interface, with chondrocyte proliferation occurring in neonatal rabbit knee joints above the subchondral plate.^{15,18} These macroscopic tissue changes during growth may be described biomechanically by growth strains.¹⁹ Thinning of cartilage during postnatal development is due to mineralization in the deep zone and advancement of the tidemark separating the deep and calcified cartilages. In conjunction with these axial growth processes, articular cartilage expands tangentially along with the underlying subchondral bone. Both axial and tangential growth processes may generate internal stresses²⁰ and affect the material quality of adult articular cartilage. Thus, a detailed understanding of the shape of the cartilage-bone interface during development would provide invaluable insight into the biomechanics of cartilage maturation and macroscopic joint size and shape.

The length and shape of long bones during *in vivo* development is in part contributed through the differential growth and remodeling of articular and growth plate cartilages. Both begin as a homogenous condensation of chondrocytes that undergo proliferation, hypertrophy, and mineralization in distinct zones to form a highly organized structure at maturity.^{10,21–26} In the growth plate, rates of chondrocyte proliferation, hypertrophy, and matrix production in different zones have been related to longitudinal bone growth.^{26–29} Differences in these rates at specific bone and joint locations give rise to differential elongation rates, such as those observed at opposite ends of long bones and between different joints. Additional variations in articular cartilage growth rates and directions by age

and species produce the wide range of joint shapes and relative proportions of anatomical features (ie. lateral and medial condyle proportions in the knee).

Current knowledge of the macroscopic shape of joints is based on measurements of cartilage and bone geometries from gross specimens, plain radiographs, magnetic resonance imaging (MRI), and computed tomography (CT). However, the complexities of joint shape are challenging to represent by 2-D or best-fit 3-D measurements.^{30,31} While the distal femur has been characterized by measurements such as anterior-posterior length, medial-lateral width, and intercondylar height,^{32,33} these parameters are confounded by the size of the knee, and more so in skeletally immature knees, making it difficult to distinguish shape changes during the developmental growth process. Statistical shape modeling (SSM)^{34,35} is one technique that can concisely quantify complex shapes in a limited number of independent parameters based on a sample population. This method can automatically identify corresponding anatomical regions between samples with high reproducibility,³⁶ making it ideal for studying shape changes during growth and development. Both 2-D and 3-D SSMs have been used to analyze joint shape and bone density as risk factors for the development of osteoarthritis^{36–42} and osteoporotic fractures,^{43–45} but have yet to be applied to the growth of healthy joints.

The hypothesis of this study was that shape changes of the distal femur at the cartilage-bone interface vary differentially with age and anatomical region during postnatal growth and development. The aims were to (1) illustrate and qualitatively compare the shape changes of the cartilage-bone interface during normal development in humans and C57BL/6 wild-type mice, (2) establish a statistical shape model and determine shape parameters for the growth of the mouse distal femur from postnatal day 12 to 120, and (3) determine growth strain rates of the cartilage-bone interface. Quantification of the shape plasticity throughout growth and development will provide invaluable insight into *in vivo* developmental biomechanics of the knee. In addition, quantitative models of the developing knee are useful as design targets for tissue engineering that extends to the joint scale, as well as and developing new technologies for clinical diagnosis and treatment of skeletal disorders.

MATERIALS AND METHODS

Sample Preparation and Imaging

With IRB approval, clinical computed tomography (CT) scans of six patients (range: 3.9–11.9 years; mean: 8.2 years) with asymptomatic knees were obtained at 0.4–0.6mm in-plane resolution and 0.63mm slice thickness (GE Lightspeed VCT, GE Healthcare, USA).

The structure of mouse knee joints was assessed by micro-computed tomography (μ CT) and histology. With IACUC approval, both hindlimbs of twenty-one C57BL/6 male mice, $n=3$ each at 12, 16, 20, 24, 30, 60, and 120 days post-natal, were harvested, fixed in 10% neutral buffered formalin, and scanned intact using μ CT at $(9\mu\text{m})^3$ isotropic voxel resolution (SkyScan 1076, SkyScan, Belgium; 70kVp, 140 μ A, 1750ms exposure). Following μ CT, one femur at each age point was randomly selected for histological processing. Proximal and distal femora were decalcified in 10% formic acid and paraffin-embedded. 5 μ m thick sagittal sections were obtained approximately through the center of the medial femoral condyle of the distal femur. Sections were stained with Safranin-O and digitized at 20X magnification with Aperio ScanScope (Aperio Technologies, Vista, CA).

