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Clinical Investigations

Dual X-ray Absorptiometry and Bone Ultrasonography in Patients with Rett Syndrome

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Abstract. This study evaluated bone status and bone turnover in 82 females (ages 2–21 years) with the Rett Syndrome (RS) and 82 age-matched controls. Bone mineral density (BMD) by dual X-ray absorptiometry (DXA) at the ultradistal and proximal radius and ultrasonographic (QUS) parameters at the calcaneus [speed of sound(SOS), broadband ultrasound attenuation(BUA), and stiffness] and at the phalanxes (amplitude dependent speed of sound: AD-SOS) were measured. We also measured serum calcium, phosphate, 25-hydroxyvitamin D, and biochemical markers of bone turnover. DXA and QUS parameters were significantly lower in patients with RS compared with controls and, among RS alone, in those treated with anticonvulsants and in those who are nonambulatory. Ambulatory RS patients showed QUS and DXA parameters significantly greater than nonambulatory patients but significantly lower than controls. Patients with RS treated with anticonvulsants presented QUS and DXA parameters lower than those of other RS. In RS patients, walking significantly influences BMD-UD, BMD-P, SOS, BUA, and Stiffness. Serum 25-hydroxyvitamin D was significantly lower in RS than in controls. These results suggest that ambulatory status, to a major extent, and anticonvulsant therapy certainly play an important role in the reduction of bone mass and bone quality, but they cannot completely explain the altered bone status. Whatever the cause, girls with RS present abnormal bone status with an increase in the risk of fracture.

Key words: Rett syndrome — Bone ultrasonography — Bone mineral density

A progressive neurological disorder in girls, defined as Rett Syndrome (RS), was first reported by Andreas Rett in 1966, but it is only since 1983 that a series of reports on its clinical entity and prevalence have provided international recognition for RS [1–4].

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The incidence among females has been estimated to be 1 in 10,000–15,000 in Scotland [5]; the prevalence of the phenotype among females is estimated at 1:10,000–1:22,000 [6]. RS accounts for 2–3% of severely mentally handicapped [6, 7] and perhaps 10% of profoundly handicapped females [8]. Despite its importance, however, the pathogenesis of RS remains obscure. It is most likely to be an X-linked dominant neurological disorder, lethal in hemizygous males [1], but this is still being debated. A mutation in the gene (MECP2) encoding X-linked methyl-CpG-binding protein 2 (MeCP2) has been recently identified as the cause of some cases of RS [9]. The MeCP2 can bind methylated DNA and has been implicated as a key player in assembling transcriptional silencing complexes [10]. These data establish RS as the first human disease caused by defects in a protein involved in DNA methylation [11]. It has been recently reported that phenotype variability in Rett is only partially dependent on the kind of MECP2 mutation, and that other mechanisms should be investigated to explain the variable recovery in speech and hand use [12].

The syndrome consists of profound psychomotor retardation, with the absence of speech, hand use, and reduced brain growth and, in some cases, ataxia. The patients appear to be normal at birth and during the first months of life, but in their second year of life, important deficits are identified. The development of major motor milestones occurs later than normal; i.e., sitting, standing, and walking, and speech development of patients is delayed compared with siblings [13]. The initial clinical manifestations are a deceleration of head growth with a consequent microcephaly, loss of communications skills and purposeful hand use, stereotypes, grossmotor dysfunction, epilepsy, bruxism, abnormal breathing, and severe scoliosis. Loss of acquired hand use and speech by three years of age is followed by severe mental retardation [14]. The clinical manifestations have been classified in to four stages with various designations:

preregression (in which there are subtle delays in psychomotor development); regression; postregression ambulatory and postregression nonambulatory [15, 16]. There are now established criteria for diagnosis [17]. In addition, a number of skeletal abnormalities have been reported in subjects with RS which include the presence of short fourth metacarpals and metatarsals and a short ulna [18–20]. It has also been demonstrated that patients with RS have reduced bone density with increased fracture risk [18, 21, 22].

