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Skeletal Dynamics of Down Syndrome: A Developing Perspective

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Abstract

Individuals with Down syndrome (DS) display distinctive skeletal morphology compared to the general population, but disparate descriptions, methodologies, analyses, and populations sampled have led to diverging conclusions about this unique skeletal phenotype. As individuals with DS are living longer, they may be at a higher risk of aging disorders such as osteoporosis and increased fracture risk. Sexual dimorphism has been suggested between males and females with DS in which males, not females, experience an earlier decline in bone mineral density (BMD). Unfortunately, studies focusing on skeletal health related to Trisomy 21 (T21) are few in number and often too underpowered to answer questions about skeletal development, resultant osteoporosis, and sexual dimorphism, especially in stages of bone accrual. Further confounding the field are the varied methods of bone imaging, analysis, and data interpretation. This review takes a critical look at the current knowledge of DS skeletal phenotypes, both from human and mouse studies, and presents knowledge gaps that need to be addressed, differences in research methodologies and analyses that affect the interpretation of results, and proposes guidelines for overcoming obstacles to understand skeletal traits associated with DS. By examining our current knowledge of bone in individuals with T21, a trajectory for future studies may be established to provide meaningful solutions for understanding the development of and improving skeletal structures in individuals with and without DS.

Keywords

Trisomy 21; Down syndrome; Skeletal abnormalities; Genetic animal models; Developmental modeling; Osteoporosis

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Conflict of Interest

The authors declare no conflict of interests.

1. Introduction: all individuals with Down syndrome have bone abnormalities

Down syndrome (DS) resulting from trisomy of chromosome 21 (Trisomy 21 or T21) is the most viable trisomy and occurs in about one in 800 live births [1]. All individuals with DS have hallmark skeletal defects such as craniofacial abnormalities and short stature resulting from disproportionately shortened legs [2-4]. They are also predisposed to reduced bone mineral density (BMD), increased fracture risk, increased atlanto-odontoid distance, and stenosis, which may cause apnea and dysphagia [2, 5, 6]. In this review, we critically examine the current literature on the appendicular skeleton of individuals with DS due to its translational value in terms of fracture risk assessment and breakage. We highlight gaps in understanding, identify shortcomings in research strategies and methodologies, review current and potential treatment strategies, discuss models of DS used to study skeletal abnormalities, recommend guidelines for analysis, and propose future research directions. Craniofacial features are not discussed because of the significant differences between the development of craniofacial and appendicular bone: a large part of craniofacial skeleton develops from paraxial mesoderm and neural crest whereas the appendicular skeleton is formed from somitic lateral plate mesoderm. The study of the skeleton in individuals with DS is limited compared to other bone disorders in normal individuals such as osteosarcoma, postmenopausal osteoporosis, and osteopenia. Studying differences in the skeleton caused by T21 will not only benefit those with DS, the lessons learned may be applied to bone deficiencies that affect the general population.

Medical advances and increased standards of care have contributed significantly to increasing the expected lifespan of individuals with DS from 9 years of age in 1929 compared to over 60 years of age currently [7, 8]. Infant mortality of T21 has been substantially reduced, one-year survival significantly increased, and survival into adulthood considerably increased. Increased lifespan is attributed to improvements in correcting congenital heart conditions occurring in about 40% of individuals with DS, the widespread availability of antibiotics to treat respiratory infections common in individuals with DS due to immune dysregulation, improved home care, and fewer individuals with T21 residing in institutions [7, 9-11]. Complications such as fracture and nonunion will likely rise with increased life expectancy within this population.

Skeletal studies of DS appear disparate and heterogenous in their methodology. Features unique to DS, such as shortened long bones, absent or rudimentary 12^{th} thoracic rib pairs, short stature, and small digits are not always reported [2, 4, 12, 13]. Methods to measure unique skeletal features associated with DS, including dual x-ray absorptiometry (DXA) postnatally and ultrasonography in utero, also differ widely between studies. Heterogeneity and lack of data reported adds confusion to the field; reports may include raw or adjusted values, T- or Z-scores, or percentiles, and rarely utilize biochemical analysis or cellular studies to analyze bone formation and resorption.

2. Unique features of skeletal abnormalities in DS

The most noticeable feature of the appendicular skeleton in individuals with DS is short stature. Individuals with DS have a shorter period of bone growth than normal individuals and maximal height in individuals with DS is attained at ~15 and ~16 years in females and males with DS, respectively, compared to ~17 and ~18 years in females and males in the general population, respectively [14-17]. Puberty is both precocious and incomplete in individuals with DS with peak height velocity occurring at an earlier stage of puberty for both males and females. The mean age of menarche in females with DS does not seem to significantly differ from normal individuals [18]. Individuals with DS attain peak bone mass at an earlier age and experience bone loss sooner and at a higher rate than the general population [19].

The early development of bone abnormalities and anticipated extended lifespan of individuals with DS may predispose them to age-related complications such as increased fracture risk [20, 21]. Studies in the past were often too underpowered to discern age- or sex-related defects in the skeleton. These studies were not directed toward ages when bone accrual and growth mechanisms are affected by T21 and also utilized large age ranges. While bone defects such as short stature are outwardly apparent, the mechanisms causing structural and strength differences in bone are not completely understood nor explored in human studies. The most comprehensive studies to date show significant differences in BMD between individuals with DS beginning in their second and third decades of life but do not include enough individuals older than 60 or younger than 20 years of age to draw meaningful conclusions about bone health at those ages [22, 23]. Deficits in bone formation at younger ages may place individuals with DS at significant risk of bone-related complications at older ages. As opposed to age-related osteoporosis in the general population, skeletal defects in DS arise due to dysregulated developmental processes. One study showed that bone resorptive processes mediated by osteoclasts were unaffected in individuals with DS, while bone formation processes mediated by osteoblasts were significantly reduced [24]. These data suggest bone formation processes may drive the skeletal abnormalities associated by DS [24]. Understanding genetic, molecular, and cellular mechanisms governing skeletal development in DS is critical to prevent skeletal deficits from forming.

3. Methods to quantify bone in individuals with DS

Dual X-ray Absorption (DXA) is used to measure bone density and other bone parameters noninvasively, including whole body or areas of interest such as the lumbar spine, wrist, or femoral neck. DXA provides a measure of bone mineral content (BMC) that is divided by the two-dimensional area of the bone to yield areal BMD, as opposed to computed tomography (CT) which yields a volumetric BMD measure [25]. BMD measurements obtained from DXA that are adjusted to account for the size of the bone are reported as bone mineral apparent density (BMAD) [19, 24]. Control BMD measurements to compare with individuals with DS are often obtained from cohorts of normal individuals at peak bone mass. Without adjusting for the reduced size of the bones of individuals with DS, the data can be deceiving. For example, Costa et al. 2018 reports a downward trend in lumbar spine

BMD from age 15-64 while Carfi et al. 2017 reports little or no decline in lumbar spine BMAD from age 20-69 [19, 22]. Garcia-Hoyos et al. reported that areal BMD was significantly lower than age-matched individuals in the general population but volumetric (v)BMD fell into normal range [26]. A subsequent study of the same population by Garcia-Hoyos using 3D-DXA found that individuals with DS had lower vBMD than age matched controls [27]. While the trends of areal BMD and vBMD are similar between age-matched adult studies (reduced in the DS population compared to general population), adjusted BMD (vBMD or BMAD) has a stronger correlation to bone strength than areal BMD and should be the preferred measurement [28]. The BMD value from individuals with DS should be adjusted depending on the age and size of the subjects to draw accurate conclusions.

The most frequently reported measurements of BMD in individuals with DS are of the lumbar spine (vertebral fractures are the most common manifestation of osteoporosis) and femoral neck (hip fractures are the most debilitating manifestation of osteoporosis) [29]. Total hip BMD is generally considered the least reliable measurement of BMD, compared to the lumbar spine or femoral neck, due to inconsistencies in change between measurements [30, 31]. The part of bone analyzed must also be carefully considered. Trabecular BMD is a more valuable measure for predictive calculations such as fracture risk or ultimate load [28], and hand X-rays are valuable for assessing skeletal age [25].

Two methods are predominantly used to determine if significant bone deficits exist in individuals with DS as compared to the general population. Both have advantages and drawbacks depending on the comparison, which may add to the confusion about DS bone measurements. T-scores are calculated by comparing the mean BMD of the individual with DS to a subset of the healthy general population between 25 and 30 years of age of the same ethnicity. This measurement is valuable because it assesses how far off an individual's BMD is from their maximum potential BMD. Normal BMD T-scores are >-1, osteopenic BMD is between -2.5 and -1, and osteoporotic BMD is <-2.5. Z-scores are calculated to compare the BMD of an individual with DS to a subset of the healthy general population with a similar age and sex. This measurement is valuable because it assesses how different an individual's BMD is from healthy people of the same age and sex. T-scores are valuable for assessing when individuals with DS attain peak bone mass and the degree of bone loss, while Z-scores are valuable for assessing the effect of trisomy on bone compared to the general population [19]. Comparing studies with T- and Z-scores may be difficult because of the different emphases of these scores.