Gross Morphology

Human and mouse CT images were qualitatively analyzed for morphological changes at the cartilage-bone interface during developmental growth in 2-D coronal and sagittal planes through the center of the load-bearing region of the condyle and in 3-D reconstructions.

Distal femora were assessed for contour of the condyles, posterior condyle prominence, medial versus lateral femoral condyle (MFC/LFC) size, and trochlear ridge development, as well as growth plate location and morphology. Contour of the opposing tibial plateau and joint space width between the distal femoral and proximal tibial secondary ossification centers (SOCs) were also noted.

Histology

Safranin-O sections were compared to matching μ CT sections of mouse femora to interpret the cartilage-bone interface of the SOC relative to the overlying articular cartilage. In addition, histology sections at 20x were assessed for chondrocyte hypertrophy and organization.

Image Processing

All CT and μ CT scans were imported into Mimics (Materialise, Belgium) for surface segmentation and 3-D reconstruction. Left femora were flipped in orientation to match right femora. Cartilage-bone interfaces were identified by thresholding, segmented, and exported as point clouds.

Width and Thickness Measurements

Trans-epicondylar widths were determined in human and mouse distal femora as a measure of growth. In 3-D reconstructed models, trans-epicondylar width was measured as the nearest distance from the edge of the lateral epicondyle bone to the edge of the medial epicondyle bone. In the mouse, the overall length of the femur was also determined from the most proximal point on the femoral head to the most distal point on the femoral condyles.

Thicknesses of the articular surface and growth plate were calculated from histology images. Articular cartilage was defined from the articular surface and the calcified cartilage tidemark, or at the beginning of large hypertrophic cells for young joints with no tidemark. Growth plate cartilage was defined from the top of the reserve zone to the bottom of hypertrophic zone chondrocytes. Since articular and growth plate cartilage contours were highly curved in the mouse distal femur, making it difficult to estimate thickness directly, cartilage thickness was calculated as the area of the cartilage divided by the average width of the articular or growth plate cartilage.

Statistical Shape Modeling (SSM)

Both femora of fifteen mice, three each at days 12, 24, 30, 60, 120, were used as the initial training samples for SSM. Training samples were rigidly registered and isotropically scaled⁴⁶ to the largest-volume femur (120 day sample). Point-to-point correspondences between coordinates of each femur were defined automatically, following previously established methods⁴⁷ by first constructing a normalized average atlas shape and then extrapolating points, or **landmark coordinates**, in corresponding locations to each femur. The distal femur contained 412 landmarks, located from the femoral condyles to the proximal edge of the trochlea.

The statistical shape model was built from the landmarks of the training samples using principal component analysis.^{34,47} A mean shape was calculated from training sample landmarks, and deviations of each shape from the mean were determined. Singular value decomposition of the covariance matrix was performed to obtain the eigenvectors and corresponding eigenvalues (in descending order). The eigenvectors represent the **modes of variation** within the training set, analogous to the principal axes of an ellipse. The eigenvalues represent the **variance** explained by each mode, or the amount of contribution of each mode to overall joint shape variation. Using the modes of variation from the model,

the *shape parameters*, $\mathbf{b}$, of each sample was calculated by $\mathbf{x} = \bar{\mathbf{x}} + \mathbf{P}\mathbf{b}$, where $\bar{\mathbf{x}}$ is the mean shape, $\mathbf{P} = (\mathbf{p}_1 | \mathbf{p}_2 | \dots | \mathbf{p}_t)$ are the first t modes of variation that explain >90% of the total shape variance, and $\mathbf{x}$ is the sample shape. Shape parameters are analogous to principal radii of an ellipse. Parameters were normalized to one standard deviation of the mode, calculated as the square root of the corresponding eigenvalue.

Additional samples at days 16 and 20 were analyzed by applying the SSM. Samples were segmented in Mimics, rigidly aligned to the same reference femur from the training set, and non-rigidly aligned to the model atlas shape to extrapolate landmarks. Shape parameters were then determined as described above.

