Elderly-onset calcinosis of hyperphosphataemic familial tumoral calcinosis/hyperostosis-hyperphosphataemia syndrome: the role of comorbid scleroderma

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Summary

A 73-year-old woman with type 2 diabetes mellitus was referred to our department for glycaemic control. Physical examination revealed two subcutaneous hard masses around the left shoulder and the right hip joint. The patient could not fully extend her fingers because of skin sclerosis in both hands. Laboratory studies showed hyperphosphataemia and a high ratio of renal tubular maximum reabsorption of phosphate to glomerular filtration rate. There were no abnormalities in serum calcium, creatinine, alkaline phosphatase, and intact parathyroid hormone levels, whereas serum fibroblast growth factor 23 was low. Hyperphosphataemic familial tumoral calcinosis/hyperostosis-hyperphosphataemia syndrome (HFTC/HHS) was diagnosed using whole genome sequencing that revealed a novel frameshift beyond the 584th threonine located in the lectin domain of UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 3 associated with a duplication of the 1748th thymine in the coding region of the corresponding gene. Furthermore, anti-nuclear, anti-centromere, and anti-cardiolipin antibodies were positive, implying that comorbid limited type scleroderma might play a role in tumoral calcinosis (TC) development. A low phosphate diet was prescribed with phosphate-lowering medications, including aluminium hydroxide, acetazolamide, and sevelamer hydrochloride. The patient displayed a decrease in serum phosphate levels from 6.5 to 5.5 mg/dL 10 months after the initiation of treatment, but her TC had not improved during treatment for more than 1 year. This case was interesting because the patient with HFTC/HHS exhibited TC despite being over her 60s, and subsequent scleroderma might contribute to the specific clinical course. When HFTC/HHS presents with elderly-onset TC, the involvement of comorbidities in exacerbating TC should be considered.

Learning points

• HFTC/HHS occurs on an autosomal recessive basis, but its clinical course and manifestations differ significantly throughout the cases.
• HFTC/HHS may be undiagnosed until later in life because of its rarity, unfamiliarity, and phenotype diversity; therefore, HFTC/HHS should be included in the differential diagnosis of elderly patients with unexplained hyperphosphataemia or ectopic calcinosis.
• Comorbidities, including rheumatologic disorders, may contribute to developing HFTC/HHS-associated calcinosis.
Background

Ectopic calcinosis occurs when calcium phosphate salts, including hydroxyapatite and amorphous calcium phosphate, accumulate within body parts other than bone and teeth (1). Ectopic calcinosis is classified into five forms: metastatic calcinosis, dystrophic calcinosis, idiopathic calcinosis, iatrogenic calcinosis, and calciphylaxis (1). Several rheumatologic disorders, such as systemic lupus erythematosus, dermatomyositis, scleroderma, mixed connective tissue disease, and Calciosis/Raynaud’s phenomenon/Oesophageal dysmotility/Sclerodactyly/Telangiectasia (CREST) syndrome may cause dystrophic calcinosis in the absence of systemic mineral imbalance (1). Approximately 25% of scleroderma patients have ectopic calcinosis in the skin, termed calcinosis cutis, due to local inflammation and altered arteriole flow (2).

Fibroblast growth factor 23 (FGF23), a 227 amino acid peptide hormone, is secreted by osteoblasts, osteocytes, and erythroid precursor cells (3). FGF23 reduces the expression of type 2a and 2c sodium-phosphate cotransporters in the apical membrane of renal proximal tubular cells, suppressing phosphate reabsorption in the site and promoting urinary phosphate excretion (3, 4). FGF23 also inhibits intestinal phosphate absorption by modifying vitamin D-metabolising enzymes and decreasing 1α,25-dihydroxyvitamin D₃ levels (3, 4). Hyperphosphataemic familial tumoral calcinosis/hyperostosis-hyperphosphataemia syndrome (HFTC/HHS) is an autosomal recessive disease characterised by FGF23 deficiency or resistance due to homozygous or compound heterozygous inactivating mutations in the FGF23, UDP-N acetyl-alpha-D-galactosamine: polypeptide N-acetylgalactosaminyltransferase 3 (GALNT3), or Klotho (3, 4). Serum FGF23 levels depend on the underlying genetic abnormalities (3, 4), and bone mineral density (BMD) results vary significantly in patients with HFTC/HHS (5, 6). They have also been shown to suffer from various dental problems (7).