While DXA is a convenient and powerful tool, it cannot provide cellular related measurements for bone accrual, remodeling, or homeostasis. Bone biopsies and postmortem samples would reveal much about the cellular activity in the bones of individuals with DS, but the invasive nature of collecting a sample may be both stressful and painful. To date, a single report of a postmortem histological evaluation of a lumbar spine sample from a 49-year old male with DS reported that there were no osteoclasts present in the cross-section and surmised that bone resorption was impaired [32]. This report is often cited as if it were a study of many individuals with DS, but because it only sampled one individual and because of known variability in individuals with DS, more biopsies and postmortem samples should be performed in individuals with DS to validate the findings.

Biomarkers such as procollagen 1 amino-terminal peptide (P1NP) and C-terminal telopeptide (CTx) are used to measure bone formation and resorption, respectively. Before being conjugated into the bone matrix, the N- and C-terminals of procollagen are cleaved through enzymatic reactions in the osteoblast resulting in deposition of collagen and release of P1NP into the bloodstream. P1NP is a reliable bone formation biomarker and is currently being developed for clinical application. CTx, on the other hand, is released into the blood stream by osteoclastmediated degradation of collagen. While CTx is a specific and sensitive biomarker for bone resorption, measurements must be obtained in a fasting state because food intake decreases serum CTx concentrations [33]. P1NP serum concentrations in individuals with DS were significantly reduced compared to controls, but CTx levels were not significantly reduced [24]. Based on this information, it was hypothesized that the cellular defect in adults with DS lies not in osteoclast activity and resorption, but in decreased osteoblast activity and bone formation [24].

A major limitation of skeletal research in DS is the heterogeneity of analysis methods across studies. Large numbers of individuals in relevant age groups are seldom compared, sexes are sometimes pooled, measurements not always adjusted for size, and data presentation is not standardized (Supplemental Table 1). Some examples of these cases are as follows: 1) A study sampled only 22 individuals with DS, had no control group, pooled sexes, and presented only raw areal BMD data [34]. 2) A study sampled 94 individuals, compared male with female, presented serum hormone concentrations, areal BMD and Z-scores, but pooled all individuals with intellectual disability [35]. 3) A study sampled only 10 males and 9 females with DS, 7 males and 7 females without DS, and presented only Z-scores [36]. Because a thorough study of all ages is difficult, age, BMD, and other raw measures obtained from each subject should be included in the study instead of only adjusted values. Presented as such, data may be compared to previous studies, values may be adjusted according to current and future specifications, and individuals may be grouped appropriately by biological sex and age. Future studies should utilize DS outreach programs connecting caregivers of individuals with DS to each other for the recruitment participants of specific ages to studies [37].

4. Bone growth in individuals with DS

4.1 Prenatal bone growth

Two major hallmarks of DS, craniofacial abnormalities and reduced long bone length, are visible via ultrasonography performed during the second trimester but are considered soft markers rather than diagnostic markers for DS [38-40]. Nuchal thickening is the most common sonographic marker for DS, followed by shortened humerus. Femur length is a sensitive soft marker, but is the least correlated with DS and has the highest false positive rate [40, 41]. Shortened humerus and femur were the highest correlated sonographic markers. Unlike sonographic detection in the second trimester, only 9% of T21 fetuses had long bone shortening below the 5^{th} percentile during the first trimester and there was no correlation between nuchal thickening and long bone shortening [38]. The value of a single soft marker is low, but multiple aberrant soft markers suggest the need for further testing.

These findings suggest that some sonographic markers are present in early fetal development, may become more apparent over time, and lead to DS skeletal defects.

4.2 Childhood to Adolescence

The timing and velocity of bone growth in children with DS is different from the general population. Reduced bone mass accrual during childhood results in lower bone mineral density and bone area in the upper and lower limbs, predisposing adolescents to an increased risk of fragility fractures and osteoporosis [2, 21, 34, 35, 42, 43]. Growth velocity is severely affected from six months to three years of age and again at puberty [14]. Around seven years of age, the skeletal age of individuals with DS is delayed compared to chronological age; growth of the secondary ossification center is delayed until approximately eight years of age [42]; and bone growth of the appendicular skeleton during puberty in individuals with DS is stunted [14], all of which contribute to the shortened stature of children with DS. By the age of 15, however, DS skeletal age has advanced beyond chronological age and individuals with DS have reached their maximum height, compared to the general population who reach maximum height between 17-20 years of age [34, 42].

4.3 Reaching Peak Bone Mass

Peak bone mass, defined as the highest bone density value achieved during a lifetime, is generally considered to be attained near the end of the second decade of life shortly after closure of the epiphyseal growth plates [16, 17, 44]. Growth plate closure is sexually, spatially, and temporally dependent. Androgens drive periosteal expansion while suppressing endosteal contraction and estrogens drive endosteal contraction while suppressing periosteal expansion. This interplay of sex hormones drives a sexual dimorphism in bone resulting in endosteal bone accrual in pubescent girls and periosteal bone accrual in pubescent boys. Periosteal mineral apposition and expansion of the outer diameter of bone is more mechanically advantageous than endosteal mineral apposition and contraction of the inner diameter of bone [45]. Estrogen signals the closure of the growth plate in boys and girls and due to the higher production of estrogen in girls, the growth period ends earlier than in boys. Women therefore attain peak bone mass and maximal height earlier than men and have a lower overall peak bone mass. Peak axial skeletal bone mass in the general population is attained near the end of the second decade, but peak bone mass of the hip is attained between 14 and 18 years of age [16, 17]. Peak mass of the appendicular skeleton in the general population is normally attained near the end of the second decade of life but may be reached as early as 18 and as late as 35 years of age [44].

Individuals with DS reach peak bone mass 5-10 years earlier than the general population [19]. Hypogonadism is a common feature of DS, but more common in males than females. Testosterone levels in males with DS have been reported as similar or slightly lower than males in the general population [35, 46-48]. Males and females with DS approaching peak bone mass have significantly reduced BMAD in the lumbar spine at 20 years of age but not significantly lower femoral BMAD compared to the general population [22].

Reduced physical activity may contribute to low BMD in individuals with DS [34]. Adolescents with DS are less active than the general population, with an average of less than

60 minutes of moderate to vigorous activity each day [49, 50]. Low physical activity in adolescents with DS compared to the rest of the population has been correlated with low BMD, especially in females with DS [36, 51, 52]. Regular physical activity during adolescence increases skeletal gain by 30% compared to inactivity [53]. Application of physical activity programs in individuals with trisomy increased BMD and lean muscle mass [52, 54]. Mechanical stimulus may have a lesser effect on bone strength in individuals with DS, suggesting a defect in the mechanostat; however, the success of long-term physical activity programs focused on increasing lean muscle mass and BMD contradict that argument [52, 53]. Again, the major complication in studies of this nature is the lack of statistical power due to small sample sizes and pooling sexes. Future studies should include more participants, grouped by sex, with controls from the general population, and utilize long-term programs that promote active lifestyles.

4.4 Beyond Peak Bone Mass

Bone loss in the general population begins in the third decade of life after achieving peak bone mass [20, 44], and in individuals with DS, it may begin sooner because they reach peak bone mass 5-10 years earlier [19]. Following peak bone mass, bone remodeling results in a net negative balance as the basic multicellular unit (BMU) consisting of osteoclasts and osteoblasts resorbs more bone than is formed [20, 55-57]. Trabeculae in cancellous bone become less numerous while thickness is unchanged or increased and cortical bone becomes more porous [58]. In normal individuals, trabecular bone loss begins around age 30 and cortical bone loss begins around age 50 [20, 59, 60]. Only a single postmortem report exists that confirms trabecular thinning in the lumbar spine of a middle aged man with DS [32]. Women experience exaggerated bone loss following menopause, which highlights the importance of estrogen in bone homeostasis [60]. Estrogen represses osteoclastogenesis and prevents endosteal bone resorption [17, 60, 61]. Women with DS lose BMD in the femur at a similar rate as the general population; although they have significantly reduced BMD in the lumbar spine, there is not a high rate of bone loss in the lumbar spine like the general population [19, 22, 23].

Lumbar spine BMAD is significantly reduced in individuals with DS compared to normal individuals in both sexes but does not decline with age as dramatically as in the general population. Normal females experience a dramatic reduction in lumbar spine BMAD after menopause, nearly matching the BMAD of females with DS after 50 years of age. Sexual differences become more pronounced at middle age. Femoral neck BMAD in males with DS declines sharply across all age groups as a consequence of aging whereas females with DS do not begin to experience a sharp decline in femoral neck BMAD until after 40 years of age [19, 22]. Delayed decline in BMAD in the femoral neck of females until after menopause suggests a protective effect of estrogen in females with DS.

By advanced age, the differences in BMAD in the lumbar spine and femoral neck between individuals with DS and the general population have not been shown to be significantly different [22]. It is likely that vBMD differences have not reached significance levels in the literature due to the low sample size for individuals with DS who are 50 and beyond [22]. It

is surprising that despite having BMD deficits throughout their lives, females with DS have a similar BMD in the lumbar spine as females in the general population at advanced age [19].

