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Panostotic Expansile Bone Disease With Massive Jaw Tumor Formation and a Novel Mutation in the Signal Peptide of RANK

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Abstract

Precise regulation of bone resorption is critical for skeletal homeostasis. We report a 32-year-old man with a panostotic expansile bone disease and a massive hemorrhagic mandibular tumor. Originally from Mexico, he was deaf at birth and became bow-legged during childhood. There

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Disclosures

All authors state that they have no conflicts of interest.

was no family history of skeletal disease. Puberty occurred normally, but during adolescence he experienced difficulty straightening his limbs, sustained multiple fractures, and developed a bony tumor on his chin. By age 18 years, all limbs were misshapen. The mandibular mass grew and protruded from the oral cavity, extending to the level of the lower ribs. Other bony defects included a similar maxillary mass and serpentine limbs. Upon referral at age 27 years, biochemical studies showed serum alkaline phosphatase of 1760 U/L (Nl: 29-111) and other elevated bone turnover markers. Radiography of the limbs showed medullary expansion and cortical thinning with severe bowing. Although the jaw tumors were initially deemed inoperable, mandibular mass excision and staged partial maxillectomy were eventually performed. Tumor histopathology showed curvilinear trabeculae of woven bone on a background of hypocellular fibrous tissue. Fibrous dysplasia of bone was suspected, but there was no mutation in codon 201 of *GNAS* in samples from blood or tumor. His clinical and radiographic findings, elevated serum markers, and disorganized bone morphology suggested amplified receptor activator of NF-κB (RANK) signaling, even though his disorder differed from conditions with known constitutive activation of RANK signaling (eg, familial expansile osteolysis). We found a unique 12-base pair duplication in the signal peptide of *TNFRSF11A*, the gene that encodes RANK. No exon or splice site mutations were found in the genes encoding RANK ligand or osteoprotegerin. Alendronate followed by pamidronate therapies substantially decreased his serum alkaline phosphatase activity. This unique patient expands the phenotypes and genetic basis of the mendelian disorders of RANK signaling activation.

Keywords

PANOSTOTIC EXPANSILE BONE DISEASE; OSTEOCLASTS; RANK SIGNALING; OSTEOPROTEGERIN

Introduction

Receptor activator of NF-κB (RANK), on the surface of osteoclast (OC) precursor cells and OCs, interacts with RANK ligand (RANKL) in a process critical for OC differentiation and activation.[1] After the discoveries of these proteins and of the RANKL decoy receptor, osteoprotegerin (OPG), studies in mice identified the importance of RANK/RANKL/OPG in osteoclastogenesis and the regulation of skeletal remodeling.[1-4]

Elucidation of the genetic bases for several rare heritable diseases involving RANK/RANKL/OPG signaling underscored the significance of this pathway in humans.[5] A subset of these disorders is caused by either constitutive activation of RANK or deficiency of OPG. The disorders in this subset manifest clinically with accelerated bone turnover and sometimes with focal bone lesions. One such disorder, familial expansile osteolysis (FEO; Online Mendelian Inheritance in Man [OMIM] #174810[6]) presents with early-onset deafness, destruction of adult dentition, and progressive expansile lytic lesions of the limb bones causing pain, deformity, and fracture.[5] In 2000, Hughes and colleagues identified an activating 18-base pair (bp) tandem insertional duplication in exon 1 of *TNFRSF11A*, the gene that encodes RANK, as the culprit mutation in FEO.[7] Expansile skeletal hyperphosphatasia (ESH), seemingly distinct from FEO by its progressive hyperostotic

widening of long bones and its painful cystic lesions in the hands instead of large osteolytic lesions, as well as its periodic hypercalcemia, is caused by a 15-bp tandem duplication in exon 1 of *TNFRSF11A*.[8] Another allelic disorder is early-onset Paget's disease of bone (PDB2; OMIM #602080), described in Japanese and Chinese kindreds and caused by 27-bp duplication in this gene.[7,9] PDB2 includes involvement of the mandible, maxilla, and small bones of the hands. These 18-, 15-, and 27-bp heterozygous tandem insertion duplication mutations in *TNFRSF11A* in FEO, ESH, and PDB2, respectively, involve an overlapping region within the signal peptide of RANK. Lastly, juvenile Paget's disease (JPD; OMIM #239000) typically results from homozygous inactivating mutations in *TNFRSF11B*, the gene that encodes OPG.[10] In these JPD patients, deficiency of this RANKL decoy receptor amplifies RANK signaling, leading during childhood to painful, fragile bones, skeletal deformities, and deafness.

We report a 32-year-old man with panostotic expansile bone disease, congenital deafness, and massive jaw tumors. Together with the extreme severity and additional distinct features of his phenotype compared with the known heritable diseases of constitutive RANK activation, he demonstrated a unique heterozygous duplication mutation within the sequence encoding the signal peptide of RANK.

Case Report

Medical history and physical examination

A then-27-year-old man was transferred to the otolaryngology service of the University of California, San Francisco (UCSF) Medical Center from a nearby hospital for management of a hemorrhagic, basketball-sized mandibular tumor. Deaf and mute, he communicated with family members using an ad hoc sign language. His medical history was obtained with family members' assistance through medical interpreters of their native Spanish.

He was born in Mexico to nonconsanguineous parents after an uncomplicated pregnancy. Although he was deaf at birth, this was never evaluated further. His infancy was otherwise unremarkable, and he began walking at age 14 months.

He was one of 12 children. There was no family history of skeletal abnormality among his siblings, his mother and her 12 siblings, and his father and his 5 siblings. A paternal uncle and his 2 children were deaf but reportedly without skeletal abnormalities.

Growth and eruption of his deciduous teeth were normal. However, his mother noted their delayed and gradual shedding, without replacement by permanent teeth.

At approximately age 4 years, his family noted that his thighs splayed outward mildly as his lower limbs developed bowing deformities. At first, this did not interfere with his physical activity as a child. At approximately age 12 years, his bow-leggedness caused an abnormal gait, and he developed difficulty straightening his limbs. Doctors in their Mexican community initially reassured the family, but then he began to suffer falls and to sustain multiple fractures. His mother reported that some of the fractures were confirmed radiographically at a local clinic, but doctors prescribed only bed rest and caution and did not cast the limbs. Meanwhile, a bony tumor appeared on his chin. His family recalled no

inciting dental procedure, trauma, or infection. The tumor grew progressively, involving the inside and outside of his mandible.

Height was average for age, and puberty was reported to occur normally.

At age 18 years, the patient moved with his mother and several siblings to the US. By that time, all of his limbs were misshapen, and he had diffuse skeletal pain. The mandibular mass interfered with, but did not completely prevent, eating or swallowing. It bled chronically.

At two medical centers in Arizona, he was treated repeatedly in the emergency room for fractures and hospitalized on multiple occasions for severe anemia associated with the bleeding mandibular mass. He had become nonambulatory. According to his medical records, a diagnosis of McCune-Albright syndrome (MAS) was considered based on his skeletal phenotype. However, no endocrinopathy was identified, and no biopsy or genetic testing of bone tissue was performed. The mandibular mass was deemed inoperable, and he was provided hospice care with management of his chronic skeletal pain. He passed a kidney stone of unknown composition at age 21 years.

During a trip to California, the mandibular mass bled. He was evaluated in a local hospital and then transferred to the UCSF Medical Center for further management.

