Bone Robusticity in Two Distinct Skeletal Dysplasias Diverges from Established Patterns

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Abstract

Achondroplasia is a heritable disorder of endochondral bone formation characterized by disproportionate short stature. Osteogenesis imperfecta is a heritable bone and connective tissue disorder characterized by bone fragility. To investigate bone morphology of these groups, we retrospectively reviewed 169 de-identified bone age films from 20 individuals with achondroplasia, 39 individuals with osteogenesis imperfecta and 37 age- and sex-matched controls (matched to historical measurements from the Bolton-Brush Collection). We calculated robustness (Tt.Ar/Le) and relative cortical area (Ct.Ar/Tt.Ar) from measurements of the second metacarpal, which reflect overall bone health. Relative cortical area is a significant predictor of fracture risk and correlates with robustness at other sites. Individuals with osteogenesis imperfecta had relative cortical area values above and robustness values below that of the control population. Bisphosphonate treatment did not significantly impact either robustness or relative cortical area. In contrast to that reported in the unaffected population, there was no sexual dimorphism found in osteogenesis imperfecta robustness or relative cortical area. We suggest that the underlying collagen abnormalities in osteogenesis imperfecta override sex-specific effects. Individuals with achondroplasia had robustness values above and relative cortical area values below that of the control population. Sexual dimorphism was found in achondroplasia robustness and relative cortical area values. **Clinical significance:** Identifies morphologic trends in two distinct skeletal dysplasia populations (osteogenesis imperfecta and achondroplasia) to better understand development of bone robusticity and slenderness in humans. Understanding these patterns of bone morphology is important to predict how individuals will respond to treatment and to increase treatment effect.

Key words: Achondroplasia, Osteogenesis Imperfecta, Bone Robustness, Bone Morphology, Bisphosphonate Treatment

Introduction

Variation in morphologic parameters (e.g. bone length and width) contributes to the stiffness and strength of the skeleton. Robusticity (total area/bone length) is a trait that describes the relationship between transverse expansion and longitudinal growth in long bones (i.e. expansion at the growth plate versus periosteal growth)[1]. The second metacarpal, which has been used to determine overall bone health since the 1960s[2], has multiple muscle attachments that apply sufficient forces to establish mechanically functional structures with varying robustness through compensatory changes in morphology [3]. Additionally, the second metacarpal has been

shown to be a significant predictor of fracture risk at the hip[4] and vertebrae[5] and its robustness has been correlated with that at other sites, i.e. femur and tibia [6, 7].

Ideally, bones which fall towards the very robust end of the spectrum compensate by having a proportionally thinner cortex to minimize mass[3]. Therefore, robust bones should have a low mineral content. Bones which fall toward the narrow or slender end of the external size spectrum compensate by having a proportionally thicker cortex to maximize stiffness and strength. Therefore, slender bones should have a higher mineral content. The relationship between external bone size and relative cortical area and tissue-mineral density is an adaptive biological process that involves bone cells adjusting traits (e.g., cortical area, mineralization) in a highly coordinated manner so the resultant set of traits has sufficient stiffness to support the loads incurred during daily activities (e.g., walking, running, standing, sitting)[1].

We postulate that the adaptation process during growth varies in fundamentally different ways among individuals, which results in the acquisition of specific sets of traits that are predictable based on bone robustness. Because homeostatic buffering mechanisms during growth control the degree of compensation that can be expressed in a population, we expect that genetically heterogeneous individuals would not show random trait sets (length, external size, internal size), but rather a narrow range of functionally adapted sets of traits. This has been confirmed in a recent study using hand radiographs purchased from the Bolton-Brush collection. The Bolton-Brush collection includes 330 longitudinally acquired hand radiographs from 24 healthy white girls and 31 healthy white boys collected in the 1930s in Cleveland, OH. [8] That research demonstrated that the inter-individual variation in bone robustness was largely determined by 2 years of age. There was a sex-specific difference in that prepubertal girls had

more slender metacarpals with proportionally thicker cortices compared with prepubertal boys. Boys and girls with robust diaphyses had proportionally thinner cortices, thereby minimizing overall mass, whereas children with slender diaphyses had proportionally thicker cortices to maximize stiffness[8] consistent with weight-bearing long bones[1].

