Bone density and metabolism in subjects with microdeletion of chromosome 22q11 (del22q11)

Stefano Stagi 1, Elisabetta Lapi 2, Eleonora Gambineri 1, Cristina Manoni 1, Maurizio Genuardi 2, Gloria Colarusso 1, Camilla Conti 1, Francesco Chiarelli 3, Maurizio de Martino 1 and Chiara Azzari 1

1Paediatric Endocrinology Unit, Department of Paediatrics, 2Genetics and Molecular Medicine Unit, Anna Meyer Children’s Hospital, University of Florence, Viale Pieraccini 24, 50139 Florence, Italy and 3Department of Paediatrics, University of Chieti, via dei Vestini 5, 66100 Chieti, Italy

(Correspondence should be addressed to S Stagi; Email: stefano.stagi@yahoo.it)

Abstract

Introduction: Although hypoparathyroidism with hypocalcaemia is one of the most frequent clinical features of monoallelic microdeletion of chromosome 22q11 (22q11DS), bone mass and metabolism have not yet been assessed in these patients.

Design: This study aimed to evaluate bone mass and metabolism in a cohort of patients, both children and adults, with 22q11DS.

Methods: In twenty-eight patients with 22q11DS (median age 12.5, range 6.1–42.8 years), serum levels of ionised and total calcium, phosphate, parathyroid hormone (PTH), 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, osteocalcin and bone-specific alkaline phosphatase (BSAP), and urinary deoxypyridinoline concentrations were evaluated. In these patients, bone mineral density (BMD) was evaluated by dual-energy X-ray absorptiometry (DXA) examination, and volumetric BMD (bone mineral apparent density (BMAD)) was calculated.

The data obtained from paediatric and adult patients were compared with two age-, sex- and body size-matched healthy subject control groups.

Results: Patients with 22q11DS showed a reduced BMAD Z-score compared with controls (P<0.001). These patients also had significantly lower ionised (P<0.001) and total calcium (P<0.05) levels as well as lower PTH levels (P<0.05), compared with the controls. In particular, children and young patients with 22q11DS had significantly lower serum osteocalcin levels (P<0.001), BSAP levels (P<0.001) and urinary deoxypyridinoline concentrations (P<0.001) than controls. These results were not confirmed in adults.

Finally, patients with hypoparathyroidism and/or hypocalcaemia at the time of the study showed significantly lower ionised (P<0.001) and total calcium levels (P<0.001), PTH levels (P<0.05), BSAP levels (P<0.001), osteocalcin levels (P<0.001) and urinary deoxypyridinoline concentrations (P<0.001), compared with patients without hypoparathyroidism and/or hypocalcaemia. Nonetheless, the BMAD Z-score did not show substantial differences between these two groups.

Conclusions: Subjects with 22q11DS have a significant reduction in bone mass that appears to be more severe in adults who have already attained peak bone mass than in children who are still growing. Therefore, we suggest a close monitoring of bone mass and metabolism in 22q11DS patients.

Introduction

Microdeletion of chromosome 22q11.2 (22q11DS) is a relatively common genetic condition, occurring with an incidence of 1 out of 4000 live births (1). It is characterised by a highly heterogeneous phenotypic expression, and more than 100 different phenotypes have been described; the most commonly occurring phenotypes are DiGeorge syndrome, velocardiofacial syndrome (VCFS) and conotruncal anomaly face syndrome (1).

As a consequence of the microdeletion, there is a congenital failure in the development of the derivatives of various pharyngeal arches and pouches (2), with highly variable clinical features (3) encompassing congenital cardiac defects, hypocalcaemia, immunodeficiency from thymic hypoplasia, palate anomalies and velopharyngeal dysfunction, cognitive impairment and minor facial dismorphism (2).

Hypocalcaemia is the most frequent feature of 22q11DS, occurring in nearly 60% of the patients; this condition is invariably due to hypoparathyroidism, which is caused by the aplasia or hypoplasia of the parathyroid glands (3, 4). Usually hypoparathyroidism manifests itself during the neonatal period (5); however, late-onset appearance of symptomatic
hypocalcaemia has also been reported in adolescence and adulthood (5).

Dual-energy X-ray absorptiometry (DXA) is one of the mainstays in the evaluation of bone diseases and disorders, and is the most widely used technique for measuring bone mineral density (BMD) as well as bone mineral density (BMD) in children because of its low cost, accessibility and ease of use (6).

As BMD measurements are influenced by bone size and short children will have a lower BMD than their age-matched peers with normal stature, bone mineral apparent density (BMAD) must be calculated (7, 8). Many previous studies that used DXA have reported reduced BMD measurements for patients with short stature and/or genetic syndromes associated with short stature; this result could be explained by reduced height because BMD is a measurement of area and these patients have reduced height, which negatively affects their BMD values (9–13).

