



Review

# Skeletal Characteristics of Children and Adolescents with Turner Syndrome

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**Abstract:** Turner syndrome (TS) is a chromosomal disorder characterized by a short stature and gonadal dysgenesis, the latter of which requires estrogen replacement therapy (ERT) to induce and maintain secondary sexual characteristics. Insufficient ERT is associated with compromised skeletal health, including bone fragility, in adults with TS. In particular, estrogen insufficiency during adolescence is critical because the acquisition of a defective bone mass during this period results in impaired bone strength later in the life. In addition to bone mass, bone geometry is also a crucial factor influencing bone strength; therefore, a more detailed understanding of the skeletal characteristics of both bone mass and geometry during childhood and adolescence and their relationships with the estrogen status is needed to prevent compromised skeletal health during adulthood in TS. Although a delay in the initiation of ERT is associated with a lower bone mineral density during adulthood, limited information is currently available on the effects of ERT during adolescence on bone geometry. Herein, we summarize the current knowledge on skeletal characteristics in children and adolescents with TS and their relationships with estrogen sufficiency, and discuss the potential limitations of the current protocol for ERT during adolescence in order to achieve better skeletal health in adulthood.

**Keywords:** turner syndrome; bone mineral density; bone geometry; estrogen replacement therapy; children



**Citation:** Kawai, M.; Hasegawa, Y. Skeletal Characteristics of Children and Adolescents with Turner Syndrome. *Endocrines* **2022**, *3*, 476–487. <https://doi.org/10.3390/endocrines3030038>

Academic Editors: Emanuela Greco and Maria Felicia Faienza

Received: 8 June 2022

Accepted: 28 July 2022

Published: 2 August 2022

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## 1. Introduction

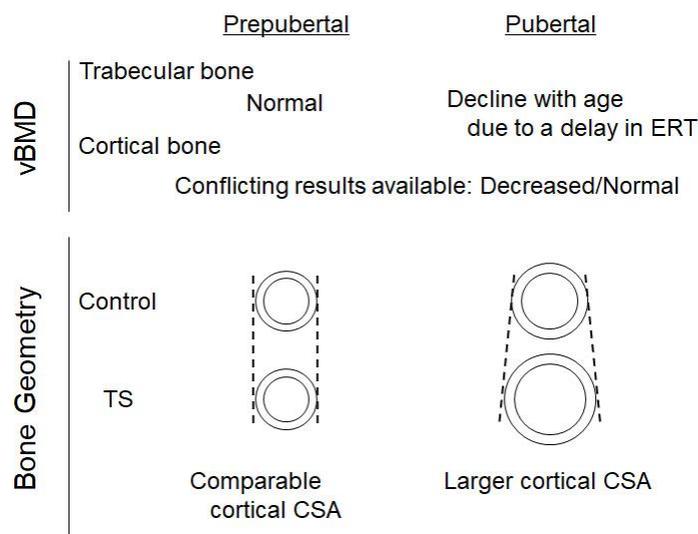
Turner syndrome (TS) is caused by the complete or partial loss of one X chromosome and its prevalence is approximately 1 out of 2000 live-born females [1,2]. Although the clinical characteristics of children with TS vary, a short stature and gonadal dysgenesis are highly prevalent and clinically evident [1,2]. Estrogen insufficiency by gonadal dysgenesis is associated with inappropriate maturation of secondary sexual development; therefore, current guidelines for the clinical care of TS recommend the initiation of estrogen replacement therapy (ERT) with low-dose estradiol between 11 and 12 years old, with gradual increases to an adult dose over 2–3 years, where appropriate [1].

