Normal Bone Density and Fat Mass in Heterozygous SERPINF1 Mutation Carriers

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Context: Homozygous mutations in SERPINF1 cause deficiency of pigment epithelium-derived factor (PEDF) and lead to osteogenesis imperfecta (OI) type VI, but it is not known whether heterozygous mutations in SERPINF1 cause a phenotype.

Objective: In the present study, we therefore assessed family members of individuals with OI type VI and compared the results of SERPINF1 mutation carriers with those of noncarriers of SERPINF1 mutations.

Setting: This study was conducted at a metabolic bone clinic of a pediatric orthopedic hospital.

Subjects: The study population comprised 29 family members (age range 8–89 y; 18 females, 11 males) of patients with a diagnosis of OI type VI. Eighteen individuals were heterozygous for SERPINF1 mutations, but the others did not carry a mutation.

Main Outcome Measures: PEDF expression was assessed in skin fibroblasts from four heterozygous SERPINF1 mutation carriers. Skeletal characteristics and body composition were measured using dual-energy X-ray absorptiometry and peripheral quantitative computed tomography. Serum samples were used to quantify markers of bone metabolism, lipid status, and PEDF.

Results: Carriers of heterozygous stop or frame shift mutations in SERPINF1 had low SERPINF1 transcript levels. Mean PEDF serum concentrations were significantly lower in the carrier group than in the noncarriers (P = .04). However, no group differences were found with regard to areal bone density at the lumbar spine and total body, volumetric bone density at the radius and tibia, body composition, lipid status, and markers of bone metabolism.

Conclusion: Heterozygous SERPINF1 mutation carriers had no detectable abnormalities in fat and bone, despite decreased PEDF expression. (J Clin Endocrinol Metab 99: E2446–E2450, 2014)
There is presently no information on whether heterozygous mutations in SERPINF1 cause a phenotype. Some genetic defects that lead to severe bone fragility disorders in the homozygous state also cause bone disease in heterozygous carriers. For example, homozygous defects in LRP5 lead to osteoporosis-pseudoglioma syndrome, whereas heterozygous LRP5 mutations can give rise to osteoporosis (10). Similarly, homozygous mutations in WNT1 cause a severe form of OI, but heterozygous mutation carriers can have early-onset osteoporosis (11, 12). In the present study, we therefore assessed family members of individuals with OI type VI and compared the results of SERPINF1 mutation carriers with those of noncarriers of SERPINF1 mutations.

Materials and Methods

Subjects
The study population comprised 29 family members (age range 8–89 years, 11 females, 18 males) of patients with a diagnosis of OI type VI. Members of eight families were examined. All study participants were assessed at the Shriners Hospital for Children (Montreal, Quebec, Canada). The study was approved by the Institutional Review Board of McGill University, and informed consent was obtained from the participants or, for minors, their legal guardians. Assent was obtained from children and adolescents aged 7–17 years.

Height, weight, and body mass index were converted to age- and sex-specific z-scores on the basis of reference data published by the Centers for Disease Control and Prevention (13).

Bone densitometry
Lumbar spine areal bone mineral density (aBMD; grams per square centimeter) was determined in the anterior-posterior direction at the lumbar spine (L1-L4) using a Hologic QDR Discovery device (Hologic Inc). Total body dual-energy X-ray absorptiometry (DXA) was performed with the same device to measure total body aBMD, lean body mass, and fat mass. Percentage body fat was calculated as the ratio between fat mass and total body mass. Results were transformed to age-specific z-scores, using reference data provided by the densitometer manufacturer.

Forearm peripheral quantitative computed tomography (XCT-2000; Stratec Inc) was performed at the 4% site of the radial metaphysis to measure trabecular volumetric BMD (vBMD) and at the 65% site of the radial diaphysis to measure cortical vBMD, as described elsewhere (14, 15). Gender-specific z-scores were calculated based on reference data established by one of the authors (14, 15).

Biochemical measurements
Serum samples were obtained between 8:00 and 10:00 AM after an overnight fast. Serum PEDF was quantified by an ELISA (BioVendor Laboratory Medicine Inc) (16). Serum levels of intact PTH were analyzed using a chemiluminescent immunoassay (Access immunoassay systems; Beckman Coulter Canada). Serum amino-terminal propeptide of type I collagen, cross-linked C-telopeptide of type I collagen (CrossLaps; Immunodiagnostic Systems), and 25-hydroxyvitamin D were measured by immunochemiluminescence assays on an IDS-iSYS automated analyzer (Immunodiagnostic Systems).

Sequence analysis of SERPINF1
The SERPINF1 exons harboring the mutation of the OI type VI patient in each family were amplified by PCR in genomic DNA. The mutations were previously described (7). The sequencing reaction was performed using a BigDye Terminator cycle sequencing kit (Applied Biosystems). The nucleotide sequence was determined using an Applied Biosystems 3100 DNA sequencer. Patient sequences were compared with a GenBank reference sequence (NM_002615.5).