Physical examination showed a large mass that protruded from the oral cavity and extended inferiorly as far as the lower ribs (Fig. 1*A*). A separate maxillary mass filled the oral cavity (Fig. 1*B*). His limbs were serpentine and precluded the normal use of his hands, as well as normal weight bearing on his legs. There were no café-au-lait macules, thyroid nodules, thyromegaly, or stigmata of Cushing's syndrome. Visual acuity was 20/30 bilaterally with normal intraocular pressure and no abnormalities of the anterior chambers or fundi of either eye.

Materials and Methods

Biochemical studies

Biochemical studies included serum electrolytes, calcium, phosphate, intact parathyroid hormone (PTH), 25-hydroxyvitamin D [25(OH)D], 1,25-dihydroxyvitamin D [$1,25(OH)_2D$], bone-specific alkaline phosphatase (BAP), collagen type I C-telopeptide (CTX), and osteocalcin. Urinary chemistries were undertaken at local institutions and at Quest Diagnostics Nichols Institute (San Juan Capistrano, CA, USA). Serum intact fibroblast growth factor 23 (FGF23) was measured by ELISA (Kainos, Tokyo, Japan) using a de-identified specimen at the Skeletal Clinical Studies Unit, Craniofacial and Skeletal Diseases Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health.

Radiological studies

We attempted to obtain all previous radiologic studies for review, but we were unable to acquire radiographs from the Mexican clinic where he was seen as an early adolescent. Additional radiographs and computed tomography (CT) scans were performed at UCSF and

Santa Clara Valley Medical Centers. Dual-energy X-ray absorptiometry scanning was not undertaken because of the inability to position his limbs appropriately.

Histologic evaluation

Surgical specimens from the resection of the mandibular and maxillary tumors were examined histologically using routine methods. Tissue was fixed in 10% neutral-buffered formalin and paraffin-embedded sections (4 μm) stained with hematoxylin and eosin. Tissue was examined and photographed with an Olympus BX41 microscope outfitted with Olympus UPlanFL objective lenses and an Olympus DP72 camera (Tokyo, Japan). Iliac crest bone biopsy was deemed inappropriate because of the patient's baseline bone pain and general vulnerability.

Gene mutation studies

Genetic analysis was initially performed for mutations in *GNAS*, the gene that encodes the alpha subunit of G_s, the adenylate cyclase stimulatory G protein implicated in classical MAS. Site-specific enrichment mutation analysis was performed using DNA from blood leukocytes and resected maxillary tumor bone, followed by sequencing for codon 201, located in exon 8 of *GNAS* (Center for Genetic Testing at St. Francis, Tulsa, OK, USA).[11]

Subsequently, when the clinical, biochemical, and radiographic features of his skeletal disease suggested instead a disorder involving activation of the RANK/RANKL/OPG pathway, sequencing of those genes was performed in our laboratory (Washington University, St. Louis, MO, USA). Informed consent was obtained, and the institutional review boards of UCSF and Washington University approved the study. Briefly, genomic DNA was purified from blood leukocytes using the Gentra Puregene DNA extraction kit (Invitrogen, Carlsbad, CA, USA). Exon 1 of *TNFRSF11A*, where activating duplications cause FEO and the other allelic disorders of RANK activation,[5] was amplified by PCR and sequenced according to published methods.[7] Subsequently, the remaining exons of *TNFRSF11A* were PCR-amplified and sequenced. In addition, all coding exons and adjacent mRNA splice sites of *TNFRSF11B* (OPG) and *TNFSF11* (RANKL) were amplified by PCR and sequenced in both directions using published methods and primers.[10,12] DNA sequences were evaluated using AlignX software (Vector NTI, Invitrogen), and by inspecting individual electropherograms.

Results

Biochemical findings

At referral, serum calcium concentration was 7.1 mg/dL with albumin 1.7 g/dL (corrected serum calcium 8.9 mg/dL), phosphate 2.4 mg/dL (Nl: 2.4–4.6), PTH 91 ng/L (Nl: 12–65), 25(OH)D 11 ng/mL (Nl: 20–100 for the assay), and alkaline phosphatase 1760 U/L (Nl: 29–111) (Supplemental Table S1). Estimated glomerular filtration rate was normal. There was no biochemical evidence for altered thyroid, adrenal, or pituitary function. After vitamin D supplementation for 15 months (vitamin D_3 400 IU daily), serum calcium was 8.3 mg/dL, albumin 2.9 g/dL, phosphate 3.8 mg/dL, 25(OH)D 24 ng/mL, and alkaline phosphatase 2398 U/L. Serum BAP level was >575 mcg/L (Nl: 8.4–29.3), CTX 1644 pg/mL (Nl: 87–1200),

$1,25(OH)_2D$ 45 pg/mL (Nl: 15–60), and intact FGF23 60 pg/mL (Nl: 10–50). Twenty-four-hour urinary calcium level was 34 mg (Nl: 100–300), phosphorus 0.6 g (Nl: 0.4–1.3), and creatinine 0.7 g (Nl: 0.8–2.0). Serum uric acid concentration was 10.2 mg/dL (Nl: 3.7–7.7). Later, when serum 25(OH)D was 33 ng/mL, CTX was 2950 pg/mL. After an episode of nephrolithiasis, stone composition analysis revealed 50% ammonium hydrogen urate and 50% carbonate apatite, suggestive of underlying hyperuricosuria and prior urinary tract infections.

Radiological findings

Sagittal CT delineated the large sclerotic masses of his mandible and maxilla (Fig. 2*A*), and radiographs and additional CT projections defined the tumors further (Supplemental Fig. S1). Comparison of skull radiographs from ages 20 and 31 years revealed increased expansion of the diploic space with areas of increased and decreased sclerosis (Fig. 2*B, C*). The lucent areas contained predominantly fat, based on Hounsfield units (HU). CT of the right petrous bone showed that the middle-ear cavity contained a small rudimentary fused ossicle. There was no cochlea, and a large cyst occupied the otic capsule (Fig. 2*D*). After debulking of the maxillary and mandibular tumors, CT of the face showed orbital and maxillary distortion by the osteoblastic mass (Supplemental Fig. S2).

Radiographic skeletal surveys revealed severe dysplasia involving all bones examined. Comparison of radiographs and CT scans from ages 20 through 31 years showed progressive loss of trabeculae and thinning of cortices (Figs. 3-5). The bones became increasingly lucent from fat (based on HU) and increasingly deformed. Imaging of the hips documented marked coxa vara (Fig. 4*B*). Chest radiography and CT showed a bell-shaped thorax with wide deformed clavicles and ribs (Supplemental Fig. S3). Abdominal CT identified a nonobstructing 3-mm right renal calculus.

Surgical management and histopathologic findings

After careful planning by his surgeons, the patient first underwent mandibular mass excision with tracheotomy for airway maintenance. Five months later, a partial maxillectomy was performed and subsequently additional maxillary debulking. He tolerated the procedures well. No unusually rapid healing or exuberant callus formation was noted.

Histopathology of the surgical specimens was reviewed by a bone pathologist (AEH). Formalin-fixed, paraffin-embedded, and hematoxylin-and-eosin–stained sections showed a fibro-osseous lesion composed of curvilinear and round trabeculae of woven bone in a background of hypocellular fibrous tissue. The fibrous tissue contained a sparse population of fusiform, cytologically bland, spindle cells (Fig. 6). A few trabeculae of bone demonstrated more active turnover with osteoblastic rimming and OC resorption. The normal cellular components of bone (OCs, osteoblasts) were identified by routine histopathologic examination.[13] Cytologic atypia, including nuclear hyperchromasia, high nuclear to cytoplasmic ratio, and cellular and nuclear pleomorphism, was absent. Mitotic activity was low throughout the lesion, averaging <1 mitotic figure per 10 high-magnification (400×) microscopic fields. Diagnostic considerations, based on histomorphology and anatomic site, included juvenile ossifying fibroma and fibrous

dysplasia. These impressions remained when the surgical specimens were reexamined after additional laboratory and genetic evaluation.

