CLINICAL REPORT

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A Co-Occurrence of Osteogenesis Imperfecta Type VI and Cystinosis

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Osteogenesis imperfecta type VI (OI type VI) is a rare autosomal recessive disorder caused by mutations in the *SERPINF1* gene that encodes pigment epithelium-derived factor (PEDF). Cystinosis is an autosomal recessive lysosomal transport disorder caused by mutations in the *CTNS* gene. Both *SERPINF1* and *CTNS* are located on chromosome 17p13.3. We describe an individual presenting with both OI type VI and cystinosis. The patient was diagnosed with cystinosis at the age of 11 months and OI type VI on bone biopsy at the age of 8 years. He has sustained over 30 fractures during his lifetime, and at the age of 19 years entered end-stage renal disease and subsequent renal transplant. An Affymetrix 6.0 array was used to look for areas of loss of heterozygosity on chromosome 17. Sequencing of the *SERPINF1* and *CTNS* genes was performed, followed by quantitative PCR and Western blot of PEDF to characterize the identified mutation. A 6.58 Mb region of homozygosity was identified on the Affymetrix 6.0 array, encompassing both the *SERPINF1* and *CTNS* genes. Sequencing of the genes identified homozygosity for a known pathogenic *CTNS* mutation and for a novel in-frame duplication in *SERPINF1*. Skin fibroblasts produced a markedly reduced amount of *SERPINF1* transcript and PEDF protein. This patient has the concurrent phenotype of two rare recessive diseases, cystinosis and OI type VI. We identified for the first time an in-frame duplication in *SERPINF1* that is responsible for the OI type VI phenotype in this patient. © 2012 Wiley Periodicals, Inc.

Key words: recessive osteogenesis imperfecta; SERPINF1; pigment epithelium-derived factor; cystinosis

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INTRODUCTION

Osteogenesis imperfecta (OI) is the most common heritable bone fragility disorder and in the majority of cases is caused by dominant mutations in *COL1A1* or *COL1A2*, the two genes that encode collagen type I alpha chains [Dalgleish, 1998]. These chains undergo extensive posttranslational modification in the endoplasmic reticulum. Defects in a number of proteins that participate in posttranslational modification, chaperoning, and intracellular trafficking of type I procollagen, lead to recessive OI [Forlino et al., 2011; van Dijk et al., 2012].

OI type VI differs from other types of OI in that there is a mineralization defect in bone tissue that leads to the accumulation of unmineralized osteoid, as seen in osteomalacia [Glorieux et al., 2002]. These characteristic features of OI type VI are readily apparent on bone histology, but there are no distinguishing radiological signs of OI type VI, and parameters of calcium and phosphorus metabolism are within normal limits. Patients with OI type VI usually do not have fractures or bone deformity at birth, but typically sustain their first fracture after the age of 6 months. The disease is characterized by a large number of fractures thereafter. Compared to other types of OI, bisphosphonate treatment appears to have less effect on fracture rates in OI type VI [Land et al., 2007].

Recent research demonstrated that OI type VI is caused by loss-of-function mutations in *SERPINF1* [Homan et al., 2011]. *SERPINF1* is located on 17p13.3 and codes for pigment epithelium-derived factor (PEDF), a secreted 50 kDa protein that is almost ubiquitously expressed [Filleur et al., 2009]. It is highly expressed in bone cells but its function in bone is not well characterized