Growth Maps: Principal Strain Rates and Directions

Principal surface strain rates between ages were calculated from corresponding landmark coordinates of the average shape at each age. Here, landmark coordinates represented non-scaled shapes. The two-dimensional components of Green's strain⁴⁸ E_{ij} were calculated from $ds^2 - ds_0^2 = 2E_{ij}dX_idX_j$ for $i, j = 1:2$, where $ds_0^2 = dX_idX_j$ is the squared segment length of a pair of landmarks at the first age point, and ds^2 is the squared length at the second age point. Maximum principal strain rates and directions were calculated from the Green's strain components.⁴⁸

Strain rates were mapped in 3-D onto the triangulated surfaces of the average shape at each age point. Surface patches intersecting the transverse plane and sagittal plane through the medial condyle were determined, and the 3-D principal directions of those patches were projected onto each plane to illustrate strain directions along the 2-D surface contour.

Statistics

Differences in mode parameters between age points were assessed by a one-way ANOVA with post-hoc Tukey test. All data are expressed as mean $\pm$ SD.

RESULTS

Gross Morphology of the Developing Distal Femur: Human and Mouse

The overall size and shape of both human (Figure 1A, **L=lateral**, **M=medial**, A=anterior, P=posterior. Figure 2A) and mouse (Figure 1B, L=lateral, M=medial, A=anterior, P=posterior. Figure 2B) distal femoral cartilage-bone interface changed markedly over the evaluated growth period, as visualized by μ CT in coronal (Figure 1-a) and sagittal (Figure 1-b) planes, and in 3-D reconstructions (**L=lateral**, **M=medial**, A=anterior, P=posterior. Figure 2).

In the human, the femoral growth plate was situated just proximal to the posterior edge of the condyles and was relatively flat in the transverse plane (Figure 1A-b, **L=lateral**, **M=medial**, A=anterior, P=posterior. Figure 2A-b,c). At age 4, femoral condyle contours were round in both coronal and sagittal planes (Figure 1Ai). At age 7, the posterior condyle was prominent, and the sagittal contour became elliptical. The MFC appeared slightly larger than the LFC. By age 12, the load-bearing region of the femoral condyles flattened in the coronal plane, while the sagittal profile remained elliptical (Figure 1Aiii). A prominent intercondylar notch was observed in the coronal view. Extension of the lateral trochlear ridge was evident at day 7 and prominent by day 12 (**L=lateral**, **M=medial**, A=anterior, P=posterior. Figure 2A-a). As the lateral trochlea developed, the lateral side of the growth plate became larger than the medial side (**L=lateral**, **M=medial**, A=anterior, P=posterior. Figure 2A-b). Joint space width between femoral and tibial epiphyseal bone decreased noticeably with age (Figure 1A-a). At the opposing joint surface, proximal tibia SOC was

round at age 4, developed flat plateaus by age 7, and had concave plateaus by age 12 (Figure 1A-a).

In the mouse, growth plates were also situated proximal to the posterior edge of the condyle but had an undulating shape in the transverse plane, with four regions that extended convex towards the articular surface (**L=lateral, M=medial, A=anterior, P=posterior**. Figure 2B-b,c). At postnatal day 12 (Figure 1Bi-b), the femoral SOC had a rounded distal contour and an undulating proximal contour matching the growth plate geometry. Between days 16 and 24, SOC's expanded radially as femoral condyles extended outward, and the geometry of the epiphysis interlocked with the metaphysis (Figure 1Bii). At day 30, the posterior condyle became prominent, and the load-bearing regions of the condyles were flattened in the coronal plane (Figure 1Biii). The MFC also extended more medially compared to the LFC. By day 60, the shape of the knee stabilized. Prominence of the lateral trochlear ridge, an evolutionary feature of bipedalism,⁴⁹ was not observed in mice. Similar to humans, joint space width between femoral and tibial epiphyseal bone decreased with age (Figure 1B-a). In contrast to humans, mouse growth plates remained approximately symmetrical in the medial-lateral direction throughout development (**L=lateral, M=medial, A=anterior, P=posterior**. Figure 2B-b). Proximal tibial SOC's were round at day 12, flattened with two plateaus at day 20, concave towards the distal femur at day 30, and fully developed by day 60 (Figure 1B-a).