After the operations, there was mild regrowth of the maxillary mass, but his oral function remained much improved (Fig. 1*C*). The tracheostomy remained, prophylactically, but he was able to eat and breathe by mouth.

Gene mutation analyses

In samples of affected bone from the maxillary tumor and on peripheral leukocyte DNA, site-specific enrichment for the two known MAS mutations was performed.[11] Subsequent confirmatory sequencing of codon 201 of the *GNAS* gene detected no mutation, making MAS and non-MAS panostotic fibrous dysplasia of bone unlikely.[14]

Concerning the RANK/RANKL/OPG pathway, no exon or splice site mutations in the genes encoding RANKL (*TNFRSF11*) or OPG (*TNFRSF11B*) were identified in peripheral leukocyte DNA. However, sequencing demonstrated a unique heterozygous tandem insertion duplication mutation in exon 1 of *TNFRSF11A*, which encodes the signal peptide of RANK (Fig. 7). This 12-bp duplication is distinct from the larger signal peptide sequence duplications of FEO (18-bp duplication), ESH (15-bp duplication), and PDB2 (27-bp duplication). No other mutations were found in the remaining *TNFRSF11A* exons.

Medical treatment

The patient had chronic diffuse bone pain, which he rated 7 out of 10 in severity on a Likert scale[15] without scheduled opioid medication, and 2.5 out of 10 with pain medication. Potential antiresorptive medications were considered, along with plans for close biochemical and clinical surveillance during such therapy (see Discussion). Eighteen months after his final jaw tumor debulking operation, under the care of another medical provider, alendronate 70 mg weekly by mouth was initiated. Subsequently, his serum alkaline phosphatase declined precipitously, from 2588 U/L to 606 U/L after 4 months, 488 U/L after 10 months, 285 U/L after 13 months, and 163 U/L after 20 months (Supplemental Table S1). The albumin-corrected serum calcium level dropped significantly after initiation of alendronate, to 6.5 mg/dL after 4 months, but then it rose to 7.7 mg/dL after 10 months, 8.8 mg/dL after 13 months, and 9.3 mg/dL after 20 months. Calcium intake after initiation of the alendronate therapy was approximately 1300 mg/day (800 mg dietary calcium plus 500 mg as a supplement), and vitamin D supplementation was 400 IU daily. On that regimen, 25(OH)D level ranged from 12 to 25 ng/mL, and 24-hour urinary calcium level was <68 mg even while serum 25(OH)D was 25 ng/mL. The patient retrospectively reported intermittent paresthesias but no other symptoms of hypocalcemia. He did not report any dramatic change in bone pain but perhaps some mild improvement. Alendronate therapy was discontinued after 21 months because of concern about risk of esophagitis, and pamidronate 10 mg was administered intravenously. Three weeks later, his serum alkaline phosphatase level was 158 U/L and his albumin-corrected serum calcium level 9.5 mg/dL. Currently, calcium and vitamin D supplementation is 500 mg 3 times daily and 2000 IU daily, respectively.

After several episodes of nephrolithiasis (requiring bilateral ureteral stent placement, lithotripsy, and open cystolithotomy), allopurinol therapy was initiated in an effort to decrease the risk of future urate-containing kidney stones.

Discussion

Our patient has a unique skeletal disorder featuring severe, generalized, high-turnover bone disease with recurrent fractures and deformities, congenital deafness, and massive jaw tumors. We ascribe the etiology to a novel heterozygous mutation in the signal peptide of RANK (Fig. 7). Based on his physical and radiographic skeletal abnormalities, an unusual presentation of fibrous dysplasia of bone was initially considered. However, genetic analysis showed no mutation in codon 201 of *GNAS* in blood and affected bone, making fibrous dysplasia unlikely. When it was recognized that his disease might reflect a mutation in the genes encoding RANK, RANKL, or OPG, mutation analysis revealed a 12-bp duplication in *TNFRSF11A*, the gene that encodes RANK. The mutation was found in the same region as (but distinct from) the mutations that cause the rare hereditary skeletal diseases FEO, ESH, and PDB2, and would be expected to enhance RANK signaling.

Hughes and colleagues discovered that a heterozygous 18-bp tandem insertion duplication mutation in the sequence encoding the signal peptide of RANK causes FEO, and a similar heterozygous 27-bp duplication causes PDB2. Both mutations were shown to increase NF-κβ signaling in vitro.[7] Crockett and colleagues subsequently demonstrated that the heterozygous 15-bp duplication in ESH acts by the same mechanism.[16] Although we have not verified the effects of our patient's unique 12-bp duplication in cultured cell models, we predict that it behaves in a similar manner. Constitutive activation of RANK signaling appears to explain our patient's high bone turnover and disorganized bone morphology. As suggested for the three disorders of constitutive RANK activation, the mutant RANK transcript made in our patient's cells may not be degraded normally, and/or it may cause endoplasmic reticulum stress, with resulting amplification of OC activation.[17]

Despite the likelihood of shared excessive RANK activity, our patient's presentation and disease differ from FEO, ESH, PDB2, and JPD (Table 1). His generalized skeletal disease is more severe than that reported in FEO, ESH, and PDB2. He has congenital deafness, whereas deafness is of later onset in FEO and ESH. Deafness is relatively mild in PDB2 but is common in JPD. Our patient has no family members with skeletal disease, making his mutation likely sporadic. The activating mutations of RANK cause autosomal dominant disorders, whereas JPD, with its inactivating mutations of OPG, is an autosomal recessive disorder. Our patient's heterozygous mutation indicates the potential for autosomal dominant transmission.

The pathogenesis of our patient's jaw tumors remains unclear. Paget's disease of bone increases the risk of osteogenic and other types of sarcomas, and osteogenic sarcoma has been reported in FEO[5] and in a child with JPD.[18] However, histologic evaluation of our patient's tumor showed no cellular features of malignancy. Further, Sparks and colleagues identified no specific mutations in *TNFRSF11A* in Pagetic osteosarcoma or in six osteosarcoma cell lines.[19] Our patient's tumor did not have the abundant OCs seen in

tumors caused by dysregulation of RANK/RANKL signaling, such as giant-cell tumor of bone and tenosynovial giant cell tumor.[20,21] Possibly, our patient has a second, coincidental, somatic mutation causing his jaw tumors. Primary bone tumors and metastatic lesions to bone can alter RANK/RANKL/OPG signaling.[22] There is increased expression of RANKL in multiple myeloma and reduced expression of OPG.[23] Perhaps our patient's jaw tumor burden induced cytokine production that fueled his high bone turnover state and contributed to the severity of his panostotic disease. In turn, his excessive bone resorption may have released growth factors from the bone matrix (eg, bone morphogenic proteins, transforming growth factor-β) that enhanced tumor growth, creating a vicious cycle of tumor growth and osteolysis.[24]

Of interest, our patient had recurrent nephrolithiasis in the setting of low urinary calcium levels. This is likely because of chronically elevated uric acid levels—possibly owing to the high metabolic turnover of his skeleton—and compounded by dehydration. Despite the plausibility of nephrolithiasis related to accelerated bone remodeling, nephrolithiasis is not a complication of FEO, ESH, or PDB2.