The complications of TS during adulthood also vary, with compromised bone health being one of the clinically evident complications [2–6]. Gravholt et al. previously reported the clinical characteristics of 594 subjects with TS in Denmark based on data from the Danish Cytogenetic Central Register and Danish National Registry of Patients, and reported an increased incidence of osteoporosis (RR 10.12 [95% CI 2.18–30.93]) and fractures (RR 2.16 [95% CI 1.50–3.00]) [6]. Multiple lines of evidence, including meta-analyses, indicate that estrogen insufficiency is a predominant risk factor for low bone mineral density (BMD) [7–9]. In addition, the early introduction of ERT to accrue better BMD during adulthood has been suggested [10–15]. As the acquisition of an adequate bone mass

during adolescence and young adulthood is crucial for reducing the subsequent risk of osteoporosis, the appropriate introduction of ERT during adolescence is of clinical importance. Additionally, TS-associated comorbidities, including vitamin D deficiency, celiac disease, inflammatory bowel disease, and hyperthyroidism, are also risk factors for low BMD [1,16]. The genetic influence of haploinsufficiency of the *short-stature homeobox (SHOX)* gene on the skeletal characteristics in TS has also been reported [17]. Furthermore, the transcriptome analysis revealed a down-regulation of *bone morphogenic protein 2* and *insulin-like growth factor 2* expressions in human fibroblasts with 45, X karyotype compared with those with 46, XX karyotype [18], suggesting that the lack of one X chromosome may have a profound effect on bone metabolism beyond the effect of haploinsufficiency of the *SHOX* gene. These findings suggest that the bone phenotype may already be intrinsically compromised independent of estrogen deficiency in TS, which may also contribute to impaired bone mass and strength during adulthood.. These lines of evidence demonstrate the importance of a detailed understanding of the skeletal phenotypes of TS during childhood and adolescence to prevent compromised skeletal health during adulthood. In this review, we summarize current knowledge on the skeletal phenotypes of TS during childhood and adolescence and discuss the factors that affect bone mass accrual and bone geometry during these periods.

## 2. BMD in Prepubertal Children with TS

To clarify the skeletal characteristics, including BMD, of children with TS, it is important to investigate the bone mineral phenotype at ages corresponding to the prepubertal period in the general population, which is hereafter referred to as “prepubertal ages”, thereby excluding the effects of estrogen insufficiency on BMD. However, the number of studies performed during prepubertal ages is limited. A volumetric analysis is also important when evaluating BMD in children because a small bone size underestimates areal BMD (aBMD), particularly in conditions associated with small bones, such as TS. Ross et al. investigated aBMD of the lumbar spine (LS) by dual photon X-ray absorptiometry (DPA) in 78 pre- and peri-pubertal girls with TS aged between 4 and 13 years, and showed that aBMD was lower than in the controls matched for age and body mass index [19]. However, this difference was not observed after adjustments for bone age or height age, indicating that LS aBMD is underestimated in TS due to a small bone size. Högler et al. examined BMD in 83 girls with TS aged between 4 and 24 years, among whom 21 were younger than 10 years [20]. In this subpopulation during prepubertal ages, the volumetric BMD (vBMD) Z-score of LS, which mainly represents trabecular vBMD, was higher than  $-1.5$  in all individuals, whereas that of the femoral neck (FN), largely representing cortical vBMD, showed a decrease to lower than  $-1.5$  in 5 out of 21 subjects [20]. Soucek et al. assessed BMD using peripheral quantitative CT (pQCT) in 22 prepubertal children [mean (SD): 10.3 (2.2) years], among whom 8 were younger than 10 years old and had a normal trabecular vBMD of the radius [21]; cortical vBMD was lower than the lower limit of the normal reference range in 3 out of 8 subjects [21]. Nanao et al. evaluated vertebral trabecular vBMD in 21 prepubertal girls with TS aged between 4 and 9.9 years through a QCT analysis and showed that it was not decreased in TS [22]. Cortical vBMD was not assessed in this study. Collectively, these findings indicate that trabecular BMD is not affected in TS during prepubertal ages, whereas cortical vBMD decreases (Table 1) (Figure 1). Decreased cortical BMD in TS may be explained by the haploinsufficiency of the *SHOX* gene based on previous findings showing a lower cortical BMD of the forearm in 41 women with TS aged between 18–45 years than in karyotypically normal subjects with premature ovarian failure [23]. Soucek et al. analyzed BMD in 22 prepubertal girls with TS and in 10 subjects with an isolated *SHOX* gene deficiency [17]. They exhibited a similar bone geometry, indicating that the *SHOX* gene deficiency at least partly affected the skeletal phenotype in TS, independent of estrogen insufficiency [17]. Although limited information is currently available, these findings suggest that factors other than estrogen insufficiency may at least partly influence BMD in TS.



