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Bone Mineral Density in Angelman Syndrome

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Our aim was to evaluate bone mineral densitometry in patients with Angelman syndrome with or without antiepileptic therapy. Eighteen patients (9 females, 9 males), aged 4.0-24.3 years (mean age, 10.1 years), and two control groups consisting of 18 epileptic and 24 healthy patients, underwent dual-energy x-ray absorptiometry at the lumbar spine (L_1-L_4), and *z* score was evaluated for each patient; the *t* score was considered for patients aged ≥18 years. Abnormal bone mineral density was present in 8/18 (44.5%) of patients with Angelman syndrome, in 7/18 (38.9%) of the epileptic group, and in none of the healthy controls. Furthermore, a significant difference regarding mean age of patients (6 versus 15 years, $P = 0.008$, by Fisher exact test), and mean length of drug treatment (3.5 versus 11.1 years, $P = 0.005$ by Fisher exact test), appeared in the group with Angelman syndrome. Most of these patients (94.4%) were receiving antiepileptic drugs, mainly valproic acid, for many years. In conclusion, our study revealed osteopenia in almost half the children and young patients with Angelman syndrome. Dual-energy x-ray absorptiometry should be performed in all patients with Angelman syndrome, particularly if they are treated with antiepileptic drugs.

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Introduction

Angelman syndrome is a genetically determined syndrome, and its prevalence among children and young adults is estimated at 1:12-20000 [1]. It is characterized by severe psychomotor delay, prominent chin, deep-set eyes, macrostomia with protruding tongue, generally absent speech, gait ataxia with tremulous movements, and behavioral abnormalities including excessive laughter and apparent happiness, combined with hyperactivity. Peculiar electroencephalogram patterns, microcephaly, and epileptic seizures requiring anticonvulsant treatment are also present in 80% of patients with Angelman syndrome.

It is well-known that antiepileptic drugs are associated with osteopathy, with such manifestations as decreased bone mineral density, increased risk of fractures, and overt osteomalacia [2,3]. Osteopathy occurs not only in institutionalized patients, but also in ambulatory subjects [4].

Although the effects of valproic acid on bone density have received some attention [5-7], no such effects have been described in patients with Angelman syndrome. The aim of the present study was to evaluate bone mineral densitometry in patients with Angelman syndrome who are receiving anticonvulsant treatment, compared with epileptic and healthy control subjects.

Materials and Methods

Patients were enrolled from those followed in our clinic or recruited by the Italian Family Association of Angelman syndrome. Clinical diagnoses were performed according to recent consensus criteria [8]. Criteria for participation included: (1) age ≥3 years; (2) a molecular diagnosis of Angelman syndrome; and (3) informed consent from parents or caregivers. The study was conducted after receiving approval from the Ethics Committee of the Medical Faculty of Second University of Naples, Italy.

Exclusion criteria were: (1) hepatic or renal disorders, endocrinologic diseases, or malabsorption; (2) a familial history of abnormalities of bone metabolism or bone fractures; and (3) chronic treatment with drugs other than anticonvulsants (in particular, medications that can affect bone turnover).

Eighteen patients with Angelman syndrome (9 females, 9 males), aged 4-24 years (mean age, 10.1 years), with a maternal microdeletion of the 15 q11.2-13 region (16 cases), a uniparental disomy (one case), or a mutation of the imprinting center (one case), were enrolled. The mean value of body mass index was 20.2 (range, 12.6-34). The mean age at which autonomous gait was reached was 35 months (range, 23-58 months). Seventeen of 18 patients (94.4%) were treated with antiepileptic drugs for a mean period of 7.2 years (range, 6 months to 14 years)

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because of recurring generalized tonic-clonic/myoclonic seizures. All patients were receiving monotherapy (specifically, valproic acid).

Three patients had been treated previously with ethosuximide or clonazepam in addition to baseline valproic acid. Most patients had a seizure frequency ranging from 1-3 a year, and four patients had been seizure-free for >2 years. All other patients had been seizure-free for at least 6 months before the evaluation.

Severe scoliosis was found in an 18-year-old boy with a bone mineral density of less than –2.5 SDs, and mild scoliosis was present in a further five patients with osteopenia. No patient developed hypogonadism, and none had experienced previous bone fractures, except for one who had presented with an accidental wrist fracture 2 years before examination.