We initially considered denosumab therapy for our patient because denosumab targets the RANK/RANKL/OPG pathway as a neutralizing monoclonal antibody against RANK. However, Crockett and colleagues demonstrated that cells expressing wild-type RANK, but *not* cells expressing the mutant FEO, PDB2, or ESH RANK, showed RANKL-dependent activation of the signaling pathway.[16] These mutant RANKs did not localize to the plasma membrane, but rather accumulated intracellularly, where they were unavailable for activation by RANKL. With this in mind, we were concerned that treatment with denosumab might be ineffective for our patient, and we instead recommended bisphosphonate treatment.

Treatment with a bisphosphonate has been helpful for patients with the other disorders that activate the RANK/RANKL/OPG signaling pathway.[25-27] Riches and colleagues described the clinical and biochemical response to bisphosphonate therapy in 3 patients with PDB2 and found that treatment with aminobisphosphonates resulted in greater suppression of bone turnover markers and a more durable response than treatment with the first-generation bisphosphonate etidronate.[27] Clinically, however, only one of the aminobisphosphonate-treated patients experienced an improvement in bone pain, and none had clear benefit with respect to bone deformity, deafness, or tooth loss. Because of the high bone turnover in these disorders involving the RANK/RANKL/OPG pathway, the risk of hypocalcemia caused by antiresorptive drugs is significant. Indeed, a case of profound hypocalcemia after zoledronic acid treatment for JPD has been reported.[28] With alendronate therapy, our patient became hypocalcemic with a nadir albumin-corrected calcium level of 6.5 mg/dL. Fortunately, he did not experience serious clinical complications, and several months later the hypocalcemia resolved. His biochemical response to alendronate was impressive (~93% reduction in serum alkaline phosphatase level). We have now replaced the alendronate therapy with low-dose pamidronate therapy, and we have provided more robust calcium and vitamin D supplementation as we monitor for clinical and radiographic improvement. Finally, the high bone turnover could theoretically be addressed directly with a small interfering RNA (siRNA) targeting the heterozygous aberrant RANK. Further, it would be

intriguing to consider that hematopoietic stem cell transplantation could provide an avenue for treating this condition.

In conclusion, this man has a novel heterozygous tandem insertion duplication mutation in *TNFRSF11A*, within the sequence encoding the signal peptide of RANK. This predicted gain-of-function mutation likely explains his panostotic high turnover disease. Massive jaw tumors have not been reported previously with activating mutations of RANK and may be coincidental or due to a second, somatic mutation within the tumors. Also, it is possible that the tumors are exacerbating his generalized skeletal disease by producing an unidentified circulating factor. This case report expands the spectrum of mutations of RANK and the phenotypes associated with the monogenic disorders of enhanced RANK signaling.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Hsu H, Lacey DL, Dunstan CR, et al. Tumor necrosis factor receptor family member RANK mediates osteoclast differentiation, activation induced by osteoprotegerin ligand. Proc Natl Acad Sci USA. 1999; 96:3560–5.
2. Dougall W, Glaccum M, Charrier K, et al. RANK is essential for osteoclast and lymph node development. Genes Dev. 1999; 13:2412–24. [PubMed: 10500098]
3. Kong YY, Yoshida H, Sarosi I, et al. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. Nature. 1999; 397:315–23. [PubMed: 9950424]
4. Li J, Sarosi I, Yan XQ, et al. RANK is the intrinsic hematopoietic cell surface receptor that controls osteoclastogenesis, regulation of bone mass, calcium metabolism. Proc Natl Acad Sci USA. 2000; 97:1566–71. [PubMed: 10677500]
5. Whyte, MP. Mendelian disorders of RANKL/OPG/RANK signaling. In: Thakker, RV.; Whyte, MP.; Eisman, J.; Igarashi, T., editors. Genetics of bone biology and skeletal disease. London: Elsevier; 2013. p. 309-24.
6. Online Mendelian Inheritance in Man, OMIM®. Baltimore: McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University; 1995. [Internet]. Available at: http://omim.org/ [2013 June 2]
7. Hughes AE, Ralston SH, Marken J, et al. Mutations in TNFRSF11A, affecting the signal peptide of RANK, cause familial expansile osteolysis. Nat Genet. 2000; 24:45–8. [PubMed: 10615125]
8. Whyte MP, Hughes AE. Expansile skeletal hyperphosphatasia is caused by a 15-base pair tandem duplication in TNFRSF11A encoding RANK and is allelic to familial expansile osteolysis. J Bone Miner Res. 2002; 17:26–9. [PubMed: 11771666]
9. Nakatsuka K, Nishizawa Y, Ralston SH. Phenotypic characterization of early onset Paget's disease of bone caused by a 27-bp duplication in the TNFRSF11A gene. J Bone Miner Res. 2003; 18:1381–5. [PubMed: 12929927]
10. Whyte MP, Obrecht SE, Finnegan PM, et al. Osteoprotegerin deficiency and juvenile Paget's disease. N Engl J Med. 2002; 347:175–84. [PubMed: 12124406]

11. Weinstein LS, Shenker A, Geiman PV, Merino MJ, Friedman E, Spiegel AM. Activating mutations of the stimulatory G protein in McCune-Albright Syndrome. N Engl J Med. 1991; 325:1688–95. [PubMed: 1944469]
12. Sobacchi C, Frattini A, Guerrini MM, et al. Osteoclast-poor human osteopetrosis due to mutations in the gene encoding RANKL. Nat Genet. 2007; 39:960–2. [PubMed: 17632511]
13. Rosenberg, AE.; Roth, SI. Bone. In: Mills, SE., editor. Histology for pathologists. 4. Philadelphia: Lippincott Williams & Wilkins; 2012. p. 85-106.
14. Lee SE, Lee EH, Park H, et al. The diagnostic utility of the GNAS mutation in patients with fibrous dysplasia: meta-analysis of 168 sporadic cases. Hum Pathol. 2012; 43:1234–42. [PubMed: 22245114]
15. Likert R. A technique for the measurement of attitudes. Arch Psychol. 1932; 140:1–55.
16. Crockett JC, Mellis DJ, Shennan KIJ, et al. Signal peptide mutations in RANK prevent downstream activation of NF-κβ. J Bone Miner Res. 2011; 26:1926–38. [PubMed: 21472776]
17. Crockett JC, Mellis DJ, Scott DI, Helfrich MH. New knowledge on critical osteoclast formation and activation pathways from study of rare genetic diseases of osteoclasts: focus on the RANK/RANKL axis. Osteoporos Int. 2011; 22:1–20. [PubMed: 20458572]
18. Bacri D, Arush MW, Vlodavsky E, Kollander Y, Militianu D, Postovsky S. Osteogenic sarcoma in a child with familial expansile osteolysis syndrome: an accidental association? J Pediatr Hematol Oncol. 2010; 32:e50–3. [PubMed: 20168251]
19. Sparks AB, Peterson SN, Bell C, et al. Mutation screening on the TNFRSF11A gene encoding receptor activator of NF kappa B (RANK) in familial and sporadic Paget's disease of bone and osteosarcoma. Calcif Tissue Int. 2001; 68:151–5. [PubMed: 11351498]
20. Robinson D, Einhorn TA. Giant cell tumor of bone: a unique paradigm of stromal-hematopoietic cellular interactions. J Cell Biochem. 1994; 55:300–3. [PubMed: 7962160]
21. Roux S, Amazit L, Meduri G, Guiochon-Mantel A, Milgrom E, Mariette X. RANK (receptor activator of nuclear factor kappa B) and RANK ligand are expressed in giant cell tumors of bone. Am J Clin Pathol. 2002; 117:210–6. [PubMed: 11863217]
22. Buckle CH, Neville-Webbe HL, Croucher PI, Lawson MA. Targeting RANK/RANKL in the treatment of solid tumours and myeloma. Curr Pharm Design. 2010; 16:1272–83.
23. Pearse RN, Sordillo EM, Yaccoby S, et al. Multiple myeloma disrupts the TRANCE/osteoprotegerin cytokine axis to trigger bone destruction, promote tumor progression. Proc Natl Acad Sci USA. 2001; 98:11581–6. [PubMed: 11562486]
24. Weilbaecher KN, Guise TA, McCauley LK. Cancer to bone: a fatal attraction. Nat Rev Cancer. 2011; 11:411–25. [PubMed: 21593787]
25. Whyte MP, Reinus WR, Podgornik MN, Mills BG. Familial expansile osteolysis (excessive RANK effect) in a 5-generation American kindred. Medicine (Baltimore). 2002; 81:101–21. [PubMed: 11889411]
26. Demir E, Bereket A, Ozkan B, Topçu M. Effect of alendronate treatment on the clinical picture and bone turnover markers in chronic idiopathic hyperphosphatasia. J Pediatr Endocrinol Metab. 2000; 13:217–21. [PubMed: 10711670]
27. Riches PL, Imanishi Y, Nakatsuka K, Ralston SH. Clinical and biochemical response of TNFRSF11A-mediated early-onset familial Paget disease to bisphosphonate therapy. Calcif Tissue Int. 2008; 83:272–5. [PubMed: 18836673]
28. Polyzos SA, Anastasilakis AD, Litsas I, et al. Profound hypocalcemia following effective response to zoledronic acid treatment in a patient with juvenile Paget's disease. J Bone Miner Metab. 2010; 28:706–12. [PubMed: 20533067]