Finding a consistent pattern of trait sets across a population is important, because it means that individuals within this population share a common biological control which regulates functional adaptation. Further, finding a consistent pattern of trait sets across a population suggests that we can use these biomechanically-based methods to identify, in a new way, disease states or treatments that potentially alter the development of bone strength.

Specifically, understanding the biological factors and variation in these parameters in two disparate skeletal dysplasias should allow a better understanding of the inter-individual variation in biologic processes that establish mechanical function during growth and development in a non-dysplastic population. The skeletal dysplasias comprise a clinically and genetically heterogeneous group of over 400 disorders associated with abnormal bone and cartilage growth and development[9]. The two skeletal dysplasias in this study are achondroplasia and osteogenesis imperfecta. We selected these diagnoses because of their relatively high prevalence, comparatively different clinical findings, and distinct skeletal phenotypes.

Achondroplasia (ACH) is an autosomal dominant form of skeletal dysplasia caused by gain-of-function mutation of the *FGFR3* gene.[10] ACH is the most common form of non-lethal skeletal dysplasia with a prevalence of ~1/25,000 live births.[11] Characteristic features include an average-size trunk, short arms and legs with particularly short upper arms and thighs

(rhizomelia), and macrocephaly with a prominent forehead. There is a characteristic hand phenotype in that fingers are typically short (brachydactyly) and the ring finger and middle finger may diverge, giving the hand a three-pronged (trident) appearance. Individuals with Achondroplasia are not predisposed to decreased bone density and do not have a predisposition to fracture.

Osteogenesis Imperfecta (OI) is a heritable connective tissue disorder characterized by bone and tissue fragility. Osteogenesis Imperfecta (OI) occurs in approximately 1/10,000 live births.[12] The majority of cases result from dominant mutations of the *COL1a1* or *COL1a2* genes, although less common, mostly recessive forms result from mutations affecting proteins associated with collagen processing and trafficking.[13] (13) Clinical severity and phenotypic presentation ranges widely, from perinatal lethality (type II) to mild disease (type I). Phenotypically, even patients with more severe types of OI tend to have hands which fall within morphologic norms for non-skeletal dysplasia populations. The most common treatments for OI are surgical rodding[14] and bisphosphonate treatment[12].

This study aims to calculate robustness from measurements of the second metacarpal of patients with ACH, OI, and average-statured individuals in order to characterize the pattern of bone morphology and to identify differences in growth patterns that may inform clinicians on how to best treat these diseases. We hypothesize that: 1) patients with ACH will have more robust bones than average-statured patients, 2) OI patients will have similar robustness to average-statured patients, and 3) that bisphosphonate treatment will not impact bone robustness for OI patients.

Patients and Methods

This is an IRB-approved retrospective review (evidence level 3) of bone age films (AP hand/wrist). A total of 169 de-identified digital bone age films were reviewed and measured using Sectra IDS 7 PACS (Sectra AB, Linköping, Sweden). Measurements included: second metacarpal length (Le), proximal to distal end; and marrow diameters (Ma.Dm, distance between inner cortices) and outer bone diameters (B.Dm, distance between outer cortices) measured at 30%, 40%, 50%, and 60% of the total metacarpal length. Total cross sectional area ($\square R^2$, R = outer bone diameter/2), cortical area (total area - marrow area), marrow area (πr^2 (r = inner bone diameter/2), polar moment of inertia ($\pi/2$ (R^4 - r^4)), robustness (Total Area / Bone Length), and relative cortical area (RCA, cortical area /total area) were calculated to analyze metacarpal size and to estimate strength.