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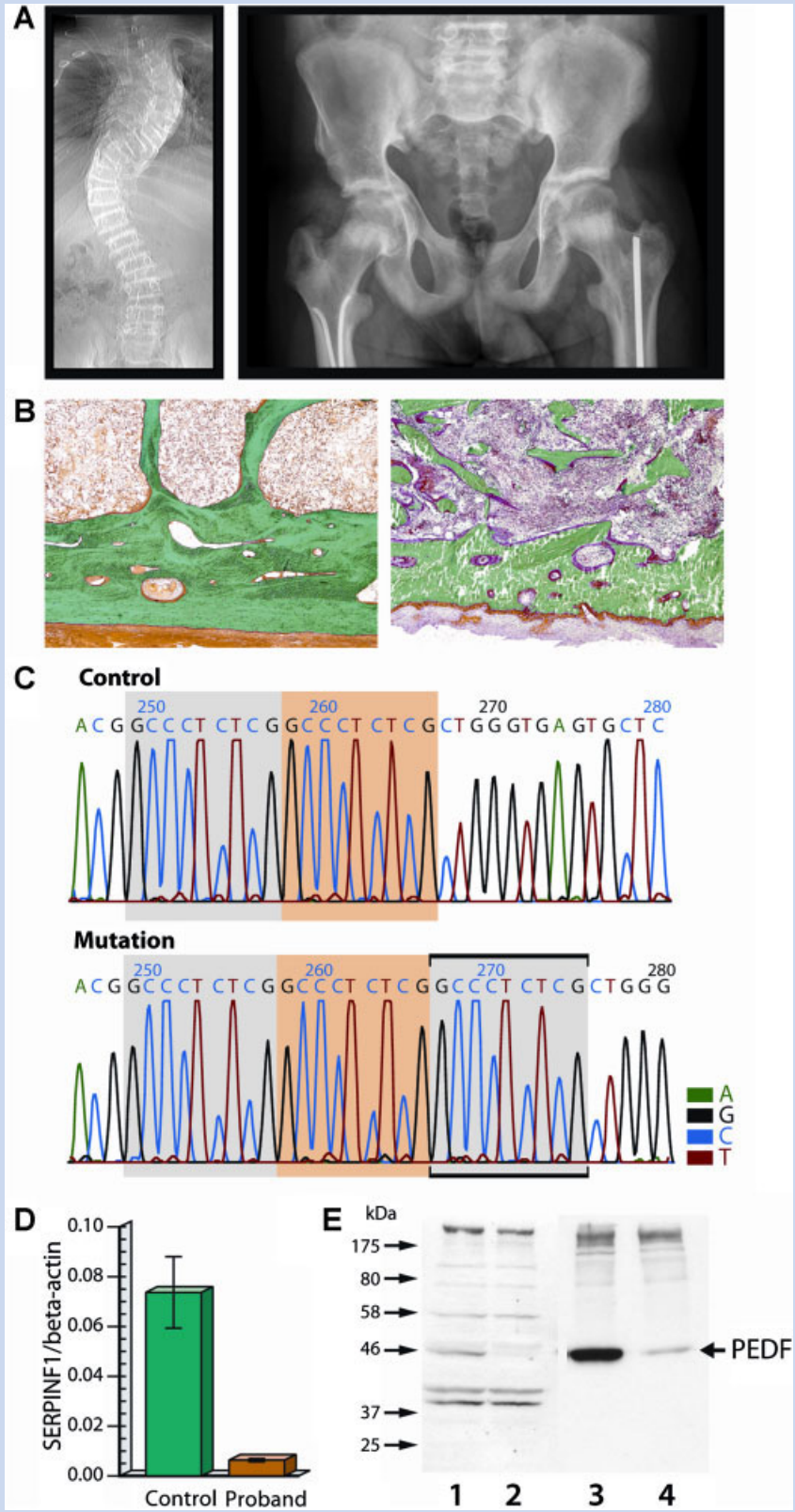
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4

FIG. 1.

[Akiyama et al., 2010]. An earlier report had found inactivating *SERPINF1* mutations in severe OI, but as bone histology had not been performed, the link to OI type VI was not made [Becker et al., 2011].

Cystinosis is an autosomal recessive disorder that affects 1 in 200,000 births. It is the most common cause of proximal renal tubular dysfunction and is due to a defect in lysosomal transport of cystine. There are three different clinical categories of cystinosis based on the age of onset and severity of symptoms. Nephropathic cystinosis is characterized by Fanconi renal tubular syndrome by age 6–12 months, and a history of failure to thrive, polyuria, and hypophosphatemic rickets with renal failure due to glomerular damage at ~10 years of age. Patients with intermediate cystinosis have a later onset of glomerular impairment and photophobia and may not have Fanconi renal tubular disease. The third type is ocular non-nephropathic cystinosis characterized by adult onset ocular defects without renal anomalies [Nesterova and Gahl, 2008].

Cystinosis is caused by mutations in the *CTNS* gene, located on 17p13.3, which encodes cystinosin, a membrane transport protein that mediates the removal of cystine from the lysosome. The variation in the clinical presentation of cystinosis is thought to reflect mutation type. Loss-of-function mutations lead to a more severe phenotype, whereas mutations that allow residual protein function cause a later presentation and a less severe course of the disease. The most common mutation associated with nephropathic cystinosis is a 57 kb deletion involving the five prime region of *CTNS* up to and including exon 10 [Nesterova and Gahl, 2008].

