Appendix
Material and Methods

The nonmyeloablative preparative regimen of reduced intensity for transplantation consisted of IV fludarabine 8.7 mg (30 mg/m² x 6 doses) on days -10 to -5; oral Busulfan 8.7 mg (30 mg/m² x 4 doses/d x 2 days) every 6 hours on days -6 and -5; and IV anti-thymocyte globulin (ATGam) (Upjohn, Kalamazoo, MI) 55 mg (10 mg/kg) on days -4 through -1 as previously reported (29). Phenytoin (Parke-Davis, USA) for seizure prophylaxis was started on day -9 and continued until day +2 post-BMT (pBMT), and oral cyclosporine for graft-versus-host-disease prophylaxis was started on day -3 at 6.25 mg every 12 hours orally and was continued for 1 year. On the day of BMT, day +0, the patient received her father’s marrow (4/6 match, B and DR mismatch) after T-cell depletion (TCD) by E rosetting for a total of 1.8 x 10⁸/kg total nucleated cells, or 1.6 x 10⁶/kg CD34⁺ cells.

On day 16 pBMT, six bone fragments (2 mm x 8-10 mm) were obtained from the donor father with a Jamshidi needle. Two fragments were inserted IP into the patient through an incision in her umbilicus under general anesthesia. Attempts to place two more fragments into her iliac crest were abandoned because it gave no resistance to the needle. Instead, the fragments were placed SC under the adjacent skin. The remaining two bone fragments were used to culture osteoblasts (30-31). Accordingly, we henceforth call this procedure “bone transplantation” (BT).

On day 12 pBT, the patient received the osteoblast-like cells (OBLCs) (1 x 10⁴/kg) IV along with additional thawed TCD donor marrow (1.4 x 10⁸/kg) and additional T-cells (10⁵/kg) in an attempt to establish bone marrow engraftment, because
her day +18 peripheral blood cytogenetics were reported as 100% host, indicating failure of hematopoietic engraftment.

**Donor bone marrow and bone harvest:** The donor was the father, the better parental match. A total of 450 cc of his marrow from multiple aspirations of his iliac crest on day -1 was T-cell depleted by E-rosetting with sheep red blood cells, and infused IV into the patient the next day (day +0). She received $1.35 \times 10^8$/kg total nucleated cells including $1.6 \times 10^6$/kg of CD34$^+$ cells with < 1 % CD3$^+$ cells. Then, on day +28 (or 12 days after the bone was harvested from the donor), the cultured OBLCs were administered IV with additional frozen-thawed bone marrow ($1.5 \times 10^8$/kg) and CD3$^+$ cells ($5.6 \times 10^5$/kg). Insertion of the bone fragments and IV infusion of cultured OBLCs into the patient was delayed because ATG (anti-Thy-1) in the non-myeloablative regimen for BT could have had detrimental effects on osteoblasts which are Thy-1$^+$ (32).

**Human Osteoblast Cultures:** Donor osteoblasts were cultured according to Robey and Termine (30) with slight modification by El-Badri and colleagues (31).

**SRY Determination and PCR Analyses:** A 5 mm specimen of iliac crest bone was washed in media to remove marrow cells and then crushed in a mortar, centrifuged twice at 1200 rpm, and the supernatants discarded. DNA extraction was by proteinase-based lysis of the washed bone. Contaminating proteins and lipids were organically extracted, followed by alcohol precipitation of high molecular weight DNA. DNA quantitation was determined by spectrophotometry (optical density 260, 280, and the 260/280 ratio) for bone, bone marrow, and peripheral blood preparations. The presence of sex-determining region Y chromosome gene (SRY) was sought using a DNA specific PCR assay (33). Two annealing temperatures (55°C vs 60°C) were used in the PCR amplification of SRY from bone. The optimal annealing temperature (60°C) was used for
the patients bone marrow, peripheral blood, and the controls. The primers for SRY (XES2) were as follows: 5’CTG TAG CGG TCC CGT TGC TGC GGT G and SRY (XES7):5’ GAC AAT GCA ATC ATA TGC TTC TGC. These primers are derived from the SRY mRNA, GenBank NM_003140. The sensitivity of the assay is 1/1000 cells (34).

PCR analysis was performed in 2006 using frozen DNA from the patient’s bone marrow and bone specimens obtained 20 months pBT (6/2001) to quantitate engraftment using standardized methods with commercially available probes for short tandem repeats (STR) as previously reported (35). Briefly, PCR amplification using the fifteen di- and tetra nucleotide repeat polymorphisms at multiple loci (AmpFLSTR® Identifiler®, Applied Biosystems; Foster City, CA) were resolved by capillary electrophoresis on an ABI 3100 automated analyzer (Applied Biosystems) and analyzed using GeneMapper software (Applied Biosystems). Informative identity markers were determined using peripheral blood specimens from donor and recipient (pre-transplant). Detection sensitivity (1% donor or recipient background) and quantitation error (± 3%) was established from standardized protocols for routine bone marrow engraftment monitoring.

**Biochemical studies:** Urine phosphoethanolamine (PEA) was assayed by Mayo Medical Laboratories, Rochester, MN. Urine PPi was kindly quantitated by Dr. Lawrence M. Ryan using a modification of the radiometric uridine disphosphoglucose pyrophosphorylase method (36). Plasma pyridoxal 5’-phosphate (PLP) was assayed by the method of Mahuren and Coburn (37). Serum bone-specific ALP (BALP) was quantitated with an Alk Phos B kit (Metra Biosystems, Mountain View, CA). DXA scans were performed using Hologic QDR-2000 and QDR-4500A instruments (Integrity Medical Systems, Inc; Ft Myers, FL).
**TNSALP gene studies:** Patient and parental DNA for *TNSALP* analysis were obtained after informed written consent approved by the Human Studies Committee, Washington University School of Medicine, St. Louis, MO. Genomic DNA was isolated and *TNSALP* exons 2-12, which cumulatively contain the entire protein coding region and adjacent mRNA splice sites, were screened for mutations (38).

**RESULTS**

**TNSALP mutation analysis:** Patient exon 5 contained a synonymous polymorphism, c.330C>T, p.S110S, whereas exons 6 and 11 contained mutations c.571G>A, p.E191K and c.1289A>G, p.N430S, respectively. This nomenclature is based on den Dunnen and Antonarakis (39). The p.E191K mutation was previously called Glu174Lys, whereas the p.N430S would have been called Asn413Ser.

The father carried the p.E191K change, which is a common mutation in our cohort of ~150 patients (38) and has been reported previously. The mother carried the p.N430S mutation, which has not been reported, nor have we previously detected it in our HPP patient population (3, 38) (and unpublished data). The paternal E191K seems to be relatively mild, because it generally leads to childhood HPP when associated with other mutations (40) (and unpublished studies). Furthermore, in a transfection study, p.E191K had ~88% of wild type TNSALP activity (40). In fact, the donor father had relatively mild hypophosphatasemia and no elevation in plasma PLP (Table). The maternal unique N430S has not been tested *in vitro* for TNSALP activity. However, the mother had more marked hypophosphatasemia as well as a distinctly elevated plasma PLP level (Table). Accordingly, her mutation likely had the greater pathogenetic role in our patient’s HPP.