Quantitative ultrasound (QUS) has recently been introduced as an alternative, radiation-free method for noninvasive assessment of skeletal status [23]. The basic principle of US measurements of bone is that the speed at which it propagates in bone and the extent of its attenuation through the bone are determined by physical properties of bone determined by bone constituents (e.g., BMD and elasticity) and by structure (e.g., bone architecture) [24]. The absence of radiation makes this technique particularly useful in children [25–27], where it has been demonstrated that ultrasound variables can discriminate between healthy and osteopenic subjects [25].

The aim of our study was to evaluate bone status, as assessed by traditional densitometry and quantitative ultrasound, and bone turnover in patients with RS.

Subjects and methods

We studied all the patients (age range 2–21 years) with RS referred to the Institute of Child Neuropsychiatry of Siena from January 1998 to May 2000. The diagnosis was made according to the internationally accepted diagnostic criteria [17]. We also studied 82 age-matched healthy controls. Informed consent was obtained according to the local ethics committee. Questionnaires completed by parents provided information on clinical data, level of mobility, use of medications, history of fracture, and calcium intake (on the basis of a food-frequency questionnaire). Twenty-nine patients with RS were nonambulatory at the time of the study; 40 were treated with anticonvulsants known to affect bone metabolism, such as diphenylhydantoin and phenobarbital; the others were either not treated or treated with different anticonvulsants. To analyze the data, the RS patients were also classified into four groups based on their mobility status (ambulatory or nonambulatory) and anticonvulsant use (anticonvulsants known to affect bone metabolism and no anticonvulsants or other anticonvulsants). In all patients we measured BMD by dual-energy X-ray absorptiometry (DXA) at the junction of the distal and middle third of the radius (BMD-MR) and at the ultradistal radius (BMD-UD) (Osteoscan, NIM, Verona, Italy); at our Institution, the *in vivo* precision of this device was 0.9%. We also measured ultrasound parameters at the heel by Achilles Plus (Lunar Corp, Madison, WI) and at phalanxes by DBM Sonic 1200 (Igea, Carpi, Italy). The Achilles measures speed of sound (SOS), broadband ultrasound attenuation (BUA), and a clinical index named Stiffness, which is not stiffness in the true biomechanical sense (i.e., bone resiliency or stress/strain), but rather an attempt by the manufacturer to define a clinical index of bone quality. Stiffness is expressed as a percentage relative to the mean value for young normals and is calculated from the mean of BUA, and SOS measurements according to the formula:

$$\text{Stiffness} = [(0.67 \times \text{BUA}) + (0.28 \times \text{SOS})] - 420.$$

At our institution, the *in vivo* precision of this instrument has a coefficient of variation of 0.3%, 1.2%, and 1.5%, respectively for SOS, BUA, and Stiffness. The DBM Sonic 1200 is an ultrasound device that measures the amplitude-dependent speed of sound (AD-SoS) through the distal metaphysis of the proximal phalanges at the last four fingers. The precision of DBM-Sonic was 0.5%.

In patients with RS and controls, blood samples were also collected to evaluate serum calcium (Ca), phosphate (P), total alkaline phosphatase (ALP), bone alkaline phosphatase (B-ALP, Tandem- R Ostase, Hybritech, CA, USA), type I procollagen carboxyterminal propeptide (PICP, Farmos Diagnostica, Finland), osteocalcin (OC, osteocalcin RIA kit, DiaSorin, MN,USA), parathyroid hormone (PTH; Technogenetics, Milano, Italy), and 25-hydroxyvitamin D [25(OH)D] (25-Hydroxyvitam D, DiaSorin, MN, USA).

Statistical Analysis

All values were expressed as mean ± SD. Two-sample analysis was performed between patients with RS and the control group and between RS walking or not and treated or not with anticonvulsants known to affect bone metabolism. Multiple regression analyses were performed for each QUS and densitometric parameter, with densitometry and QUS as dependent variables, and age, BMI, and walking or therapy with anticonvulsants, respectively, as independent variables. As walking and anticonvulsant therapy significantly influence QUS and DXA parameters, we also performed analysis of covariance for each QUS and DXA parameter with walking and therapy, respectively, as factors and age as covariate. For the statistical software we used the SPSS.