Fibroblast analyses
Skin biopsy samples were obtained at the proximal forearm using a 3-mm punch biopsy needle. Skin fibroblasts were cultured in the presence of 50 μg/mL ascorbic acid. Cells were maintained in serum-free medium for 24 hours prior to analyses. Real-time PCR was performed on cDNA using an ABI 7500 real-time PCR machine using Taqman primers specific for SERPINF1 (Hs01106934_m1) and ACTINB (Hs01060665_g1) (Applied Biosystems). The expression levels for SERPINF1 were normalized to those of ACTINB using the threshold cycle method and expressed as fold difference relative to control fibroblasts. For PEDF protein analyses in conditioned medium, 20 μg protein from control and selected carriers were separated on 10% sodium dodecyl sulfate-polyacrylamide gels. Primary antibodies were applied against PEDF (rabbit polyclonal antinmouse-PEDF; Millipore).

Statistical analyses
Differences between 2 groups were tested for significance using the unpaired t test. Group differences in dichotomous variables were tested for significance using the χ² test. All tests were two-tailed, and values of P < .05 were considered significant. Calculations were performed using SPSS version 19.0 (SPSS Inc).

Results
Six different SERPINF1 mutations were found in the carrier population, of which three led to premature stop codons (c.295C>T [p.Arg99*]; c.540dupC [p.Asn181Glnfs*22]; c.808G>T [p.Gly270*]), and three represented previously reported in-frame deletions or insertions (c.271_279dup [p.Ala91_Ser93dup]; c.440_643del [p.Lys147_Gly215delinsArg], corresponding to the deletion of exon 5; c.829_831del [p.Phe277del]). The p.Asn181Glnfs*22 and p.Gly270* variants had not been reported before, but both lead to premature stop codons and therefore are clearly pathogenic.

Skin fibroblasts were obtained from four heterozygous mutation carriers. Carriers (C) 1 and C2 were heterozygous for p.Arg99*. C3 had the p.Ala91_Ser93dup mutation. C4 carried a p.Asn181Glnfs*22 mutation. Cellular consequences of heterozygous SERPINF1 mutations were assessed in these skin fibroblasts. Quantitative real-time PCR showed that SERPINF1 transcript levels were reduced in fibroblasts with stop or frame shift mutations but...
not in fibroblasts harboring the in-frame duplication p.Ala91_Ser93dup (Figure 1A). Western blot analysis of conditioned medium suggested that fibroblasts of individuals C1, C2, and C4 but not fibroblasts of individual C3 secreted lower amounts of PEDF (Figure 1B).

Clinical characteristics of 18 carriers of SERPINF1 mutations (age from 8 to 89 y) were compared with those of 11 noncarriers (age range 8–78 y) (Table 1). The two groups did not differ with regard to height, weight, and body mass index z-scores or with regard to markers of bone and mineral metabolism. PEDF serum concentrations ranged from 2.1 to 10.1 mg/L in SERPINF1 mutation carriers without obvious difference between individual mutations, and from 2.0 to 28.3 mg/L in noncarriers (Figure 1C). This resulted in a significantly lower mean PEDF serum concentration in the carrier group (Table 1).

Serum levels of triglycerides, total cholesterol, low-density lipoprotein-cholesterol, high-density lipoprotein-cholesterol, and the ratio between total cholesterol and high-density lipoprotein-cholesterol were not different between groups (P > .3 for each parameter; data not shown).

All 18 SERPINF1 mutation carriers and all 11 noncarriers had normal aBMD results (z-scores between −2 and +2) at both the lumbar spine and the total body (Table 1). Total-body DXA in addition showed a similar percentage body fat mass z-score between the 2 groups. Peripheral quantitative computed tomography at the radius (Table 1) and at the tibia (not shown) also did not reveal significant group differences for trabecular and cortical bone density.

**Discussion**

In this study we assessed the consequences of heterozygous SERPINF1 mutations. On the cellular level, we observed

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Table 1. Clinical Characteristics and Biochemical Results in Heterozygous Carriers of SERPINF1 Mutations and Family Members Who Do Not Carry a SERPINF1 Mutation