**Figure 1.** Summary of the skeletal characteristics of TS. Trabecular volumetric bone mineral density (vBMD) is normal in Turner syndrome (TS) during the prepubertal period, whereas it declines thereafter due to a delay in the commencement of estrogen replacement therapy (ERT). Cortical vBMD is low or normal in TS, depending on the confounding variables included in the study design. The inclusion of bone size results in the normal cortical vBMD in TS. The cortical cross-sectional area (CSA) is normal in TS during the prepubertal period, but a larger CSA is observed in TS probably due to a delay in ERT commencement, which may confer bone strength. Growth hormone treatment may also be responsible for a larger CSA in TS.

Despite evidence of low cortical BMD in TS during prepubertal ages as described above, normal cortical vBMD has also been recently reported [24]. As cortical BMD is affected by bone size [25], Soucek et al. recently performed height-adjusted measurements of cortical vBMD and found that cortical vBMD was normal during prepubertal ages [24] (Table 1) (Figure 1). Although this issue has not yet been examined in detail, these findings suggest that cortical and trabecular BMD are not compromised in TS during prepubertal ages, which implies the lack of an influence of *SHOX* haploinsufficiency on bone mass during prepubertal ages. Because of these discrepancies, further accumulation of evidence is needed to obtain a more detailed understanding of the skeletal characteristics in TS, particularly cortical bone BMD, during prepubertal ages, in addition to the influence of *SHOX* haploinsufficiency on the bone phenotype.

**Table 1.** Bone mineral density and geometry in children and adolescents with Turner syndrome.

Author	Year	Age (Years) #	Bone Mineral Density				Bone Geometry
			Number	Methods	Trabecular Bone ##	Cortical Bone ##	
Prepubertal							
Ross et al. [19]	1991	4–13	78	DPA DXA	Lower LS aBMD by DPA, but the difference disappeared after adjustments for bone age or height age	Lower aBMD of the wrist after adjustments for chronological age, bone age, or BMI by SPA	na
Nanao et al. [22]	2002	4–6.9	5	QCT	Similar LS vBMD to age-matched controls	na	na
		7–9.9	8				
		10–12.9	15				
Högler et al. [20]	2004	10.46 ± 3.25	51	DXA	LS vBMD Z score higher than –1.5 in 44 out of 51	FN vBMD Z-score higher than –1.5 in 38 out of 51	na
		<10 *	21		LS vBMD Z score higher than –1.5 in all subjects	FN vBMD Z-score higher than –1.5 in 16 out of 21	

Table 1. Cont.