Rapid joint-scale growth occurred from ages 4 to 12 in the human and during the first 30 days in mice. Trans-epicondylar width in the human distal femur (Figure 3A) increased linearly from age 4 to 12 ($R^2=0.95$; 4 y.o., 45.3mm; 12 y.o., 78.9mm), while trans-epicondylar width in the mouse femur (Figure 3B) increased up to day 30, after which it plateaued (12 day, 1.9mm; 30 day, 2.7mm; 120 day, 2.8mm). Overall femur length in the mouse increased at a slower pace and did not plateau until day 60 (Figure 3C). Femur length at day 12 was approximately 50% that of day 120. The final length and volume of the mouse femur at day 120 were $15.2\pm0.2\text{mm}$ and $47.0\pm4.3\text{ mm}^3$, respectively.

Histology

In C57BL/6 mice, both articular and growth plate cartilage stained intensely with Safranin-O at all ages, with zonal and age-associated variations in cellular organization (Figures 1B-c, 4).

At day 12, articular cartilage regions contained randomly distributed chondrocytes, with large hypertrophic chondrocytes at the secondary ossification center (Figure 4Ai). By day 30, the zonal architecture of articular cartilage was apparent with underlying bone formation, and by day 60, the articular cartilage was fully formed with a continuous subchondral bone plate.

Growth plate cartilage exhibited a distinct architecture with reserve, proliferative, and hypertrophic zones that decreased in height with age (Figure 4-ii). At day 12, hypertrophic chondrocytes on the periphery of the SOC that stained with Safranin-O were not visible in μCT (Figures 1B-b,c, 4A). By day 20, bony regions in histology sections corresponded well with mineralized regions observed in μCT , and proliferative zone chondrocyte nuclei stained prominently (Figure 4B). At day 60, hypertrophic chondrocytes were essentially absent from the growth plate (Figure 4D).

Articular and growth plate cartilage of the opposing proximal tibia showed similar age-dependent patterns of cellular organization and matrix staining (Figure 4-iii).

Cartilage Thickness

Articular cartilage thickness in the mouse decreased with age and plateaued after day 60 (Figure 5). Thickness measurements from histology at days 12 and 120 were 0.17mm and 0.03mm, respectively. Growth plate cartilage thickness also decreased with age and did not plateau by day 120. Growth plate thickness at days 12 and 120 were 0.56mm and 0.10mm, respectively. Both articular and growth plate cartilage thickness were inversely correlated to overall femur length (articular cartilage, $R^2=0.93$; growth plate, $R^2=0.94$; data not shown).

Statistical Shape Model: Mouse Distal Femur Cartilage-Bone Interface

Irrespective of size, 11 modes of variation accounted for >90% of the total shape variation during developmental growth of the distal femur. The first 5 modes of variation accounted for 83% of total shape variation and described mid-condyle outward extension, posterior condyle upward extension, the relative size and medial extension of the MFC, varus/valgus rotation, and trochlea protrusion and intercondylar notch width, respectively (Figure 6). Modes 6 to 11 accounted for 7% of the total shape variation and described minor shape changes of the condyles and trochlear groove.

Shape parameters for Modes 1, 2, 3, and 5 were significantly different between age points in the distal femur (Figure 7, Table 1, $p<0.01$). From day 12 to 60, the femoral condyles underwent mid-condyle extension (decrease in Mode 1 parameter from 1.42 to -0.93), after which Mode 1 parameter plateaued. The posterior condyle region extended outward from day 12 to day 30 (decrease in Mode 2 parameter from 1.25 to -0.76), and then retracted relative to the rest of the distal femur up to day 120. The relative size of the MFC (Mode 3 parameter) peaked at day 20, and slowly decreased up to day 120. Intercondylar notch width (Mode 5 parameter) widened from day 12 to 24, then became narrow up until day 60, and widened again at day 120.

Mode 1 parameters, describing mid-condyle extension, were linearly correlated with femur length, articular cartilage thickness, and growth plate cartilage thickness ($R^2=0.96$, 0.83 , and 0.94 , respectively; data not shown).