In any case, there is little data available about bone metabolism and mass in these patients; therefore, the purpose of this study was to evaluate BMAD and bone metabolism parameters in a cohort of patients with 22q11DS.

Subjects and methods

We have studied a cohort of 28 patients with 22q11DS (19 females and 9 males; median age 12.5, range 6.1–42.8 years), containing both children (17 patients, 11 females and 6 males; median age 9.6 years; range 6.1–16.6 years) and adults (11 patients, 8 females and 3 males; median age 26 years; range 16.75–42.8 years) who were recruited from July to October 2006 at Meyer Children’s Hospital in Florence, Italy.

Ethical approval was obtained from the ethics committee of the Meyer Children’s Hospital. Written informed consent was obtained from parents or patients according to age and ability to assent.

Case definition and study protocol

Between 1994 and 2004, a diagnosis of 22q11DS was obtained for all subjects at the Genetics and Molecular Medicine Unit of the A. Meyer Children’s Hospital using a fluorescence in situ hybridisation (FISH) test. Of these, 26 patients showed a de novo deletion at the 22q11.2 level, whereas 2 patients (mother and child) showed a familial deletion. All of the patients showed typical 22q11.2 microdeletions that were ~3 Mb in size.

Based on the literature concerning the major clinical features of patients with 22q11DS, the presence or absence of the following features in each patient was recorded: congenital cardiac defects; palate anomalies, including cleft palate or velopharyngeal insufficiency; craniofacial dysmorphism; congenital hypocalcaemia and hypoparathyroidism; thymic hypoplasia or a history of recurrent infections; and cognitive/learning difficulties and behavioural abnormalities.

Participants or their parents were asked to fill out a questionnaire, which was then reviewed by the medical staff during the baseline examination. The questions related to current and past medications, especially vitamin D and/or calcium intake, familial and personal bone fracture history, dietary habits and physical activity.

For all subjects, data on height, height velocity, pubertal staging, weight and body mass index (BMI) were collected and, when appropriate, bone age was determined. According to their growth velocity, pubertal staging and/or bone age, the patients were divided into two groups: i) paediatrics (patients that had not reached the adult height) and ii) adults (patients that had reached adult height).

The investigation consisted of a fasting blood sampling that was analysed to determine the following measurements: serum concentrations of creatinine, albumin, calcium (total and ionised), phosphate, 25-hydroxyvitamin D (25[OH]D) and 1,25-dihydroxyvitamin D (1,25[OH]2D); plasma levels of parathyroid hormone (PTH); and markers of bone formation (bone-specific alkaline phosphatase (BSAP) and osteocalcin) and bone resorption (urinary deoxypyridinoline).

All patients under observation were free of congenital or acquired bone disease, and we excluded all subjects who were using any drug known to affect bone turnover markers at the time of the study. Furthermore, we only considered 22q11DS patients who had not been using calcium supplementation or vitamin D treatment for at least 1 year prior to the beginning of the study.

Using an activity questionnaire, physical activity was assessed with a modified activity score composed of the scores for sports/leisure activities (0, <2 or >2 h/week), as previously described (14).

Calcium dietary intake was assessed with the semi-quantitative validated food frequency questionnaire (14). Selection of items was based on the food composition diet, frequency of use and relative importance of food items as a calcium source. The questionnaire included the following food items: milk and dairy products, including calcium-enriched items such as yoghurt, cheese and chocolate. Items such as eggs, meat, fish, cereals, bread, vegetables and fruits were also included.

The data obtained were compared with two age-, sex- and body size-matched healthy subject control groups for paediatric (67 subjects, 46 females and 21 males, mean age 9.9 ± 3.1 years) and adult patients (81 subjects, 58 females and 23 males, mean age 25.1 ± 5.9 years).

For every patient, we selected four to seven control subjects that matched the following criteria: age ±12 months, height ±10 cm, weight ±2.0 kg and equivalent pubertal stage. Controls were randomly selected from a population survey of healthy Caucasian
inhabitants in Tuscany with no rheumatic, endocrine or metabolic diseases, some of whom were seen for non-inflammatory musculoskeletal complaints at the Paediatric Rheumatology Unit of our hospital. Informed consent was obtained from all subjects and/or parents.

**Study and laboratory methods**

Height was measured using a wall-mounted stadiometer, and weight was measured to the nearest 0.1 kg. All of the measurements were carried out by the same trained staff members. The coefficient of variation (CV) values were <1% for these measurements.

BMI was calculated as weight divided by height squared (kg/m²). Age-related reference values for height, bone age and BMI were obtained from a wide sample of Italian children (15).