We describe a 19-year-old man with OI type VI and cystinosis caused by homozygous mutations in *SERPINF1* and *CTNS* respectively; these genes are located 1.9 Mb apart on 17p13.3.

CLINICAL REPORT

Our patient was born by spontaneous vaginal delivery after an unremarkable pregnancy. He required some resuscitation but was discharged with no other reported difficulties. He had normal developmental milestones until 8 months of age when he was noted to have a rapid decline in appetite, weight loss, vomiting, and subsequent decline in milestones.

At 11 months he presented to the emergency room with a left femur fracture from falling out of his crib. X-ray analyses identified healing fractures of the right 9th and 10th rib, cortical irregularity on the right proximal femoral metaphysis and diffuse moderately severe osteopenia. Urinalysis suggested a proximal tubulopathy with generalized excretion of amino acids as well as elevated glucose (5.5 mmol/L), protein (0.3 g/L), and ketones (+1). Renal ultrasound showed renal and parenchymal echogenicity consistent with medical renal disease and minimal left caliectasis. Diethylene-triamine-penta-acetic acid scan showed symmetrical renal function with normal glomerular filtration rate. He had elevated leukocyte cystine content and on ophthalmic examination he was noted to have corneal cystine crystals. Treatment with cystagon was started and metabolic control was good.

By the age of 7 years, he had seven fractures, one of which required placement of an intramedullary rod (Fig. 1A). Dual energy X-ray absorptiometry of the lumbar spine revealed a bone mineral density at 4.5 standard deviations below the mean for age. He was treated with pamidronate infusion every 3 months. Spine X-rays at the age of 17 years revealed marked scoliosis (Fig. 1A). Fibroblast analysis showed normal expression of type I procollagen on the protein level. A transiliac bone biopsy sample (Fig. 1B) indicated a markedly increased amount of unmineralized osteoid (osteoid volume per bone volume 16.2%; normal: 0.6–4.6%) [Glorieux et al., 2000]. Both the thickness of the osteoid seams (osteoid thickness 12.1 μm; normal: 3.7–8.1 μm) and the percentage of trabecular bone surface covered by unmineralized osteoid (osteoid surface per bone surface 59%, normal: 3–55%) were elevated. The patient had received two courses of tetracycline prior to bone biopsy in order to label the sites of active bone formation, but the label was too blurred for quantification, indicating a mineralization defect. These characteristic bone histological and histomorphometric findings led to the diagnosis of OI type VI.

In the years following the start of pamidronate treatment, bone mineral density increased to within normal limits. However, he continued to have a large number of fractures, as is generally seen in patients with OI type VI [Land et al., 2007]. By 19 years of age, he had sustained over 30 long-bone fractures as well as progressive kyphosis and scoliosis that required spinal fusion. He is currently mobile without aid and is 159 cm tall (<3rd centile). In addition, at the age of 17 years renal function began to decrease and at the age of 19 years he had bilateral open nephrectomy and a living-relative renal transplant.

Other notable findings at 19 years include normal audiology exam, corneal crystals that continue to increase, no epithelial changes of the cornea, no myopathy, no cardiac complications, no endocrine complications, decreased pulmonary function, and normal leukocyte cystine content (maintained by medication).

Genetic investigations were performed at the age of 19 years. Chromosome microarray analysis performed using the Affymetrix 6.0 array identified a 6.58 Mb stretch of homozygosity (coordinates:

FIG. 1. (*Overleaf*) A: Radiographs at the age of 17 years. The left panel shows marked scoliosis and sclerosis of vertebral end plates due to bisphosphonate treatment. The right panel shows bilateral femoral intramedullary rods and areas of sclerosis in metaphyses as a consequence of bisphosphonate treatment. B: Iliac bone sample of the patient at the age of 8 years (*right panel*) and of a 9-year-old male control (*left panel*). In the patient, mineralized bone (green color) is to a large extent covered by unmineralized osteoid (red/orange color). C: Region of *SERPINF1* exon 3 showing the normal sequence in a control (*upper panel*), and the 9 bp duplication c.271_279dupGCCCTCTCG in the patient (*lower panel*). The same 9 bp sequence is repeated twice in the control and three times in the patient (indicated by background colors). The line above and below the chromatogram indicates the duplicated sequence. D: Real-time PCR quantification of *SERPINF1* transcript normalized against beta-actin. E: Western blot of fibroblast proteins with PEDF antibody: *Lane 1*: control cell extract; *lane 2*: cell extract from patient; *lane 3*: control medium; *lane 4*: medium from patient. Ponceau staining demonstrated equal amounts of protein in lanes 1 and 2 as well as in lanes 3 and 4 (not shown).