Hyperphosphataemia with normocalcaemia in HFTC/HHS patients is associated with widespread bulky metastatic calcinosis, called tumoural calcinosis (TC), around joints and areas under pressure, such as the shoulder, elbow, and hip, which limits the mobility of joints (3, 4, 5). TC in HFTC/HHS patients generally occurs by 20 years of age but is also variable for its onset and severity, even among patients sharing common genetic alterations, suggesting that the disease penetrates at different rates in each individual (5).

This article describes an elderly patient with HFTC/HHS caused by a novel frameshift mutation in GALNT3. The patient had concurrent scleroderma, which may have adversely affected the clinical course of HFTC/HHS, including the exacerbation of TC.

Case presentation

A 73-year-old woman was referred to our hospital with uncontrolled hyperglycaemia. The patient was diagnosed with type 2 diabetes mellitus (T2DM) at 64 years of age and had been taking alogliptin benzoate (12.5 mg once daily) for 6 years. At the first visit, the patient had diabetic microangiopathies, including simple retinopathy and macular degeneration with visual impairment and nephropathy with microalbuminuria but was free of peripheral neuropathy. She had no history of fragility fractures. Her younger sister died of breast cancer, and her younger brother suffered from T2DM, hypertension, and dyslipidaemia. A review of the patient’s information revealed no family history of electrolyte imbalance, ectopic calcinosis, ischaemic heart disease, stroke, renal failure, or autoimmune endocrinopathy, including parathyroid disease. The patient had experienced gradual deterioration in the range of motion of her right hip joint without sudden pain. Walking and utilising the toilet gradually became more complex over the past few years. Clinical signs of systemic inflammation were absent apart from chronic fatigue.

Investigation

The patient’s height was 150.2 cm, weight was 42.1 kg, and body mass index was 18.7. Physical examination revealed that blood pressure was 116/66 mmHg and pulse rate was 89 beats per minute, regular. Large subcutaneous hard masses were palpable around the left shoulder (Fig. 1A) and the right hip joint (Fig. 1B). The patient could not fully extend her fingers because of skin sclerosis in both hands.

Laboratory examination revealed high serum phosphate levels (6.5 mg/dL, reference range (RR): 2.7–4.6 mg/dL) with a high percentage tubular reabsorption of phosphate (95%, RR: 80–94%) and a high ratio of maximal tubular reabsorption of phosphate to glomerular filtration rate (> 5.0 mg/dL; RR: 2.3–4.3 mg/dL) (Table 1). There were no abnormalities in the serum calcium corrected for albumin (9.6 mg/dL, RR: 8.8–10.1 mg/dL), magnesium (2.0 mg/dL, RR: 1.9–2.5 mg/dL), creatinine (0.49 mg/dL, RR: 0.46–0.79 mg/dL), and alkaline phosphate levels (246 IU/L, RR: 106–322
IU/L); however, the calcium–phosphorus product was elevated (62.4, RR: 23.8–46.5). Serum 1α,25-dihydroxyvitamin D3 level was inappropriately high (84.4 pg/mL, RR: 20.0–60.0 pg/mL), even though hyperphosphataemia was present. Serum intact parathyroid hormone level was in the lower one-third of the reference range (16 pg/mL, RR: 10–65 pg/mL), and serum intact FGF23 level was decreased (11.7 pg/mL, RR: 19.9–52.9 pg/mL). Insurance coverage restrictions in our country prevented the measurement of serum FGF23 C-terminal level. No apparent abnormalities in the other basal blood hormone levels and acid-base balance were present (Table 1). C-reactive protein (CRP) levels were persistently elevated (2.15–4.55 mg/dL, RR: <0.3 mg/dL), but leucocytosis was absent (white blood cell count 8600/mm³, RR: 3300–8600/mm³). The glycated haemoglobin level was 7.2% (RR: 4.6–6.2%).