Bone age, when appropriate, was evaluated through radiographs of the left hand and wrist, and then calculated according to the Greulich & Pyle method (16). We considered it unnecessary to perform radiographs when the subjects had reached adult height (growth velocity <1 cm/year with complete pubertal development).

Height, bone age and BMI were normalised for chronological age by conversion to SDS. SDS values were calculated according to the following formula: (patient value—mean of age-related reference value)/s.d. of the age-related reference value.

Pubertal staging was carried out according to Tanner & Whitehouse’s criteria (17).

All laboratory measurements were performed on blood samples collected after overnight fasting and on a 24-h urinary collection. Serum levels of calcium, phosphate, creatinine and albumin were measured in all samples by the standard autoanalyser method routinely used for daily practice.

Normal serum concentrations of total calcium are 2.2–2.7 mmol/l for children and 2.2–2.6 mmol/l for adults. Normal blood concentrations of phosphate are 1.4–1.7 mmol/l from 2 to 12 years and 1.09–1.4 mmol/l from 12 to 16 years of age; the normal adult range is 0.8–1.45 mmol/l.

Blood-ionised calcium concentrations were measured within a few minutes of sampling with an ICA Kit (McLendon Clinical Laboratories, Chapel Hill, NC, USA). The normal range is 1.18–1.32 mmol/l.

Serum intact (1–84) PTH concentrations were measured with a two-site chemiluminescent immunoassay (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). The inter-assay CV was 10%. The normal range is given as 0.9–5.4 pmol/l.

Serum 25(OH)D and 1,25(OH)₂D were determined according to a competitive binding protein assay (Nichols Diagnostics). The inter-assay CV was 8%. The normal range is stated as 9.2–45.2 ng/ml for 25(OH)D and 19.9–67 pg/ml for 1,25(OH)₂D.

A commercially available RIA kit was used to measure serum osteocalcin levels (CIS Diagnostici S.p.A., Tronzano Vercellese, Italy). The sensitivity of the method was 0.50 ng/ml.

Urinary deoxypyridinoline concentrations were measured by high-resolution chromatography in a fluid environment (Medical System, Genova, Italy). Deoxypyridinoline values were expressed in nM for mM of nocturnal 12-h urinary creatinine. The intra- and inter-assay CV of RIA and IRMA methods were <9.8%.

The serum level of BSAP was measured by immunoassay (Metra Biosystems, Mountain View, CA, USA) with a sensitivity of 0.7 U/l and a CV of 3.9–5.8%.

In all the patients, lymphocyte subpopulations were measured by flow cytometry (FACScan cytofluorimeter; Becton Dickinson, San José, CA, USA) with the use of fluorescein- or phycoerythrin-labelled human MABs (anti-CD3, anti-CD19, anti-CD3+4+, anti-CD3+8+, anti-CD3–16+56+; Becton Dickinson).

In all patients, BMC (g) and BMD (g/cm²) of the lumbar spine (L1–L4) were measured by DXA (Delphi-A System, Hologic, Inc., Waltham, MA, USA).

BMD was expressed as Z-scores (that is, the difference between the value of the patient and the normal value for age divided by the s.d. of the normal patient group). Average BMD values for L2–L4 were used for calculations.

The DXA instrument’s software calculates BMD by dividing the BMC by the area of the projection surface of bones (areal BMD; g/cm²). This does not take into account the actual bone volume, which is strictly related to body size (weight and height), a particularly important aspect when evaluating a growing skeleton. Different methods of correction have been proposed for pathologies where a smaller-than-normal body size may be present (7, 8), such as 22q11DS (18).

Therefore, for estimation of the respective volumetric density, which is usually referred to as the BMD, the following formula from Kröger et al. (8) was used:

\[
\text{BMAD} = \text{BMD}_{L2-L4} \times \left( \frac{4}{\pi \times \text{width}} \right),
\]

expressed in (g/cm³)

Bone width was the real mean width of these vertebrae. It was calculated from the dimensions that were manually read off with the ruler from the picture of the spine that was included in the printout of the results of each measurement. If \(A2\), \(A3\) and \(A4\) are the real projected areas of the respective vertebrae, \(h\) is their depicted total height and \(b\) is their depicted mean width, then

\[
\text{Bone width} = \sqrt{\frac{A2 + A3 + A4}{h}} \times b
\]

Each measurement was taken along the vertebral body at three locations (upper, middle and lower parts of the...
the vertebra) by the same researcher, and the mean from these measurements was used; the intra-observer 
CV was 1.0%.

This model was validated by in vivo volumetric data obtained from magnetic resonance imaging of the 
lumbar vertebrae (19).

Also, patients’ BMADs were expressed as Z-scores 
(that is, the difference between the value of the patient 
and the normal value for age divided for S.D. of the 
normal patients group).