6688–6584638 bp) on chromosome 17 that includes the *SERPINF1* and *CTNS* genes.

Sequence analysis of the exons and flanking intronic sequences of the *SERPINF1* gene revealed a novel homozygous 9 bp in-frame duplication, c.271_279dupGCCCTCTCG (p.Ala91_Ser93dup), in exon 3 (Fig. 1C), which adds three amino acids to the PEDF protein sequence. The carrier status of the patient's parents was assessed for this mutation and both were confirmed to be heterozygous for this mutation.

The patient had a PEDF serum concentration of 0.2 mg/L (normal: 4.7–32.5 mg/L) [Stejskal et al., 2010]. Real-time PCR on cDNA from the patient's skin fibroblasts showed markedly decreased levels of *SERPINF1* transcript (Fig. 1D). To evaluate PEDF protein expression, Western blot analysis was conducted on lysates of patient fibroblasts and on culture medium. Patient fibroblasts had low levels of *SERPINF1* transcript and very little PEDF protein expression compared to controls, both in the cell lysate and in the culture medium (Fig. 1E). In the control, the bulk of PEDF was chiefly detected in the media, with low but detectable level in the cell extract. In the OI type VI patient, the secreted PEDF was markedly reduced by at least 50-fold, and correspondingly not detectable in the cell lysate.

Sequence analysis of the exons and flanking intronic sequences of the *CTNS* gene revealed a homozygous missense mutation, c.613G>A (p.Asp205Asn), in exon 8. Both parents of the patient were confirmed to be heterozygous for this mutation.

A detailed description of the methods can be found in the Supplementary notes.

DISCUSSION

Here, we describe the first patient with OI type VI and cystinosis, two autosomal recessive disorders. Sequence analysis identified a novel in-frame duplication in exon 3 of the *SERPINF1* gene which adds three amino acids to the PEDF protein sequence. PEDF is a member of the serpin superfamily and is an inhibitor of angiogenesis. It also has anti-proliferative capabilities and a pro-differentiation function [Akiyama et al., 2010]. PEDF is widely expressed in fetal and adult tissues including adult brain, spinal cord, plasma, lung, eye, heart, and bone. An in vitro model suggests that PEDF may decrease bone resorption by inhibiting osteoclasts, the main cell responsible for bone resorption [Akiyama et al., 2010]. PEDF may also act on bone through antagonizing the action of vascular endothelial growth factor [Broadhead et al., 2010]. Nevertheless, it must be acknowledged that none of the presently known PEDF functions explains the occurrence of a mineralization defect in the bone tissue of patients who have loss-of-function mutations in *SERPINF1*.

The duplication observed in our patient affects a highly acidic region of the PEDF protein that has a high concentration of aspartic and glutamic acid residues thought to have an important role in binding receptors or other cofactors [Simonovic et al., 2001]. The addition of three amino acids may significantly affect the ability of PEDF to bind these factors and may decrease protein function. In vitro assays confirmed that this duplication significantly reduces PEDF protein expression and therefore suggest that the mutation is pathogenic for this patient's OI type VI phenotype. To date, 7 *SERPINF1* mutations have been reported and all are homozygous stop or frameshift mutations. This is the first in-frame duplication reported in the *SERPINF1* gene that is associated with OI.

The clinical OI symptoms of our patient differ somewhat from a recent report of OI cases associated with mutations in the *SERPINF1* gene [Becker et al., 2011]. In contrast to this report, our patient is still mobile at the age of 19 years, although he is short and had progressive scoliosis. Cultured fibroblasts from our patient expressed some PEDF protein, which may account for the slightly less severe skeletal phenotype compared to previously published cases caused by mutations leading to premature termination of the protein [Becker et al., 2011].