Patients with Angelman syndrome were compared with two control groups: the first consisted of 18 children with cryptogenic or idiopathic epilepsies, all of them treated with valproic acid as monotherapy; the second comprised 24 healthy children. Both control groups matched the study group in terms of age, sex, body mass index, pubertal age, geographic area, and familial dietary calcium intakes.

At entry, each patient was evaluated for age, sex, weight, height, body mass index, and pubertal stage according to Tanner's classification. In the same day, a blood sample was obtained to evaluate serum calcium, phosphorus, alkaline phosphatase, total proteins, transaminases, gamma-glutamyl-transferase, urea, creatinine, glucose, total red and white cells, platelet count, iron, transferrin, parathormone, osteocalcin, calcitonin, vitamin D, and serum levels of antiepileptic drugs. Furthermore, the ratio of urine creatinine/calcium and phosphorus excretion was evaluated. All parents and caregivers were asked to complete a questionnaire, to assess diet (i.e., calcium, carbohydrate, lipid, and protein intake, and total amount of daily calories), daily motor activity, and previous bone fractures. A neurologic examination, electroencephalogram recordings while awake and asleep, frequency and type of seizures, age at seizure onset, age at onset of antiepileptic therapy, duration of therapy, and type and number of antiepileptic drugs were determined in all patients. Bone density was determined by dual-energy x-ray absorptiometry at the lumbar spine (L_1-L_4), using a Hologic QDR 1000 densitometer (Hologic, Inc., Waltham, MA). Individual bone mineral density values were expressed as g/cm^2 and as *t* and *z* scores.

Children underwent examination of the lumbar spine (L_1-L_4) while they were in supine position, with partial elevation of the lower limbs to decrease lumbar lordosis. When necessary, oral niaprazine (30 mg/dose) or intramuscular prometazine (25-50 mg/dose) was given about 30 minutes before the examination, to decrease motor hyperactivity.

Based on World Health Organization criteria, osteopenia was diagnosed when bone mineral density values were between 1-2.5 SDs below the mean value, compared with age-, weight-, and sex-matched normal control subjects, whereas osteoporosis was characterized by bone mineral density values of <2.5 SDs [9] (Fig 1). The pubertal status of both the study group and control patients was determined by physical examination using Tanner's classification.

The *z* score consisted of the SD compared with persons of the same age, and was used in all cases except for young adults, in whom the