Author	Year	Age (Years) #	Bone Mineral Density				Bone Geometry
			Number	Methods	Trabecular Bone ##	Cortical Bone ##	
Soucek et al. [21]	2011	10.3 ± 2.2	22	pQCT	Similar vBMD of the radius to age-matched controls	Lower vBMD of the radius than age-matched controls	Higher radial CSA than age-matched controls after adjustments for height. Lower cortical thickness than age-matched controls
		<10 *	8		Normal vBMD of the radius in all subjects	Lower vBMD of the radius than the lower limit of the age-matched reference in 3 out of 10	CSA above the lower limit in all subjects. Cortical thickness: the lower half of the normal range in 6, lower than the lower limit in 1
Pitukcheewanont et al. [26]	2011	11.9 ± 3.3	22	DXA pQCT	Similar LS vBMD to age-matched controls after adjustments for weight, height, skeletal age, and pubertal stage	Similar femoral vBMD with age-matched controls after adjustments for weight, height, skeletal age, and pubertal stage	Similar cortical bone CSA to age-matched controls after adjustments for weight, height, skeletal age, and pubertal stage
Prepubertal/Pubertal							
Shaw et al. [27]	1997	4–17	18	DXA	Lower LS aBMD than the age-matched controls, but the difference disappeared after adjustments for body weight and pubertal status	na	na
Holroyd et al. [28]	2010	12.7 ± 3.8 7–19	22	DXA pQCT	Similar LS BMAD and radial vBMD Z-scores as the age- and Tanner-stage-matched controls	Lower FN BMAD and radial vBMD Z-scores than the age- and Tanner-stage-matched controls	A similar radial cortical thickness Z-score as the age- and Tanner-stage-matched controls
Soucek et al. [24]	2018	10.0 ± 2.2 13.5 ± 1.5 16.1 ± 0.4	15 14 3	pQCT	Normal height-specific z-score of the radius vBMD during the prepubertal period. Age-dependent decrease in the radial vBMD Z-score thereafter	Normal vBMD during prepubertal ages, followed by a decrease during peripubertal ages. Thereafter, an increase was observed during pubertal initiation/induction	Comparable height-specific CSA during prepubertal period. The height-specific CSA Z-score showed an age-dependent increase
Pubertal							
Högler et al. [20]	2004	13.89 ± 2.23	16	DXA	Age-dependent decrease during the pre- and peripubertal, and pubertal periods	The FN vBMD Z-score is constant during the pre- and peripubertal, and pubertal periods	na
		18.98 ± 2.40	16				
Soucek et al. [21]	2011	14.3 ± 1.7 17.4 ± 1.2	25 20	pQCT	Lower radial vBMD Z-score than the reference value	Lower radial vBMD Z-score than the reference value	Similar height-adjusted CSA as the reference value

BMD: bone mineral density; BMI: body mass index; BMAD: bone mineral apparent density; aBMD: areal BMD; vBMD: volumetric BMD; CSA: total cross-sectional area; SPA: single photon absorptiometry; DPA: dual photon absorptiometry; DXA: dual energy X-ray absorptiometry; QCT: quantitative computed tomography; pQCT: peripheral QCT; LS: lumbar spine; FN: femoral neck; na: not available. #: Age is shown as the mean ± standard deviation, a range, or both. ##: Data on aBMD of the lumbar spine by DXA are regarded as trabecular bone BMD, whereas those on the femoral neck are regarded as cortical bone BMD. \*: subpopulation of those younger than 10 years old collected based on the figures reported in the study.

### 3. BMD in Peripubertal and Pubertal Girls with TS

#### 3.1. Effects of Estrogen Insufficiency on the BMD of the Trabecular Bone

The majority of girls with TS show a lack of or an insufficient progression of puberty [2]; therefore, assessing BMD during peripubertal and pubertal periods needs caution, because many studies have defined prepubertal girls as those without the spontaneous initiation of puberty, resulting in the inclusion of subjects with a delayed onset of puberty. Accordingly, herein, we utilize the term “peripubertal and pubertal ages” to describe the ages corresponding to the peripubertal and pubertal periods, respectively, in the general population. The term “peripubertal” is herein defined as a period during which puberty is about to commence.

Shaw et al. investigated LS aBMD using dual energy X-ray absorptiometry (DXA) in 18 girls with TS aged between 4 and 17 years, and reported that LS aBMD was lower in TS girls than in the age-matched controls; however, decreases in LS aBMD were not observed after adjustments for body weight and pubertal status [27]. As LS aBMD mainly represents trabecular bone density, these findings indicate that the pubertal status, namely, estrogen sufficiency, affects trabecular aBMD during the peripubertal and pubertal ages. To clarify the effects of pubertal status on BMD, Pitukcheewanont et al. investigated the LS bone mineral apparent density (BMAD), a volumetric calculation of DXA-based BMD, and LS vBMD using pQCT in 22 prepubertal TS with a mean (SD) age of 11.9 (3.3) and found that these parameters were similar between the TS patients and controls after adjustments for weight, height, skeletal age, and pubertal stage [26]. Consistent with these findings, Holroyd et al. examined LS BMAD and trabecular vBMD of the radius in 22 TS girls with a mean (SD) age of 12.7 (3.8), which included 12 prepubertal girls, and found these parameters were similar between the TS girls and the age- and Tanner-stage-matched controls [28]. Therefore, pubertal status is an important factor affecting trabecular BMD in TS during pubertal ages (Table 1) (Figure 1).