Taken together, female biological sex may offer a protective effect against the trisomyrelated BMD loss after peak bone mass observed in males with DS. Males and females with DS experience a decline in lumbar spine and femoral neck BMD and BMAD at similar rates after reaching peak bone mass with little effect of menopause on females [19, 22]. Females with DS were found to have a less dramatic loss of BMD after menopause compared to females in the general population, suggesting a protective effect of trisomy. Males with DS generally have lower values compared to females with DS at all sites measured and it has been concluded that male biological sex predisposes individuals with DS to worse skeletal abnormalities than females [19].

Increased attention in the last few decades has contributed immensely toward improving the quality of life and lifespan of individuals with DS. The current standard of care for the general population can be applied to individuals with DS to improve quality of life, but there are features and processes unique to DS that must be unraveled to provide better personalized care. Not only are DS bone studies few in number, but the sample sizes are too small for specific age groups to characterize specific developmental or age-related phenotypes. Skeletal abnormalities caused by T21 arise during prenatal development as delayed growth and never catch up with the general population, but no study to date has determined what cellular or developmental mechanisms are dysregulated during that time. Future studies should analyze skeletal development in male and female children with DS in age groups before, during, and after puberty using standardized methods for imaging bone and quantifying serum metabolites indicative of bone cell activity. Because individuals with DS are living longer, skeletal aging in DS should also be given more attention by assessing the skeletal health of individuals with DS who are reaching advanced age. The benefits of this research would be two-fold: the lessons learned would not only enhance the quality of life in the DS population, but may also apply to the general population.

5. Sexual dimorphisms in DS bone

Males and females with DS have distinct skeletal differences compared to the general population. Because of low sample sizes in DS bone studies, it was perceived that males and females with DS had similar skeletal measures and consequently data were pooled to increase statistical power for comparison between DS and non-DS [26, 36, 52]. Studies with higher power revealed that males with DS begin losing BMD in the femur much earlier than females with DS, suggesting a protective effect of female biological sex in terms of maintaining BMD [19, 22, 23, 62]. Some have suggested that bone loss in males is due to low estradiol instead of testosterone [63, 64]. Further supporting this notion is that most females with DS use contraceptives and do not experience bone loss until after menopause, which begins on average at 46 years of age in females with DS [22, 23, 65]. Contraceptives are commonly used by females with DS to mitigate menstrual cramps, behavioral changes, and to prevent pregnancy in the event of sexual abuse, but the effect on bone density in this population has not been described [66, 67]. There may also be an age- and sex-specific response to physical activity [50].

6. Fracture risk vs rates in DS

Nearly all bone studies on DS suggest an increased fracture risk for myriad reasons (reduced BMD, reduced BMC, reduced physical activity, reduced visual perception/acuity, reduced coordination) [2, 19, 22, 24, 51, 68]. Despite the unanimous agreement that DS predisposes individuals to increased fracture risk at all ages, little data are available to confirm if individuals with DS have a correlative relationship with fracture risk [22, 23, 62]. A study of individuals with DS living in a clinic found that about 50% of the residents with DS and Alzheimer disease had experienced a long bone fracture and about 20% had experienced a vertebral fracture [69]. An analysis of 75 men and women with DS over the age of 18 as compared to 76 age and sex matched controls found an 11% as compared to 12% prevalence of fractures in individuals with DS as compared to controls [26]. Some information on skeletal health in both young and old individuals can be obtained from recent surveys including from the DS-Connect registry [37]. Of the 3490 people who responded to the DS-Connect survey on health conditions of individuals with DS (https://dsconnect.nih.gov/, accessed 8/21/2019), 521 (267 males) or 15% indicated that they had been diagnosed with a skeletal problem and 63 were unsure. When answering which skeletal problems they had, most reported atlanto-axial instability, fractures, osteopenia or osteoporosis (Table 1). In a more specific skeletal health questionnaire, 51/189 (25 males) or 27% of individuals with DS reported that they had a fracture or broken bone, and 7/189 (4%) reported they had been diagnosed with osteoporosis. Four individuals reported that they had used a vitamin D supplement, two each a calcium supplement or bisphosphonate medication, and one physical therapy as therapies for osteoporosis. Most people reported a fracture of either a leg or arm bone (Table 2) and most fractures occurred in individuals under 20 years of age (Table 3).

Data obtained from DS-Connect the questionnaire suggest that most fractures occurred in younger individuals, either due to the bone deficits present at early ages in individuals with DS or because of the lack of data from older individuals with DS. Additionally, not many individuals with DS had been diagnosed with osteopenia or osteoporosis, likely because they had not been tested (only four of eight people who responded said they had ever had a DXA scan). These limited data illustrate the need for a comprehensive understanding of skeletal deficits in DS throughout the lifetime.

7. Hypothesized cellular basis of skeletal abnormalities in humans with DS

The currently accepted view of the underlying cellular cause of bone phenotypes in DS is limited by the scope and sample size of current studies. Individuals with DS have significantly reduced P1NP compared to the normal population, and reduced but not significantly different CTx serum concentrations [24, 26]. These findings suggest that the underlying cellular process that disrupts bone homeostasis and promotes osteoporotic phenotypes is reduced bone formation and, to a lesser degree, reduced bone resorption. Yet, data presented to support the claims that individuals with DS have impaired bone formation but not bone resorption must be critically assessed. Serum biomarker data were pooled from males and females with DS ranging from 19 to 51 years of age, which may provide a

suitable comparison between the general population and the DS population, but because bone development and homeostasis is dynamic, this methodology does not provide insight into whether or not the defects observed occur only at a specific age or only in a specific sex [24]. The supposition that bone resorption is repressed in individuals with DS is supported by the analysis of one middle-aged individual with DS [32]. Other studies citing this article found that bone resorption was not significantly reduced in individuals with DS [24, 26]. Osteoblasts were found to be fewer in number and osteoclasts completely absent in the histological evaluation. P1NP was significantly reduced, matching the reduced osteoblasts, but CTx should be reduced if there are fewer osteoclasts. A reported interassay variability of 7.4 to 35.6% when measuring CTx may explain why the data failed to reach significance. It may also be possible that because data were pooled from a wide age range that the data does not match the histological evaluation of the lumbar spine from the 49-year old male with DS. There may be an age-dependent reduction in osteoblasts and osteoclasts as a result of trisomy that is impossible to detect when data from a wide age range are pooled.

The dynamic nature of bone during development and aging reveals that processes observed during fetal, child, adolescent, adult, and aging adults are unique to each age. Thus, sampling from a wide range of ages and pooling the data to test a general hypothesis complicates the understanding of what processes are actually defective at each age. Also, because bone cells respond differently to sex hormones, samples obtained from males and females should be examined and reported separately. Bone biopsies would provide the most powerful insight on the cellular processes governing skeletal growth and homeostasis in individuals with DS. Although less powerful, measurement of serum biomarkers is a less painful and more plausible method for examining skeletal homeostasis. P1NP and CTx should be measured in individuals at different ages to evaluate bone formation and resorption processes during critical milestones of skeletal development such as: puberty, peak height, peak bone mass, middle age, and advanced age. This experiment would help define when bone growth and decline occur and when certain therapies will benefit individuals with DS.

8. Mouse models of the DS skeleton

Mouse models of DS recapitulate some T21 skeletal phenotypes and have been used to better understand the ontogeny and progression of skeletal deficits and test potential therapies to improve DS bone. Much of what we know about the molecular and genetic defects in DS comes from the study of mouse models, but in the study of bone there are several caveats to consider. Although the growth plate never closes and both longitudinal and appositional growth slow dramatically at puberty, skeletal maturity in mice is attained between four and six months of age. Unlike humans, the growth plate of long bones never fully closes and longitudinal bone growth continues slowly after sexual maturity. Another difference between human and murine cortical bone is the absence of osteons in murine bone [70].

While there are diverse mouse models of DS, [71, 72], the Ts(17^{16})65Dn (Ts65Dn) mouse is the most well-studied model of DS and was used to characterize appendicular skeletal deficits in DS [73-75]. Ts65Dn mice contain a small extra translocation chromosome

homologous to human chromosome 21 (Hsa21) with 94 genes conserved in both humans and mice [76]. The freely segregating chromosome also contains ~35 extraneous protein coding Mmu17 genes that are not homologous to Hsa21, and subfertile Ts65Dn males are often used for experimental procedures, reserving the fertile females for colony maintenance [71]. Despite these caveats, Ts65Dn mice display several bone phenotypes that resemble those found in humans with DS including craniofacial deficits, small size, reduced BMD and trabecular and cortical skeletal deficits [3, 73, 77, 78]. Mechanical strength is also reduced in Ts65Dn mice which parallels increased fracture risk in humans with DS [79]. The appendicular skeletons of adolescent Ts65Dn mice exhibit reduced bone formation rate, reduced mineral apposition rate, and fewer osteoblasts and osteoclasts. P1NP, a bone formation marker, is unaffected at 3 months, but significantly depressed at 24 months [77, 79].