Twenty individuals with Achondroplasia (5 months-58 years), 39 patients with OI (1 year 9 months- 67 years) and 37 modern controls (4 year 6 months-21 years) were included in this study. Their demographic data derived from chart review are in Table I. This sample of modern controls (diagnosed with leg length discrepancy) matched historical measurements from the Bolton-Brush Collection (ages 3 months to 16 years). Chronological age, as opposed to bone-age, was used to compare groups given that standard growth curves have been established for controls, but not for the Achondroplasia or OI populations. Non-parametric Kruskal-Wallis tests were used to compare differences in bone characteristics between study groups with statistical significance accepted at $p \leq 0.05$.

Table I: Place here, please.

Results

Individuals with ACH had robustness values above ($p<.0.001$) and relative cortical area (RCA) values below ($p<.0.001$) that of the control population (Figure I-a, b). This robust

phenotype was consistent with the reduced longitudinal metacarpal growth seen in ACH. ACH cortices tended to be thinner ($p<.0.001$) than those of the control population. Sexual dimorphism in robustness ($p<0.01$) and RCA values ($p<0.05$) were found in ACH, similar to that reported in the unaffected population.

RCA values for individuals with OI were higher than the control group ($p<.0.001$) and OI displayed decreased robustness across all types ($p<.0.001$) (Figure I-a, b). Individuals with OI fell into the most slender tertile for robustness of the control population (Table II) and had no increase in absolute cortical thickness averages compared to control population. Sexual dimorphism was not significant for either robustness or RCA.

Figure I: Place here, please

Table II: place here, please.

Nineteen of the 39 individuals with OI (17 males, 22 females) received bisphosphonate treatment ±2 years of their bone age X-ray. This included 28.6% of OI Type I (4/14), 75% of OI Type III (9/12), 50% of OI Type V (1/2); 100% of OI Type VIII (1/1); 50% of OI Type IX (1/2); 100% of unknown recessive OI (2/2), and 42.9% of OI Type IV(2/7). There were no significant differences in robustness and RCA between treated and non-treated individuals. All had robustness values below and RCA values above the control population.

Discussion

This is the first study of bone robustness in two skeletal dysplasia populations, Achondroplasia and OI. Understanding the changes in bone surfaces (periosteal, endosteal) individually and relative to each other provides important insight into the structural basis of the strength deficits that may indicate how and when to treat to improve bone strength in OI and

ACH. We also considered the effects of bisphosphonate treatment on bone morphology in OI, which has not been done before.

This study shows that the ACH population has a pattern of more robust bones compared to control populations, while the OI population tended to be slightly less robust compared to control populations. The inverse relationship between robustness and RCA for the ACH and OI populations was consistent with the control populations. The increased metacarpal length in patients with OI compared to patients with ACH ($p<.0.001$) suggests that differences in robustness between the two can be attributed in part to the difference in longitudinal metacarpal growth (Table II).

The reduced bone mass of OI seems to come from suppressed periosteal expansion (lower Tt.Ar) and not failure to accumulate bone mass, given that RCA was appropriate for slender phenotype. The lack of increase in absolute cortical thickness averages in OI does not follow the expected pattern seen in the control population, but may be consistent with bone fragility characteristic of OI. Because slender bones are structurally weaker than wide bones, individuals with OI, therefore, have both a material and structural problem contributing to their overall strength deficit. Further, the lack of sexual dimorphism in the OI population is in contrast to that reported in unaffected populations[8]. Given the mechanistic link between type 1 collagen and bone strength, we suggest that the underlying collagen abnormalities in OI may override sex-specific effects on bone development. In general, slender bones rely on osteoblasts for new bone deposition, whereas robust bones rely on osteoclasts for continued marrow expansion during growth. Therefore, it is not surprising that bisphosphonate treatment, which affects osteoclasts more than osteoblasts, did not appreciably change morphologic parameters in the diaphyses of

individuals with OI. These findings suggest that future treatments for OI should focus on periosteal expansion (not suppressing resorption) to strengthen long bones.