No abnormality was on the chest radiograph 10 years earlier (Fig. 1C); however, a calcified mass was noted around the left shoulder at the first visit to our department (D, an arrow). Radiographs of the overlying skin. (C, D) A chest radiograph demonstrates no abnormal findings 10 years earlier (C); however, a calcified mass was noted around the left shoulder at the first visit to our department (D, an arrow).

The clinical and laboratory findings suggested HTFC/HHS due to a germline mutation of GALNT3 or FGF23 rather than a Klotho mutation because the patient had low serum intact FGF23 level (3, 4). Indeed, whole genome sequencing analysis revealed a novel thymine duplication of the 1748th in the GALNT3 coding region that caused a frameshift beyond the 584th threonine located in the lectin domain of UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 3 (GalNAc-T3) (Reference genome: hg38, Gene_ID: 2591, Transcript_ID: NM_004482, Coordinates (gDNA/cDNA/protein): chr2:g.165749773dupA/c.1748dupT/p.T584Hfs*47).

Figure 1
(A, B) Photographs show that swellings were evident around the left shoulder extending to the upper back (A, arrows) and the right hip joint (B, arrows), with dilated blood vessels but no redness or pigmentation of the overlying skin. (C, D) A chest radiograph demonstrates no abnormal findings 10 years earlier (C); however, a calcified mass was noted around the left shoulder at the first visit to our department (D, an arrow).

Table 1  Laboratory evaluations of the patient upon presentation to our department.

<table>
<thead>
<tr>
<th>Investigation</th>
<th>Value</th>
<th>Reference</th>
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<tr>
<td>Serum albumin (g/dL)</td>
<td>3.8</td>
<td>4.1–5.1</td>
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<td>Serum calcium (mg/dL)</td>
<td>9.4</td>
<td>8.8–10.1</td>
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<td>Serum phosphate (mg/dL)</td>
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<td>2.7–4.6</td>
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<td>Serum creatinine (mg/dL)</td>
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<td>Serum ALP (IU/L)</td>
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<tr>
<td>%TRP (%)</td>
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<td>80–94</td>
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<tr>
<td>Tmp/GFR (mg/dL)</td>
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<tr>
<td>1,25-(OH)2-D3 (pg/mL)</td>
<td>84.4</td>
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</tr>
<tr>
<td>Intact PTH (pg/mL)</td>
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<td>10–65</td>
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<tr>
<td>FGF23 (pg/mL)</td>
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<tr>
<td>Base excess (mmol/L)</td>
<td>–1.3</td>
<td>–2.3 to 1.3</td>
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ALP, alkaline phosphatase; %TRP, percentage tubular reabsorption of phosphate; 1,25-(OH)2-D3, 1α,25-dihydroxyvitamin D3; ACTH, adrenocorticotropic hormone; FGF23, fibroblast growth factor 23; IGF-1, insulin-like growth factor 1; PTH, parathyroid hormone; Tmp/GFR, ratio of maximal tubular reabsorption of phosphate to glomerular filtration rate; TSH, thyroid-stimulating hormone.
Furthermore, extensive autoantibody screening was conducted to rule out the involvement of rheumatologic disorders in the patient’s skin sclerosis and elderly-onset calcinosis. The high titres of antinuclear antibody (×320, RR: <×40), anti-centromere antibody (260 U/mL, RR: 0–9.9 U/mL), and anti-cardiolipin IgG antibody (12.4 U/mL, RR: 0–9 U/mL) indicated that limited type scleroderma was present simultaneously with HTFC/HHS. All other autoantibody titres were within the reference range, including anti-double-stranded DNA antibody (<10 U/mL, RR: <12 U/mL), anti-Sm antibody (<1.0 U/mL, RR: <10 U/mL), anti-Jo-1 antibody (<1.0 U/mL, RR: <10 U/mL), antitopoisomerase I (anti-scleroderma-70) antibody (<1.0 U/mL, RR: <10 U/mL), and anti-ribonucleoprotein antibody (<2.0 IU/mL, RR: <10 U/mL). Lupus anticoagulant was considered unharmful (the silica clotting time screen ratio 1.00, RR: ≤1.16). In addition, there was no evidence of pulmonary hypertension, deep vein thrombosis, or interstitial pneumonia based on cardiac ultrasonography, lower extremity venous ultrasonography, and chest CT imaging.

