CLINICAL RESEARCH ARTICLE

Bone status in relation to ambulatory performance in girls with Rett syndrome: a 10-year longitudinal study

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BACKGROUND: Low bone mass is a frequent and early complication of girls with Rett syndrome. As a consequence of the low bone mass, Rett patients are at an increased risk of fragility fractures. This study aimed to investigate the long-term influences of mobility on bone status in girls with Rett syndrome.
METHODS: In 58 girls with Rett syndrome, biochemical parameters and quantitative ultrasound parameters at phalanges (amplitude-dependent speed of sound: AD-SoS and bone transmission time: BTT) were measured at baseline and after 5 and 10 years. The subjects were divided into two groups: nonambulatory ($n = 28$) and ambulatory ($n = 30$).
RESULTS: In nonambulatory Rett subjects, the values of AD-SoS and BTT were significantly lower than in ambulatory Rett subjects at each time point. However, during the 10-year follow-up both ambulatory and nonambulatory Rett patients showed a similar worsening in their bone status.
CONCLUSION: This longitudinal study suggests that both ambulatory and nonambulatory Rett subjects present a progressive deterioration of bone status as assessed by quantitative ultrasound parameters, and the ambulatory impairment and the nutritional status seem to play a key role in the deterioration of bone status.

INTRODUCTION

Rett's syndrome is a genetic disorder that causes severe cognitive and physical impairments. In its classic form, it appears to affect almost exclusively females with an incidence of approximately 1 in 10,000 female births.[1,2] Approximately 96% of classic Rett's syndrome cases have mutations in the gene which encodes MeCP2, whereas other forms are largely associated with other genetic mutations, such as CDKL5 in the early onset seizure variant and FOXG1 mutations in the congenital variant. The MeCP2 is a multifunctional nuclear protein, with potentially important roles in chromatin architecture, regulation of RNA splicing and active transcription.[3]

Patients with Rett syndrome appear to develop normally up to 6–18 months of age. They typically achieve normal neurodevelopmental milestones, from gross and fine motor functions to social communication skills. The head circumference of Rett girls is normal at birth; however, it begins to decelerate in its growth at 2–3 months of age.[4] Distinctive aspects contributing to the diagnosis include developmental regression, with accompanying loss of hand skills, mobility skills, and speech and stereotypical hand movements. As the syndrome progresses, social withdrawal and loss of language become apparent with features reminiscent of autism. The onset of mental deterioration is accompanied by loss of motor coordination and the development of ataxia and gait apraxia. Associated features such as microcephaly, respiratory/ autonomic abnormalities, seizures, scoliosis, growth deficits, and early hypotonia are highly prevalent.

Clinical data show that, along with neurological defects, females with Rett's syndrome frequently have marked decreases in bone mineral density (BMD).[5–15] As a consequence of the low bone mass, Rett girls are at an increased risk of fragility fractures and it has been reported that 25–40% of Rett girls have experienced fracture at some time during their lives.[8,10,14,16] However, clinical data on the course of skeletal development and the factors that influence bone status in Rett girls are scarce. Hitherto, dual-energy X-ray absorptiometry (DXA) at axial skeleton, the "gold standard" for evaluating bone mineral density both in adults and children, has only been carried out in few studies on subjects with Rett Syndrome. This may be explained by the fact that Rett subjects often present involuntary muscle contractions or uncontrollable movements which make it necessary to lightly sedate the girls before the scan, to prevent repetitive involuntary movements which could invalidate the analysis. For this reason, there is a growing interest in using Quantitative Ultrasound (QUS) as an alternative method for noninvasive assessment and monitoring of skeletal status.[16] The attractiveness of the use of QUS for bone measurements in children and adolescents lies in its lack of ionizing radiation, its ease of use, portability, and low cost. Moreover QUS, namely QUS at phalanges, seems to be less influenced by motion artifacts than central DXA.[17] In particular, phalangeal QUS measurements have shown the ability to reveal changes due to skeletal growth, aging, and diseases. There are also some longitudinal studies carried out by using QUS in healthy children and adolescents, in survivors of malignant bone tumors and acute lymphoblastic leukemia, in subjects with renal insufficiency and in subjects with genetic disorders.[18,19]

The aim of this longitudinal study was twofold:

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1. to evaluate bone status, as assessed by quantitative ultrasound over a 10-year period in Rett's girls;
2. to investigate the long-term influences of mobility on bone status in girls with Rett syndrome.