In adults, DXA measurements are usually reported as 
T-scores (number of S.D.S from the mean BMD of a 
reference group of normal gender-matched individuals 
in the age range (third decade) during which BMD 
peaks). However, T-scores are not applicable for 
individuals under the age of 20 years; therefore, for 
comparisons and correlations between groups, all 
measurements of BMAD were reported as age- and 
gender-matched Z-scores.

Quality control was regularly performed by using a 
phantom to ensure the reliability of the densitometer. 
All BMD measurements were performed by the same 
operator and were carried out on the same DXA 
instrument using a standardised protocol of measure-
ment. The CV was 0.64% for BMC and 1.0% for lumbar 
spine BMD and BMAD.

Statistical analysis

Statistical analyses were performed using SPSSX (SPSSX 
Inc., Chicago, IL, USA). Summaries of continuous 
variables are given as mean ± S.D. or median and 
range, depending on whether the data were normally 
distributed or not. To compare differences, we used the 
Student’s t-test and Mann–Whitney U test, depending 
on the distribution of the analysed variable. The χ²-test 
and Fisher’s exact test were used to examine associ-
tions between dichotomous variables. Spearman’s 
(rank) correlation test was used to determine the 
correlation coefficients. A multiple stepwise regression 
was used to determine the variables (age (years), sex 
(M:F), serum PTH concentrations, ionised and total 
calcium, phosphate, 25[OH]D and 1,25[OH]₂D levels, 
serum osteocalcin levels, urinary deoxypyridinoline 
concentrations, quantitative assessment of physical 
activity (h/week), calcium intake (mg/day) and BSAP 
levels) that may correlate independently with BMD and 
BMAD Z-score values. P values <0.05 were considered 
statistically significant.

Table 1 Baseline characteristics of 22q11DS patients and controls.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>22q11DS</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children (n)</td>
<td>9.5 ± 3.2 (17)</td>
<td>9.9 ± 3.1 (67)</td>
<td>NS</td>
</tr>
<tr>
<td>Adults (n)</td>
<td>27.0 ± 8.5 (11)</td>
<td>25.1 ± 5.9 (81)</td>
<td>NS</td>
</tr>
<tr>
<td>Height (SDS)</td>
<td>-0.6 ± 0.7</td>
<td>-0.1 ± 0.7</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Children</td>
<td>-0.5 ± 0.7</td>
<td>-0.0 ± 0.8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Adults</td>
<td>-0.7 ± 0.8</td>
<td>-0.2 ± 0.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>BMI (SDS)</td>
<td>0.0 ± 0.7</td>
<td>-0.2 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>Children</td>
<td>0.1 ± 0.7</td>
<td>-0.2 ± 0.8</td>
<td>NS</td>
</tr>
<tr>
<td>Adults</td>
<td>-0.2 ± 0.6</td>
<td>-0.2 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>Bone age (SDS)</td>
<td>-0.1 ± 1.3</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Calcium intake (mg/day)</td>
<td>790 ± 260</td>
<td>825 ± 302</td>
<td>NS</td>
</tr>
<tr>
<td>Children</td>
<td>725 ± 210</td>
<td>760 ± 195</td>
<td>NS</td>
</tr>
<tr>
<td>Adults</td>
<td>2.41 ± 1.22</td>
<td>3.15 ± 1.41</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Serum intact PTH (pmol/l)</td>
<td>2.37 ± 1.36</td>
<td>3.27 ± 1.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Children</td>
<td>2.51 ± 0.97</td>
<td>3.08 ± 1.52</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Adults</td>
<td>28.62 ± 4.71</td>
<td>92.18 ± 20.83</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Osteocalcin (ng/ml)</td>
<td>7.53 ± 3.12</td>
<td>8.54 ± 2.55</td>
<td>NS</td>
</tr>
<tr>
<td>Children</td>
<td>57.9 ± 19.5</td>
<td>101.8 ± 28.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adults</td>
<td>13.3 ± 10.5</td>
<td>16.5 ± 8.6</td>
<td>NS</td>
</tr>
<tr>
<td>Serum 25(OH) vitamin D (ng/ml)</td>
<td>35.1 ± 17.2</td>
<td>30.5 ± 15.6</td>
<td>NS</td>
</tr>
<tr>
<td>Children</td>
<td>32.0 ± 16.3</td>
<td>28.7 ± 14.0</td>
<td>NS</td>
</tr>
<tr>
<td>Adults</td>
<td>39.5 ± 18.3</td>
<td>33.8 ± 14.5</td>
<td>NS</td>
</tr>
<tr>
<td>Serum 1,25(OH)₂ vitamin D (pg/ml)</td>
<td>51.9 ± 16.1</td>
<td>42.0 ± 20.7</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Children</td>
<td>46.8 ± 14.6</td>
<td>41.2 ± 18.0</td>
<td>NS</td>
</tr>
<tr>
<td>Adults</td>
<td>54.5 ± 17.9</td>
<td>43.0 ± 19.2</td>
<td>NS</td>
</tr>
<tr>
<td>Urinary deoxypyridinoline (nM/mM creatinine)</td>
<td>16.6 ± 11.53</td>
<td>42.17 ± 14.76</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Children</td>
<td>9.43 ± 3.22</td>
<td>13.67 ± 4.78</td>
<td>NS</td>
</tr>
</tbody>
</table>

Results

The main auxological features and laboratory charac-
teristics of the patients are summarised in Tables 1 and 2.