Growth Maps: Strain Rates and Directions

Surface growth strains per day in the distal femur were highest at the condyles at day 12 and decreased with time (Figure 8). Between days 16–20, strain rates were higher in the MFC than LFC, with maximum strains of 0.12 per day. Between days 20–24, strain rates were highest on the medial side of the LFC and the intercondylar notch, and between days 24–30, on the lateral side of the MFC. After day 30, strain rates were small (<0.03 per day).

In general, directions of maximum strain were similar between all ages in the transverse and sagittal planes (Figure 9). Strain directions in the transverse plane between days 12 and 16 were most variable, with strains in both directions along the surface contour. Along both sides of the epicondyles, directions of maximum strain primarily pointed up toward the trochlea (Figure 9A). At the load-bearing surface of the medial condyle, principal strains were directed medially. Strain directions in the lateral condyle were more variable, with direction vectors pointing medial and lateral between days 12 and 30, and lateral after day 30. In the sagittal plane (Figure 9B), a distinct transition point was visible near the center of the load-bearing surface of the MFC (and LFC, not shown) at all ages, where strains posterior to this location pointed toward the posterior condyle, and strains anterior to the location pointed anteriorly toward the trochlea. Surface strains of the trochlea pointed proximally, away from the condyles.

DISCUSSION

Overall development of the distal femur cartilage-bone interface was similar between humans and mice, with subtle differences in condyle and trochlea morphology between the two species (Figures 1–3). Distal femur size increased linearly up to age 12 in humans and day 30 in mice, with trans-epicondylar widths of 78.9 mm and 2.7 mm, respectively. In both species, the distal femoral SOC began with a rounded contour, followed by protrusion of the condyles and trochlear ridges and the appearance of the intercondylar notch. Using statistical shape modeling, it was possible to represent these intricate and complex 3-D size and shape changes with 11 modes of variation and corresponding shape parameters (Figures 6, 7). From day 12, mouse mid-condyles extended outward up to day 60, associated with a decrease in Mode 1 parameter from 1.42 to -0.93 , as posterior condyle regions extended up to day 30 and then partially retracted (Mode 2, Figure 7). Concomitantly, the relative size and medial extension of the MFC increased and peaked at day 24 (Mode 3), with related trends in intercondylar notch width (Mode 5). Mode 1 shape parameters (mid-condyle extension) were highly correlated with overall femur length and the thickness of articular and growth plate cartilage (Figure 5). In addition, growth strain vectors decreased with age and were consistent with shape parameters (Figures 8, 9). Principal strain directions demonstrated that trochlear and shaft strains pointed proximally, while a sharp transition point in strain directions existed at the center of the MFC load-bearing region. Together, these results quantitatively illustrate how the cartilage-bone interface takes form and expands during postnatal development and growth.

As with all animal and modeling studies, a number of limitations exist in the study design and interpretation of results. Quantitative assessment of shape changes during development was performed with C57BL/6 mice, a commonly used wild-type laboratory strain. The ages chosen for this study cover the range of mouse development starting from the appearance of the secondary ossification center as detectable by μ CT, to puberty (~ 30 days), skeletal maturity (~ 60 days), and up to early adulthood.^{50–52} Human development was observed up to puberty (age 12) for comparison. While inherent differences in anatomy exist between humans and mice, conclusions of the model are applicable to human development, as both species undergo the same general sequence of development that leads to functional adaptation and skeletal maturity. In the growth model, statistical shape analysis was used to describe shape-related variations of the mouse distal femur, irrespective of joint size. As such, shape parameters from the model quantified relative, and not absolute, changes in proportions of anatomical features. In addition, surface strain rates between ages were calculated by assuming a perfect anatomical correspondence between landmark coordinates of samples extrapolated during statistical shape modeling (extrapolation precision was within 0.03mm).