The very robust bones of the ACH population arise from suppressed longitudinal growth. Lower than expected RCA for the increased robustness, suggests ACH patients fail to accumulate sufficient bone mass (Ct.Ar). Presumably, the reduced RCA comes from excessive marrow space expansion (almost no difference in Ma.Ar between ACH and control). Sexual dimorphism was found in both robustness and RCA for the ACH patients in this study, suggesting that without the dual material and structural problem of OI, this patient population retains the sexual dimorphism reported in unaffected populations. Treatment for individuals with ACH developing osteoporosis should focus on targeting marrow space expansion.

This study lays the groundwork for analysis of potential treatments and their effect on bone morphology both in OI and other osteopenic/osteoporotic populations, as well as in Achondroplasia and other forms of dwarfism. The x-rays measured in this study were taken as a standard-of-care measure, but there is always a concern about radiation exposure. These disparate growth patterns would be expected to result in different responses to clinical treatments (anabolic versus anti-catabolic), depending on sex, age, and robustness. Although measurements conducted by different researchers were tested and found to have statistically insignificant differences, there remains potential for inter-measurer discrepancies. A larger sample size is needed to ensure the reliability of population means and confirm the trends observed. Specifically, additional studies are needed to test whether the underlying collagen abnormalities in OI override sex-specific effects on bone strength and development. In addition, a larger sample size and additional longitudinally acquired x-rays for individual patients would allow for observation of any aging effect on bone growth patterns in OI and ACH populations. Lastly,

genetic and environmental factors define the variation in subperiosteal expansion relative to longitudinal growth by 2 years of age in unaffected populations.[8] Additional patients will allow validation of this in the dysplastic populations.

Acknowledgements

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Legends List:

Figure I: Individual patient measurements for Achondroplasia and Osteogenesis Imperfecta. Plots compare Tt.Ar/Le or robustness (a, d) and Ct.Ar/Tt.Ar or RCA (b, e) by diagnosis across chronological age against control values. RCA plotted by sex and diagnosis across chronological age (c, f).

Table I: Demographic data for the three study populations.

	Total (n)	Male	Female
Achondroplasia	**20**	**10**	**10**
OI (All types)	**39**	**17**	**22**
OI Type I	14	4	10
OI Type III	12	6	6
OI Type IV	7	5	2
OI Type V	2	1	1

OI Type VIII	1	0	1
OI Type IX	3	1	1
OI Unknown Recessive	1	0	1
Control	**37**	**22**	**15**

Table II: Average measurements and calculations for second metacarpal (mean ± stdev) reported for total (males and females), males, and females; all ages included by diagnosis.

	Control			**ACH**		
	Total	**Male**	**Female**	**Total**	**Male**	**Female**
Le. (mm)	56.8 ± 9.43	60.0 ± 8.81	53.6 ± 9.06	28.9 ± 14.8	29.6 ± 14.9	28.2 ± 14.2
Tt.Ar (mm^2)	40.4 ± 11.5	44.2 ± 11.5	35.5 ± 9.48	27.9 ± 12.8	28.9 ± 11.6	25.7 ± 14.5
Ct.Ar (mm^2)	27.7 ± 8.36	31.2 ± 8.25	24.2 ± 6.97	16.5 ± 11.6	16.4 ± 10.8	16.3 ± 12.7
Marrow Area (mm^2)	12.7 ± 4.20	14.0 ± 4.38	11.4 ± 3.57	11.4 ± 5.60	12.5 ± 5.90	9.33 ± 5.02
Polar Moment of Inertia (mm^4)	253 ± 141	311 ± 149	192 ± 103	124 ± 135	122 ± 117	119 ± 160
Cortical Thickness (mm)	1.57 ± 0.31	1.67 ± 0.29	1.45 ± 0.28	1.05 ± 0.59	1.03 ± 0.59	1.10 ± 0.61
RCA	0.68 ± 0.05	0.69 ± 0.05	0.68 ± 0.05	0.57 ± 0.16	0.54 ± 0.17	0.60 ± 0.14
Tt.Ar/Le. (mm)	0.70 ± 0.14	0.75 ± 0.14	0.66 ± 0.11	1.01 ± 0.29	1.07 ± 0.32	0.92 ± 0.23

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Introduction

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Results

Individuals with ACH had robustness values above ($p<.0.001$) and relative cortical area (RCA) values below ($p<.0.001$) that of the control population (Figure I-a, b). This robust phenotype was consistent with the reduced longitudinal metacarpal growth seen in ACH. ACH cortices tended to be thinner ($p<.0.001$) than those of the control population. Sexual dimorphism in robustness ($p<0.01$) and RCA values ($p<0.05$) were found in ACH, similar to that reported in the unaffected population.