No statistically significant differences were found 
between our group of patients with 22q11DS and the 
control group regarding BMI SDS, history of fractures

and calcium intake; however, a statistically significant 
difference was found regarding height (–0.6 ± 0.7 vs 
–0.1 ± 0.7; P < 0.05; Table 1).

In particular, 3 (1 male and 2 females; 10.7%) of 28 
participants had experienced a fracture before the 
study. All were post-traumatic fractures, and there 
were no statistically significant differences with respect 
to controls (9.9%). In addition, no significant differences 
were found in calcium intake between 22q11DS patients 
and controls (children 790 ± 260 vs 825 ± 302 mg/day; 
adults 725 ± 210 vs 760 ± 195 mg/day: P = NS).

Patients with 22q11DS showed a reduced BMAD 
Z-score compared with controls (–0.90 ± 1.01 vs 
0.01 ± 0.87; P < 0.001; Fig. 1a); this result was also 
evident when the subjects were divided into two groups 
of either paediatric (–0.66 ± 0.98 vs 0.01 ± 0.81; 
P < 0.05; Fig. 1b) or adult patients (–1.51 ± 0.85 vs 
–0.02 ± 0.76; P < 0.001; Fig. 1c).

Patients with 22q11DS showed significantly lower 
ionised (0.99 ± 0.07 vs 1.24 ± 0.04 mmol/l; P < 0.001) 
and total calcium levels (2.29 ± 0.13 vs 2.53 
± 0.13 mmol/l; P < 0.05) compared with the controls,
Table 2 Main characteristics of patients with 22q11DS.