Skeletal studies on the Ts65Dn mouse model have produced data for mice aged 6, 12, 16, and 104 weeks. At all ages, percent bone volume, trabecular number, and separation were significantly lower in appendicular skeleton of Ts65Dn mice compared to euploid controls. Trabecular thickness is not significantly affected in any studies at any age [73, 77, 79]. Cortical measures such as cross-sectional area, cross-sectional perimeter, and mean polar moment of inertia in femurs from 6 and 16 weeks of age are also significantly decreased [73, 79]. Trisomic 6-week-old mice have significantly decreased ultimate force, stiffness, energy to failure, and toughness compared to euploid controls. Cross-examination of studies indicates that osteoclast area and number in the appendicular skeleton are increased in 6 week old, but decreased in 12 week old Ts65Dn mice compared to euploid mice [77, 79]. Supporting this observation, TRAP 5b and P1NP serum biomarkers for bone resorption and formation, respectively, are significantly decreased at 2 years, but not 12 weeks, suggesting a shift in osteoblast and osteoclast activity [77]. These data suggest that increased osteoclast activity at 6 weeks shifts the homeostatic balance of resorption and formation activity toward increased resorption and a net bone loss. Osteoblast and osteoclast activity are influenced by osteocytes embedded in bone. Old or dying osteocytes produce fewer secretory factors that govern osteoblast and osteoclast activity which compromises both bone repair and response to anabolic signals [80]. Net bone (and osteocyte) loss results in reduced signaling and may explain decreased activity at later timepoints (12 weeks and 2 years). Together, these data support the hypothesis that defects in the adult skeleton of Ts65Dn mice result from defective cellular processes earlier in development and not as a result of aging.

DYRK1A, a gene found in three copies in humans with DS and Ts65Dn mice, significantly contributes to many DS phenotypes including skeletal malformations [81, 82]. *Dyrk1a* transgenic mice (increased copy number of just *Dyrk1a*) exhibited significantly reduced bone mass, an osteoporotic phenotype, including percent bone volume, reduced trabecular skeletal parameters but not reduced cortical bone thickness [83]. The osteoporotic phenotype of *Dyrk1a* transgenic mice was characterized by osteoblast deficiencies, resulting in the low bone mass phenotype [83], suggesting that *Dyrk1a* alone has an important role in the DS trabecular phenotype. Returning *Dyrk1a* to two functional copies in otherwise trisomic 6 week old Ts65Dn mice (Ts65Dn,*Dyrk1a*+/−) rescued many of the femoral trabecular parameters, including BV/TV, to euploid levels [79]. Cortical bone was increased, though

not at euploid levels, and periosteal perimeter was not corrected in Ts65Dn,*Dyrk1a*+/− mice, suggesting that other trisomic genes besides *Dyrk1a* influence cortical phenotypes. Trisomic *Dyrk1a* does not, however, play a significant role in developing prenatal skeletal abnormalities in Ts65Dn embryos [84].

Ts1Rhr mice are trisomic for 33 genes orthologous to Hsa21 in the “Down syndrome critical region” (DSCR). Overexpression of the 33 DSCR genes was insufficient to cause DS like craniofacial dysmorphology found in Ts65Dn mice or individuals with DS [85, 86]. Bone abnormalities were examined in male and female Ts1Rhr mice at 3 and 16 weeks of age [86]. Using DXA, no differences in spine, femur, or tibia BMD were found at 3 weeks of age between Ts1Rhr and euploid mice, regardless of sex. Tibia BMD was significantly lower and spine BMD was significantly higher in Ts1Rhr mice at 16 weeks in both sexes. It may be that Ts1Rhr contained too few trisomic genes to significantly affect skeletal phenotypes at early ages, or significant differences in skeletal structures may have been seen using additional methodologies including microCT and mechanical testing.

9. Potential treatments of bone abnormalities in humans with DS

Some interventions to improve skeletal structure in individuals with DS have been attempted and others may have promise. Individuals with DS generally have reduced physical and cardiovascular fitness, reduced general muscle strength, lower BMC and BMD, and usually live a sedentary lifestyle. BMI is usually not significantly different between children with DS and children without intellectual disability, but is more likely to be significantly different in adolescents and adults [51]. Exercise intervention in individuals with DS has produced mixed results. Participants displayed significant improvements after weight and strength training. A combined cardiovascular and strength training program involving aerobic activity and weight training resulted in improvements in aerobic activity and anaerobic power [51]. Cardiovascular training reduced fat mass and lengthened time to exhaustion, but other measures were not significant, suggesting that longer periods of training and higher training intensity may result in improving cardiovascular health [51].

Vitamin D and calcium are key components of bone health that are consistently lower when measured in individuals with DS [43, 52, 62]. Vitamin D deficiency resulting from poor intake or malabsorption has a detrimental effect on bone quality, resulting in lower BMC and BMD, during periods of bone mass accrual, typically during puberty and adolescence [43, 52, 87]. Despite vitamin D supplementation by 94% of the individuals with DS that were sampled, their plasma vitamin D concentrations were still considered insufficient [62]. Individuals with DS who were given calcium and vitamin D supplements for one year, responded with a marked improvement in serum vitamin D concentrations, decreased serum parathyroid hormone (PTH), and decreased osteocalcin [88]. It is generally agreed that individuals with DS need supplementation of vitamin D and calcium, but a standard intake for each age has not yet been established [43].

Epigallocatechin 3-gallate (EGCG) inhibits the Hsa21 protein DYRK1A in vitro [89], and has been a candidate molecule for treatment of DS phenotypes [90]. Low doses of about 9mg/kg/day pure EGCG improved trabecular but not cortical bone phenotypes in the femur

of Ts65Dn (and euploid) mice at 6 weeks of age [79]. Mineralization defects inherent to Ts65Dn mice were improved with low doses of pure EGCG, but there was no translational effect on bone strength. Pure EGCG doses of 50mg/kg/day were detrimental to bone architecture and strength in Ts65Dn mice [91]. When two different green tea extracts containing EGCG were given to Ts65Dn mice, they seemed to improve trabecular structure and percent bone volume, although the given concentration of EGCG was less than anticipated by EGCG content on the label [92]. Bone strength in mice given the green tea extract containing EGCG was reduced. After 12 months of treatment with green tea extracts containing EGCG, there was reduced bone mass in the individuals with DS, ages 16-34 years, especially in females [93, 94]. EGCG has very low bioavailability; the small amount of EGCG that is absorbed from the gut to the bloodstream has an elimination half-life of about 3.4 hours and is undetectable after 24 hours [95]. The effects of EGCG on skeletal parameters may be dependent on concentration of EGCG and other catechins that are included in the treatment [90, 96].

The skeletal defects observed in DS may not be a consequence of increased resorption, and thus would limit the utility of bisphosphonates that are the most commonly prescribed drug for preventing bone loss in the general population. Bisphosphonates may, however, be effectively utilized in combination with anabolic agents to abrogate a plateau effect during treatment or bone loss after cessation of treatment. Because the source of skeletal abnormalities in DS may arise from defects in bone accrual, parathyroid hormone (PTH) and parathyroid hormone related protein (PTHrP) analogs such as abaloparatide and teriparatide may be more appropriate for treatment in DS due to the anabolic effect when administered intermittently [24, 97]. Continuous exposure stimulates receptor activator of κ-B ligand (RANKL) production in osteoblasts and osteocytes, promoting osteoclastogenesis and bone resorption. Conversely, intermittent exposure inhibits sclerostin production and stimulates osteoblastogenesis and bone formation [98, 99]. Treatment of the Ts65Dn DS mouse model with intermittent doses of PTH increased %BV/TV, mineral apposition rate, bone formation rate, and the number of osteoblasts [77]. This strategy for abrogating bone loss in individuals with DS is not recommended because DS predisposes individuals to an increased risk acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML), which are exacerbated by PTH receptor activation [100, 101]. Studies demonstrating cancer growth and metastases as a result of PTH treatment, however, were performed in immune-compromised mice receiving injections of human breast cancer cells [102, 103]. More work must be done to demonstrate the potential harm or benefit of PTH in individuals with DS before it is used as a treatment to improve bone health.

Denosumab, a RANKL antibody designed to inhibit RANK-RANKL signaling that stimulates osteoclast differentiation, osteoclast activity, and bone metastasis [104, 105], has not been administered to individuals with DS or mouse models of DS, but may be safer than PTH or PTHrP analogs because it is used to treat osteosarcomas, the very disease that is exacerbated by PTH or PTHrP analog treatment. The long-term effects of Denosumab treatment in postmenopausal women has been well studied and has demonstrated that the drug is well tolerated, reduces fracture risk, reduces cancer metastasis and bone loss associated with cancer treatments, and increases BMD without plateauing over 10 years [104-108]. Long-term treatment side effects are limited to increased risk of infection and

osteonecrosis of the jaw [105]. Cessation of treatment is correlated with increased fracture risk of the lumbar spine and rapid loss of BMD [109]. Like bisphosphonates, the action of denosumab is anti-resorptive, and may limit the efficacy of treatment in individuals with DS.