PATIENTS AND METHODS

Study population
We studied 58 patients affected by Rett syndrome, referred to the Department of Paediatric Neuropsychiatry, at the University Hospital of Siena (Italy) from January to December 2016. Inclusion criteria were a follow-up of at least 10 years, and complete clinical, biochemical, and QUS data at baseline and at each annual check-up. The diagnosis of Rett syndrome was made according to the internationally accepted diagnostic criteria.[1,2] The patients with severe cardiac or pulmonary complications or with a life expectancy of <24 months were also excluded. The study was approved by the Ethics Committee for human investigation of our Institution and informed consent was obtained according to the rules of the Ethics Committee. Questionnaires completed by parents provided information on clinical data, level of mobility, use of anticonvulsants or calcium/vitamin D supplements, and history of fracture of the Rett patients. In our subjects, MECP2 mutations were present in 55 subjects (94.8%), no information about genetic status was available for the other three subjects. At the time of the baseline evaluation, 28 (48.3%) patients were nonambulatory whereas the other 30 subjects were ambulatory or presented a mild ambulatory impairment (51.7%).

After baseline evaluation of biochemical parameters, Rett subjects with 25-Hydroxyvitamin D levels <20 ng/ml, were supplemented with oral cholecalciferol (600–1000 IU/day after the first year of life (1–18 years).[20] After cholecalciferol supplementation, serum vitamin-D levels increased significantly.

Nutritional status evaluation
The Waterlow score, a nutritional risk score, was utilized to identify Rett girls at risk of malnutrition. The Waterlow classification measures of H/A are calculated according to the following formula: H/A = [observed height/standard height (for same age and sex)] × 100.

The criteria for the diagnosis of malnutrition were for H/A, <85% severe, 85–89% moderate, and 90–95% mild under-nutrition.[21] Cases below 95% were evaluated as undernutrition Rett subjects.

Ultrasonographic measurements
In all subjects, QUS parameters were evaluated at phalanges by using a QUS device (Bone Profiler, IGEA, Italy). The device used is based on the transmission of ultrasound signal through the distal end of the first phalangeal diaphysis in the proximity of the condyles of the last four fingers of the hand. Bone Profiler measures the amplitude-dependent speed of sound (AD-SoS, m/s) and some parameters derived from the analysis of the graphic trace of the QUS signal.[22] AD-SoS depends on the signal amplitude because it is calculated by considering the time when the electrical signal, generated by the ultrasound mechanical wave at the receiving probe, reaches an amplitude of 2 mV.[22] Among the parameters derived from the analysis of the QUS graphic trace, we have considered the bone transmission time (BTT, μs) which is the difference between the time when the first peak of the signal received attains its maximum and the time that would have been measured if only soft tissue and not bone was present between the transducers. Therefore, BTT, unlike AD-SoS, is largely independent of ultrasound attenuation and soft tissue bias, and it depends almost exclusively on bone properties. AD-SoS and BTT were measured in the non-dominant hand, and the final result is the average AD-SoS and BTT of the last four fingers. The AD-SoS and BTT values of Rett patients and controls were converted to

Z-scores using the normative data obtained from a reference paediatric Italian population.[23] In our Institution, the precision of AD-SoS and BTT evaluated in children was 0.7% and 0.8% respectively. In addition, the standardized coefficient of variation (sCV) was calculated for each QUS parameter according to the formula: sCV = CV%/range/mean, where range was the difference between the 5th and the 95th percentile of the population. The sCVs were 3.7% for AD-SoS, and 2.6% for BTT. The precision assessed in five Rett patients measured five times on 1 day by the same operator (C.C.) by repositioning has given similar results (CV = 0.5% and 0.8% for AD-SoS and BTT, respectively).