<table>
<thead>
<tr>
<th>Pz</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Height (SDS)</th>
<th>BMI (SDS)</th>
<th>Pubertal staginga</th>
<th>Neonatal hypocalcaemia (at the study time)</th>
<th>Hypoparathyroidism</th>
<th>Heart malformations</th>
<th>Immunologic screening</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>12.6</td>
<td>-1.0</td>
<td>-0.7</td>
<td>G3 PH3 AH1</td>
<td>-</td>
<td>-</td>
<td>Transposition of the great arteries</td>
<td>T cells immunodeficiency</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>42.8</td>
<td>-2.5</td>
<td>-0.2</td>
<td>B5 PH5 AH3</td>
<td>+</td>
<td>-</td>
<td>Tetralogy of Fallot</td>
<td>T cells immunodeficiency</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>16.0</td>
<td>-0.9</td>
<td>0.2</td>
<td>G4 PH4 AH3</td>
<td>-</td>
<td>-</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>6.75</td>
<td>-0.4</td>
<td>0.2</td>
<td>B1 PH1 AH1</td>
<td>-</td>
<td>-</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>29.1</td>
<td>-1.4</td>
<td>-0.7</td>
<td>B5 PH5 AH3</td>
<td>-</td>
<td>-</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>7.6</td>
<td>-0.6</td>
<td>0.0</td>
<td>B1 PH1 AH1</td>
<td>-</td>
<td>-</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>9.8</td>
<td>-0.7</td>
<td>0.3</td>
<td>G1 PH1 AH1</td>
<td>-</td>
<td>-</td>
<td>Bicuspid aortic valve</td>
<td>Normal</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>26.0</td>
<td>-1.1</td>
<td>0.8</td>
<td>B5 PH5 AH3</td>
<td>+</td>
<td>+</td>
<td>Ventricular septal defect and patent foramen ovale</td>
<td>Normal</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>7.1</td>
<td>0.2</td>
<td>0.3</td>
<td>B1 PH1 AH1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Normal</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>26.6</td>
<td>0.1</td>
<td>-0.2</td>
<td>B5 PH5 AH3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Normal</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>17.4</td>
<td>-0.4</td>
<td>-0.2</td>
<td>G4 PH4 AH3</td>
<td>-</td>
<td>-</td>
<td>Bicuspid aortic valve</td>
<td>Normal</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>9.6</td>
<td>-0.4</td>
<td>1.1</td>
<td>B2 PH2 AH1</td>
<td>-</td>
<td>+</td>
<td>Tetralogy of Fallot</td>
<td>T cells immunodeficiency</td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>6.1</td>
<td>-0.8</td>
<td>0.9</td>
<td>B1 PH1 AH1</td>
<td>-</td>
<td>-</td>
<td>Atrial septal defect</td>
<td>Normal</td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>7.25</td>
<td>0.2</td>
<td>-0.5</td>
<td>B1 PH1 AH1</td>
<td>+</td>
<td>-</td>
<td>Normal</td>
<td>T cells immunodeficiency</td>
</tr>
<tr>
<td>15</td>
<td>F</td>
<td>25.25</td>
<td>-0.5</td>
<td>1.1</td>
<td>B5 PH5 AH3</td>
<td>+</td>
<td>+</td>
<td>Tetralogy of Fallot</td>
<td>Normal</td>
</tr>
<tr>
<td>16</td>
<td>F</td>
<td>6.1</td>
<td>0.4</td>
<td>0.6</td>
<td>B1 PH1 AH1</td>
<td>+</td>
<td>+</td>
<td>Tetralogy of Fallot</td>
<td>T cells immunodeficiency</td>
</tr>
<tr>
<td>17</td>
<td>F</td>
<td>8.4</td>
<td>-0.9</td>
<td>-0.7</td>
<td>B1 PH1 AH1</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Normal</td>
</tr>
<tr>
<td>18</td>
<td>M</td>
<td>12.33</td>
<td>-0.4</td>
<td>0.6</td>
<td>G2 PH2 AH1</td>
<td>+</td>
<td>+</td>
<td>Supravalvular aortic stenosis</td>
<td>Normal</td>
</tr>
<tr>
<td>19</td>
<td>F</td>
<td>39.25</td>
<td>0.4</td>
<td>-0.8</td>
<td>B4 PH5 AH3</td>
<td>-</td>
<td>+</td>
<td>Double aortic arch</td>
<td>Normal</td>
</tr>
<tr>
<td>20</td>
<td>F</td>
<td>9.9</td>
<td>-1.5</td>
<td>-0.5</td>
<td>B2 PH2 AH1</td>
<td>+</td>
<td>+</td>
<td>Ventricular septal defect and aortic coarctation</td>
<td>Normal</td>
</tr>
<tr>
<td>21</td>
<td>F</td>
<td>10.1</td>
<td>-0.7</td>
<td>0.2</td>
<td>B3 PH3 AH1</td>
<td>+</td>
<td>-</td>
<td>Tetralogy of Fallot</td>
<td>T cells immunodeficiency</td>
</tr>
<tr>
<td>22</td>
<td>F</td>
<td>9.6</td>
<td>0.7</td>
<td>1.4</td>
<td>B1 PH1 AH1</td>
<td>+</td>
<td>+</td>
<td>Tetralogy of Fallot</td>
<td>T cells immunodeficiency</td>
</tr>
<tr>
<td>23</td>
<td>M</td>
<td>16.6</td>
<td>-1.9</td>
<td>-1.3</td>
<td>G3 PH4 AH2</td>
<td>+</td>
<td>-</td>
<td>Atrial and ventricular septal defects</td>
<td>T cells immunodeficiency</td>
</tr>
<tr>
<td>24</td>
<td>M</td>
<td>6.25</td>
<td>0.1</td>
<td>0.3</td>
<td>G1 PH1 AH1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Normal</td>
</tr>
<tr>
<td>25</td>
<td>F</td>
<td>16.75</td>
<td>-0.8</td>
<td>-0.9</td>
<td>B4 PH5 AH3</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>T cells immunodeficiency</td>
</tr>
<tr>
<td>26</td>
<td>F</td>
<td>16.8</td>
<td>-0.5</td>
<td>-0.2</td>
<td>B5 PH5 AH3</td>
<td>+</td>
<td>-</td>
<td>Ventricular septal defect</td>
<td>Normal</td>
</tr>
<tr>
<td>27</td>
<td>M</td>
<td>25.7</td>
<td>-0.9</td>
<td>-0.8</td>
<td>G5 PH5 AH3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Normal</td>
</tr>
<tr>
<td>28</td>
<td>M</td>
<td>31.3</td>
<td>-0.7</td>
<td>-0.3</td>
<td>G5 PH5 AH3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Normal</td>
</tr>
</tbody>
</table>

*aAH, axillary development; PH, pubic hair development; B, breast development; G, male genital development.*
whereas their phosphate levels were normal. Our patients also had significantly lower PTH levels compared with those of the controls (2.41 ± 1.22 vs 3.15 ± 1.41 pmol/l; P < 0.05).