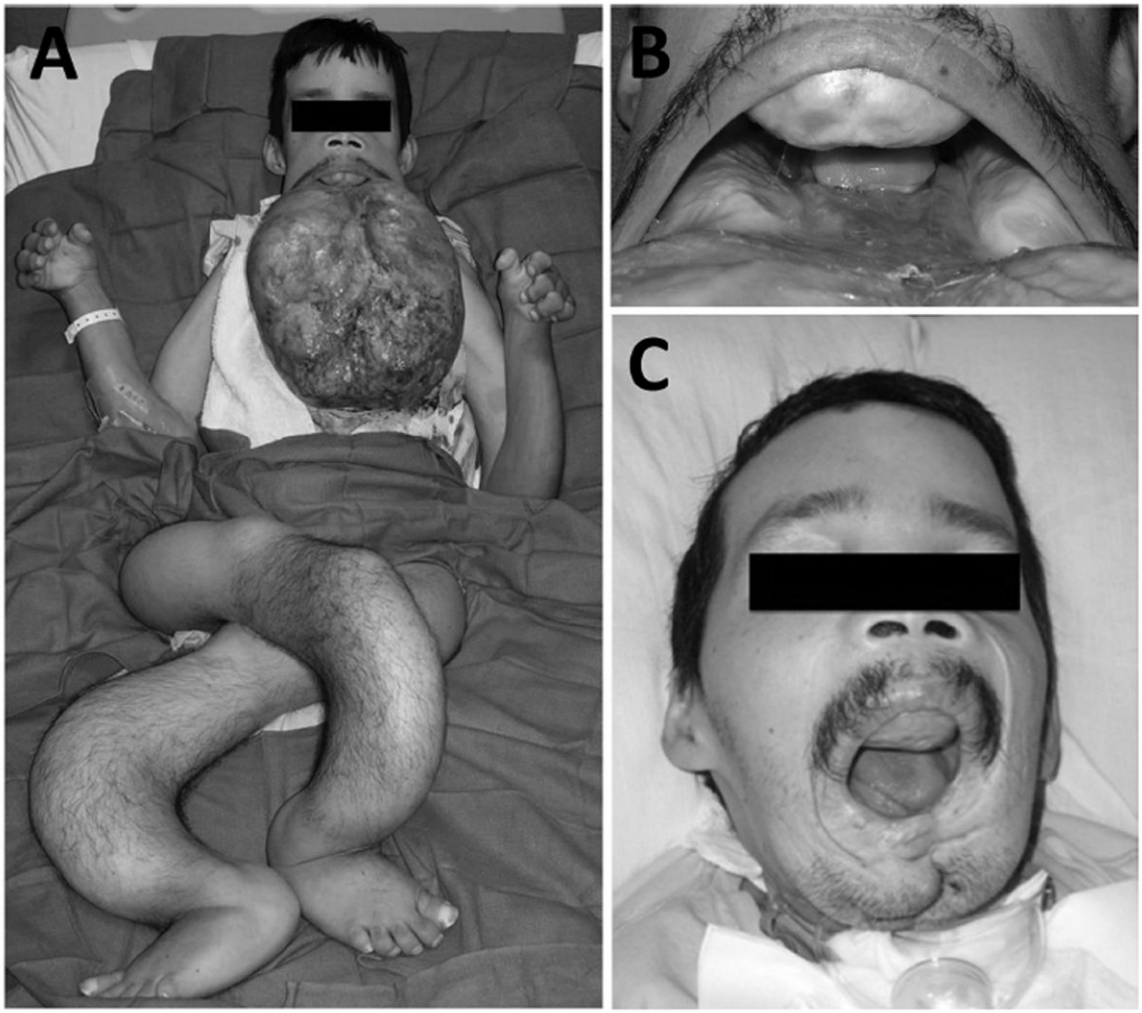

Fig. 1.

At age 27 years, a large mandibular mass protrudes from the oral cavity of our patient and extends to the lower ribs. All limbs are deformed (*A*). A separate maxillary mass nearly fills the oral cavity (*B*). After sequential debulking operations for both masses, there has been mild regrowth of the maxillary mass (*C*), but our patient's oral function is much improved in that he is able to eat and swallow comfortably.