Biochemical parameters
In all subjects, at baseline and at each follow-up visit, fasting blood samples were collected under fasting conditions to evaluate serum calcium, serum phosphate and 25-hydroxyvitam D (25OHD). Serum 25OHD was determined by a radioimmunometric method (25-hydroxyvitam D, DiaSorin, MN). In our Institution, the intra- and inter-assay coefficients of variation for 25OHD were 6.8% and 9.2%, respectively.

Statistical analysis
The variables normally distributed were expressed as mean ± SD. Differences between the prevalence of undernutrition Rett subjects among different groups were assessed with the chi-square test. A two-tailed p value < 0.05 was considered statistically significant. For QUS parameters, the absolute changes over time for each Rett subject were expressed as a percentage of the baseline values. Two-tailed paired t-tests and Wilcoxon matched-pairs signed-ranks tests were used when appropriate, to compare the changes at each time point with the baseline values. Two-tailed Student's t-test and Mann–Whitney U-test were used to compare the difference between subject groups. Separate multiple linear regression models (method: Stepwise) were used to assess independent predictors of AD-SoS and BTT at baseline and the time points follow-up, while age, weight, height, 25OHVitD, fracture, scoliosis, ambulatory capacity, nutritional status were included as independent variables in the models. For each model, the regression coefficients (b-coefficients) and their 95% confidence intervals were described. A p-value < 0.05 was considered statistically significant.

All statistical tests were performed using SPSS 10.1 statistical software (SPSS 10.1).

RESULTS
The clinical characteristics of the ambulatory and nonambulatory Rett subjects at baseline and after 5 and 10 years are reported in Table 1. All anthropometric parameters were lower in the nonambulatory with respect to ambulatory patients, both at baseline, after 5 years and also at the end of the study; however only BMI reached statistical significance ($p < 0.05$) at the 10-year point. Moreover, according to the Waterlow classification, malnutrition status was lower in nonambulatory with respect to ambulatory subject at every time, but reached statistical significance at baseline. Also, birth weight was lower in nonambulatory Rett girls with respect to the ambulatory subjects, while the age at menarche was similar in ambulatory and nonambulatory Rett girls. No differences between the two groups in biochemical parameters and 25OHD were observed. Finally, in nonambulatory Rett subjects, the values of AD-SoS and BTT were significantly lower than in ambulatory Rett subjects at each time point.

Scoliosis was found in 16 (57.1%) out of the 28 nonambulatory Rett subjects, but only in 13 (43.3%) out of 30 ambulatory Rett subjects. At baseline visit, only one ambulatory Rett girl (3.3%) experienced a fracture episode, while eight nonambulatory Rett subjects (28.6%) experienced a fracture episode. During the

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Table 1. Clinical characteristics, bichemical, and ultrasonographic parameters at baseline, after 5 and 10 years in nonambulatory and ambulatory Rett girls