Sclerostin, a protein coded by the *SOST* gene, is secreted by osteocytes. When detected by osteoblasts, the Wnt pathway is suppressed and RANKL production increases. The net effect is downregulation of osteoblast activity and increased osteoclast activation[110]. *Sost* knockout mice were found to have high bone mass with no response to mechanical loading, which recapitulated the human condition. High bone mass accrual was attributed to increased Wnt/β-catenin signaling[111]. Short- and long-term treatment in mice increased overall BMD, cortical, and trabecular measures. When sclerostin antibody was given to 8-week-old Ts65Dn mice for 28 days, it increased osteoblast activity and normalized BMD to euploid levels, improved trabecular architecture, and some cortical measurements [112]. Romosozumab (sclerostin antibody trade name) is a humanized monoclonal antibody that has undergone phase III clinical trials that concluded it was an effective anabolic drug with few side effects [110] that may be a candidate therapy in individuals with DS.

10. Conclusions

The majority of research on individuals with DS in past decades has largely been directed towards cognitive health [72, 90] and comprehensive skeletal examinations are lacking. Current data suggest some phenotypes in DS are a result of a defect in development and homeostasis as opposed to typical aging mechanisms [73], and this, at the least, contributes to DS bone deficits. Although cellular mechanisms for DS skeletal deficits have been discussed [24, 26, 73, 84, 113], no genetic or molecular mechanism has been identified as the absolute cause of all aberrant bone phenotypes in DS. Additionally, different mechanisms with adverse effects on bone may come into play at different ages in individuals with T21. As such, future studies should focus on ages critical to bone development or ages following peak bone mass during which rapid loss of BMD is observed to determine mechanisms responsible for developmental and homeostatic defects. As individuals with DS increase in average age, they will likely be subject to more effects of old age, including osteoporosis and skeletal fractures. Understanding the developmental bases of these bone phenotypes may help to prevent the adverse effects of weak bones later on in life; understanding additional adverse mechanisms affecting bone later in life and how they compound these developmental abnormalities may also be necessary.

It appears that the mechanisms of bone loss in individuals with DS are different from the general population, and there is a need for further study. Mouse models of DS have established that skeletal defects arise before peak bone mass, and these studies have led to proposed cellular mechanisms for DS skeletal deficits [73, 77, 79, 84]. Yet, different mechanisms for trisomic skeletal deficits have been proposed and these disparities may be a function of the skeletal ages studied when the mechanisms were proposed. Studies in humans with DS and DS mouse models should be conducted in a longitudinal manner, and assume trisomic skeletal abnormalities are a snapshot of a dynamic system, rather than considering deficits to be constant at all ages. It is likely that specific genetic and molecular mechanisms are differentially regulated in an age-dependent manner during development.

Future studies should focus on physiologically relevant ages in terms of skeletal health and development and clearly state the reasoning for choosing a specific age.

Past studies in individuals with DS have provided an incomplete story with gaps and inconsistencies because skeletal features associated with DS are uncharacterized, studies are underpowered and often do not have sufficient individuals at important skeletal ages, and measurement methods do not follow a standardized protocol. The variation in phenotypes and results from individuals with DS further confound the data available, making comparisons between studies difficult. Because of the wide variability associated with DS phenotypes, sample sizes should be adequately powered. Future studies may consider grouping individuals by their skeletal age, as measured by hand and wrist x-rays, rather than their chronological age. Developmental studies and lumbar spine measurements may determine the difference between the BMD of the subject and peak bone mass. Height should be measured to determine the difference between the subject's height and peak height. Studies on the aging DS population should measure both lumbar spine and femoral neck/hip, as the BMD in these regions may decline in an age-and sex-dependent manner. Finally, both male and female data should be analyzed separately to characterize unique sexually dimorphic differences in the DS population. Male biological sex may be a risk factor for skeletal defects in DS; conversely, female biological sex seems to be protective, especially at early ages. It may be that the somewhat "protective effect" observed in females with DS could be attributed to contraceptive use, but this has not been explored. Incorporating the aforementioned changes to future studies will propel the field closer to developing therapeutics to improve bone health and the quality of life in individuals with DS as their lifespan nears that of the general population.

Increased rigor should be applied to skeletal studies to tease out genetic and molecular mechanisms; microCT and mechanical testing provide many measurements that assess bone quality much better than BMD, as provided by DXA. Skeletal phenotypes should be characterized utilizing different DS mouse models and include both sexes to facilitate more accurate detection of gene-phenotype correlations. Elucidation of defective mechanisms in mouse models of DS may be extrapolated to humans, offering the potential for treatment and improved quality of life.

While it is likely that aberrant skeletal development is modulated by a network of genes, a single trisomic gene such as *DYRK1A* may cause a number of adverse skeletal phenotypes and manipulation or reducing the activity of the gene may dosage improve skeletal phenotypes [79]. Relevant skeletal gene expression should be characterized at the ages of interest to identify genetic or molecular mechanisms that may be responsible for the emergent phenotypes. Validation of DYRK1A as a pharmacological target suggests that pharmacological inhibition with more specific and bioavailable inhibitors may yield greater improvements. A correlation between a genetic mechanism and a phenotypic outcome during development will allow for a genetic normalization prior to the emergence of the aberrant phenotype, then a pharmaceutical inhibition of the causative protein before adverse phenotypes develop [90].

Currently, there is not enough information on bone deficits in the DS population to suggest comprehensive strategies to improve skeletal phenotypes. Future studies should prioritize sample sizes with adequate power in both sexes at ages relevant to bone development in children, adolescents, and individuals at advanced age. Dysfunctional developmental mechanisms leading to defective bone architecture as well as potential later stage skeletal degeneration occur at time points that have not yet been defined. Therefore, treatment strategies must be preceded by the identification of a developmental or later stage window that is dysregulated by trisomy before specific molecular targeting with bioavailable pharmacological agents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

[1]. de Graaf G, Buckley F, Skotko BG, Estimates of the live births, natural losses, and elective terminations with Down syndrome in the United States, Am J Med Genet A 167A(4) (2015) 756–67. [PubMed: 25822844]

[2]. Baptista F, Varela A, Sardinha LB, Bone mineral mass in males and females with and without Down syndrome, Osteoporos Int 16(4) (2005) 380–8. [PubMed: 15365695]

[3]. Richtsmeier JT, Baxter LL, Reeves RH, Parallels of craniofacial maldevelopment in Down syndrome and Ts65Dn mice, Dev Dyn 217(2) (2000) 137–45. [PubMed: 10706138]

[4]. Barden HS, Growth and development of selected hard tissues in Down syndrome: a review, Hum Biol 55(3) (1983) 539–76. [PubMed: 6227546]

[5]. Ali FE, Al-Bustan MA, Al-Busairi WA, Al-Mulla FA, Esbaita EY, Cervical spine abnormalities associated with Down syndrome, Int Orthop 30(4) (2006) 284–9. [PubMed: 16525818]

[6]. Stanley MA, Shepherd N, Duvall N, Jenkinson SB, Jalou HE, Givan DC, Steele GH, Davis C, Bull MJ, Watkins DU, Roper RJ, Clinical identification of feeding and swallowing disorders in 0-6 month old infants with Down syndrome, Am J Med Genet A 179(2) (2019) 177–182. [PubMed: 30588741]

[7]. Bittles AH, Glasson EJ, Clinical, social, and ethical implications of changing life expectancy in Down syndrome, Dev Med Child Neurol 46(4) (2004) 282–6. [PubMed: 15077706]

[8]. Esbensen AJ, Johnson EB, Amaral JL, Tan CM, Macks R, Differentiating Aging Among Adults With Down Syndrome and Comorbid Dementia or Psychopathology, Am J Intellect Dev Disabil 121(1) (2016) 13–24. [PubMed: 26701071]

[9]. Englund A, Jonsson B, Zander CS, Gustafsson J, Anneren G, Changes in mortality and causes of death in the Swedish Down syndrome population, Am J Med Genet A 161A(4) (2013) 642–9. [PubMed: 23436430]

[10]. Bittles AH, Bower C, Hussain R, Glasson EJ, The four ages of Down syndrome, Eur J Public Health 17(2) (2007) 221–5. [PubMed: 16857692]

[11]. Irving CA, Chaudhari MP, Cardiovascular abnormalities in Down's syndrome: spectrum, management and survival over 22 years, Arch Dis Child 97(4) (2012) 326–30. [PubMed: 21835834]

[12]. Schut PC, Ten Broek CMA, Cohen-Overbeek TE, Bugiani M, Steegers EAP, Eggink AJ, Galis F, Increased prevalence of abnormal vertebral patterning in fetuses and neonates with trisomy 21, J Matern Fetal Neonatal Med 32(14) (2019) 2280–2286. [PubMed: 29353507]

[13]. Maymon R, Tovbin Y, Dreazen E, Weinraub Z, Herman A, All five digits of the hands of fetuses with Down syndrome are short, Ultrasound Obstet Gynecol 23(6) (2004) 557–60. [PubMed: 15170795]

[14]. Myrelid A, Gustafsson J, Ollars B, Anneren G, Growth charts for Down's syndrome from birth to 18 years of age, Arch Dis Child 87(2) (2002) 97–103. [PubMed: 12138052]

[15]. Zemel BS, Pipan M, Stallings VA, Hall W, Schadt K, Freedman DS, Thorpe P, Growth Charts for Children With Down Syndrome in the United States, Pediatrics 136(5) (2015) e1204–11. [PubMed: 26504127]

[16]. Weaver CM, Fuchs RK, Skeletal Growth and Development, Basic and Applied Bone Biology (2014) 245–260.