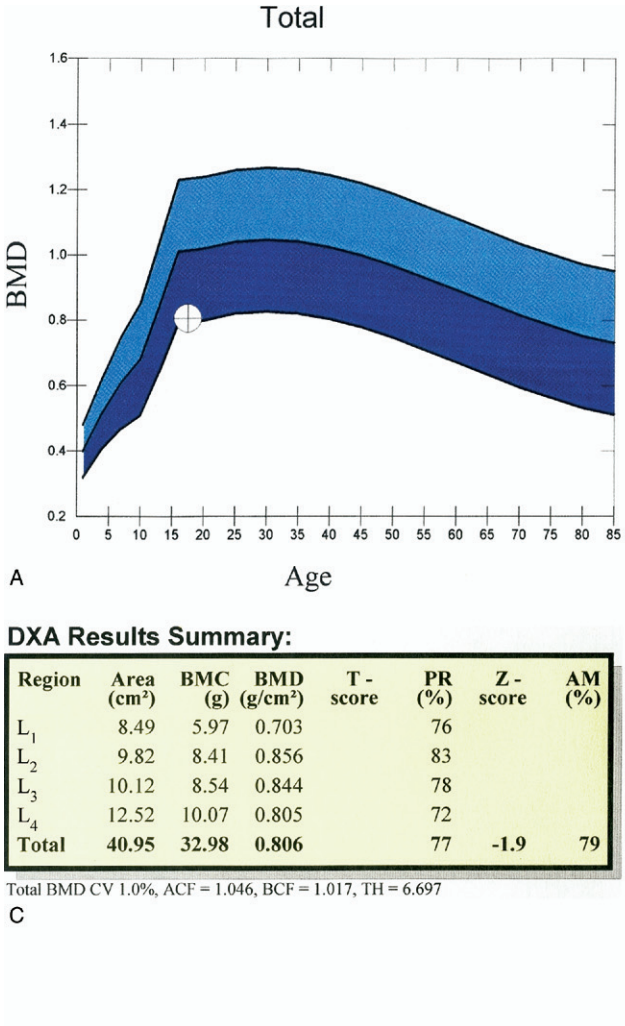

Figure 1. Dual-energy x-ray absorptiometry (DXA) at the lumbar spine (L_1-L_4) reveals osteopenia in a 16-year-old girl with Angelman syndrome. Abbreviations: BMD = Bone mineral density; BMC = Bone mineral content; PR = Percentage rates; AM = Age matched; BMD CV = Bone mineral density coefficient of variation.

Table 1. Comparison of clinical characteristics, bone mass density and *z* score among patients with Angelman syndrome, with epilepsy without Angelman syndrome, and healthy controls

	Angelman Syndrome Group	Epilepsy Without Angelman Syndrome	Healthy Group
Number of patients	18	18	24
Sex	9 female, 9 male	8 female, 10 male	13 female, 11 male
BMI (mean value, range)	20.29 (12.62-34.0)	18.91 (12.24-24.03)	20.27 (14.1-32.65)
Mean age (years)	10.05 (3-22)	10.72 (3-22)	10.62 (3-22)
Prepuberty	10	9	14
Puberty	8	9	10
BMD (mean value range)	0.65† (0.352-1.019)	0.73 (0.481-1.215)	0.75† (0.449-1.387)
Z-score (mean value range)	−0.62* (−3.35/+1.61)	−0.30 (−2.13/+2.68)	0.28* (−0.93/+2.64)

* $P = 0.03$ (by Student *t* test).
† $P = 0.04$ (by Student *t* test).

Abbreviations:
BMD = Bone mineral density
BMI = Body mass index

t score was evaluated. The *t* score represents the number of S.D.s from a reference group of young subjects who reached peak bone mass. Normative data for sex, age, weight, and pubertal stage were generated by the same densitometer.

Data are expressed as mean ± SD. All results were analyzed using SPSS version 10 (SPSS, Chicago, IL). Comparisons of continuous variables between subgroups of subjects were performed using the Student *t* test (two-tailed) or Fisher exact test. One-way analysis of variance was performed to determine the significance of any difference between the mean values of the three groups (those with Angelman syndrome, patients with epilepsy, and healthy patients). A simple correlation was used to assess the relationship between body-mass density *z* score and blood biochemical indices of mineral metabolism. Significance was set at $P < 0.05$.

Results

Bone Mineral Density

Overall, there was no statistical difference between those with Angelman syndrome, patients with epilepsy, and healthy patients regarding body mass index, mean age, and pubertal age. Conversely, a significant statistical difference was found between bone mass density and *z* score in the Angelman syndrome group and in healthy patients (Table 1).

An abnormal bone mineral density was present in 8/18 (44.5%) of patients with Angelman syndrome, in 7/18 (38.9%) of the group with epilepsy, and in none of the healthy control subjects (Table 2). Furthermore, mean age (6 versus 15 years, $P = 0.008$ by Fisher exact test) and mean length of drug treatment (3.5 versus 11.1 years, $P = 0.005$ by Fisher exact test) were significantly different between Angelman syndrome patients with normal and abnormal bone mineral density values.

All but one of the patients with Angelman syndrome had gained autonomous gait by the time of dual-energy x-ray absorptiometry evaluation. The daily lifestyle and motor activity characterized by hypermotoric behavior, motor im-

Table 2. Comparison of clinical parameters and bone mass density values among epilepsy patients with and without Angelman syndrome