Table 1. Clinical characteristics of patients with RS and of controls

	RS Patients	Controls	*P*
N	82	82	—
Age (years)	11.6 ± 6.3	11.0 ± 4.8	n.s.
Weight (kg)	33.9 ± 17.8	43.8 ± 15.4	<0.001
Height (cm)	132.5 ± 21.5	155.1 ± 25.4	<0.001
Ca (mg/dl)	9.2 ± 0.5	9.1 ± 0.6	n.s.
P (mg/dl)	3.9 ± 0.9	3.8 ± 0.6	n.s.
T-ALP (UI/l)	382.1 ± 293.5	364.4 ± 230.2	n.s.
B-ALP (μg/l)	55.4 ± 42.0	53.4 ± 37.5	n.s.
PICP (ng/ml)	324.2 ± 156.3	312.4 ± 180.5	n.s.
OC (ng/ml)	15.0 ± 17.7	13.4 ± 16.9	n.s.
PTH (pg/ml)	60.1 ± 27.5	58.5 ± 21.9	n.s.
25OHD3 (ng/ml)	10.0 ± 7.3	23.5 ± 8.1	<0.001
SOS (m/s)	1461.8 ± 46.4	1530.8 ± 23.9	<0.001
BUA (dB/MHz)	67.5 ± 18.8	98.4 ± 16.9	<0.001
Stiffness (%)	34.5 ± 24.7	74.5 ± 17.65	<0.001
AD-SOS (m/s)	1910.9 ± 85.4	1936.6 ± 81.9	<0.01
BMD-UD (mg/cm^2)	252.1 ± 90.6	324.3 ± 79.4	<0.001
BMD-P (mg/cm^2)	524.8 ± 145.5	567.9 ± 113.3	<0.001

All biochemical parameters were evaluated in serum

Results

Clinical characteristics and biochemical, QUS, and DXA data of our patients and of the control group are reported in Table 1. The RS patients were significantly shorter in height and lower in weight than the control group. Among biochemical parameters, serum CA, P, ALP, and PTH did not show any significant difference between the two groups; serum B-ALP, OC, and PICP were higher in RS even though without statistical significance; in contrast, serum [25(OH)D] was significantly lower in RS than in controls.

Table 2. Densitometric and ultrasonographic parameters of patients with RS ambulatory and of patients with RS nonambulatory

	RS ambulatory	RS nonambulatory	*P*
N	53	29	
SOS (m/s)	1475.3 ± 48.1	1437.1 ± 32.6	<0.001
BUA (dB/MHz)	71.9 ± 19.9	59.4 ± 13.3	<0.001
Stiffness (%)	41.3 ± 25.1	22.1 ± 17.2	<0.001
AD-SOS (m/s)	1923.8 ± 84.2	1887.9 ± 83.9	<0.05
BMD-UD (mg/cm^2)	271.9 ± 82.9	216.0 ± 94.4	<0.001
BMD-P (mg/cm^2)	560.7 ± 122.9	459.8 ± 162.0	<0.001

Table 3. Multiple regression analysis for each QUS and densitometric parameters, with densitometric and QUS as dependent variables, and walking as independent variables, after correction by age and BMI.

	Walking	
Parameter	Coefficient (*P*)	R^2 adjusted
BMD-UD	32.0 (0.05)	0.38
BMD-P	59.5 (0.02)	0.52
SOS	26.7 (0.003)	0.39
BUA	8.2 (0.03)	0.30
Stiffness	12.9 (0.006)	0.39
AD-SOS	Not in the model	