In accordance with this notion, an age-dependent decrease in trabecular BMD has been reported in peripubertal children without the spontaneous or medically induced initiation of puberty. Höglér et al. showed that the LS vBMD Z-score did not decrease during prepubertal ages, but showed an age-dependent reduction thereafter [20]. Similar findings were also reported by Nanao et al., namely, the trabecular vBMD of LS decreased with age in TS during these ages [22]. These findings indicate that a delay in the induction of puberty impairs the acquisition of trabecular BMD. A previous study demonstrated that individuals with spontaneous puberty had better BMD than those with induced puberty, and this was attributed to the age at which ERT was initiated generally being later than that of the onset of puberty in those with spontaneous puberty [29]. To clarify the importance of the administration of estrogen for trabecular BMD during this period, Soucek et al. longitudinally investigated the effects of ERT based on the currently recommended protocol on trabecular vBMD of the radius using pQCT for 6 years [24]. They included 15 prepubertal TS girls with a mean age (SD) of 10.0 (2.2) years for whom ERT was initiated at a mean age (SD) of 12.0 (0.9) years. At the end of the study (mean age [SD]: 15.9 [2.3] years), the height and age-adjusted vBMD Z-scores were not reduced at the initiation of ERT, but decreased from  $-0.11$  to  $-1.5$  during the 6-year follow-up, which indicated that the current protocol to induce puberty may not be sufficient to maintain the adequate acquisition of trabecular BMD during adolescence (Table 1) (Figure 1).

### 3.2. Effects of Estrogen Insufficiency on the BMD of Cortical Bone

Höglér et al. showed that the mean (SD) FN vBMD Z-scores during the pubertal and postmenarchal periods were  $-0.30$  (0.78) and  $-0.77$  (1.49), respectively [20]. These values did not show age-dependent changes during these periods [20]. Holroyd et al. also reported a lower FN BMAD and cortical vBMD of the radius in TS than in the age- and Tanner-Stage-matched controls [28]. A pQCT-based analysis also revealed reduced cortical vBMD [21]. Taken together, these findings indicate that cortical vBMD is impaired in TS during the peripubertal and pubertal ages; however, there is conflicting evidence to show similar cortical vBMD between the TS patients and controls [24,26]. As cortical vBMD is affected by bone size [25], Soucek et al. recently performed height-adjusted measurements of cortical vBMD and found that cortical vBMD was not significantly decreased during the peri- and pubertal periods [24]. Similar to pre-pubertal children, discrepant findings have been reported on this issue (Table 1) (Figure 1). This difference may be caused by the protocols used in studies, i.e., the inclusion of confounding variables. Therefore, the further accumulation of evidence is needed to confirm whether cortical BMD is impaired in TS during the peri- and pubertal periods.

#### 4. Bone Geometry and Strength in TS during Pre-Pubertal and Pubertal Periods

Although BMD is an important surrogate marker for bone strength, other parameters of bone geometry also have significant impacts on bone strength; therefore, analyses of the bone geometry provide additional insights into the skeletal phenotype in children with TS. Cortical bones generally show age-dependent increases in the total cross-sectional area (CSA) and cortical thickness, and this is associated with increases in the strength–strain index (SSI) during puberty [25,30]. Pitukcheewanont et al. evaluated cortical bone geometry in 22 prepubertal TS with a mean (SD) age of 11.9 (3.3) years and found that the cortical bone CSA was similar to that of the controls after adjustments for weight, height, skeletal age, and pubertal stage [26]. Soucek et al. investigated 22 prepubertal girls with TS with a mean (SD) age of 10.3 (2.2) years and found a lower CSA Z-score in the TS group than in the controls, and this difference was not observed after adjustments for height. Therefore, the inclusion of prepubertal girls with delayed puberty may have affected the findings obtained. In a subpopulation of eight children younger than 10 years old, the cortical CSA was above the lower limit of the reference values in all of the subjects [21]. These findings indicate that cortical bone geometry, particularly cortical bone size, was unlikely affected during prepubertal ages in TS (Table 1) (Figure 1).