	Angelman Syndrome Group		Epilepsy Without Angelman Syndrome	
	Normal BMD	Abnormal BMD	Normal BMD	Abnormal BMD
Number of patients	10 (55.5%)	8 (44.5%)	11 (61.1%)	7 (38.9%)
Sex	6 female, 4 male	3 female, 5 male	4 female, 7 male	4 female, 3 male
BMI (mean value, range)	18 (12.6-24.6)	23.2 (16.0-34.0)	19.47 (12.98-23.92)	18.04 (12.24-24.03)
Mean age (years)	6*	15*	10.64	10.86
Mean duration of AED therapy (years)	3.5†	11.1†	7.36	6.67
Monotherapy	9	8	11	7
Prepuberty	8	2	5	4
Puberty	2	6	8	1
BMD (mean value, range)	0.62 (0.352-1.019)	0.70 (0.533-0.997)	0.78 (0.537-1.215)	0.66 (0.481-0 893)
Z score (mean value, range)	0.36 (0.91/+1.61)	−1.85 (−3.35/−1.29)	0.44 (−0.99/+2.68)	−1.46 (−2.13/−1)

* $P = 0.008$ by Fisher exact test.
† $P = 0.005$ Fisher exact test.

Abbreviations:
AED = Antiepileptic drug
BMD = Bone mineral density
BMI = Body mass index

Table 3. Comparison of vitamin D levels and other biochemical parameters of bone metabolism among patients with and without Angelman syndrome, and healthy controls

	Angelman Syndrome Group	Epilepsy Without Angelman Syndrome	Healthy Group
1-25(OH) vitamin D (ng/mL)	29.5 (14.1-58.0)	30.6 (12.0-77.9)	24.5 (11.8-69.0)
Osteocalcin (ng/mL)	30.4 (3.4-141.0)	28.8 (2.4-94.0)	26.8 (7.0-90.1)
PTH (pg/mL)	40.0 (12.0-99.0)	35.1 (5.5-84.5)	30.8 (3.3-60.8)
Calcitonin (pg/mL)	6.5 (1.0-13.5)	6.6 (0.1-19.3)	5.0 (0.1-12.9)
Alkaline phosphatase (IU/L)	452.0 (131.0-787.0)	417.2 (50-837)	399 (112-700)
Serum calcium (mg/dL)	9.0 (7.0-11.3)	9.5 (9.0-10.2)	10.0 (9.0-12.3)
Serum phosphorus (mg/dL)	4.9 (3.8-7.0)	4.6 (3.5-6.0)	4.3 (3.0-5.9)

One-way analysis of variance, no significance.
Abbreviation:
PTH = Parathormone

pairment, and gait ataxia were considered almost to overlap in all patients. In addition, patients were considered homogeneous in terms of geographic area (southern regions of Italy), sunlight exposure, and dietary habits, with no relationship with bone absorptiometry values.

Biochemical Parameters

No significant differences in vitamin D levels and other biochemical parameters of bone metabolism were found among the three groups. Vitamin D levels and other parameters did not correlate with bone mineral density, either in the overall study group or in the three subgroups, and did not correlate with duration, dosage, and plasma concentrations of antiepileptic drug therapy (Table 3).

Discussion

This preliminary study indicated that abnormal bone mineral density was present in about half of the patients of this series with Angelman syndrome. Osteopenia was documented in all patients except for one, who manifested osteoporosis.

An overlapping number of patients with abnormal bone mineral density were also found in the group with epilepsy, but in none of the healthy controls. The only significant difference was found between the mean values of bone mineral density and z score of patients with Angelman syndrome and healthy control subjects; this difference was not discovered between epileptic and healthy control subjects.

These data militate against any significant pathogenic role of the genetic syndrome itself, and highlight the role played by anticonvulsant treatment. Nonetheless, there seems to be a trend for patients with Angelman syndrome to have a somewhat lower bone mineral density compared with the epileptic group.

Low bone mineral density was previously described in young adults with mental retardation with or without antiepileptic drug therapy or genetic syndromes such as Down or Rett syndrome [10-15], cerebral palsy and epilepsy [16], or epilepsy alone and anticonvulsant treatment [2]. To our knowledge, this is the first report on an abnormal mineral bone density in a series of children and young adults with Angelman syndrome. Generalized osteoporosis was recently described in a 13-year-old girl with Angelman syndrome with unusual, marked limb deformities and brachydactyly [17].

In our series, abnormal bone densitometry values were found in children with a significantly longer total time on antiepileptic drugs and older than Angelman-syndrome patients with normal absorptiometry parameters. Regarding drug therapy, it is noteworthy that all our patients had been treated with valproate as monotherapy. Only a few patients had received short-term treatment with additional anticonvulsant drugs (clonazepam or ethosuximide).

