Dysosteosclerosis: Clinical and Radiological Evolution Reflecting Genetic Heterogeneity

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ABSTRACT
Dysosteosclerosis (DSS), the term coined in 1968 for ultrarare dysplasia of the skeleton featuring platyspondyly with focal appendicular osteosclerosis, has become generic by encompassing the genetic heterogeneity recently reported for this phenotype. We studied four unrelated Turkish patients with DSS to advance understanding of the new nosology. Patient 1 suffered femur fractures beginning at age 1 year. DSS was suspected from marked metaphyseal osteosclerosis in early childhood and subsequently platyspondyly accompanying patchy osteosclerosis of her appendicular skeleton. She harbored in SLC29A3, in 2012 the first gene associated with DSS, a unique homozygous duplication (c.303_320dup, p.T102_107dupYFESYL). Patient 2 presented similarly with fractures and metaphyseal osteosclerosis but with no platyspondyly at age 2 months. She was homozygous for a novel nonsense mutation in SLC29A3 (c.1284C>G, p.Tyr428X). Patient 3 had ocular disease at age 2 years, presented for short stature at age 11 years, and did not begin to fracture until age 16 years. Radiographs showed mild platyspondyly and focal metaphyseal and femoral osteosclerosis. She was homozygous for a unique splice site mutation in TNFRSF11A (c.616+3A>G). Patient 4 at age 2 years manifested developmental delay and frequent infections but did not fracture. He had unique metadiaphyseal splaying and osteosclerosis, vertebral end-plate osteosclerosis, and cortical thinning of long bones but no mutation was detected of LRRK1, or CSF1R associated with DSS. We find that DSS from defective SLC29A3 presents earliest and with fractures. DSS from compromised TNFRSF11A can lead to optic atrophy as an early finding. Negative mutation analysis in patient 4 suggests further genetic heterogeneity underlying the skeletal phenotype of DSS. © 2022 The Authors. JBMR Plus published by Wiley Periodicals LLC on behalf of American Society for Bone and Mineral Research.

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Introduction
Dysosteosclerosis (DSS or DOS), considered an osteopetrosis (OPT) when first described in 1934 by Ellis,1 was named in 1968 by Spranger and colleagues2 to emphasize its patchy osteosclerosis accompanied by platyspondyly with vertebral endplate thickening (OMIM %.224300).3 During infancy and early childhood the osteosclerosis of DSS is widely distributed but especially pronounced in expanded metaphyses, whereas diaphyses are broad and radiodense or radiolucent.4–10 Then, these features of DSS evolve.4–6,10 Expanded osteoclastic metaphyses become osteopenic, sometimes including portions of the diaphyses where the cortex may be thin.4,6,10 Cranial nerve palsies and developmental delay can occur.6–10 Skin changes are sometimes noted.1–7 By middle age, there can be diffuse osteosclerosis of cranial bones, mandible, maxilla,
Diagnosing DSS may be delayed because its radiographic hallmarks can go undocumented or misunderstood. In 2010, we reported that DSS in early childhood represents an “osteoclast-poor” form of OPT wherein some heritable defect transiently abrogates osteoclastogenesis.\(^\text{4,5}\)

Delineation of the etiology of DSS began in 2012 when we reported two girls who harbored biallelic mutations of the nucleoside transporter gene “solute carrier family 29 member 3” (SLC29A3).\(^\text{5}\) The following year, Sule and colleagues\(^\text{12}\) found no SLC29A3 defect in one patient\(^\text{6,10}\) thereby suggesting genetic heterogeneity for DSS. In 2016, Iida and colleagues\(^\text{13}\) associated “leucine rich repeat kinase 1” (LRRK1) with osteoclastic metaphyseal dysplasia (OMD; OMIM \# 615198)\(^\text{3}\) featuring characteristics of DSS\(^\text{5,13–17}\). In 2018, two genes underlying autosomal recessive OPT\(^\text{18,19}\) became associated with DSS\(^\text{20,21}\): (i) “tumor necrosis factor receptor superfamily member 11A” (TNFRSF11A) that encodes receptor activator of NF-κB (RANK) (OMIM \# 612301),\(^\text{3}\) and (ii) “T-cell immune regulator 1” (TCIRG1) that encodes a component of the vascular proton (H\(+\))-pump necessary for osteoclasts (OCs) to produce hydrochloric acid (OMIM \# 259700).\(^\text{3}\) Most recently, in 2019, biallelic mutations of “colony-stimulating factor 1 receptor” (CSF1R)\(^\text{22}\) were associated with dysostosesclerosis-Pyle disease (DSS-PD), also called “brain abnormalities, neurodegeneration, and dysostosclerosis” (BANDOS: OMIM \# 618476).\(^\text{3}\)

Herein, we characterize the phenotype of four young unrelated Turkish patients with DSS. Two carried novel homozygous SLC29A3 defects, one a novel homozygous TNFRSF11A change, and one for whom no mutation was found.

