Low bone mineral density is a common feature of Zellweger spectrum disorders

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ABSTRACT

Patients with Zellweger Spectrum Disorders (ZSDs) have impaired peroxisome biogenesis and severe, multisystem disease. Although the neurologic symptoms of ZSD tend to be the most prominent, patients also have hepatic, renal and adrenal impairment. Little is known about bone health in patients with ZSD, particularly those with mild or moderate presentation. We investigated 13 ZSD patients who had strikingly abnormal bone mineral density for age. DXA scans showed mean lumbar and femoral neck Z-scores of −3.2. There were no major differences between ambulatory and nonambulatory patients, and no biochemical abnormalities consistent with rickets or vitamin D deficiency were seen. Cyclic bisphosphonate therapy in one ZSD patient was successfully used to increase in bone mineral density. Although the etiology of bone disease in this condition is unknown, we speculate that altered signaling through the PPARγ pathway or deficient plasmalogens in patients with ZSD disrupts osteogenesis, resulting in poor bone formation and poor mineralization. Further investigation into the pathogenic mechanisms of bone disease in ZSD and the role of peroxisomal metabolism in osteogenesis may yield insights into the pathology of bone disease and suggest novel treatment options.

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1. Introduction

Peroxisomal Biogenesis Disorders (PBD) are rare, multisystem genetic diseases with a wide range in clinical severity. These disorders can be divided into two clinical categories: Zellweger spectrum disorder (ZSD) and rhizomelic chondrodysplasia punctata (RCDP). ZSD has historically been further subdivided into Zellweger syndrome (ZS), neonatal adrenoleukodystrophy (NALD), and infantile Refsum disease (IRD) [19], corresponding to severe, intermediate and milder phenotypes, respectively. Once thought to be clinically distinct entities, it is now understood that ZSDs share common biochemical and genetic abnormalities with overlapping phenotypes. Owing to their common etiologies, the preferred term to use is ZSD, which is further divided into severe, intermediate and milder. Patients with severe ZSD are the most homogeneous group and do not usually survive beyond the first year of life. They present in the neonatal period with profound hypotonia, dysmorphic facial features, seizures, and hepatic dysfunction [6]. They have developmental defects of the brain (neuronal migration defects), kidney (renal cysts) and bone (chondrodysplasia punctata limited to the hips and knees), indicating an early insult in fetal development. They generally achieve little or no developmental progress before their death. In contrast, patients with intermediate and milder ZSD frequently survive for much longer periods of time, achieving a variable level of intellectual function, expressive speech and independent ambulation [16]. These patients do not have structural defects of the brain, but typically show a progressive course over time due to ongoing peroxisome dysfunction, resulting in sensorineural hearing loss and vision loss due to pigmentary retinopathy. They may also develop liver dysfunction, adrenal insufficiency, peripheral neuropathy, cerebellar dysfunction, and leukodystrophy, the latter which can result in neurodegeneration and possibly death [17].

ZSD is caused by mutations in the family of PEX genes, encoding various peroxin proteins that are required for the assembly of functional peroxisomes [3]. To date, sixteen mammalian PEX genes have been identified and mutations in twelve have been implicated in ZSD [12]. Mutations in this group of disorders cause dysfunctional peroxisomes which results in a spectrum of metabolic abnormalities, including accumulation of very long chain fatty acids and branched-chain fatty acids;
deficient plasmalogens and increased bile acid metabolite precursors. The constellation of metabolic abnormalities is thought to be responsible for the clinical phenotype of ZSD.

We report a series of patients with intermediate and milder ZSD, who show a strong trend toward low bone mineral density associated with pathologic fractures. Our studies suggest that ongoing peroxisome dysfunction over time adversely affects bone mineralization.

2. Methods

Thirteen patients with confirmed PEX mutations and/or biochemical evidence of ZSD involving multiple peroxisome pathways (VLCFA oxidation, phytanic acid oxidation, and plasmalogen synthesis), who were clinically in the intermediate or milder category of ZSD were evaluated at the University of Nebraska Medical Center. Routine history and physical examinations were performed. Dual-energy X-ray absorptiometry (DEXA) was performed, and patients had standard biochemical evaluations to investigate metabolic bone disease.