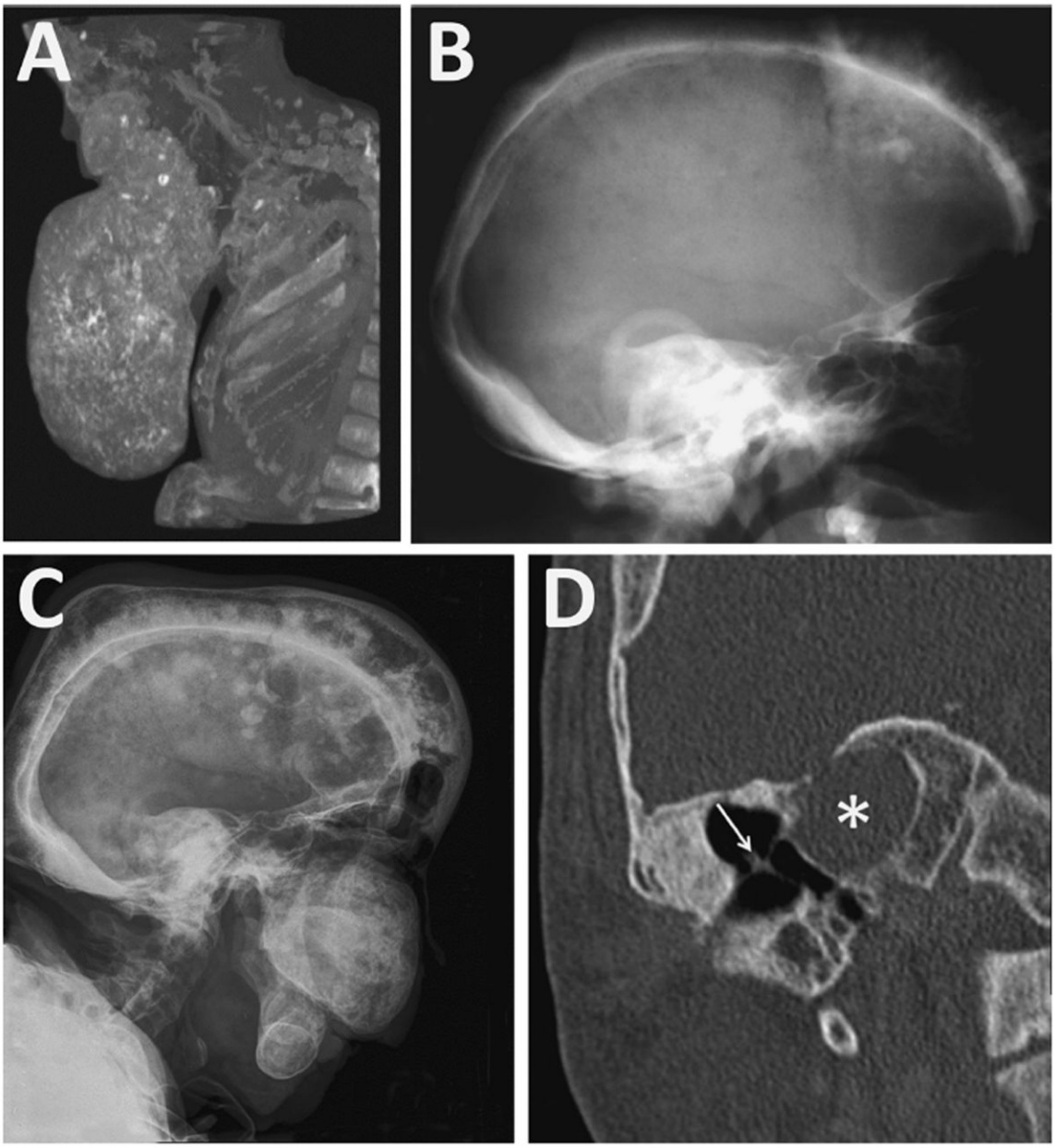

Fig. 2.

Sagittal CT (*A*) at age 26 years demonstrates the large osteoclerotic mass. Lateral skull radiographs at age 20 years (*B*) and age 31 years (*C*, postoperative) show increased expansion of the diploic space with areas of increased and decreased sclerosis. The lucent areas predominantly contain fat, based on Hounsfield units. The maxilla has a large osteosclerotic mass. CT of the right petrous bone (*D*) shows the middle ear cavity, which contains a small rudimentary fused ossicle (arrow). There is no cochlea, and a large cyst occupies the otic capsule (*).

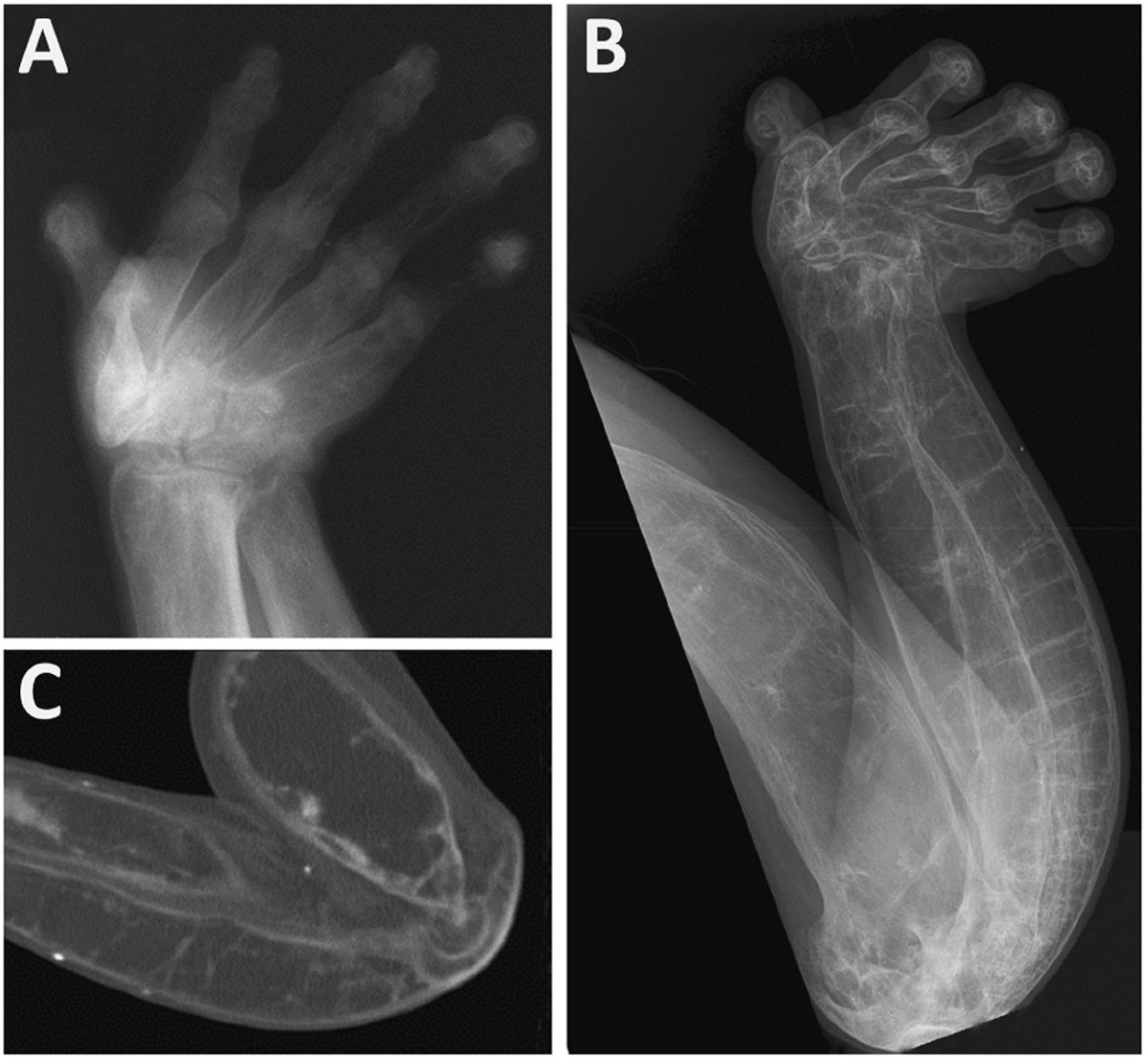

Fig. 3.

Hand radiograph at age 20 years (*A*) and upper extremity radiograph (*B*) and CT scan (*C*) both at age 31 years demonstrate progressive loss of trabeculae, thinning of the cortices, and right-angled trabeculae in the radius and ulna. The forearm bones and humerus contain a great deal of fat, based on Hounsfield units. The metacarpals and phalanges have become thinner, more deformed, and more cystic appearing.