Children and young subjects with 22q11DS showed significantly lower serum osteocalcin levels (28.62 ± 4.71 vs 92.18 ± 20.83 ng/ml; P < 0.001) and urinary deoxypyridinoline concentrations (16.64 ± 11.53 vs 42.17 ± 14.76 nM/mM creatinine; P < 0.001) compared with age-matched controls, but this result was not seen when the adult groups were compared (osteocalcin, 7.53 ± 3.12 vs 8.54 ± 2.55 ng/ml; P = NS; urinary deoxypyridinoline, 9.65 ± 3.02 vs 12.93 ± 4.97 nM/mM creatinine; P = NS; Figs 2 and 3 respectively). Children and young subjects with 22q11DS also showed significantly lower serum osteocalcin levels (28.62 ± 4.71 vs 92.18 ± 20.83 ng/ml; P < 0.001) and urinary deoxypyridinoline concentrations (16.64 ± 11.53 vs 42.17 ± 14.76 nM/mM creatinine; P < 0.001) compared with patients without hypoparathyroidism and/or hypocalcaemia. Nonetheless, there were no substantial differences in BMAD Z-scores between these two groups (P = NS). No differences in BMAD Z-score were also evident when the patients were divided according to the presence of congenital cardiac defects and/or a history of recurrent infections and immunodeficiency (P = NS).

Among the 28 patients, 25(OH)D values were <10 ng/ml (deficient) in none of the patients, 10–20 ng/ml in 3 patients (11%) and >20 ng/ml in 25 patients (89%), with a mean of 35.1 ± 17.2 ng/ml (controls: 30.5 ± 15.6 ng/ml; P = NS). Additionally, 1.25(OH)2D values were also in the normal range in 89% of patients, with a mean of 51.9 ± 16.1 pg/ml (controls: 42.0 ± 20.7 pg/ml; P < 0.05); in the group, one patient showed low 1.25(OH)2D levels (2.8%) and two patients showed high 1.25(OH)2D levels (5.6%).

The quantitative assessment of physical activity in patients with 22q11DS and controls showed significant differences between the two groups; the percentage of
current physical activity levels was significantly lower for patients with 22q11DS than for controls (0 h/week group 51 and 25% respectively; <2 h/week group 36 and 49% respectively; >2 h/week group 13 and 26% respectively). Even if the dimensions of the subgroups are limiting for reliable statistical results, there were differences in BMAD Z-score between the patients with a current physical activity level >2 h/week and the other groups (−0.79 ± 0.86 vs −1.36 ± 0.93; P = NS). The last group of patients was in better physical condition.

Spearman’s rank correlation test showed that in patients with 22q11DS, BMAD Z-score values displayed a significant inverse correlation with age (r = −0.53; P < 0.005; Fig. 4). Both BSAP and osteocalcin levels also showed a significant correlation with total calcium values (r = 0.47; P < 0.05). PTH correlates significantly with ionised calcium (r = 0.41; P < 0.05) and osteocalcin (r = 0.62; P < 0.005).

The multiple regression analysis included age, sex, PTH, ionised and total calcium levels, phosphate levels, 25(OH)D and 1,25(OH)2D levels, serum osteocalcin levels, urinary deoxypyridinoline concentrations, quantitative assessment of physical activity, calcium intake and BSAP levels; this analysis did not identify significant predictors of a lower BMAD Z-score.

Discussion

Our study shows that subjects with 22q11DS have a lower bone mass, probably due to reduced bone modelling during childhood, which may cause a reduced bone mass peak with a significant risk of impaired bone mass in adulthood.

The aetiology of bone impairment in 22q11DS patients may be multifactorial, and we suspect that additional longitudinal data will be necessary to fully ascertain the importance of the many variables that are involved in conditioning a lower bone mass in these patients.

Hypocalcaemia is a frequent manifestation in subjects with 22q11DS, particularly in patients of neonatal age, although it may also be observed later in life (20). This disorder is present in 49–60% of patients with a confirmed del22q11 (4), and patients with the phenotypic characteristics of the DiGeorge anomaly are more likely to have clinical evidence of hypocalcaemia (70%) than patients with VCFS (13–22%) (21).

This mild or transient hypocalcaemia may frequently be missed because it is probably asymptomatic, and a systematic screening is required for its detection (20). Commonly, however, with an increase in dietary calcium intake, the remaining parathyroid activity supplies sufficient PTH to meet metabolic demands, even if a recurrence of hypoparathyroidism may be precipitated during the periods of increased metabolic demand (18). Therefore, a long-term follow-up should be given to 22q11DS patients with normocalcaemia because of the potential for evolution to hypocalcaemic hypoparathyroidism (22).

A prolonged deficiency in calcium metabolism may cause bone quality alterations (23). Calcium intake has been shown to correlate with bone density in healthy children (23), and maintaining adequate calcium intake during childhood and adolescence is necessary to attain a normal peak bone mass, which may be important in reducing the risk of fractures and osteoporosis later in life (24).