	Nonambulatory (N = 28)			Ambulatory (N = 30)		
	Baseline	5 years	10 years	Baseline	5 years	10 years
Age (yrs)	5.1 ± 2.1	9.7 ± 3.5	14.6 ± 3.4	5.9 ± 3.2	10.9 ± 3.4	15.8 ± 3.4
Birth weight (kg)	2.944 ± 0.350			3.143 ± 0.387*		
Weight (kg)	19.2 ± 4.2	29.4 ± 5.2	33.9 ± 9.2	22.9 ± 12.1	31.8 ± 13.6	38.8 ± 13.6
Height (cm)	104.9 ± 14.1	129.1 ± 8.5	140.6 ± 10.6	113.5 ± 21.5	131.9 ± 14.4	142.7 ± 8.2
BMI (kg/m^2)	16.3 ± 0.1	16.7 ± 1.3	16.8 ± 2.9	16.4 ± 3.5	17.4 ± 4.6	18.8 ± 5.2*
Waterlow score[a]	16/28 (57.1%)	20/28 (71.4%)	16/28 (85.7%)	9/30 (30.0%)*	19/30 (63.3%)	23/30 (76.6%)
Age Menarche (yrs)	11.7 ± 2.8			11.9 ± 2.4		
Calcium (mg/dl)	9.5 ± 0.5	9.4 ± 0.6	9.6 ± 0.4	9.5 ± 0.5	9.6 ± 0.6	9.5 ± 0.5
Phosphorous (mg/dl)	5.2 ± 0.8	5.0 ± 0.7	4.9 ± 0.6	5.0 ± 0.7	5.1 ± 0.6	4.8 ± 0.6
25OHD (ng/ml)	15.2 ± 13.2	24.3 ± 14.7	28.1 ± 18.9	16.2 ± 10.8	26.7 ± 16.8	26.5 ± 16.3
AD-SoS (m/s)	1805.5 ± 43.1	1846.9 ± 21.2	1865.3 ± 36.8	1840.5 ± 137.2*	1886.5 ± 88.5*	1906.2 ± 66.6*
AD-SoS Z-score	−2.27 ± 1.06	−2.39 ± 1.14	−3.15 ± 0.30	−1.59 ± 1.67*	−1.96 ± 1.87*	−2.68 ± 1.40*
BTT (µs)	0.35 ± 0.19	0.60 ± 0.18	0.53 ± 0.19	0.51 ± 0.23*	0.73 ± 0.27	0.88 ± 0.22*
BTT Z-score	−1.91 ± 0.30	−2.01 ± 0.15	−3.27 ± 0.28	−1.10 ± 1.78*	−1.52 ± 2.84*	−2.68 ± 1.70*

Values are expressed as mean ± SD
*$p < 0.05$ between nonambulatory and ambulatory groups
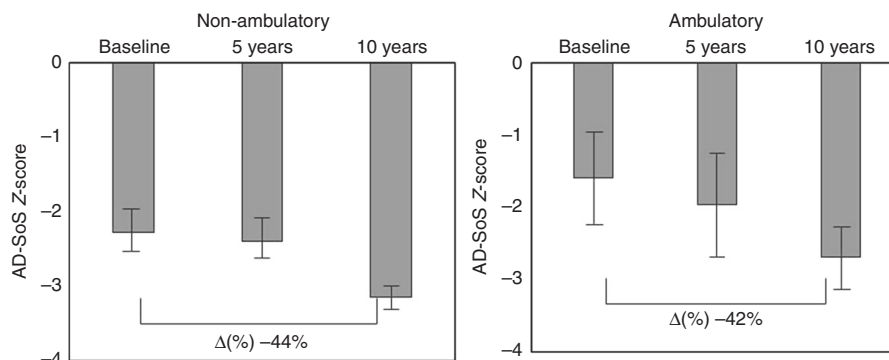[a]Categorial variable (chi-squared test)

Fig. 1 Percent change from baseline of AD-SoS Z-score in nonambulatory and ambulatory Rett girls

10-year follow-up, new fractures occurred only in three non-ambulatory Rett girls (one at humerus, one at tibia, and one at femoral diaphysis). However, it is important to point out that all the fractures had been caused by minimal traumas.

During the longitudinal study, each of the two groups showed a similar pattern, in fact the nonambulatory and the ambulatory Rett subjects presented a worsening of their bone status, and AD-SoS and BTT Z-score markedly decreased (Figs. 1 and 2). In fact, at years 5 and 10, the difference in Z-score between the nonambulatory group and ambulatory group was significant for both AD-SoS and BTT.