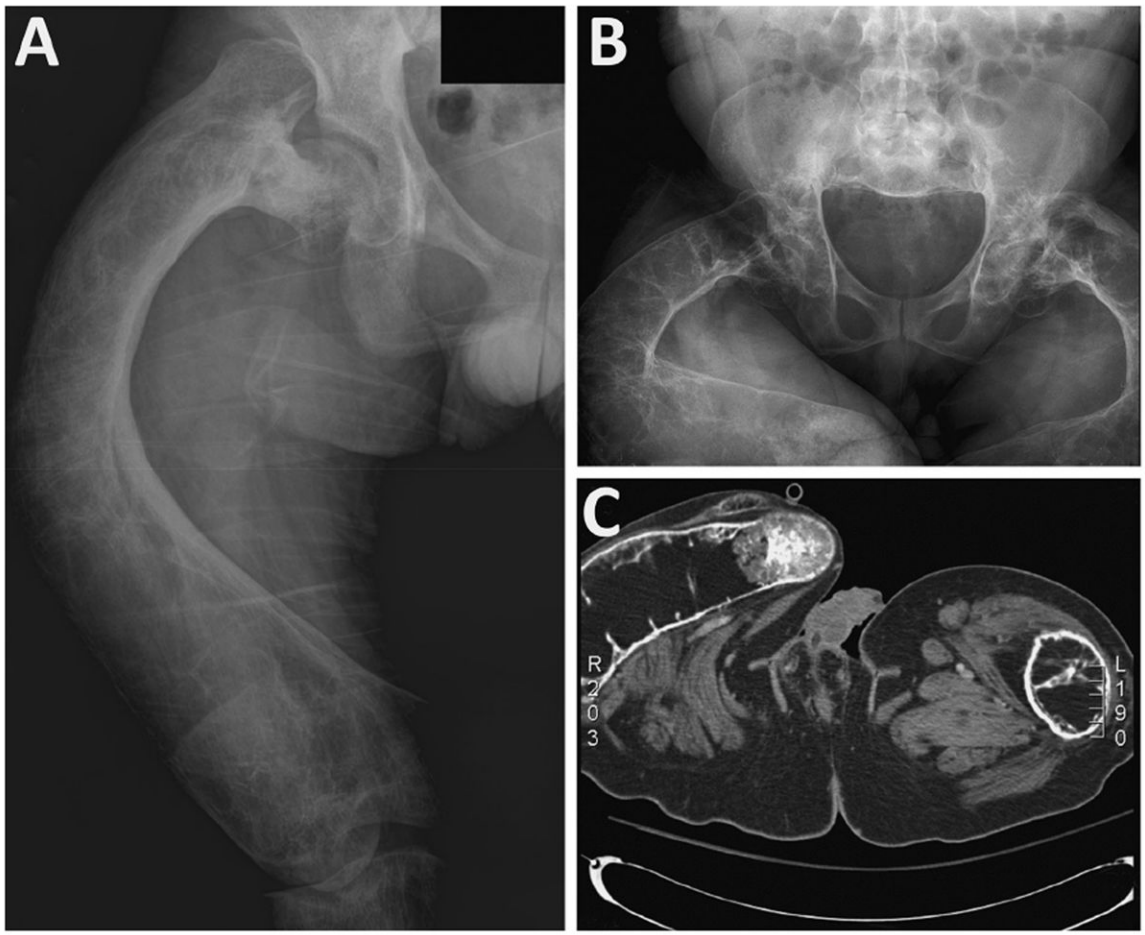

Fig. 4.

AP radiograph of the right femur at age 21 years (*A*) and pelvis and femurs at age 31 years (*B*) show wide osteosclerotic femora with inner thick cortices, which become much more cystic with thin cortices. There is marked coxa vara. A CT (*C*) shows the fat-filled femora with thin cortices, thick right-angled struts of bone, and osteosclerotic areas.

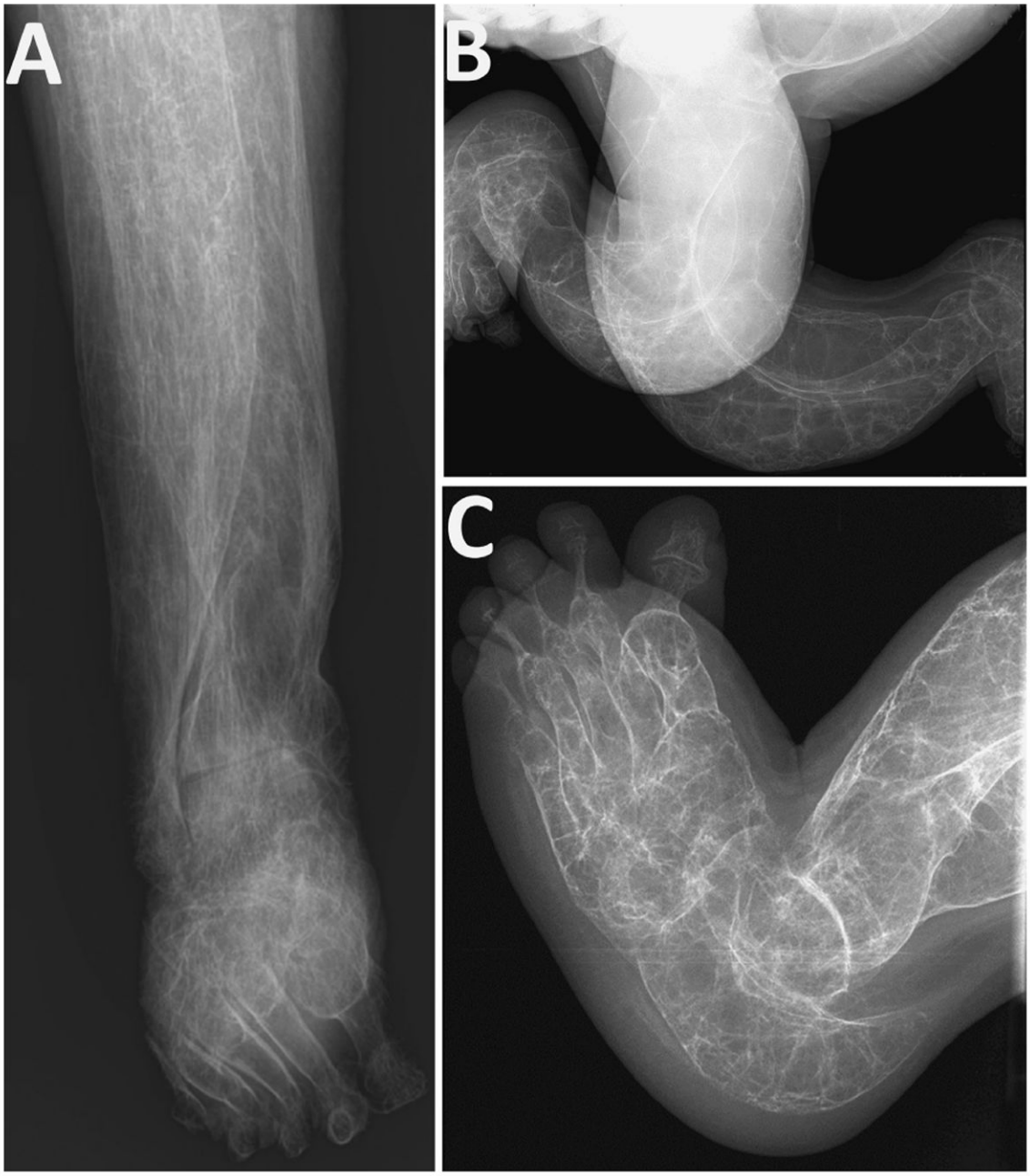

Fig. 5.

Right leg radiograph (*A*) at age 21 years and lower extremities (*B*) at age 31 years show a wide osteosclerotic tibia and fibula with multiple coarse trabeculae. The bones become far more lucent from fat (based on Hounsfield units) with fewer but thick trabeculae, thin cortices, and more deformity. Right foot radiograph (*C*) at age 30 years shows markedly lucent expanded bones with thin cortices and disorganized trabeculae in the tibia and fibula.