The growth and attainment of shape in the distal femur was similar in humans and mice, even with substantial differences in growth plate morphology. Trans-epicondylar width increased in humans from age 4 up to puberty, comparable to that of mice between days 12 to 30 (Figure 3), and as observed to plateau similarly after puberty.⁵³ In both species, femoral condyles developed from a rounded contour in sagittal and coronal planes to elliptical and flattened contours (Figure 1, 2). As condyle shape and relative proportions of the MFC to LFC changed, growth plate morphology did not vary noticeably, supporting the theory that growth plates primarily contribute to longitudinal bone growth while radial expansion of the SOC affects joint shape, with final shape modulated by biomechanical loading.^{17,26,54,55} However, lateral extension of the human growth plate occurred in conjunction with prominence of the lateral trochlear ridge, suggesting that the distal femoral growth plate may have a role in dictating trochlear morphology. The observed similarities

and differences between human and mouse cartilage-bone interfaces serve as a basis for the interpretation and generalization of statistical shape modeling results.

This is the first study to quantitatively describe shape variations, irrespective of size, throughout postnatal developmental in MFC/LFC proportion, extension of the condyles, and intercondylar notch width. The plateau of shape parameters around day 60 corresponded well with the cessation of femur growth, finalization of zonal organization in articular and growth plate cartilage, and stabilization of distal femur geometry (Figures 1–4, 7). However, during the rapid lengthening phase, the distal femur underwent many healthy variations in shape that were not directly related to femur length and chondrocyte organization. Extension of the posterior condyle peaked around day 20 and decreased afterward, similar to the relative size of the MFC. In contrast, intercondylar notch width widened from day 12 to 20, narrowed up to day 60, and became wider again at day 120. It is unclear how functional adaptation or pre-programmed differential growth played a role in defining these intermittent shapes. One of the most striking changes during pediatric skeletal development in humans is the reorientation of the tibio-femoral angle from $>15^\circ$ varus at birth to 10° valgus around 3 years of age, and finally decreasing to $\sim 6^\circ$ valgus by 6–7 years of age, with related growth of the MFC.^{56,57} Similar angular remodeling changes in the mouse may be related to the observed variations in MFC size and shape described above. Analysis of these intermittent developmental shapes may also provide insight into questions such as why certain intercondylar notch shapes predispose the joint to osteoarthritis in adults,⁵⁸ but not in adolescents.

This is also the first study to provide a direct estimate of growth strain vectors. Growth maps highlighted the spatially-distinct shape changes throughout development and corresponded well with shape parameters. As strain rates were higher in the MFC than LFC between days 16–20, Mode 3 parameters for relative MFC size peaked (large MFC:LFC size), and as strain rates were high on the medial side of the LFC between days 20–24, Mode 5 parameters for notch width decreased (narrow width). The alternating patterns of higher strain rate on the MFC and LFC surfaces are reminiscent of adaptive shaping of the femoral condyles as the body responds to internal and external factors. Temporal and joint-specific variations during growth and development have previously been observed in the dynamics of collagen remodeling^{59–61} as well as biomechanics of articular cartilage.^{6,7,11,62} However, little is known about the spatial distribution of matrix components during growth in the knee, especially between the medial and lateral femoral condyles. It would be interesting in future studies to investigate the relationship between developmental shape changes within the MFC and LFC and the properties of articular cartilage in those regions of highest change.

Principal strain directions in the transverse and sagittal planes illustrated directional patterns of growth that have previously only been assumed or qualitatively described.⁶³ The general pattern of principal directions remained fairly consistent between ages, even as strain rates decreased with age. One of the more intriguing findings from this study was the distinct transition point in principal directions located within the load-bearing region of the condyle in the sagittal view (Figure 9B). This location may be the central point of mid-condyle growth and extension, as surface strains around it were directed in opposite directions. Strains on the sides of the epicondyles in the transverse view were directed up towards the trochlea (Figure 9A), which may be related to the emergence of trochlear ridges. Along the load-bearing regions of the MFC in the transverse view, strains were directed medially, matching the medial extension and growth of the MFC as described by the shape parameters (Figures 7, 9A). These unique strain direction patterns of the developing distal femur serve as snapshots in time through which *in vivo* joint development and the mechanisms behind cartilage structural maturation can be better understood.