The homozygous c.613G>A *CTNS* mutation found in our patient has previously been described in a compound heterozygote of Italian descent [Shotelersuk et al., 1998]. Our patient developed end-stage renal disease at the age of 19, whereas the compound heterozygote, who did not have cysteamine therapy, developed end-stage renal disease at the age of 15. In vitro assays have shown that the c.613G>A mutation does not affect the amount of cystinosin in the lysosomal membrane, but rather the ability of the protein to transport cystine across the membrane [Kalatzis et al., 2004], consistent with the hypothesis that missense mutations may allow for residual protein function resulting in later onset of end-stage renal disease, as observed in these patients.

In conclusion, this patient has the concurrent phenotype of two rare recessive diseases, cystinosis and OI type VI. Further, we identified for the first time an in-frame duplication in *SERPINF1* that is responsible for the OI type VI phenotype in this patient.

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REFERENCES

Akiyama T, Dass CR, Shinoda Y, Kawano H, Tanaka S, Choong PF. 2010. PEDF regulates osteoclasts via osteoprotegerin and RANKL. Biochem Biophys Res Commun 391:789–794.

Becker J, Semler O, Gilissen C, Li Y, Bolz HJ, Giunta C, Bergmann C, Rohrbach M, Koerber F, Zimmermann K, de Vries P, Wirth B, Schoenau E, Wollnik B, Veltman JA, Hoischen A, Netzer C. 2011. Exome sequencing identifies truncating mutations in human SERPINF1 in autosomal-recessive osteogenesis imperfecta. Am J Hum Genet 88:362–371.

Broadhead ML, Akiyama T, Choong PF, Dass CR. 2010. The pathophysiological role of PEDF in bone diseases. Curr Mol Med 10:296–301.

Dalgleish R. 1998. The human collagen mutation database. Nucleic Acids Res 26:253–255.

Filleur S, Nelius T, de Riese W, Kennedy RC. 2009. Characterization of PEDF: A multi-functional serpin family protein. J Cell Biochem 106:769–775.

Forlino A, Cabral WA, Barnes AM, Marini JC. 2011. New perspectives on osteogenesis imperfecta. Nat Rev Endocrinol 7:540–557.

Glorieux FH, Travers R, Taylor A, Bowen JR, Rauch F, Norman M, Parfitt AM. 2000. Normative data for iliac bone histomorphometry in growing children. Bone 26:103–109.

Glorieux FH, Ward LM, Rauch F, Lalic L, Roughley PJ, Travers R. 2002. Osteogenesis imperfecta type VI: A form of brittle bone disease with a mineralization defect. J Bone Miner Res 17:30–38.

Homan EP, Rauch F, Grafe I, Lietman C, Doll JA, Dawson B, Bertin T, Napierala D, Morello R, Gibbs R, White L, Miki R, Cohn DH, Crawford S, Travers R, Glorieux FH, Lee B. 2011. Mutations in SERPINF1 cause osteogenesis imperfecta type VI. J Bone Miner Res 26:2798–2803.

Kalatzis V, Nevo N, Cherqui S, Gasnier B, Antignac C. 2004. Molecular pathogenesis of cystinosis: Effect of CTNS mutations on the transport activity and subcellular localization of cystinosin. Hum Mol Genet 13:1361–1371.

Land C, Rauch F, Travers R, Glorieux FH. 2007. Osteogenesis imperfecta type VI in childhood and adolescence: Effects of cyclical intravenous pamidronate treatment. Bone 40:638–644.

Nesterova G, Gahl W. 2008. Nephropathic cystinosis: Late complications of a multisystemic disease. Pediatr Nephrol 23:863–878.

Shotelersuk V, Larson D, Anikster Y, McDowell G, Lemons R, Bernardini I, Guo J, Thoene J, Gahl WA. 1998. CTNS mutations in an American-based population of cystinosis patients. Am J Hum Genet 63:1352–1362.

Simonovic M, Gettins PG, Volz K. 2001. Crystal structure of human PEDF, a potent anti-angiogenic and neurite growth-promoting factor. Proc Natl Acad Sci USA 98:11131–11135.

Stejskal D, Karpisek M, Svestak M, Hejduk P, Sporova L, Kotolova H. 2010. Pigment epithelium-derived factor as a new marker of metabolic syndrome in Caucasian population. J Clin Lab Anal 24:17–19.

van Dijk FS, Byers PH, Dalgleish R, Malfait F, Maugeri A, Rohrbach M, Symoens S, Sistermans EA, Pals G. 2012. EMQN best practice guidelines for the laboratory diagnosis of osteogenesis imperfecta. Eur J Hum Genet 20:11–19.