However, reduced levels of physical activity are also considered to be important. From the answers given by patients and/or parents using an activity questionnaire, 22q11DS patients participate in less physical activity than controls. This behaviour may be due to associated disorders such as heart defects or neuropsychological
impairment. High physical activity has been reported to be associated with high BMD in healthy individuals (25). The effects of physical activity on bone turnover are well known (26). Bone strength is regulated by mechanical loads, especially muscle forces. In children and adolescents, lean body mass and bone mass are highly related (6).

Bone mass accounts for 75–85% of the variance in the ultimate strength of bone tissue, and a low BMD may lead to an impaired bone mass with an increased risk of fracture (6). Our data, nevertheless, seem to show that 22q11DS patients may not have a significantly greater risk of bone fractures than individuals in the general population.

Bone mass is under strong genetic control, with heritability estimated to be >50%; this feature is likely determined by complex interactions between genetic and environmental factors throughout fetal development, childhood and adult life (27). Other authors have obtained suggestive evidence for a quantitative trait locus (QTL) affecting lumbar spine BMD variation on chromosomes 22q11–12 (27). The QTL on 22q11 is novel and does not overlap with major QTLs reported by other studies (27).

Therefore, the 22q11 region could be a specific site that is important for conditioning the heritability of a lower BMD in the general population, and, if so, this aspect may explain some of our results.

In 22q11DS patients, BMAD appears to be a useful parameter that can also be utilised to estimate the effective bone density. Correction for weight and height may be necessary because DXA underestimates BMD in short subjects by measuring a real rather than volumetric bone density. In fact, volumetric density has been considered to be a more accurate estimate of bone density in smaller individuals and those with small bones (9). Nevertheless, many previous studies that were conducted in patients with short stature or genetic syndromes associated with short stature did not correct the DXA data, which led to many errors in the interpretation of the results because of false diagnoses of lower bone mass. Today, more attention is paid to this problem, and techniques such as the use of BMAD have allowed researchers to determine a correct BMD for these patients (9, 13).

Interestingly, our data show that 22q11DS children and youth have both reduced bone formation (evaluated by the study of the BSAP and osteocalcin) and bone resorption (evaluated by the study of the urinary deoxypyridinoline) markers. These data were not confirmed in adult patients with 22q11DS.

Several studies conducted in both animals and adult humans have shown the presence of increased bone mass in subjects with hypoparathyroidism, although some data obtained from postnatal hypoparathyroid (PTH−/−) animals demonstrated a reduction in bone turnover associated with an increase in trabecular bone volume (28). Therefore, some studies demonstrate a physiological anabolic role for PTH, underpinning its importance in inducing bone accrual (29).

PTH binds to cells of the osteoblastic lineage (28), enhances both bone formation and bone resorption (28), and appears to exert discrete effects on trabecular bone in the intrauterine and postnatal environments (30). PTH contributes to maintenance of normal extracellular fluid calcium levels, at least in part by enhancing trabecular bone resorption (30).

Therefore, we may hypothesise that reduced PTH levels in childhood, even if this phenomenon is frequently latent and may or may not be associated with variable hypocalcaemia, may determine a lower level of bone modelling, which subsequently results in reduced bone accrual and bone mass peak. However, other data, especially long-term data, will be necessary to understand this phenomenon.

This is also of particular importance because childhood is a critical time for bone development and mineralisation (31), and childhood and adolescence are crucial periods of life for the attainment of an optimal bone mass (31). Increases in bone mass and maximal bone mass accrual occur in early mild puberty and slow down in late puberty (32).

A reduced bone accrual may predispose subjects with 22q11DS to a higher risk for impaired bone mass later in life, differentiating the results of a congenital hypoparathyroidism from those of a secondary and acquired hypoparathyroidism, especially at the onset of adulthood.

In conclusion, the present study indicates that subjects with 22q11DS have a significant reduction in bone mass, particularly in adulthood. Furthermore, bone metabolism appears to be altered in these patients, especially in the paediatric age groups. We therefore speculate that factors associated with deletion of 22q11, for example hypoparathyroidism, hypocalcaemia, reduced physical activity, presence of cardiac defects or recurrent infections, may cause reduced bone remodelling and bone mass. Therefore, we suggest that bone mass and metabolism in 22q11DS patients should be closely monitored. More studies are needed to confirm our data and to evaluate eventual treatments that may ameliorate the bone mass peak and reduce the risk of osteoporosis in patients with this syndrome.

Declaration of interest
The authors declare that there are no conflicts of interest that could be perceived as prejudicing the impartiality of the research reported herein.

Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.
Acknowledgements
The authors are grateful to Dr Giampiero Igli Baroncelli (University of Pisa) for his useful help and counselling.

References