Discrete data elements included bone mineral density at the lumbar spine and femoral neck with data normalized where possible for age, gender, ethnicity, and height. Descriptive statistics were performed on normalized data derived by database provided by DXA device manufacturer (Hologic, Bedford, MA) or based on internal validation by reference laboratory (ARUP Laboratories, Salt Lake City, UT). All DXA data was interpreted per International Society for Clinical Densitometry (ISCD) standards for pediatric patients by a Certified Clinical Densitometrist (ETR). Descriptive statistics were also performed on biochemical data and normal ranges are given where appropriate. Families completed the PedsQL quality of life questionnaire at patient appointments, and each subset score was recorded. Physical score subscores were compared to Lumbar Z-score with Pearson Correlation score for patients in whom both data elements were available.

This research was approved by the University of Nebraska Medical Center Institutional Review Board.

3. Results

3.1. Patient characteristics

Thirteen ZSD patients (8 males and 5 females) with mild or moderate phenotypes were studied. The mean age of the cohort was 7.2 years (range of 1.8–17.9 years) (Table 1). Four patients (#1, 8, 12 and 13) had experienced fractures, and three of these fractures were likely related to underlying bone fragility. All patients had biochemical evidence of peroxisome dysfunction with elevated plasma VLCFA and phytanic acid, and decreased RBC plasmalogens. Ten of the patients also had genotyping performed. PEX1 mutations were seen in 9 of them and PEX12 mutations were carried by one subject (#12). Among the patients with PEX1 mutations, 8 carried the common c.2528G→A (p.Gly843Asp) mutation that is associated with a milder ZSD phenotype. One patient (#3) was homozygous for this mutation, whereas the other patients were compound heterozygotes.

3.2. Bone density studies and biomarkers

All 13 patients had physical examinations and DXA scans (Table 2). DXA of the lumbar spine was performed on L1 through L4. Bone mineral density varied between 0.200 and 0.776 g/cm². Z-scores for patients who were greater than two years of age ranged between 0 and −5.5 (mean = −3.19 ± 1.48, n = 10) (Table 3). Nine of the ten patients for whom normal age- and gender-matched Z-scores were available had bone mineral densities that were low for age (Z-score < −2).

DXA of the femoral neck showed bone mineral density between 0.140 and 0.656 g/cm². Z-score for patients greater than five years of age ranged between −1.8 and −4.2 (mean = −3.25 ± 0.87; n = 6). Five of six patients for whom normal age- and gender-matched Z-scores were available had bone mineral densities that were low for age (Z-score < −2).

Comparison of mean bone mineral density Z-scores of ambulatory and nonambulatory patients showed no significant difference at the lumbar spine (−3.18 versus −3.20) and only modest difference at the femoral neck (−3.08 versus −3.60). For additional evaluation of the potential relationship between disuse and low bone mineral density, PedsQL physical score data was compared with lumbar Z-score. In the nine patients in whom both data points are recorded, Pearson correlation analysis showed r = 0.281, implying a weak correlation between the physical score and normalized bone density. The coefficient of determination, r² = 0.079, implied that 7.9% of the variance in normalized bone density is attributable to items measured in the physical score. There was also no correlation between bone density and age at the lumbar spine (r = 0.017). Insufficient data was present for similar analysis of femoral neck density for either age or PedsQL data.

Consideration of malabsorption of fat-soluble vitamins, due to the requirement for peroxisomal enzymes to synthesize mature bile acids, prompted evaluation of 25-OH vitamin D levels in all patients. Mean serum 25-OH vitamin D was 61 ± 33 ng/mL (range 27–126 ng/mL). No patients had vitamin D deficiency as defined by serum 25-OHD levels below 20 ng/mL and one patient had vitamin D insufficiency as defined by serum 25-OHD levels between 21 and 29 ng/mL [8]. Vitamin D supplementation was managed by local providers and in some cases was part of a fat soluble supplement such as AquaDEKs and in some cases was standard ergocalciferol or cholecalciferol. Supplementation ranged between 400 IU and 2000 IU and information was not available for all patients. Mean serum calcium level was 9.74 mg/dL and mean serum phosphorus level was 4.63 mg/dL with no patients exhibiting abnormal serum levels of either.