In Table 2, we reported multiple linear regression analysis of predictors of QUS parameters in Rett subjects. The analysis was performed by including age, weight, height, 25OHD, fracture, scoliosis, ambulatory capacity, and nutritional status in the model as independent variables. At baseline, in Rett subjects, AD-SoS was predicted primarily by height and weight, whereas BTT was predicted by height. Moreover, at 5 years, AD-SoS was predicted by weight, height, and ambulatory capacity, while BTT was predicted only by height. Finally, at 10 years, AD-SoS was predicted only by ambulatory capacity, while BTT was predicted only by height.

DISCUSSION

To the best of our knowledge, this is the first study in Rett subjects to evaluate the changes in bone mineral status over a 10-year period by using QUS. The main finding of this study was that the Rett subjects present a decrease in ultrasonographic bone parameters, suggesting a progressive deterioration of bone status. These findings are in agreement with the few previous studies carried out to date by other authors[1,5–15] who evaluated bone mineral status by DXA or QUS in both children and young adults affected by Rett syndrome. Clinical data on the natural course of skeletal development and influencing factors in females with Rett syndrome are poor. Moreover, most of the studies aimed to assess the changes in bone status in Rett patients were cross-sectional.

The decision for using QUS at phalanges was prompted by the consideration that the ability of QUS to detect a reduced bone mineral status has been confirmed also in other studies carried out on healthy children or on pediatric populations with mineral disorders or chronic diseases.[14,23,24] Moreover, the advantages for the use of QUS in the assessment of bone status in children and adolescents lie in its lack of ionizing radiation, ease of use, portability, and low cost.
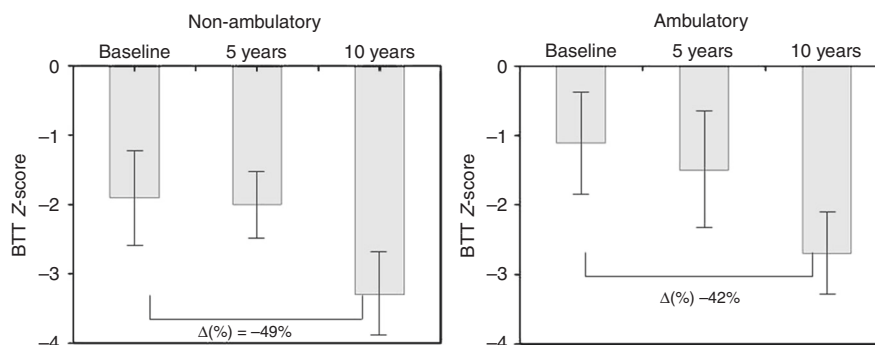
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Fig. 2 Percent changes from baseline of BTT Z-score in nonambulatory and ambulatory Rett girls

	Variable		standardized coefficient, b	95% CI	p
Table 2. Multiple linear regression analysis of predictors of QUS parameters in Rett girls at baseline and after 5 and 10 years					
Baseline	AD-SoS (m/s)				
		Height (cm)	4.352	2.105 to 6.600	<0.0001
		Weight (kg)	5.786	0.99 to 10.58	0.019
	BTT (μs)				
		Height (cm)	0.009	0.006 to 0.012	<0.0001
5 years	AD-SoS (m/s)				
		Height (cm)	5.188	2.867 to 7.509	<0.0001
		Weight (kg)	4.18	1.547 to 6.814	0.003
		Ambulatory	43.874	5.453 to 82.294	0.026
	BTT (μs)				
		Height (cm)	0.011	0.006 to 0.015	<0.0001
10 years	AD-SoS (m/s)				
		Ambulatory	51.298	12.933 to 89.662	0.01
	BTT (μs)				
		Height (cm)	0.029	0.002 to 0.056	0.036
Whole set of variables included into the models: age, weight, height, BMI, 25OHVitD, fracture, scoliosis, and ambulatory capacity					