[17]. Bellido T, Gallant KMH, Hormonal Effects on Bone Cells, Basic and Applied Bone Biology (2014) 299–314.

[18]. Arnell H, Gustafsson J, Ivarsson SA, Anneren G, Growth and pubertal development in Down syndrome, Acta Paediatr 85(9) (1996) 1102–6. [PubMed: 8888926]

[19]. Costa R, Gullon A, De Miguel R, de Asua DR, Bautista A, Garcia C, Suarez C, Castaneda S, Moldenhauer F, Bone Mineral Density Distribution Curves in Spanish Adults With Down Syndrome, Journal of clinical densitometry : the official journal of the International Society for Clinical Densitometry (2018).

[20]. Almeida M, Aging mechanisms in bone, Bonekey Rep 1 (2012).

[21]. Barnhart RC, Connolly B, Aging and Down syndrome: implications for physical therapy, Physical therapy 87(10) (2007) 1399–406. [PubMed: 17712035]

[22]. Carfi A, Liperoti R, Fusco D, Giovannini S, Brandi V, Vetrano DL, Meloni E, Mascia D, Villani ER, Manes Gravina E, Bernabei R, Onder G, Bone mineral density in adults with Down syndrome, Osteoporos Int 28(10) (2017) 2929–2934. [PubMed: 28685282]

[23]. Tang JYM, Luo H, Wong GHY, Lau MMY, Joe GM, Tse MA, Ip P, Wong ICK, Lum TYS, Bone mineral density from early to middle adulthood in persons with Down syndrome, Journal of intellectual disability research : JIDR (2019).

[24]. McKelvey KD, Fowler TW, Akel NS, Kelsay JA, Gaddy D, Wenger GR, Suva LJ, Low bone turnover and low bone density in a cohort of adults with Down syndrome, Osteoporos Int 24(4) (2013) 1333–8. [PubMed: 22903293]

[25]. Allen MR, Krohn K, Skeletal Imaging, Basic and Applied Bone Biology (2014) 93–113.

[26]. Garcia-Hoyos M, Garcia-Unzueta MT, de Luis D, Valero C, Riancho JA, Diverging results of areal and volumetric bone mineral density in Down syndrome, Osteoporos Int 28(3) (2017) 965–972. [PubMed: 27838733]

[27]. Garcia Hoyos M, Humbert L, Salmon Z, Riancho JA, Valero C, Analysis of volumetric BMD in people with Down syndrome using DXA-based 3D modeling, Arch Osteoporos 14(1) (2019) 98. [PubMed: 31494745]

[28]. Taton G, Rokita E, Wrobel A, Korkosz M, Combining areal DXA bone mineral density and vertebrae postero-anterior width improves the prediction of vertebral strength, Skeletal Radiol 42(12) (2013) 1717–25. [PubMed: 24081424]

[29]. Ensrud KE, Epidemiology of fracture risk with advancing age, J Gerontol A Biol Sci Med Sci 68(10) (2013) 1236–42. [PubMed: 23833201]

[30]. Ma XH, Zhang W, Wang Y, Xue P, Li YK, Comparison of the Spine and Hip BMD Assessments Derived from Quantitative Computed Tomography, Int J Endocrinol 2015 (2015) 675340. [PubMed: 26273301]

[31]. Rosenthall L, Range of change of measured BMD in the femoral neck and total hip with rotation in women, J Bone Miner Metab 22(5) (2004) 496–9. [PubMed: 15316872]

[32]. Grimwood JS, Kumar A, Bickerstaff DR, Suvarna SK, Histological assessment of vertebral bone in a Down's syndrome adult with osteoporosis, Histopathology 36(3) (2000) 279–80. [PubMed: 10809596]

[33]. Kuo TR, Chen CH, Bone biomarker for the clinical assessment of osteoporosis: recent developments and future perspectives, Biomark Res 5 (2017) 18. [PubMed: 28529755]

[34]. Angelopoulou N, Souftas V, Sakadamis A, Mandroukas K, Bone mineral density in adults with Down's syndrome, Eur Radiol 9(4) (1999) 648–51. [PubMed: 10354878]

[35]. Center J, Beange H, McElduff A, People with mental retardation have an increased prevalence of osteoporosis: a population study, American journal of mental retardation : AJMR 103(1) (1998) 19–28. [PubMed: 9678227]

[36]. Matute-Llorente A, Gonzalez-Aguero A, Gomez-Cabello A, Vicente-Rodriguez G, Casajus JA, Decreased levels of physical activity in adolescents with down syndrome are related with low bone mineral density: a cross-sectional study, BMC endocrine disorders 13 (2013) 22. [PubMed: 23826810]

[37]. Peprah EK, Parisi MA, Kaeser L, Bardhan S, Oster-Granite M, Maddox YT, DS-Connect: A Promising Tool to Improve Lives and Engage Down Syndrome Communities Worldwide, Glob Heart 10(4) (2015) 337–40. [PubMed: 26271554]

[38]. Longo D, DeFigueiredo D, Cicero S, Sacchini C, Nicolaides KH, Femur and humerus length in trisomy 21 fetuses at 11-14 weeks of gestation, Ultrasound Obstet Gynecol 23(2) (2004) 143–7. [PubMed: 14770393]

[39]. Bromley B, Lieberman E, Shipp TD, Benacerraf BR, Fetal nose bone length: a marker for Down syndrome in the second trimester, J Ultrasound Med 21(12) (2002) 1387–94. [PubMed: 12494981]

[40]. Bromley B, Lieberman E, Shipp TD, Benacerraf BR, The genetic sonogram: a method of risk assessment for Down syndrome in the second trimester, J Ultrasound Med 21(10) (2002) 1087–96; quiz 1097-8. [PubMed: 12369663]

[41]. Nyberg DA, Souter VL, El-Bastawissi A, Young S, Luthhardt F, Luthy DA, Isolated sonographic markers for detection of fetal Down syndrome in the second trimester of pregnancy, J Ultrasound Med 20(10) (2001) 1053–63. [PubMed: 11587012]

[42]. de Moraes ME, Tanaka JL, de Moraes LC, Filho EM, de Melo Castilho JC, Skeletal age of individuals with Down syndrome, Special care in dentistry : official publication of the American Association of Hospital Dentists, the Academy of Dentistry for the Handicapped, and the American Society for Geriatric Dentistry 28(3) (2008) 101–6.

[43]. Hawli Y, Nasrallah M, El-Hajj Fuleihan G, Endocrine and musculoskeletal abnormalities in patients with Down syndrome, Nat Rev Endocrinol 5(6) (2009) 327–34. [PubMed: 19421241]

[44]. Mora S, Gilsanz V, Establishment of peak bone mass, Endocrinol Metab Clin North Am 32(1) (2003) 39–63. [PubMed: 12699292]

[45]. Ammann P, Rizzoli R, Bone strength and its determinants, Osteoporos Int 14 Suppl 3 (2003) S13–8. [PubMed: 12730800]

[46]. Peters A, Culley W, Urinary levels of testosterone and epitestosterone in Down's syndrome (mongolism), Clin Chim Acta 25(2) (1969) 199–203. [PubMed: 4308304]

[47]. Pueschel SM, Orson JM, Boylan JM, Pezzullo JC, Adolescent development in males with Down syndrome, Am J Dis Child 139(3) (1985) 236–8. [PubMed: 3156491]

[48]. Hsiang YH, Berkovitz GD, Bland GL, Migeon CJ, Warren AC, Gonadal function in patients with Down syndrome, Am J Med Genet 27(2) (1987) 449–58. [PubMed: 2955699]

[49]. Fox B, Moffett GE, Kinnison C, Brooks G, Case LE, Physical Activity Levels of Children With Down Syndrome, Pediatr Phys Ther 31(1) (2019) 33–41. [PubMed: 30507856]

[50]. Matute-Llorente A, Gonzalez-Aguero A, Vicente-Rodriguez G, Sardinha LB, Baptista F, Casajus JA, Physical activity and bone mineral density at the femoral neck subregions in adolescents with Down syndrome, J Pediatr Endocrinol Metab 30(10) (2017) 1075–1082. [PubMed: 28902628]

[51]. Gonzalez-Aguero A, Vicente-Rodriguez G, Moreno LA, Guerra-Balic M, Ara I, Casajus JA, Health-related physical fitness in children and adolescents with Down syndrome and response to training, Scand J Med Sci Sports 20(5) (2010) 716–24. [PubMed: 20456681]

[52]. Ferry B, Gavris M, Tifrea C, Serbanoiu S, Pop AC, Bembea M, Courteix D, The bone tissue of children and adolescents with Down syndrome is sensitive to mechanical stress in certain skeletal locations: a 1-year physical training program study, Res Dev Disabil 35(9) (2014) 2077–84. [PubMed: 24878629]

[53]. Robling AG, Fuchs RK, Burr DB, Mechanical Adaptation, Basic and Applied Bone Biology (2014) 175–204.