**Patients and Methods**

**Patients**

The principal clinical and radiological features and results from mutation analyses of our four patients are detailed below and summarized in Table 1.

**Patient 1**

Patient 1, 23 years-of-age, was homozygous for a novel defect in SLC29A3. In 2015, we briefly reported her features of DSS and unique mutation.\(^\text{23}\) Her parents were first-degree cousins. At age 4 months, severe anemia (hemoglobin [Hb] 3 mg/dL) was attributed to OPT despite no hemolysis or extramedullary hematopoiesis. Subsequently, folic acid supplementation (serum folate 2.9 ng/mL, normal [NI] 2.0–9.0) corrected this problem. Fracturing began at about age 1 year. Shedding of her deciduous teeth was delayed. Neurodevelopmental milestones were normal with good grades at school. Four of at least seven femoral breaks required surgery, including one to remove metal plates. A supracondylyar specimen of femur reportedly showed focal, irregular, lytic, and dysplastic enchondal bone adjacent to mature compact bone. Skeletal scintigraphy at age 12 years revealed symmetrically increased radionuclide uptake attributed to increased osteoblastic activity at the proximal and distal femurs and at the proximal humeral and tibial metaphyses. Sequential dual-energy X-ray absorptiometry (DXA) demonstrated (Lunar Prodigy; GE Healthcare, Piscataway, NJ, USA) markedly elevated but decreasing areal bone mineral density (aBMD) at the L\(_1\)-L\(_4\) spine; +10.8 at age 7 years, +6.3 at age 10 years, +6.4 at age 15 years, +8.7 at age 17 years, +7 at age 22 years, and +5.6 at age 23 years. Bone turnover markers (BTMs) spanning ages 11 to 23 years included elevated urinary deoxypyridinoline (DPD; Immulite\textsuperscript{\textregistered} 2000, Siemens Healthcare Diagnostic Products Ltd., Llanberis, Gwynedd, UK), but normal serum alkaline phosphatase (ALP; Hitachi 917; Roche Diagnostics International Ltd., Mannheim, Germany), C-terminal telopeptide (CTX; Cobas e411; Roche Diagnostics International Ltd., Rotkreuz, Switzerland), and procollagen type 1 N-terminal propeptide (P1NP) (Cobas e411; Roche Diagnostics International Ltd., Rotkreuz, Switzerland). Serum osteocalcin (OCN) levels (Immulite\textsuperscript{\textregistered} 2000, Siemens Healthcare Diagnostic Products Ltd., Llanberis, Gwynedd, UK) ranged from low to high (Table 2).

Referral at age 17 years was for recurring fractures. Her height was 146 cm (−2.8 standard deviation [SD]), arm span 159 cm; and arm span-height difference 12.7 cm (NI −6.3 to +6.5). She weighed 53 kg (−0.7 SD). Mid-parenteral height was 156 cm, and her healthy 30-year-old and 28-year-old sisters were 158 cm and 161 cm tall, respectively. Disproportionate short stature included a short trunk, but prior femoral fractures explained her nearly normal upper-lower segment ratio of 0.8 (NI 0.88–1.1). Mild prognathism, prominent nose, short philtrum, small maxillary lateral incisors, and broad shoulders were apparent. At age 18 years, hypertrichosis/hirsutism (Ferriman-Gallwey score = 15) was without hyperandrogenemia or menstrual irregularity. Audiology and fundus and visual field examinations were normal. DSS became suspected when her radiographs revealed marked metaphyseal sclerosis that became increasingly generalized from age 1–15 years (Fig. 1A–D). Patchy diaphyseal osteosclerosis, platspondyly with “sandwich vertebrae,” and thickening and osteosclerosis of her calvarium were striking (Fig. 1E–H). Then, accompanying the decline in spinal aBMD, long bone osteosclerosis diminished, leaving scarce and lacy sclerotic areas within an osteopenic background. At age 23 years, her long bones were under-modeled and osteopenic, whereas fractures had healed with osteosclerosis (Fig. 1E).