According to the literature, valproic acid was previously linked to low bone-mass density in pediatric patients [2,6,7], though the data are somewhat controversial. In fact, bone mineral density was not significantly impaired in some series [7,18,19], or was only fairly abnormal in others [6,20,21]. The length of drug treatment, however, was significantly correlated with low bone mineral density. Sheth [6] and Tsukahara et al. [21] pointed out that a period >18 months on valproic acid and, in general, a long-term period significantly affected body mineral density. Overall, valproate and carbamazepine seem to cause less severe effects than phenytoin, phenobarbital, and primidone [7]. Whereas phenytoin, phenobarbital, and primidone are associated with a higher risk of abnormal laboratory data (i.e., low levels of vitamin D) and clinical rickets, mainly in institutionalized patients with refractory symptomatic epilepsies, valproate and carbamazepine may cause only a mildly abnormal bone mineral density without changes of laboratory tests.

The mechanism by which valproic acid affects bone-mass density remains to be more clearly defined, because this drug does not seem to influence vitamin D metabolism, but may act upon insulin-like growth factor I [22]. This issue remains controversial [23].

In addition, valproic acid is not a significant inducer of hepatic enzymes, and would not be expected to reduce bone mineral density by this mechanism. However, valproate was associated with reversible Fanconi syndrome, suggesting that this drug may cause renal tubular dysfunction with an increased urinary loss of calcium and phosphorus [6]. In our patients (n = 6) who received combination therapy with valproic acid, this drug was given as the first treatment option and for the longest period, although a role of combination therapy cannot be ruled out. Nonetheless, the only patient with osteoporosis was an 18-year-old male who had been receiving valproate for 12 years. He manifested severe scoliosis as well.

Lower motor activity, together with valproic-acid therapy, may presumably play a role in the decreasing *z* scores in patients with Angelman syndrome, compared with epileptic patients without Angelman syndrome.

Abnormal values of bone mineral density were reported in patients with syndromic (i.e., Rett and Down syndromes) and nonsyndromic mental retardation. Most of these patients, both adults and children, were receiving antiepileptic therapy, which explains why low bone mineral density may be attributable to different cofactors such as mental retardation, antiepileptic drugs, and decreased motor activity. Cerebral palsy and insufficient exposure to sunlight, mainly in institutionalized patients, also play an important role [6].

Our series is homogeneous in terms of geographic area and lifestyle, absence of a family history of bone disease, and absence of an increased risk of bone fractures. Epileptic seizures were rare or controlled in all patients. Furthermore, all children had almost overlapping daily motor activity. The only patient who could not walk independently at age 5 years manifested osteopenia. In addition, all but one of our patients exhibited a normal body mass index. The patient with obesity (body mass index, 34) manifested osteopenia. It is noteworthy that this patient caused a light, though not significant, increase of the mean body mass index value in the group of children with low bone mineral density. The mild scoliosis found in five patients seems more likely linked to the Angelman syndrome itself [1] than to the slightly abnormal bone density. It cannot be determined to what extent osteoporosis played a role in the only patient with severe scoliosis.

None of the children had intercurrent, chronic medical conditions that might affect bone metabolism and thereby confound the results of this study. These conditions would have included liver disease, kidney disease, or conditions requiring prolonged corticosteroid therapy. To minimize potential bias, bone mineral density was evaluated at $\geq$3 years of age, because bone mineral density reaches its peak within the first 3 years of life [24].

In conclusion, the preliminary data of this trial revealed osteopenia in almost half of the children and young patients in this series with Angelman syndrome. Similar data came from the control group with epilepsy, thus confirming the key role played by anticonvulsant therapy. None of these patients exhibited any clinical problems linked to bone disorders or a personal or family history of bone fractures. Consequently, the exact clinical significance of dual-energy x-ray absorptiometry values and their relationships to an increased risk of bone fractures remain to be defined. None of our patients had ever received supplements of calcium/phosphate or vitamin D, and that could reasonably limit or preclude the development of bone impairment in these children. Careful skeletal assessment and monitoring of patients with Angelman syndrome who receive valproic acid are recommended.

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