### Table 1

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age</th>
<th>Sex</th>
<th>Mutation</th>
<th>VLCFA</th>
<th>Phytanic acid</th>
<th>RBC plasmalogens</th>
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<tr>
<td>11</td>
<td>1 year</td>
<td>M</td>
<td>ND</td>
<td>↑↑</td>
<td>↑↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>5</td>
<td>2 years</td>
<td>M</td>
<td>ND</td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>10</td>
<td>2 years</td>
<td>M</td>
<td>PEX1 c.2528 G→A (Gly843Asp)/c.2098dupT</td>
<td>↑↑</td>
<td>↑↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>6</td>
<td>3 years</td>
<td>F</td>
<td>PEX1 c.2528 G→A (Gly843Asp)/c.1865_1866insCAGTGGCA</td>
<td>↑↑</td>
<td>↑↑</td>
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</tr>
<tr>
<td>12</td>
<td>3 years</td>
<td>M</td>
<td>PEX12 c.1–26 G→A;Ac.102 A→T(p.Arg34Ser);c.1–26 G→A;Ac.102 A→T(p.Arg34Ser)</td>
<td>↑↑</td>
<td>↑↑</td>
<td>↑↑</td>
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<tr>
<td>8</td>
<td>4 years</td>
<td>F</td>
<td>PEX1 c.2098dupT (p.Ile706fs)/p.Leu1256_Ala1283del</td>
<td>↑↑</td>
<td>↑↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>9</td>
<td>4 years</td>
<td>M</td>
<td>PEX1 c.2528 G→A (Gly843Asp)/c.382 C→T</td>
<td>↑↑</td>
<td>↑↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>4</td>
<td>6 years</td>
<td>F</td>
<td>PEX1 c.2528 G→A (Gly843Asp)/c.2098dupT</td>
<td>↑↑</td>
<td>↑↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>2</td>
<td>6 years</td>
<td>F</td>
<td>PEX1 c.2528 G→A (Gly843Asp)/c.2098dupT</td>
<td>↑↑</td>
<td>↑↑</td>
<td>↑↑</td>
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<tr>
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<td>7 years</td>
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<td>↑↑</td>
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<tr>
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<td>↑↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>3</td>
<td>17 years</td>
<td>F</td>
<td>PEX1 c.2528 G→A (Gly843Asp)/PEX1 c.2528 G→A (Gly843Asp)</td>
<td>↑↑</td>
<td>↑↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>1</td>
<td>17 years</td>
<td>M</td>
<td>ND</td>
<td>↑↑</td>
<td>↑↑</td>
<td>↑↑</td>
</tr>
</tbody>
</table>

Patient number is sequentially assigned based on the order they were enrolled, and age at first evaluation is given. Biochemical testing provided is defined as either elevated or decreased compared to normal reference ranges. ND, not determined. VLCFA, very long chain fatty acids.
Parathyroid hormone levels were measured in eight patients with a mean level of 24 pg/mL and a range of 14–36 pg/mL. Two patients exhibited mild hypoparathyroidism, which correlated to elevated 25-OH vitamin D levels (92 and 126 ng/mL) and were presumed to be related to suppressive effects of 1,25-OH vitamin D on parathyroid hormone production.

Bone turnover was estimated by measurement of serum beta-crosslinked c-telopeptides (CTX) and serum osteocalcin. Results are normalized for age and gender. Serum CTX ranged between 208 and 1310 pg/mL (n = 7), all of which were normal and suggest typical osteocalcitic function. Serum osteocalcin ranged between 21 and 50 ng/mL (n = 8) with six of the eight patients had decreased levels for age. These results suggest a primarily hypoproliferative disease.

Bisphosphonate therapy was commenced in three patients (1, 3, and 13) included in this cohort with low bone density. Two of the patients (#1 and #13) had sustained a pathologic fracture and one of the patients had onset of fatigue and regression in ambulatory ability (#3). Patients were treated with intravenous pamidronate at yearly doses between 3 mg/kg and 6 mg/kg. Adequate follow up data is only available on one patient (#13), who has now been treated for five years. In that time period, the patient has had an increase in lumbar bone mineral density of 98.2% from 0.233 g/cm² (Z-score of −4.8) to 0.461 g/cm² (Z-score of −2.8), and an increase in femoral neck density of 52.2% from 0.359 g/cm² (Z-score of −3.7) to 0.546 g/cm² (Z-score of −3.1). This patient has not suffered additional fractures.