**Patient 2**

Patient 2 was homozygous for a different novel SLC29A3 mutation. Her parents were second-degree cousins. Fetal ultrasound indicated no antenatal fractures or deformities. Pregnancy and delivery were uneventful and birth weight was 3270 g. At age 2 months, a fibular fracture prompted referral for “osteogenesis imperfecta.” Height, weight, and head circumference were 57.5 cm (−0.22 SD), 4.7 kg (−0.97 SD), and 38.5 cm (−0.64 SD), respectively. Frontal bossing, open anterior (3 × 4 cm) and posterior (0.5 × 0.5 cm) fontanelles, gray-blue sclera, mild hypertichosis, and restricted elbow extension were present. Growth was good with appropriate neurodevelopmental milestones at age 6 months. Audiology, ophthalmology, metabolic and heavy metal screening, and BTMs were normal except for elevated urinary DPD (Table 2). Metaphyseal osteosclerosis was not apparent at age 19 days, but at age 2 months was documented in her femurs, proximal humeri, and metacarpals when referred to our clinic. At age 6 months, platyspondyly and “sandwich vertebrae” were absent on spinal radiographs (Fig. 2A–F).

**Patient 3**

Patient 3, 21 years old, harbored a unique homozygous mutation in TNFRSF11A. Her parents were consanguineous. At age 2 years, nystagmus, pale optic disks, and increased retinal vessel tortuosity were noted. She presented to us at age 10 years with short stature; height 121 cm (−3.3 SD) and weight 23.6 kg (−2.2 SD). Body proportions were normal (upper-lower segment ratio 1.04
[NI 0.86–1.08], and arm span/height difference 3.4 cm [NI −7.8 to +6.1)]. Family members were not short, and reportedly without heritable diseases. Pycnodysostosis was suspected because her facial dysmorphism included proptosis, beaked nose, malar hypoplasia, and short philtrum. Nystagmus had been first noticed at age 2 months, and was present at age 18 years when cranial and orbital tomography showed bilateral proptosis and thickening of optic nerve sheath indicating optic canal narrowing. Poor oral hygiene, crowded teeth, and multiple caries were present. Immunoglobulin levels were not measured because there had been no recurrent infections. Femoral fractures, three requiring surgery, began at age 16 years. Adult height was 138 cm (−4.2 SD) with mid-parenteral height 154 cm (−1.55 SD). Arm span was 151 cm, arm span/height difference 12.2 cm (NI −6.3 to +6.5 cm), and upper-lower segment ratio 0.88 (NI 0.88–1.1) showing that her short stature principally reflected her short trunk. Cranial bones and ribs were osteosclerotic (Fig. 3A,C). Platspondyly with vertebral endplate osteosclerosis was present (Fig. 3C2). Long bones featured osteopenia, thin cortices, and wide metaphyses (Fig. 3E-F). Pycnodysostosis seemed unlikely because hand radiographs lacked osteosclerosis or acroosteolysis, and her osteopenic long bones featured localized osteosclerosis consistent with DSS (Fig. 3B,E). DXA of L1–L4 revealed elevated aBMD and Z-scores increasing with age; +8.1 at 18 years, +9.3 at 19 years, and +10.7 at 21 years. BTMs spanning 17 to 21 years old were normal (Table 2). In 2017, we identified her homozygous TNFRSF11A mutation in our research laboratory (see Mutation analyses). Then, in 2018, Guo and colleagues(20) reported her the first example of DSS from TNFRSF11A mutation.

Patient 4

Patient 4, 11 years old, revealed in our research laboratory no mutation in any of the five genes (SLC29A3, TNFRSF11A, LRRK1, SLC11A1, CLN3).

| Table 1. Genetic, Clinical, and Radiographic Findings of Our Four Patients |
|------------------------|------------------------|------------------------|------------------------|
| Subject | Patient 1 | Patient 2 | Patient 3 | Patient 4 |
| Genetic defect | SLC29A3(c.303_320dup) | SLC29A3(c.1284C>G) | TNFRSF11A(c.616+3A>G) | None identified |
| Parental consanguinity | + | + | + | – |
| Sex | Female | Female | Female | Male |
| Age at presentation | 4 months | 2 months | 10.6 years | 27 months |
| Complication | Anemia | Fracture | Short stature | Hypercalcemia |
| Age last visit | 23 years | 6 months | 21 years | 4.5 years |
| Birth weight (gestational age) | 4 kg (term) | 3270 g (term) | 3010 g (term) | 2200 g (36 weeks) |
| Height (SD) | 146.3 cm (−2.8 SD)b (final) | 62.5 cm (−1.1) | 138 cm (−4.2 SD)b (final) | 90 cm (−3.8) |
| Target height (SD) | 156 cm (−1.21) | 158 cm (−0.87) | 154 cm (−1.55) | 173 cm (−0.52) |
| Age of first fracture (# of fractures) | 12 months (>7) | 2 months (1) | 17 years (4) | – |
| Skin changes | Hypertrichosis | Hypertrichosis | None | Eczema |
| Optic atrophy | – | – | + | – |
| Developmental delay | – | – | – | Severe |
| Recurrent infections | – | – | – | + |
| Dental problems | Delayed shedding of deciduous teeth | NA | Frequent carries | Discoloration |
| Radiographs | Platspondyly | + | + | + |
| Erlenmeyer flask deformity | + | + | + | + |
| Diaphyseal osteopenia and thin cortices | + | + | + | + |
| Osteosclerosis | Vertebral | + | + | + |
| Rib | Mild | – | – | – |
| Pelvic bone | + | + | + | + |
| Calvariae | Mild | – | – | – |
| Metaphyseal | + | – | – | – |
| Diaphyseal | – | – | – | – |
| Diaphyseal focal sclerosis (femur) | Few and far between | – | – | – |
| Changing with age | + | + | + | + |
| Hand and forearm bones | + | + | + | + |