**Treatment**

The patient underwent nutritional counselling and a low phosphate diet restricting phosphate intake to less than 600 mg/day, which failed to ameliorate her hyperphosphataemia. Phosphate-lowering medications, including aluminium hydroxide (1 g three times daily), acetazolamide (250 mg once daily), and sevelamer hydrochloride (500 mg three times daily), were initiated. Surgical intervention for TC was reserved for the patient due to potential risks, including poor wound healing, infection, and chronic drainage. Neither nonsteroidal anti-inflammatory drugs nor glucocorticoids were prescribed to alleviate low-grade inflammation. Scleroderma affected both fingers’ distal parts of the metacarpophalangeal joint with skin sclerosis, but no apparent organ damage was evident. Therefore, the treatment for scleroderma was not given.

**Outcome and follow-up**

Periodic monitoring of renal function and serum bicarbonate ensured no severe side effects throughout the treatment process. Ten months after the treatment...
began, serum phosphate levels were decreased by 5.5 mg/dL. The patient’s energy levels, appetite, and general well-being appear unaltered during the treatment. The patient underwent chest CT imaging in order to identify the presence of scleroderma-related interstitial pneumonia. The imaging could also help follow-up on her TC around the left shoulder (3, 4), where there was no apparent treatment response regarding the reduction in TC size 1 year and 3 months after the initiation of treatment for hyperphosphataemia.

Discussion

The present case report describes a 73-year-old patient with HFTC/HHS caused by a frameshift mutation in GALNT3. At the first visit, the patient manifested TC around the left shoulder and the right hip joint. However, a chest radiograph 10 years earlier showed no noticeable calcified mass. Therefore, the patient was likely to be asymptomatic until her 60s and the bulky calcinoses had developed within a decade.

GalNAc-T3 encoded by GALNT3 catalyses O-glycosylation of FGF23 at the 178th threonine in the subtilisin-like proprotein convertase site, which facilitates FGF23 secretion in an intact active form by protecting from furin degradation (8). GalNAc-T3 consists of three parts: catalytic domain, linker domain, and lectin domain, and a lectin-mediated substrate-binding mechanism plays a pivotal role in the site-specific O-glycosylation of FGF23 (8). The patient had a frameshift beyond 584th threonine located in the GalNAc-T3 lectin domain (p.T584Hfs*47) caused by the 1748th thymine duplication in the GALNT3 coding region (c.1748dupT). This frameshift is likely a novel mutation that was not previously noted in several published databases describing the diversity of human pathogenic genomes, such as Online Mendelian Inheritance in Man (https://omim.org), The Human Gene Mutation Database (HGMD) (https://hgd.m.cf.ac.uk/ac/index.php), ClinVar (https://ncbi.nlm.nih.gov/clinvar), and dbSNP (https://ncbi.nlm.nih.gov/snp/).

This study did not conduct an in vitro analysis to confirm the impact of the GALNT3 frameshift mutation on the GalNAc-T3 structure. An alternative approach using the bioinformatics method with MutationTaster2021 software (https://genecascade.org/MutationTaster2021/#transcript) suggests that p.T584Hfs*47 in GalNAc-T3 could be harmful, as the truncated protein with an abnormal C-terminus would likely undergo nonsense-mediated mRNA decay. A lack of GalNAc-T3-mediated O-glycosylation of FGF23 resulted in intact FGF23 being cleaved into inactive fragments (8), which is in good agreement with the patient’s biochemical profile identical to HFTC/HHS patients carrying inactivating GALNT3 variants, including an inappropriately low serum intact FGF23 level despite her hyperphosphataemia (3, 4). Additionally, there were no causal variants in the other known candidate genes for HFTC/HHS, such as FGF23 (Gene_ID: 8074) and Klotho (Gene_ID: 9365). Therefore, the patient’s frameshift mutation appeared to be pathogenic, could affect intact FGF23 dynamics, and would have a detrimental impact on HFTC/HHS development.