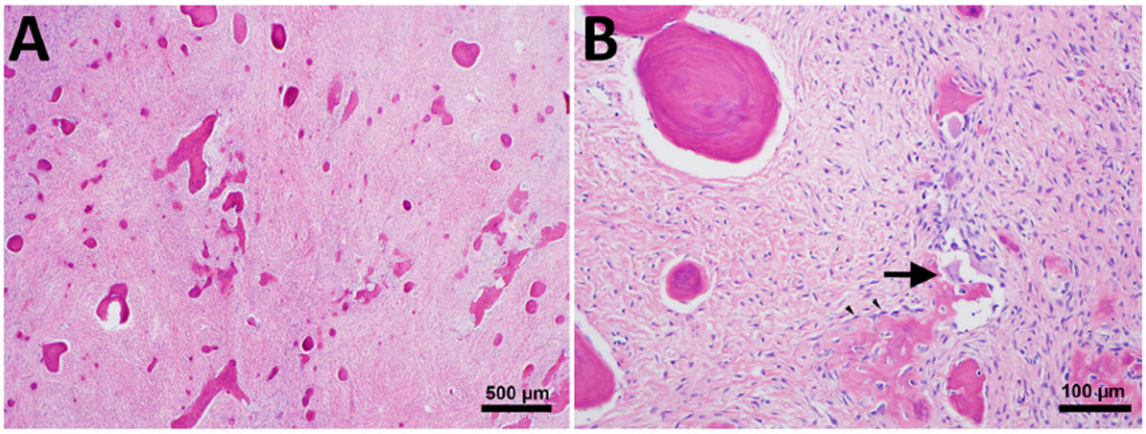

Fig. 6.

Histopathology of the maxillary tumor shows curvilinear trabeculae of woven bone on a background of hypocellular fibrous tissue (*A*, original magnification 40 ×). At higher magnification (*B*, original magnification 200 ×), the majority of the bone is dense without osteoblastic rimming, although there are scattered areas of active turnover with osteoblasts and occasional osteoclasts (arrow). There is no cytologic atypia to suggest malignancy.

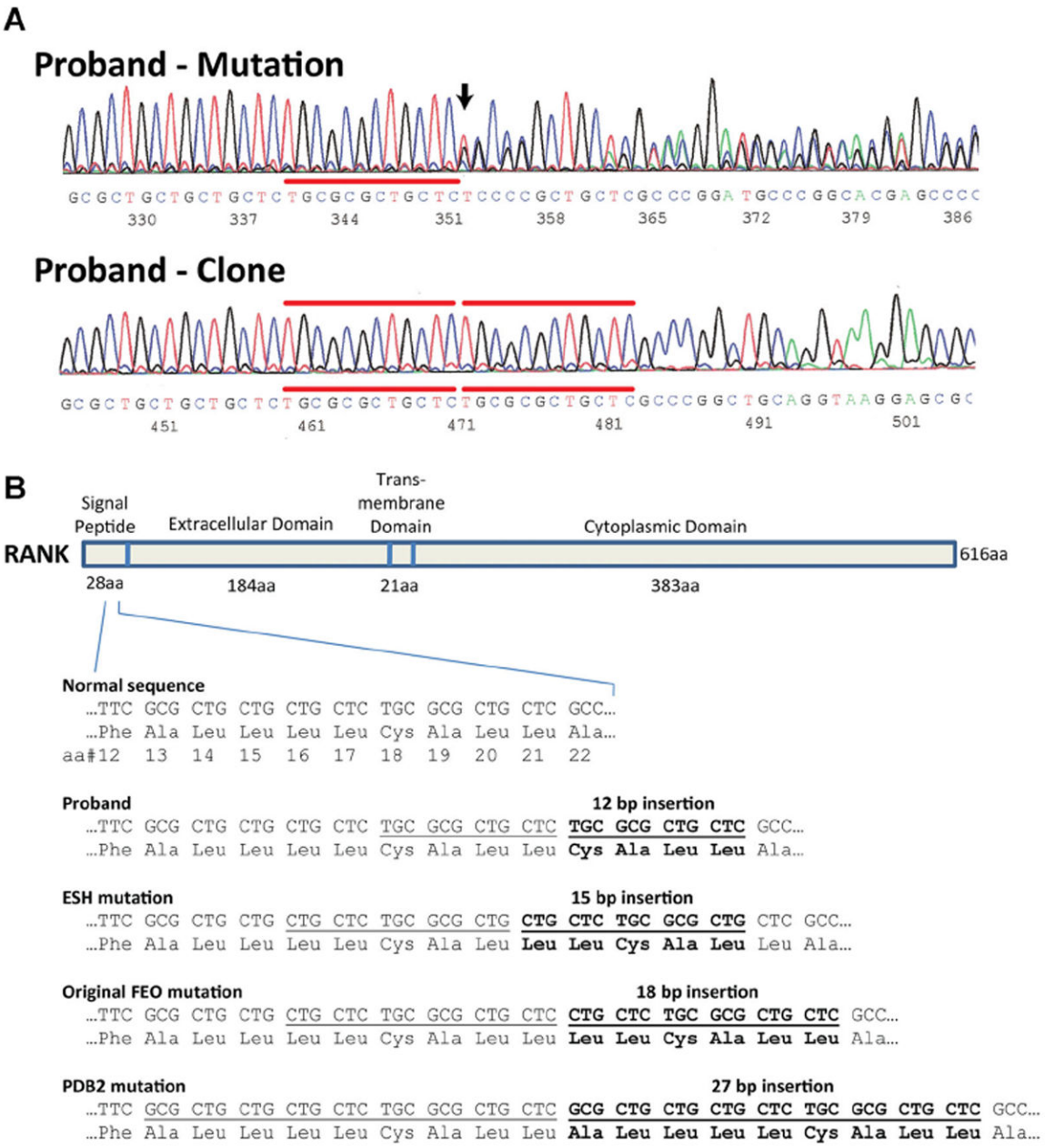

Fig. 7.

Electropherogram (*A*) and sequence (*B*) demonstrated a unique duplication in the signal peptide of RANK encoded by gene *TNFRSF11A*. The 12-bp duplication is distinct from the duplications of ESH (15-bp duplication), FEO (18-bp duplication), and PDB2 (27-bp duplication).

Table 1

Clinical Features of the Heritable Disorders of RANK/RANKL/OPG Signaling Activation

Feature	FEO	PDB2	ESH	JPD	Our Patient
Mutated protein	RANK	RANK	RANK	OPG	RANK
Deafness in childhood	+/−	−	+	+	+
Hypercalcemia	−	+	+	−	−
Large expansile lytic lesions	+	−	−	−	?[a]
Panostotic disease	−	+/−	+	+	+
Jaw tumor formation	−	−	−	−	+
Nephrolithiasis	−	−	−	+	+
Inheritance	Autosomal dominant	Autosomal dominant	Autosomal dominant	Autosomal recessive	?[b]

FEO=familial expansile osteolysis; PDB2=early-onset Paget's disease of bone; ESH=expansile skeletal hyperphosphatasia; JPD=juvenile Paget's disease; RANK=receptor activator of nuclear factor-κB; OPG=osteoprotegerin.

[a] It is unknown whether our patient previously had large lytic lesions that progressed and became his panostotic disease.

[b] The presence of our patient's mutation in the heterozygous state indicates the potential for autosomal dominant transmission of the defect.