4. Discussion

Metabolic bone disease in ZSD has not previously been recognized in the literature. In our patients of varying ages, low bone mineral density is common and should be considered a phenotypic feature of intermediate and milder ZSD patients. Our cohort of patients is too small to determine if there is any genotype–phenotype relationships, and with a lack of normalized data for very young children, it is unclear whether the phenotype is of consistently low bone mass, or whether bone health continues to deteriorate with age, although the present data does not show a clear relationship between Z-score and age.

Patients with various neurological or neuromuscular disorders have been found to have low bone mass. Cerebral palsy, although etiologically heterogeneous, has been studied most extensively. Low bone mineral density and pathologic fractures have been well described [18]. Likewise, patients with Duchenne muscular dystrophy are frequently found to have low bone mineral density for age and fractures, with the added concern of chronic glucocorticoid therapy in this patient group [15]. In both disorders, the lack of movement, particularly resistance against gravity is the primary initiator of bone disease. Likewise, patients with ZSD frequently are nonambulatory or have altered ambulation. In our cohort, six patients were noted to have either complete or partial community ambulation and were considered ambulatory. Four additional patients were noted to have either no weight-bearing or only partial weight-bearing and were judged to be nonambulatory. With this comparison, mean bone mineral density Z-scores of ambulatory and nonambulatory patients were not different at the lumbar spine (−3.18 versus −3.20) and only modestly different at the femoral neck (−3.08 versus −3.60). Additional analysis of PedsQL data suggested only weak correlations between physical abilities and lumbar BMD Z-score. These data argue against osteoporosis as the sole driver of bone disease in this cohort.

Malabsorption of fat-soluble vitamins is a major concern in the nutritional management of this condition. As a result, ZSD patients are typically supplemented with additional vitamins A, D, E, and K. Vitamin D deficiency as a major driver of bone pathology in this population is a compelling one. However, in our cohort, no patients had vitamin D deficiency as judged by 25-OH vitamin D level less than 20 ng/dL, so this is unlikely to be the cause of low bone mineral density. Likewise, our patients had normal serum calcium and phosphorus. No unusual restrictions in dietary calcium or phosphorus intake were observed, which argues against Vitamin D deficiency as a major contributor to low bone mineral density in patients with ZSD.

The pathogenic effects of peroxisomal dysfunction on bone may be related to one or more of the known metabolic abnormalities ascribed to ZSD patients or may arise from unknown metabolic consequences of peroxisomal dysfunction. In RCDP, the extreme bone abnormalities are felt to be due to profound deficiency in plasmalogens, which may have specific impact on the production and proliferation of articular cartilage [2]. In RCDP this mechanism may cause disease by impairing enchondral bone ossification leading to the identifiable phenotype. It
is therefore reasonable to presume that low plasmalogens found in ZSD may also be involved in nascent bone disease, but may be more subtle at birth, identifiable as chondrodysplasia punctata only in ZSD patients with severe phenotype. Other peroxisomal metabolic abnormalities are also present which are not present in RCDP, and these may further influence the nature or severity of different bone disease in ZSD. Increases in phytanic acid may also be partly reflected in the phenotype of Adult Refsum Disease, in which patients exhibit osteoprosis and also in some instances have short fourth metacarpals.

Osteogenesis is the process by which new bone is formed. Although it continues throughout the life span, childhood and adolescence are critical times during which active, sustained osteogenesis occurs. This process is complex and multiple regulatory mechanisms exist to influence the effects of osteoblasts and osteoclasts. Three such pathways are of particular interest.