Our data show that in Rett patients, the ambulatory impairment represents one of the factors of the changes in bone mineral status as evaluated by QUS. In a previous longitudinal study, Gonnelli et al. reported that the changes in QUS bone parameters, namely AD-SoS and BTT, were significantly influenced by the changes in ambulatory impairment, and the worsening in bone status was greater in Rett patients who were nonambulatory at baseline and who presumably were suffering from a more severe disease.[7] In fact, the ability to walk, similarly to other phenotype characteristics, was in part, related to the specific MECP2 mutations[25] and may or may not decline with age.[26] In fact, Foley et al. reported that in Rett subjects with the ability to walk, motor skills scores remained stable over a 4-year observation period.[26] It is known that participation in physical activity has been associated with numerous health benefits in relation to energy balance, physical fitness, and psychological well-being. In particular, high levels of physical activity are associated with benefits to bone mass, bone structure and muscle strength, which are traits associated with low fall and fracture risks. Nevertheless, participation in walking-based physical activity is problematic for those with a severe neurological conditions such as those in Rett subjects.[27] However, the findings of our study suggest that in Rett patients, the presence and the severity of the disease may have a greater influence on bone fragility with respect to the other risk factors, such as ambulatory capacity, BMI, presence of scoliosis, and use of antiepileptics. Our study is in accordance with the previous study by Shapiro et al.

which showed that bone mass was correlated with marginal significance to clinical severity and ambulation but not to scoliosis or anticonvulsant use.[9] At present, the mechanisms by which the genetic mutation may influence bone status and fracture risk have not yet been clarified. Some studies on genetic mutations associated with Rett syndrome have documented that these may have varying relationships with growth and bone acquisition.[10]

Our findings show that the greater involvement of the bone status in nonambulatory Rett subjects is not due only to the ability to walk, but in addition to disease impairments, puberty, and environment factors, such as nutritional status, could also have contributed. A previous study by Downs et al. that analyzed the influences of age, walking ability, scoliosis, and the severity of epilepsy in Rett subjects, observed that compared with those unable to walk, maintenance of walking capacity is associated with less marked progression of scoliosis.[28] In contrast, the study by Motil et al. reported that increased total daily energy expenditure associated with repetitive, involuntary movements does not explain the alterations in growth and body composition of girls with Rett syndrome.[29]

However, the early deceleration in height, weight, and BMI that characterizes the natural history of growth failure and undernutrition in Rett subjects could contribute to the bone status deterioration.

In fact, in Rett population, the reduced BMI may also be partly explained by feeding problems, oromotor dysfunction, and

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gastrointestinal dysmotility that may often contribute to limited dietary intake.[29,30] In this study, the mean BMI at baseline was similar in the ambulatory and nonambulatory group of Rett subjects. After 10 years, BMI in the ambulatory group was significantly higher with respect to the nonambulatory group. These findings concur with previous research, which reported a strong relationship between higher BMI and earlier onset of puberty in the general population and the converse, low BMI with late puberty. This has important implications for adequate nutrition management in Rett syndrome, given the high importance of a normal puberty for appropriate bone acquisition.[31,32]

Our study presents some limitations, firstly this study was carried out in a relatively small group of Rett patients. Another limitation is represented by the evaluation of the hand phalanges not being weight-bearing bone, may not entirely express skeletal changes in weight-being parts of the skeleton. In addition, another limitation of this study is that nutritional status was assessed only by BMI.

CONCLUSION

In conclusion, this 10-year longitudinal study suggests that both ambulatory and nonambulatory Rett subjects present a progressive deterioration of bone mineral status as assessed by QUS parameters at phalanges. In particular, we found that the ambulatory impairment and the nutritional status seem to play a key role in the progressive deterioration of bone mineral status in Rett girls. Further prospective studies are needed in order to better define the true influence of the various factors on bone mineral status.

DISCLAIMER

The material is original, has not been previously published, and has not been submitted for publication elsewhere while under consideration.

ADDITIONAL INFORMATION

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