To obtain a more detailed understanding of the alterations in bone geometry in TS during the pubertal period, Soucek et al. performed a longitudinal analysis in TS in which ERT was initiated based on the current protocol, and found that the height-specific CSA Z-score was not altered in prepubertal girls, whereas age-dependent elevations were observed thereafter that were associated with increases in the SSI Z-score, suggesting that those with TS acquired larger bones during the pubertal period [24]. This characteristic of bone geometry may be explained by the unique effects of estrogen on the acquisition of cortical bone. Estrogen has been shown to inhibit periosteal apposition, whereas endocortical apposition is stimulated [30–33], which results in the acquisition of smaller bones with a narrower marrow cavity in girls than in boys; therefore, larger cortical bones in TS may be a reflection of the delayed administration of estrogen. In accordance with this notion, in a clinical setting of delayed puberty, the delayed increase in estrogen levels resulted in normal or larger cortical bones with a thinner cortex [30]. As larger cortical bones generally confer more strength to bones [34], the SSI Z-score is considered to be higher in the TS patients than in the controls during adolescence [24].

#### 5. Fracture Risks in Children and Adolescents with TS

The larger CSA in TS indicates that bone strength is not impaired in children with TS; however, conflicting findings are available on this issue. Ross et al. examined the fracture incidence in 78 children with TS aged between 4 and 13 years old in 1991, and found that the annual incidence of fractures was similar in the TS patients and controls (19.9/1000 in TS vs. 27.8/1000 in controls), whereas the annual incidence of wrist fractures was higher in TS (9.1/1000 in TS vs. 3.5/1000 in controls) [19]. A recent longitudinal analysis of the fracture incidence in children with TS aged between 6 and 16 years demonstrated that the fracture rate of the limbs and spine was not higher in TS individuals [24]. The discrepancy between these two studies may be due to differences in the percentage of individuals receiving ERT. In the former study, 13 out of 78 subjects received ERT briefly for a mean duration of 6 months, whereas ERT was initiated in a defined protocol in the latter study, indicating that the former may have included more individuals for whom ERT was insufficiently performed; however, this difference may not have been a major factor that created a distinct result between two studies, because the age of the subjects in the former was younger than that in the later study, which may justify the increased percentage of subjects without ERT in the former study. Therefore, further accumulation of studies is clearly necessary to unravel whether the fracture risk is increased in children and adolescence with TS.

## 6. Effects of Growth Hormone Treatment on Bone Mass and Geometry in Children with TS

Growth hormone (GH) has been widely utilized to increase adult height in TS with successful outcomes [35–39]. In addition to its growth-promoting effects, GH has been shown to exert anabolic effects on the skeleton largely through its action to induce the expression of insulin-like growth factor 1, which was based on findings obtained from individuals with GH deficiency [40–42]. Additionally, a pharmacological dose of GH in short-statured children born with SGA increased the bone mass [43]. Although the effects of GH on BMD have been extensively examined in TS, conflicting findings have been obtained. Sas et al. investigated the effects of a 7-year treatment with GH on phalangeal vBMD and reported increases after adjustments for bone age [44]. In contrast, Ari et al. compared BMD between TS children treated with or without GH, and showed that after adjustments for bone size and bone age, the aBMD of LS and the radius did not significantly differ between the two groups [45]. Aycan et al. examined the effects of a 1-year GH treatment in prepubertal girls with TS with a mean age (SD) of 9.8 (2.5) years, and showed that GH treatment did not affect the vBMD of LS [46]. Similar findings were obtained on the effects of GH on BMD during childhood and adolescence [29,47,48]. These findings indicate that GH treatment in TS girls did not have a beneficial impact on BMD. However, as discussed earlier, bone strength is not affected by BMD only, bone geometry also plays important roles. Accordingly, Nour et al. recently investigated bone geometry using high-resolution pQCT and found that GH increased the bone size and the mechanical index of the polar moment of inertia, while DXA-based BMD was unaffected [49], which implies the beneficial effects of GH on bone strength, potentially resulting in reductions in the risk of fracture. Similar findings were reported on the effects of GH on decreases in the risk of fracture in age-related osteoporosis [50]. In addition to its effects on bone geometry, GH indirectly affected bone strength by improving muscle strength. Despite these benefits of GH on bone geometry and strength, limited information is currently available and, thus, further studies are needed to elucidate the effects of GH on fracture risk in adults with TS.