In conclusion, this study characterized the macroscopic shape and *in vivo* growth strains of the distal femur at the cartilage-bone interface throughout postnatal growth. Comparisons between human and mice provided a qualitative assessment of the morphological differences between species and insight into the evolutionary mechanisms of joint development. Statistical shape modeling and resultant parameters quantitatively defined initial, final, and transient geometries of the mouse distal femur during growth. The first five modes of variation accounted for the shapes of major anatomical features such as the condyles and intercondylar notch, while latter modes were associated with fine-tuning of positions and curvatures within the joint. Growth strain maps showed highest strain rates in the femoral condyles and direction vectors that corresponded with the emergence of anatomical features. Accurate quantification of the cartilage-bone interface geometry is imperative for furthering the understanding of macroscopic mechanisms of cartilage maturation and overall joint development, as well as for developing new technologies for the diagnosis and treatment of joint disorders.

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Figure 1.

(A) Clinical CT scans of the asymptomatic human knee from (i-iii) ages 4 to 12, and (B) μ CT scans and Safranin-O sections of C57BL/6 wild-type mouse knees from (i-v) postnatal days 12–120, in (a) coronal and (b,c) sagittal views. L=lateral, M=medial, A=anterior, P=posterior.

Figure 2.

3-D reconstructions of the developing right distal femur and growth plate (**A**) in the human from ages 4 to 12 years, and (**B**) in the mouse from postnatal days 12–120. Transverse views of the (i) distal femur and (ii) growth plate, and (iii) sagittal views of the growth plate overlaid on the distal femur are shown. L=lateral, M=medial, A=anterior, P=posterior.

Figure 3. Trans-epicondylar width of (A) human and (B) mouse distal femur, and (C) mouse femur length, measured in 3-D reconstructions of individual samples.

Figure 4. Safranin-O sections of mouse distal femur (i) articular cartilage, (ii) growth plate cartilage, and proximal tibial (iii) growth plate cartilage showing distinct patterns of cellular organization at postnatal days (A) 12, (B) 20, (C) 30, (D) 60, and (E) 120. Images are oriented such that the articular surface is at the top.

Figure 5. Articular and growth plate cartilage thickness in C57BL/6 wild-type mice with age, determined in 2-D histology sections.

Figure 6.

First 5 modes of variation of the distal femur (knee) of CB57BL/6 mice. Colored solid/dashed lines indicate ± 3 standard deviations from the mean. Black lines depict major changes in shape.

Figure 7. Normalized parameters for Modes of Variation 1, 2, 3, and 5 as a function of age. Data are shown as mean $\pm$ SD.

Figure 8.

Distal femoral shape at days 12, 16, 20, 24, 30, and 60. Color maps indicate maximum principal strain rates (per day) across the knee, calculated between age intervals indicated at top and mapped onto the shape of the younger age point (i.e. maximum strain rates between days 12 and 16 are mapped on the day 12 distal femur shape). L=lateral, M=medial, A=anterior, P=posterior.

Figure 9.

Directions of maximum strain (corresponding to strains shown in Figure 8) across the right distal femur (i) transverse plane and (ii) sagittal plane through the medial condyle. Direction vectors were calculated between age intervals and mapped onto the shape of the younger age point (i.e. maximum strain directions between days 12 and 16 are depicted on the day 12 femur shape). Orientation and color of arrowhead indicate direction of the vector with respect to the horizontal axis. Gray dots are points of reference at corresponding locations. L=lateral, M=medial, A=anterior, P=posterior.

Table 1

Distal Femur Mode Parameters at Different Age Points (mean±SD)

Age (Days)	Mode of Variation				
	1	2	3	4	5
12	1.42±0.13	1.25±0.31	-0.42±0.59	-0.02±0.70	0.21±1.45
16	1.14±0.07	-0.21±0.17	1.08±0.09	-0.27±0.46	0.06±0.13
20	0.77±0.08	-0.71±0.09	1.55±0.18	0.74±0.35	-0.26±0.15
24	0.53±0.05	-0.73±0.14	0.88±0.31	0.18±0.52	-0.73±0.60
30	0.15±0.07	-0.76±0.04	0.90±0.17	-0.18±0.78	0.13±0.41
60	-0.93±0.11	0.09±0.26	-0.14±0.53	-0.65±1.42	0.95±0.86
120	-1.23±0.10	0.56±0.13	-0.45±0.24	0.67±1.16	-0.62±0.56
% Variance Explained	56.49	15.71	6.45	2.29	2.10