RCA values for individuals with OI were higher than the control group ($p<.0.001$) and OI displayed decreased robustness across all types ($p<.0.001$) (Figure I-a, b). Individuals with OI fell into the most slender tertile for robustness of the control population (Table II) and had no increase in absolute cortical thickness averages compared to control population. Sexual dimorphism was not significant for either robustness or RCA.

Figure I: Place here, please

Table II: place here, please.

Nineteen of the 39 individuals with OI (17 males, 22 females) received bisphosphonate treatment ±2 years of their bone age X-ray. This included 28.6% of OI Type I (4/14), 75% of OI Type III (9/12), 50% of OI Type V (1/2); 100% of OI Type VIII (1/1); 50% of OI Type IX (1/2); 100% of unknown recessive OI (2/2), and 42.9% of OI Type IV(2/7). There were no significant differences in robustness and RCA between treated and non-treated individuals. All had robustness values below and RCA values above the control population.

Discussion

This is the first study of bone robustness in two skeletal dysplasia populations, Achondroplasia and OI. Understanding the changes in bone surfaces (periosteal, endosteal) individually and relative to each other provides important insight into the structural basis of the strength deficits that may indicate how and when to treat to improve bone strength in OI and ACH. We also considered the effects of bisphosphonate treatment on bone morphology in OI, which has not been done before.

This study shows that the ACH population has a pattern of more robust bones compared to control populations, while the OI population tended to be slightly less robust compared to control populations. The inverse relationship between robustness and RCA for the ACH and OI populations was consistent with the control populations. The increased metacarpal length in patients with OI compared to patients with ACH ($p<.0.001$) suggests that differences in robustness between the two can be attributed in part to the difference in longitudinal metacarpal growth (Table II).

The reduced bone mass of OI seems to come from suppressed periosteal expansion (lower Tt.Ar) and not failure to accumulate bone mass, given that RCA was appropriate for slender phenotype. The lack of increase in absolute cortical thickness averages in OI does not

follow the expected pattern seen in the control population, but may be consistent with bone fragility characteristic of OI. Because slender bones are structurally weaker than wide bones, individuals with OI, therefore, have both a material and structural problem contributing to their overall strength deficit. Further, the lack of sexual dimorphism in the OI population is in contrast to that reported in unaffected populations[8]. Given the mechanistic link between type 1 collagen and bone strength, we suggest that the underlying collagen abnormalities in OI may override sex-specific effects on bone development. In general, slender bones rely on osteoblasts for new bone deposition, whereas robust bones rely on osteoclasts for continued marrow expansion during growth. Therefore, it is not surprising that bisphosphonate treatment, which affects osteoclasts more than osteoblasts, did not appreciably change morphologic parameters in the diaphyses of individuals with OI. These findings suggest that future treatments for OI should focus on periosteal expansion (not suppressing resorption) to strengthen long bones.

The very robust bones of the ACH population arise from suppressed longitudinal growth. Lower than expected RCA for the increased robustness, suggests ACH patients fail to accumulate sufficient bone mass (Ct.Ar). Presumably, the reduced RCA comes from excessive marrow space expansion (almost no difference in Ma.Ar between ACH and control). Sexual dimorphism was found in both robustness and RCA for the ACH patients in this study, suggesting that without the dual material and structural problem of OI, this patient population retains the sexual dimorphism reported in unaffected populations. Treatment for individuals with ACH developing osteoporosis should focus on targeting marrow space expansion.