## 7. Effects of ERT during Adolescence on BMD in Young Adults with TS

aBMD has been extensively examined in adults with TS [4,5,12,16,51]. Itonaga et al. analyzed LS aBMD in TS patients aged between 15 and 49 years old and found that it was lower than the age-matched reference values [12]. Han et al. investigated aBMD in 177 subjects with TS aged between 19 and 60 years, and found that 55 and 9% of subjects showed T-scores for LS aBMD that were lower than  $-1.0$  and  $-2.5$ , respectively [5]. Similarly, Freriks et al. recently evaluated BMD in adult women with TS ( $N = 150$ ) with a mean age (SD) of 31.0 (10.4) years old, and showed that 52 and 12% of subjects had osteopenia with T-scores between  $-1.0$  and  $-2.5$  and lower than  $-2.5$ , respectively [4].

These findings indicate that aBMD is decreased in adults with TS, which is partly attributable to insufficient ERT, as evidenced in multiple observational studies and meta-analyses [8,9]. However, limited information is currently available on the effects of ERT during adolescence, particularly the age at which to initiate ERT, on BMD during adulthood. Cameron-Pimblett et al. analyzed the effects of the starting age of ERT on LS aBMD in adults with TS ranging between 18.1 and 70.3 years, and found a negative association between the starting age of ERT and LS aBMD [10]. Nguyen et al. also investigated the relationship between ERT and the spinal trabecular bone score (TBS) [14], an indirect measurement of the spinal trabecular architecture calculated based on DXA images that have been negatively associated with major fracture risks [52], and showed that TBS in TS patients aged between 20 and 49 years old was adversely influenced by a delay in the initiation of ERT [14]. Similar findings were obtained in other studies [13–15,53].

Therefore, these findings clearly indicate the importance of the early introduction of ERT in TS, where appropriate; however, the inclusion of aged individuals in the study design may create a bias when interpreting the effects of ERT on BMD because multiple variables, including medication for osteoporosis and age-dependent decreases in bone mass,

may have affected BMD in aged populations. To avoid these limitations, the influence of the timing of ERT on peak bone mass needs to be examined. This strategy is also supported by the peak bone mass attained during young adulthood being an important factor affecting the risk of osteoporotic fractures later in life [54–56]. In the general population, LS aBMD has been shown to peak in women in their twenties and thirties [57]; however, age-dependent alterations in aBMD have not been examined in detail in TS. Based on these findings, we initially investigated age-dependent alterations in LS aBMD in adults with TS and found that aBMD peaked at between 30 and 34 years of age in TS, and thereafter decreased, similar to the reference population [12].