Densitometric and ultrasonographic values were significantly lower in patients with RS than in controls (Table 1). Moreover, RS nonambulatory patients showed both DXA and QUS parameters significantly lower than those who were ambulatory (Table 2). By dividing RS into two groups according to the use or non-use of anticonvulsants (anticonvulsant therapy with drugs known to alter bone and no therapy or therapy with anticonvulsants not known to alter bone), all QUS and DXA parameters were lower and markers of bone turnover were higher in the first group than in the second, even though only [25(OH)D] reached statistical significance ($P < 0.001$). By analysis of covariance, ambulatory RS patients showed QUS and DXA parameters to be significantly greater than nonambulatory, but significantly lower than controls. Similarly, patients with RS treated with anticonvulsants not known to alter bone, presented QUS and DXA parameters greater than other RS patients, but lower than controls. In RS patients, walking significantly influenced BMD-UD, BMD-P, SOS, BUA, and Stiffness, with adjusted R^2 ranging from 0.30 to 0.52 (Table 3). AD-SOS did not enter into the regression model. Moreover, anticonvulsant therapy did not show any significant influence on DXA and QUS parameters.

Discussion

Our data show that patients with RS present densitometric and ultrasonographic parameters lower than controls. These findings are in agreement with the few previous studies of other authors [18,22] which have shown a reduced mineralization in girls affected by RS, as evaluated by standard radiographs of the hand. DXA was used for the first time in patients with RS 3 years ago [21]. In fact, Hass et al. [21] found a significant reduction in BMD of the whole body and, specifically, at the lumbar spine in 20 patients with RS. The fact that we have evaluated BMD at the radius and not the whole body could seem to be one of the limits to our study; instead, we have selected this site for a lower radiation dose and for technical difficulties in measuring the whole body mass in this kind of patient. To our knowledge, these are the first data on the use of QUS in patients with RS. The absence of radiation makes the QUS particularly useful in children [25–27], where it has been demonstrated that ultrasound variables can discriminate between healthy and osteopenic subjects [25]. Moreover, it is currently accepted that QUS reflects not only bone mass, but also bone quality [23]. In fact, many *in vitro* studies have shown that SOS and BUA reflect both bone density and other properties of bone, such as elasticity and microarchitecture [28]. An *in vitro* study by Gluer et al. [28] suggested that SOS is also related to trabecular separation, and BUA to either trabecular separation or connectivity. Our data suggest that the patients with RS present not only a reduced BMD, but also an abnormal bone structure, as assessed by QUS parameters. It is not surprising that motor status (walking) influences DXA and QUS parameters; in fact, the importance of physical activity on the bone mass is well known. Osteopenia is believed to be due to the lack of physical stress on the bone necessary to induce the piezoelectric forces needed to stimulate osteoblastic activity. Children with movement disorders, such as cerebral palsy, have been shown to have decreased bone mass compared with children who have no motor impairment [29]. Conversely, positive associations have been observed in children between BMD and weight-bearing activity [30] and also between ultrasound parameters and weight-bearing activity [26]. Walking seems to influence QUS parameters at the calcaneus more than at the phalanges, probably due to the effect of mobility; unlike the calcaneus, the phalanges, is a weight-bearing bone [31]. According to other authors [32, 33], anticonvulsant therapy with drugs known to affect bone metabolism seems to play an important role in the alteration of bone status in patients with RS. In fact, RS patients treated with these drugs present higher bone turnover and lower levels of [25(OH)D] compared with RS patients not treated or treated with other anticonvulsants. Although it appears evident that motor status to a major extent and anticonvulsant therapy influence BMD and QUS parameters, these cannot completely explain the altered bone status we have observed. In fact DXA and QUS parameters were significantly lower than in controls, also in RS patients without ambulatory problems or on anticonvulsant therapy. Therefore other factors could play a role in the determination of low bone status

in patients with RS, e.g., a genetic basis, which may provide a clue to the basic pathophysiology of RS itself.

Whatever the cause, it is evident that patients with RS present reduced bone mass and altered bone quality and therefore they could be considered at risk of fracture later in life. The low levels of [25(OH)D] found in RS, namely, in those with anticonvulsant treatment known to affect bone metabolism, suggest that these subjects would benefit from vitamin D supplements.

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