Calcium hydroxyapatite is the main component of TC surgically resected from HFTC/HHS patients with GALNT3 mutations (5). Numerous foamy macrophages phagocytose hydroxyapatite crystals and release inflammatory cytokines around the calcium deposits (5). Calcinosis in scleroderma patients also comprises calcium hydroxyapatite and foamy macrophage clusters, similar histopathological characteristics to that found in HFTC/HHS calcinosis (9). However, there is no difference in serum calcium and phosphate levels between the two subgroups of scleroderma patients with and without symptomatic ectopic calcinosis (10). Furthermore, higher serum FGF23 levels correspond to increased ectopic calcinosis in female scleroderma patients (11). These findings are distinct from those in HFTC/HHS; therefore, the two diseases may additively contribute to developing ectopic calcinosis. Scleroderma is divided into two subgroups: diffuse type and limited type (2). The disease usually develops later in life, probably during their 50s–60s, and it takes 1–2 years for the diffuse type and 5–10 years for the limited type to manifest the first non-Raynaud’s phenomenon after the onset of Raynaud’s phenomenon (2). The elderly-onset limited type scleroderma partly explains the deterioration of latent calcinosis after her 60s.

Several factors, including ageing, vascular disorders, chronic ischaemia, and tissue damage, can adversely affect the development of ectopic calcinosis (1). At the first visit, the patient displayed early-stage diabetic retinopathy and nephropathy, indicating the presence of hyperglycaemia-induced microvascular injury. Scleroderma is prone to vasospasm, as shown by recurrent Raynaud’s phenomenon and may cause loss of endothelial cells and proliferation of smooth muscle cells, pericytes, and fibroblasts as early manifestations of impaired vasculature (12). Ischaemia and occlusion of the small vessels occur in the later
phase of scleroderma due to progressive vascular wall thickening by collagen accumulation and intravascular thrombus formation (12). Furthermore, the patient was negative for lupus anticoagulant but had a low titre positive for anti-cardiolipin antibody. Although she did not fulfil the criteria of laboratory data for antiphospholipid syndrome, the risk of developing arteriovenous microthrombosis was expected to be relatively high. It has been reported that subsequent scleroderma or systemic lupus erythematosus can exacerbate calciphylaxis in dialysis patients when inflammatory findings are positive (13). The patient had a low-grade inflammatory status, as evidenced by persistent high serum CRP levels. The combination of older age, improper vascular tone, structural change and remodelling in the small vessels, and long-lasting inflammation may participate in TC development in the patient.

In summary, the clinical course of HFTC/HHS varies throughout the cases; therefore, HFTC/HHS should be considered even in elderly patients with undiagnosed hyperphosphataemia or ectopic calcification. Furthermore, in late-onset HFTC/HHS cases, comorbidities may have exacerbated asymptomatic HFTC/HHS, resulting in manifestations including TC.

Declaration of interest
The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of this research.

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Patient consent
Written informed consent for clinical details and images, genetic investigation using whole genome sequencing, and the publication of this case report were obtained from the patient. The Institutional Review Board of Toshiba Rinkan Hospital approved the research proposal and consent forms (No. R04-0006).

Author contribution statement
H Iwasaki, the patient’s physician at Toshiba Rinkan Hospital, isolated genomic DNA from the patient’s peripheral venous blood using the QiAamp® Blood Midi Kit (Qiagen GmbH), sequenced the whole genome using a commercial service (Rhelixa, Inc., Tokyo, Japan) and interpreted the sequencing results. In addition, H Iwasaki conducted the case description and literature review and wrote the article.

References