[54]. Gonzalez-Aguero A, Vicente-Rodriguez G, Gomez-Cabello A, Ara I, Moreno LA, Casajus JA, A 21-week bone deposition promoting exercise programme increases bone mass in young people with Down syndrome, Dev Med Child Neurol 54(6) (2012) 552–6. [PubMed: 22409634]

[55]. Demontiero O, Vidal C, Duque G, Aging and bone loss: new insights for the clinician, Ther Adv Musculoskelet Dis 4(2) (2012) 61–76. [PubMed: 22870496]

[56]. Bellido T, Plotkin LI, Bruzzaniti A, Bone Cells, Basic and Applied Bone Biology (2014) 27–45.

[57]. Allen MR, Burr DB, Bone Modeling and Remodeling, Basic and Applied Bone Biology (2014) 75–90.

[58]. Gabet Y, Bab I, Microarchitectural changes in the aging skeleton, Curr Osteoporos Rep 9(4) (2011) 177–83. [PubMed: 21901426]

[59]. Khosla S, Pathogenesis of age-related bone loss in humans, J Gerontol A Biol Sci Med Sci 68(10) (2013) 1226–35. [PubMed: 22923429]

[60]. Manolagas SC, O'Brien CA, Almeida M, The role of estrogen and androgen receptors in bone health and disease, Nat Rev Endocrinol 9(12) (2013) 699–712. [PubMed: 24042328]

[61]. Riggs BL, The mechanisms of estrogen regulation of bone resorption, J Clin Invest 106(10) (2000) 1203–4. [PubMed: 11086020]

[62]. Costa R, De Miguel R, Garcia C, de Asua DR, Castaneda S, Moldenhauer F, Suarez C, Bone Mass Assessment in a Cohort of Adults With Down Syndrome: A Cross-Sectional Study, Intellect Dev Disabil 55(5) (2017) 315–324. [PubMed: 28972872]

[63]. Mellstrom D, Vandenput L, Mallmin H, Holmberg AH, Lorentzon M, Oden A, Johansson H, Orwoll ES, Labrie F, Karlsson MK, Ljunggren O, Ohlsson C, Older men with low serum estradiol and high serum SHBG have an increased risk of fractures, J Bone Miner Res 23(10) (2008) 1552–60. [PubMed: 18518773]

[64]. Vandenput L, Ohlsson C, Estrogens as regulators of bone health in men, Nat Rev Endocrinol 5(8) (2009) 437–43. [PubMed: 19528961]

[65]. Seltzer GB, Schupf N, Wu HS, A prospective study of menopause in women with Down's syndrome, J Intellect Disabil Res 45(Pt 1) (2001) 1–7. [PubMed: 11168771]

[66]. Wandresen G, Sgarbi F, Nisihara R, Management of contraceptives and menstrual complaints in patients with Down syndrome, Gynecol Endocrinol 35(2) (2019) 103–108. [PubMed: 30324830]

[67]. Scola PS, Pueschel SM, Menstrual cycles and basal body temperature curves in women with Down syndrome, Obstet Gynecol 79(1) (1992) 91–4. [PubMed: 1530775]

[68]. Merrick J, Koslowe K, Refractive errors and visual anomalies in Down syndrome, Downs Syndr Res Pract 6(3) (2001) 131–3. [PubMed: 11501216]

[69]. van Allen MI, Fung J, Jurenka SB, Health care concerns and guidelines for adults with Down syndrome, Am J Med Genet 89(2) (1999) 100–10. [PubMed: 10559765]

[70]. Jilka RL, The relevance of mouse models for investigating age-related bone loss in humans, The journals of gerontology. Series A, Biological sciences and medical sciences 68(10) (2013) 1209–17. [PubMed: 23689830]

[71]. Gupta M, Dhanasekaran AR, Gardiner KJ, Mouse models of Down syndrome: gene content and consequences, Mamm Genome 27(11-12) (2016) 538–555. [PubMed: 27538963]

[72]. Herault Y, Delabar JM, Fisher EMC, Tybulewicz VLJ, Yu E, Brault V, Rodent models in Down syndrome research: impact and future opportunities, Dis Model Mech 10(10) (2017) 1165–1186. [PubMed: 28993310]

[73]. Blazek JD, Gaddy A, Meyer R, Roper RJ, Li J, Disruption of bone development and homeostasis by trisomy in Ts65Dn Down syndrome mice, Bone 48(2) (2011) 275–80. [PubMed: 20870049]

[74]. Reeves RH, Irving NG, Moran TH, Wohn A, Kitt C, Sisodia SS, Schmidt C, Bronson RT, Davisson MT, A mouse model for Down syndrome exhibits learning and behaviour deficits, Nat Genet 11(2) (1995) 177–84. [PubMed: 7550346]

[75]. Blazek JD, Billingsley CN, Newbauer A, Roper RJ, Embryonic and not maternal trisomy causes developmental attenuation in the Ts65Dn mouse model for Down syndrome, Dev Dyn 239(6) (2010) 1645–53. [PubMed: 20503361]

[76]. Gardiner K, Fortna A, Bechtel L, Davisson MT, Mouse models of Down syndrome: how useful can they be? Comparison of the gene content of human chromosome 21 with orthologous mouse genomic regions, Gene 318 (2003) 137–47. [PubMed: 14585506]

[77]. Fowler TW, McKelvey KD, Akel NS, Vander Schilden J, Bacon AW, Bracey JW, Sowder T, Skinner RA, Swain FL, Hogue WR, Leblanc DB, Gaddy D, Wenger GR, Suva LJ, Low bone turnover and low BMD in Down syndrome: effect of intermittent PTH treatment, PLoS One 7(8) (2012) e42967. [PubMed: 22916188]

[78]. Roper RJ, St John HK, Philip J, Lawler A, Reeves RH, Perinatal loss of Ts65Dn Down syndrome mice, Genetics 172(1) (2006) 437–43. [PubMed: 16172497]

[79]. Blazek JD, Abeysekera I, Li J, Roper RJ, Rescue of the abnormal skeletal phenotype in Ts65Dn Down syndrome mice using genetic and therapeutic modulation of trisomic Dyrk1a, Hum Mol Genet 24(20) (2015) 5687–96. [PubMed: 26206885]

[80]. Bonewald LF, The Role of the Osteocyte in Bone and Nonbone Disease, Endocrinol Metab Clin North Am 46(1) (2017) 1–18. [PubMed: 28131126]

[81]. Arron JR, Winslow MM, Polleri A, Chang CP, Wu H, Gao X, Neilson JR, Chen L, Heit JJ, Kim SK, Yamasaki N, Miyakawa T, Francke U, Graef IA, Crabtree GR, NFAT dysregulation by increased dosage of DSCR1 and DYRK1A on chromosome 21, Nature 441(7093)(2006)595–600. [PubMed: 16554754]

[82]. Duchon A, Herault Y, DYRK1A, a Dosage-Sensitive Gene Involved in Neurodevelopmental Disorders, Is a Target for Drug Development in Down Syndrome, Frontiers in behavioral neuroscience 10 (2016) 104. [PubMed: 27375444]

[83]. Lee Y, Ha J, Kim HJ, Kim YS, Chang EJ, Song WJ, Kim HH, Negative feedback Inhibition of NFATc1 by DYRK1A regulates bone homeostasis, J Biol Chem 284(48) (2009) 33343–51. [PubMed: 19801542]

[84]. Blazek JD, Malik AM, Tischbein M, Arbones ML, Moore CS, Roper RJ, Abnormal mineralization of the Ts65Dn Down syndrome mouse appendicular skeleton begins during embryonic development in a Dyrk1a-independent manner, Mech Dev 136 (2015) 133–42. [PubMed: 25556111]

[85]. Olson LE, Richtsmeier JT, Leszl J, Reeves RH, A chromosome 21 critical region does not cause specific Down syndrome phenotypes, Science 306(5696) (2004) 687–90. [PubMed: 15499018]

[86]. Olson LE, Mohan S, Bone density phenotypes in mice aneuploid for the Down syndrome critical region, Am J Med Genet A 155(10) (2011) 2436–45.