This study lays the groundwork for analysis of potential treatments and their effect on bone morphology both in OI and other osteopenic/osteoporotic populations, as well as in Achondroplasia and other forms of dwarfism. The x-rays measured in this study were taken as a

standard-of-care measure, but there is always a concern about radiation exposure. These disparate growth patterns would be expected to result in different responses to clinical treatments (anabolic versus anti-catabolic), depending on sex, age, and robustness. Although measurements conducted by different researchers were tested and found to have statistically insignificant differences, there remains potential for inter-measurer discrepancies. A larger sample size is needed to ensure the reliability of population means and confirm the trends observed. Specifically, additional studies are needed to test whether the underlying collagen abnormalities in OI override sex-specific effects on bone strength and development. In addition, a larger sample size and additional longitudinally acquired x-rays for individual patients would allow for observation of any aging effect on bone growth patterns in OI and ACH populations. Lastly, genetic and environmental factors define the variation in subperiosteal expansion relative to longitudinal growth by 2 years of age in unaffected populations.[8] Additional patients will allow validation of this in the dysplastic populations.

Acknowledgements

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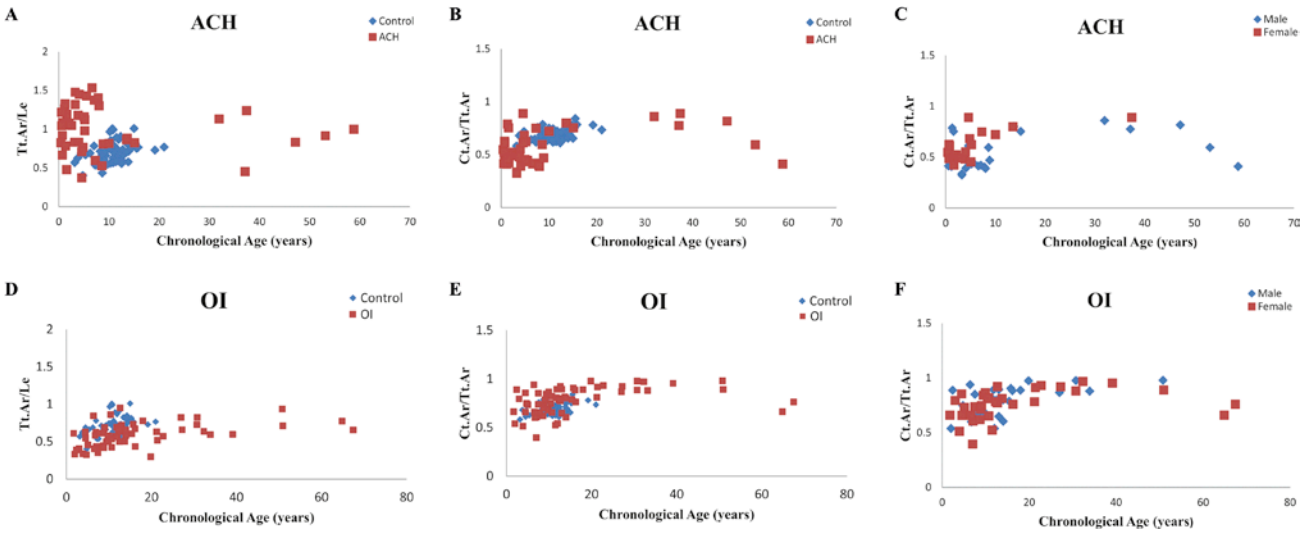
Legends List:

Table I: Demographic data for the three study populations.

Figure I: Individual patient measurements for Achondroplasia and Osteogenesis Imperfecta. Plots compare Tt.Ar/Le or robustness (a, d) and Ct.Ar/Tt.Ar or RCA (b, e) by diagnosis across chronological age against control values. RCA plotted by sex and diagnosis across chronological age (c, f).

Table II: Average measurements and calculations for second metacarpal (mean ± stdev) reported for total (males and females), males, and females; all ages included by diagnosis.



Raggio Metacarpal Figure .