<table>
<thead>
<tr>
<th></th>
<th>Carriers</th>
<th>Noncarriers</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, male/female</td>
<td>18/12</td>
<td>11/5</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>18/17.9</td>
<td>11/23.0</td>
<td>.93</td>
</tr>
<tr>
<td>Weight (z-score)</td>
<td>0.55/0.86</td>
<td>0.53/1.19</td>
<td>.96</td>
</tr>
<tr>
<td>Height (z-score)</td>
<td>−0.23/1.01</td>
<td>−0.57/0.80</td>
<td>.34</td>
</tr>
<tr>
<td>Body mass index (z-score)</td>
<td>0.67/0.79</td>
<td>0.61/0.78</td>
<td>.86</td>
</tr>
<tr>
<td>PEDF, mg/L</td>
<td>6.2/2.7</td>
<td>10.9/8.8</td>
<td>.04</td>
</tr>
<tr>
<td>P1NP, µg/L</td>
<td>43/13 to 1177</td>
<td>39/21 to 70</td>
<td>.97</td>
</tr>
<tr>
<td>CTX, µg/L</td>
<td>0.41/0.08 to 1.70</td>
<td>0.45/0.13 to 2.21</td>
<td>.14</td>
</tr>
<tr>
<td>Lumbar spine aBMD (z-score)</td>
<td>−0.04/1.04</td>
<td>0.05/1.26</td>
<td>.84</td>
</tr>
<tr>
<td>Total body aBMD (z-score)</td>
<td>0.28/1.27</td>
<td>0.90/1.09</td>
<td>.25</td>
</tr>
<tr>
<td>Percentage body fat (z-score)</td>
<td>−0.83/1.08</td>
<td>−1.03/1.09</td>
<td>.66</td>
</tr>
<tr>
<td>Radius trabecular vBMD (z-score)</td>
<td>−0.12/1.13</td>
<td>0.29/1.16</td>
<td>.36</td>
</tr>
<tr>
<td>Radius cortical vBMD (z-score)</td>
<td>−0.54/1.14</td>
<td>−0.17/1.37</td>
<td>.45</td>
</tr>
</tbody>
</table>

Abbreviations: CTX, cross-linked C-telopeptide of type I collagen; P1NP, amino-terminal propeptide of type I collagen. Results are given as mean (SD) or median (range). P values were calculated by independent t tests or U tests, as appropriate. Bold print indicates a significant P value (P < .05).
that fibroblasts with heterozygous stop or frame shift mutations in SERPINF1 express less SERPINF1 mRNA and secrete less PEDF protein than control fibroblasts. As a group, heterozygous SERPINF1 mutation carriers had lower mean serum PEDF concentrations than family members who did not carry SERPINF1 mutations. Despite the lower PEDF expression on the cellular and the systemic levels, heterozygous SERPINF1 mutation carriers had no detectable skeletal abnormality, and their fat mass and serum lipid status were similar to family members who did not carry a SERPINF1 mutation.

We used DXA, peripheral quantitative computed tomography, and serum markers of bone metabolism to search for skeletal abnormalities in heterozygous SERPINF1 mutation carriers but did not detect any differences to the results in family members without SERPINF1 mutations. It thus appears that a single functional copy of SERPINF1 is sufficient to maintain skeletal homeostasis. This is in contrast to mutations in LRP5 and WNT1, in which heterozygous mutations lead to haploinsufficiency and osteoporosis (10–12). LRP5 and WNT1 play important roles in the WNT signaling pathway, and it therefore seems that genetic defects in the WNT pathway tend to be associated with haploinsufficiency. PEDF is thought to also play a role in WNT signaling (17). However, the observation that heterozygous SERPINF1 mutation carriers did not have a bone phenotype, whereas WNT1 mutation carriers have osteoporosis suggests that the effect of PEDF on WNT signaling activity in bone cells is lower than that of WNT1. The mechanisms whereby PEDF deficiency leads to bone fragility are presently unknown.

PEDF is not only important for bone but has also been identified as a secretory product of many tissues, including liver, muscle, and fat (3). It has been proposed that PEDF acts as a negative regulator of adipogenesis (17). It is therefore reasonable to hypothesize that heterozygous SERPINF1 mutations and decreased expression of PEDF should be associated with increased fat mass. However, our data do not provide evidence for this hypothesis because family members with and without heterozygous SERPINF1 mutations had similar body fat mass and also did not differ from noncarriers in serum lipid status. This mirrors recent findings by Lakeland et al (18), who found that mice with mild overexpression (2.5-fold) of PEDF in adipocytes had normal body composition and normal serum triglycerides. It thus appears that mild decreases (as in the present study) or increases (as in the study by Lakeland et al) in PEDF have little effect on body fat mass.

It has to be acknowledged that PEDF serum concentrations in heterozygous SERPINF1 mutation carriers largely overlapped with the levels found in individuals who did not carry such mutations. It is not known what levels of PEDF are required for normal bone homeostasis. We had previously found that the average PEDF serum concentration in healthy controls was 9.0 mg/L. It appears that a level of 2 mg/L, the lowest results obtained in the present study, is compatible with normal bone health. In contrast, individuals with OI type VI consistently have PEDF serum levels below 0.3 mg/L and in most cases serum PEDF is in fact undetectable (7).

In conclusion, we found that heterozygous SERPINF1 mutation carriers did not have detectable abnormalities in fat and bone.

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Disclosure Summary: The authors have nothing to declare.

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