Abbreviation: NA, not applicable.

aRadiographs taken in older ages, osteosclerosis earlier not excluded.
bShort trunk dwarfism.
| Genetic defect (#patient/#family) | SLC29A3 (6/6)[5,21,31,32] | TNFRSF11A (4/4)[11,20,33,34] | TCF7L1 (2/1)[21] | CSF1R (11/5)[22,23,35,36] | LRRK1 (8/5)[13–17] |
|---------------------------------|--------------------------|-----------------------------|-----------------|--------------------------|-------------------|
| **Mutations**                   |                          |                             |                 |                          |                   |
| p.303_320dup (Hom)              |                          |                             |                 |                          |                   |
| p.5203P/p.536Q                  |                          |                             |                 |                          |                   |
| p.386Q(Hom)                     |                          |                             |                 |                          |                   |
| p.P391H (Hom)                   |                          |                             |                 |                          |                   |
| p.T449R (Hom)                   |                          |                             |                 |                          |                   |
| **Sex ratio**                   | F(6)/M(0)                |                             |                 |                          |                   |
| **Ethnicity (families)**        | Turkish(4), ND(1),      |                             |                 |                          |                   |
|                                 | Cameroonian (1)          |                             |                 |                          |                   |
| **Age (years [y] or months [m])** | 2y, 5y, 5.6y, 22y, 11.1y, 5.5y | 16y, 59y, 2.3y, 5.3y | 15y, 10y | 5y, 35y, 14y, 23y, 9y, 7y, 16y, 14y | 14m, 14y, 14y, 23y, 25y, 13.5y, 11.5y, 9m |
| **Short stature**               | Y(5)/N(0)/ND(1)         |                             |                 |                          |                   |
| **Skin changes**                | Y(2)/N(4)               |                             |                 |                          |                   |
| **Optic atrophy**               | Y(0)/N(2)/ND(2)         |                             |                 |                          |                   |
| **Developmental delay**         | Y(1)/N(5)               |                             |                 |                          |                   |
| **Recurrent infections**        | Y(3)/N(2)/ND(1)         |                             |                 |                          |                   |
| **Anemia/pancytopenia**         | Y(1)/N(3)/ND(2)         |                             |                 |                          |                   |
| **Hepatosplenomegaly**          | Y(0)/N(2)/ND(4)         |                             |                 |                          |                   |
| **Hypercalcemia**               | Y(1)/N(3)               |                             |                 |                          |                   |
| **Dental problems**             | Y(2)/N(4)               |                             |                 |                          |                   |
| **Long bone fractures**         | Y(5)/N(1)               |                             |                 |                          |                   |
| **Delayed fracture healing**    | Y(1)/N(0)/ND(5)         |                             |                 |                          |                   |
| **Radiographs**                 |                          |                             |                 |                          |                   |
| Osteosclerosis of calvaria      | Mild Y(6)/N(0)          | Y(4)/N(0)                   | Y(2)/N(0)       | Y(7)/N(0)/ND(1)          | Mild Y(2)/N(1)/ND(5) |
| Platsyspondyly                  | Y(6)/N(0)               |                             | Y(2)/N(0)       | Y(7)/N(0)/ND(1)          | Y(1)/N(4)/ND(3)   |
| Vertebral sclerosis             | Y(5)/N(0)/ND(1)         |                             | Y(2)/N(0)       | Y(4)/N(2)/ND(2)          | Y(6)/N(0)/ND(2)   |
| Rib sclerosis/thickening        | Y(6)/N(0)               |                             | Y(2)/N(0)       | Y(3)/N(1)/ND(4)          | Y(4)/N(0)/ND(4)   |
| Pelvis-peripheral sclerosis     | Y(3)/N(0)/ND(3)         | Y(2)/Diffuse Y(2)/N(0)     | Y(0)/N(0)/ND(2) | Diffuse Y(5)/N(2)/ND(1) | Y(4)/N(0)/ND(4)   |