These results imply the importance of investigating aBMD during ages between 30 and 34 years of age in TS patients; however, because of the paucity of data during this period, we alternatively investigated the relationship between the starting age of ERT and aBMD in young adults aged between 18 and 30 years old, during which the peak bone mass of LS appeared to be acquired in TS [11]. We also included individuals who had never been treated with anti-osteoporotic drugs, including the active form of vitamin D and bisphosphonates. Using these populations, we initially found that individuals with spontaneous menarche had higher LS aBMD than those without, suggesting that estrogen sufficiency during the pubertal period is important to accrue better LS aBMD during young adulthood. In addition, we revealed that the starting age of the adult dose of estrogen was negatively associated with LS aBMD. Although the starting age of ERT was not associated with low LS aBMD when the duration between the ages at which ERT and the adult dose of estrogen were initiated was incorporated into the study design as a confounding variable, the starting age of ERT was negatively correlated with LS aBMD, indicating that the early initiation and appropriate dose escalation of estrogen are important for the improved accrual of LS aBMD in young adulthood for patients with TS.

## 8. Future Directions and Concluding Remarks

The risk of fracture is increased in adults with TS and this has been at least partly explained by the inappropriate replacement of estrogen therapy. However, most studies examined older patients, in whom the initiation of ERT had often been delayed so as to achieve better adult height, which may have caused the insufficient acquisition of bone mass during adolescence and resulted in an increased risk of fracture later in life. Therefore, the accumulation of evidence based on the currently recommended protocol for ERT is of clinical importance because those treated with the current protocol may have better skeletal health. A recent study reported similar BMD and fracture incidence between TS individuals and controls during adolescence [24]. As the number of studies that have investigated skeletal characteristics and fracture incidence in adults with TS treated with the current protocol for ERT is limited, further investigations are required to obtain a more detailed understanding of the long-term influence of the current protocol for ERT on bone health.

Although ERT is now introduced earlier than before, there is also evidence of lower trabecular BMD in adolescence with TS [24], even when ERT is initiated based on the current protocol, which indicates that the current protocol may be suboptimal to accrue better trabecular bone mass during adolescence. Although there is lack of evidence for the skeletal sequelae for lower trabecular bone acquisition during this period, this may increase the risk of vertebral fracture later in life; therefore, further revisions to the protocol for ERT may be required. Previous studies detected serum estradiol in healthy, prepubertal girls [58–60]; therefore, the earlier initiation of ERT with a very low dose than the starting age of that in the current protocol may be physiologically more relevant to and beneficial for skeletal health. Although there is evidence to show that the earlier introduction of low-dose estrogen was not associated with better BMD than the conventional dose escalation regimen [61], the starting age of ERT in this study did not fully recapitulate the prepubertal rise in estrogen levels. Therefore, further studies are clearly required to understand the effectiveness of the prepubertal introduction of low-dose estrogen therapy on future bone health in TS.

In summary, there is concrete evidence to show that adults with TS have increased risk for osteoporosis. There is an emerging development of novel treatments for osteoporosis, such as anti-sclerostin and anti-RANKL antibodies [62–64]; therefore, applications of these new drugs may also be beneficial to these patients, partly because there is evidence to show the higher levels of sclerostin in TS [65]. However, there is consistent evidence to show that a delay in the initiation of ERT is deleterious for the acquisition of bone mass during adolescence in TS. In addition, the status of estrogen sufficiency during adulthood is also positively associated with BMD. These findings clearly indicate that the appropriate initiation and maintenance of ERT is a primary treatment to achieve better skeletal health in TS, although we need to be cautious to the contraindications for the use of estrogen such as clinical histories of breast or uterine cancer, deep vein thrombosis or pulmonary embolism, stroke or myocardial infarction, or blood clotting disorders [66].

**Author Contributions:** M.K. and Y.H. conceived the project and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** An Institutional Review Board Statement is not applicable for this study.

**Informed Consent Statement:** Written informed consent is not applicable for this study.

**Data Availability Statement:** Data are available upon request, with privacy or ethical restrictions.

**Acknowledgments:** The authors thank Satsuki Nishigaki (Osaka Women's and Children's Hospital), Tomoyo Itonaga (Department of Pediatrics, Oita University Faculty of Medicine), and Eri Koga and Hideya Sakakibara (Department of Gynecology, Yokohama City University Medical Center) for their discussions.

**Conflicts of Interest:** The authors declare no conflict of interest.

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