[87]. El-Hajj Fuleihan G, Nabulsi M, Tamim H, Maalouf J, Salamoun M, Khalife H, Choucair M, Arabi A, Vieth R, Effect of vitamin D replacement on musculoskeletal parameters in school children: a randomized controlled trial, J Clin Endocrinol Metab 91(2) (2006) 405–12. [PubMed: 16278262]

[88]. Zubillaga P, Garrido A, Mugica I, Ansa J, Zabalza R, Emparanza JI, Effect of vitamin D and calcium supplementation on bone turnover in institutionalized adults with Down's Syndrome, Eur J Clin Nutr 60(5) (2006) 605–9. [PubMed: 16391575]

[89]. Bain J, McLauchlan H, Elliott M, Cohen P, The specificities of protein kinase inhibitors: an update, The Biochemical journal 371(Pt 1) (2003) 199–204. [PubMed: 12534346]

[90]. Stringer M, Goodlett CR, Roper RJ, Targeting trisomic treatments: optimizing Dyrk1a inhibition to improve Down syndrome deficits, Mol Genet Genomic Med 5(5) (2017) 451–465. [PubMed: 28944229]

[91]. Stringer M, Abeysekera I, Thomas J, LaCombe J, Stancombe K, Stewart RJ, Dria KJ, Wallace JM, Goodlett CR, Roper RJ, Epigallocatechin-3-gallate (EGCG) consumption in the Ts65Dn model of down syndrome fails to improve behavioral deficits and is detrimental to skeletal phenotypes, Physiology & behavior 177 (2017) 230–241. [PubMed: 28478033]

[92]. Abeysekera I, Thomas J, Georgiadis TM, Berman AG, Hammond MA, Dria KJ, Wallace JM, Roper RJ, Differential effects of Epigallocatechin-3-gallate containing supplements on correcting skeletal defects in a Down syndrome mouse model, Mol Nutr Food Res 60(4) (2016) 717–726. [PubMed: 26748562]

[93]. Xicota L, Rodriguez J, Langohr K, Fito M, Dierssen M, de la Torre R, T.s. group, Effect of epigallocatechin gallate on the body composition and lipid profile of down syndrome individuals: Implications for clinical management, Clinical nutrition (2019).

[94]. De la Torre R, de Sola S, Hernandez G, Farre M, Pujol J, Rodriguez J, Espadaler JM, Langohr K, Cuenca-Royo A, Principe A, Xicota L, Janel N, Catuara-Solarz S, Sanchez-Benavides G, Blehaut H, Duenas-Espin I, Del Hoyo L, Benejam B, Blanco-Hinojo L, Videla S, Fito M, Delabar JM, Dierssen M, T.s. group, Safety and efficacy of cognitive training plus epigallocatechin-3-gallate in young adults with Down's syndrome (TESDAD): a double-blind, randomised, placebo-controlled, phase 2 trial, The Lancet. Neurology 15(8) (2016) 801–10. [PubMed: 27302362]

[95]. Mereles D, Hunstein W, Epigallocatechin-3-gallate (EGCG) for clinical trials: more pitfalls than promises?, Int J Mol Sci 12(9) (2011) 5592–603. [PubMed: 22016611]

[96]. Roper RJ, Goodlett CR, Can Green Tea Polyphenols Improve Phenotypes Associated With Down Syndrome?, in: Watson RR, Preedy VR, Zibadi S (Eds.), Polyphenols: Prevention and Treatment of Human Disease, Academic Press, London, UK, 2018, pp. 439–454.

[97]. Augustine M, Horwitz MJ, Parathyroid hormone and parathyroid hormone-related protein analogs as therapies for osteoporosis, Curr Osteoporos Rep 11(4) (2013) 400–6. [PubMed: 24078470]

[98]. Silva BC, Bilezikian JP, Parathyroid hormone: anabolic and catabolic actions on the skeleton, Curr Opin Pharmacol 22 (2015) 41–50. [PubMed: 25854704]

[99]. Park JH, Lee NK, Lee SY, Current Understanding of RANK Signaling in Osteoclast Differentiation and Maturation, Mol Cells 40(10) (2017) 706–713. [PubMed: 29047262]

[100]. Xavier AC, Ge Y, Taub JW, Down syndrome and malignancies: a unique clinical relationship: a paper from the 2008 william beaumont hospital symposium on molecular pathology, J Mol Diagn 11(5) (2009) 371–80. [PubMed: 19710397]

[101]. Rashidi A, DiPersio JF, Targeting the leukemia-stroma interaction in acute myeloid leukemia: rationale and latest evidence, Ther Adv Hematol 7(1) (2016) 40–51. [PubMed: 26834953]

[102]. Brown HK, Allocca G, Ottewell PD, Wang N, Brown NJ, Croucher PI, Eaton CL, Holen I, Parathyroid Hormone (PTH) Increases Skeletal Tumour Growth and Alters Tumour Distribution in an In Vivo Model of Breast Cancer, Int J Mol Sci 19(10) (2018).

[103]. Swami S, Johnson J, Bettinson LA, Kimura T, Zhu H, Albertelli MA, Johnson RW, Wu JY, Prevention of breast cancer skeletal metastases with parathyroid hormone, JCI Insight 2(17) (2017).

[104]. Deeks ED, Denosumab: A Review in Postmenopausal Osteoporosis, Drugs Aging 35(2) (2018)163–173. [PubMed: 29435849]

[105]. Narayanan P, Denosumab: A comprehensive review, South Asian J Cancer 2(4) (2013) 272–7. [PubMed: 24455656]

[106]. Bone HG, Wagman RB, Brandi ML, Brown JP, Chapurlat R, Cummings SR, Czerwinski E, Fahrleitner-Pammer A, Kendler DL, Lippuner K, Reginster JY, Roux C, Malouf J, Bradley MN, Daizadeh NS, Wang A, Dakin P, Pannacciulli N, Dempster DW, Papapoulos S, 10 years of denosumab treatment in postmenopausal women with osteoporosis: results from the phase 3 randomised FREEDOM trial and open-label extension, Lancet Diabetes Endocrinol 5(7) (2017) 513–523. [PubMed: 28546097]

[107]. Tsourdi E, Makras P, Rachner TD, Polyzos S, Rauner M, Mandanas S, Hofbauer LC, Anastasilakis AD, Denosumab effects on bone density and turnover in postmenopausal women with low bone mass with or without previous treatment, Bone 120 (2019) 44–49. [PubMed: 30292818]

[108]. Tsourdi E, Rachner TD, Rauner M, Hamann C, Hofbauer LC, Denosumab for bone diseases: translating bone biology into targeted therapy, Eur J Endocrinol 165(6) (2011) 833–40. [PubMed: 21852390]

[109]. Tsourdi E, Langdahl B, Cohen-Solal M, Aubry-Rozier B, Eriksen EF, Guanabens N, Obermayer-Pietsch B, Ralston SH, Eastell R, Zillikens MC, Discontinuation of Denosumab therapy for osteoporosis: A systematic review and position statement by ECTS, Bone 105 (2017) 11–17. [PubMed: 28789921]

[110]. Solling ASK, Harslof T, Langdahl B, The clinical potential of romosozumab for the prevention of fractures in postmenopausal women with osteoporosis, Ther Adv Musculoskelet Dis 10(5-6) (2018) 105–115. [PubMed: 29942362]

[111]. Lin C, Jiang X, Dai Z, Guo X, Weng T, Wang J, Li Y, Feng G, Gao X, He L, Sclerostin mediates bone response to mechanical unloading through antagonizing Wnt/beta-catenin signaling, J Bone Miner Res 24(10) (2009) 1651–61. [PubMed: 19419300]

[112]. Williams DK, Parham SG, Schryver E, Akel NS, Shelton RS, Webber J, Swain FL, Schmidt J, Suva LJ, Gaddy D, Sclerostin Antibody Treatment Stimulates Bone Formation to Normalize Bone Mass in Male Down Syndrome Mice, JBMR Plus 2(1) (2018) 47–54. [PubMed: 30283889]

[113]. Kamalakar A, Harris JR, McKelvey KD, Suva LJ, Aneuploidy and skeletal health, Current osteoporosis reports 12(3) (2014) 376–82. [PubMed: 24980541]

Highlights:

- All individuals with DS have skeletal deficits that may progress to osteoporosis
- Current research in DS bone is limited by sample sizes and varied methodologies
- Development of skeletal sexual dimorphisms in people with DS are not well defined
- Typical therapies for skeletal health may not be suitable for individuals with DS
- Gaps in knowledge and guidelines for future research in DS bone are proposed

Table 1:

Skeletal problems were diagnosed in individuals with DS responding to DS-Connect (2814 individuals provided 2886 responses (answered all that applied))

Diagnosis	Individuals	Percent
No skeletal problems	2467	88
Atlanto-axial instability	142	5
Fractures	85	3
Arthritis	77	3
Osteopenia	22	<1
Osteoporosis	15	<1
Cervical spine degeneration	15	<1
Unsure	63	2

Table 2:

Which bones have been broken (Selected all that applied) 34 people provided 39 Responses

Leg Bone	21
Arm bone	10
Hip	2
Rib/Ribcage	2
Spinal compression	1
Unsure	3

Table 3:

Age when broken bone was diagnosed (selected all that applied)

Less than a year	3
1-5	11
6-10	13
11-15	9
16-20	4
21-25	2
26-30	2
31-40	3
41-50	1
51-60	1
61-70	1
Unsure	2