Osteoblasts are responsible for forming both the organic bone matrix and its subsequent mineralization to form mature bone. Osteoblasts are derived from mesenchymal cells, which can differentiate into chondrogenic and adipogenic cell lines [4]. Two pathways appear to be very important to the maintenance of proper osteoblastogenesis and could potentially be altered in patients with PBD. The first pathway involves RUNX2, which has been previously identified as the osteogenic master switch [20]. It appears to be involved along the entire process, not only in stem cell commitment to osteoblast lineage, but also in osteoprogenitor and pre-osteoblast maturity. Germline mutations causing a loss of function of RUNX2 result in a well-described phenotype of cleidocranial dysostosis [14], which results in persistently open anterior fontanelle, absence or hypoplasia of the clavicles, mildly short stature, and increased bone fragility. Conversely, germline gain of function mutations in RUNX2 result in a different phenotype known as metaphyseal dysplasia with maxillary hypoplasia and aside from the eponymous findings can feature brachydactyly and dystrophic teeth [13]. Clearly, although RUNX2 is important for normal osteogenesis, it also requires strict regulation with detrimental consequences of either over- or underexpression.

The WNT1/beta-catenin signaling pathway has generated recent interest due to its importance in normal osteogenesis (Fig. 1). WNT1 affects two crucial aspects of bone formation. WNT, like RUNX2 is a key regulator of osteoblast development, and it appears that the stimulation of osteoblastogenesis occurs through the canonical beta-catenin signaling pathway [11]. Through this pathway, WNT appears to inhibit adipogenesis [1], which may prove to be important for full understanding of bone disease in PBD. Unlike RUNX2, however, WNT has an additional important role in promoting bone mineralization through the canonical signaling pathway by recruiting the important intermediate molecule LRPS. Loss of function mutations in WNT1 and LRPS cause low bone mass disorders. WNT1 has been implicated in patients with a form of osteogenesis imperfecta [9], while LRPS loss of function mutations have been seen in patients with osteoporosis-pseudoglioma syndrome [5]. Gain of function mutations in the LRPS gene result in varying severity of osteopetrosis, a high-bone mass disorder [23]. As seen with RUNX2, the WNT/beta-catenin pathway must be well-regulated as there are clear consequences to both over- and underexpression.

Importantly, osteogenesis appears to be strongly linked to adipogenesis. Osteoblasts and adipocytes share a common precursor stem cell, and it has become known that cell fate decisions which promote differentiation toward one cell type often do so at the expense of the other cell type [21]. We previously noted that WNT specifically inhibits adipogenesis while it stimulates osteoblastogenesis. A converse stimulatory signal exists to promote adipogenesis while inhibiting osteoblastogenesis, with the transcription factor PPARy as the main molecule of interest.

The peroxisomal proliferator-activated receptor family have a number of functions within the body, although as the name implies, among the best described is a stimulatory effect on the proliferation of peroxisomes. Although peroxisomal assembly is impaired in patients with ZSD, presumably the signaling apparatus is intact. PPARy has the function of stimulating the production of adipocytes while inhibiting osteoblast development [22] which appears to be at least partially through inhibition of RUNX2 [10]. PPARy itself is activated by fatty acids, with a net influx of fatty acids spurring adipogenesis for appropriate fat storage. Very long chain fatty acids, which are elevated in PBDs, have been specifically shown to activate PPARy and enhance adipogenesis [24], which would be presumed to happen at the expense of osteogenesis. Similarly, phytanic acid has been shown to activate PPARy and PPARx [7]. We hypothesize that PPARy activation by very long chain fatty acids and/or phytanic acid could serve as a potent inhibitor of osteoblastogenesis. This is consistent with findings of hypoproliferation of bone in our patient cohort. Greater understanding of the mechanisms for bone disease in ZSD could lead to targeted treatments that would be useful for a variety of low bone mass disorders, including ZSD.

At present, a small number of patients have been treated with cyclic bisphosphonate therapy, with insufficient information to make a recommendation for their use. Our single patient with long-term follow up has had significant increase in bone density without further pathogenic fracture. Until further data can be accumulated, any use of bisphosphonate should be undertaken by an experienced metabolic bone specialist on an individualized basis. In the absence of specific data to guide timing and frequency of surveillance, we would suggest that patients with ZSD who have a fracture of long bone which occurs without maximal trauma or who have a vertebral compression fracture should have DXA performed. Specific treatment may be considered if bone mineral density is low for age. Alternately, in patients with ZSD who have not had a fracture, it would be reasonable to perform initial DXA between three and five years of age, with screening interval dependent on initial findings.

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References