(Continues)
| Genetic defect (patient/family) | SLC29A3 (6/6) | TNFRSF1A (4/4) | TCIRG1 (2/1) | CSF1R (11/5) | LRRK1 (8/5) |
|--------------------------------|---------------|----------------|--------------|--------------|-------------|
| Metaphyseal sclerosis          | Y(5)/N(0)/ND(1) | Y(1)/N(3)    | Y(2)/N(0)   | Y(1)/N(6)/ND(1) | Y(5)/N(3) |
| Erlenmeyer flask deformity/undertubulation | Y(4)/N(2) | Y(3)/N(0)/ND(1) | Y(2)/N(0) | Y(2)/N(0) | Mild Y(6)/N(0)/ND(2) |
| Diaphyseal osteopenia and thin cortices | Y(1)/N(5) | Y(2)/N(2) | Y(0)/N(2) | Y(8)/N(0) | Y(3)/N(0)/ND(5) |
| Diaphyseal diffuse sclerosis   | Y(0)/N(5)/ND(1) | Y(2)/N(2) | Y(2)/N(0) | Y(0)/N(8) | Y(0)/N(8) |
| Diaphyseal focal sclerosis     | Y(3)/N(2)/ND(1) | Y(2)/N(2) | Y(0)/N(2) | Y(8)/N(0) | Y(6)/N(1)/ND(2) |
| Changing sclerosis with age    | Y(3)/N(0)/ND(3) | Y(0)/N(0)/ND(4) | Y(0)/N(0)/ND(2) | Y(0)/N(0)/ND(8) | Y(2)/N(0)/ND(6) |
| Normal bones of hand and forearm bones | Y(0)/N(4)/ND(2) | Y(2)/N(2) | Y(0)/N(2) | Y(0)/N(5)/ND(3) | Y(0)/N(5)/ND(3) |
| BMD Z-score (lumbar DXA)       | [+8.1 (2y), +11.9 (5y)] | [+8 (20 m), +7.1 (3.4y), +6.4 (4.5y), +5.1 (5.5y)] | [-0.6 (5.5y?)] | Brain malformations, calcifying leukoencephalopathy, epilepsy | Acroosteolysis, osteonecrosis/osteomyelitis of jaw |
| Others                         | Delayed closure of fontanelle (2), Macrocephaly (1), Melanocytic nevi | Craniosynostosis, intracranial extramedullary hematopoiesis | | | |

N = no; NA = not applicable; ND = not defined; Y = yes.

*Splice donor site mutations.

*Osteomyelitis.
TCIRG1, and CSF1R) that had come to be associated with DSS (see Discussion). His parents were unrelated. He weighed 2.2 kg when delivered at 36 weeks gestation to a 25-year-old woman who had noticed diminished fetal movement during the last week of her pregnancy. Intensive care for 10 days included respiratory support for 3 days. At age 6 months, slow motor development was reported. At age 14 months, inborn-error-of-metabolism studies were negative, and OMD was considered when radiographs showed metaphyseal osteosclerosis. Remarkably, at age 27 months, fever, cough, and vomiting accompanied unexplained severe hypercalcemia (19.8 mg/dL [NI 8.8–10.6]) with physiologically suppressed serum parathyroid hormone (PTH), hypophosphatemia, low-normal ALP, and normal 25-hydroxyvitamin D (Table 2). Intravenous hydration and pamidronate (1 mg/kg/day for 3 days) corrected the hypercalcemia, which did not recur. Frequent respiratory tract infections required four hospitalizations for pneumonia. Early developmental milestones were slightly delayed, but by age 2 years global motor retardation was apparent, and by age 4.5 years he could not sit, walk, or pronounce words. Cranial magnetic resonance imaging (MRI) was reportedly normal except for mild cortical atrophy.

When referred to us at age 28 months, his height, weight, and head circumference Z-scores were −1.8, −4.2, and −3.0, respectively. Dysmorphic facial features included a wide forehead, triangular face with open mouth, high-arched palate, yellow teeth, short nose with prominent nasal tip, long philtrum, mild hypoplasia of alae nasi, nasal obstruction, rigid pinnae, almond-shaped eyes with gray sclera, and laterally anteverted palpebral fissures (Kabuki makeup syndrome–like). Skeletal deformity was absent, but spasticity and atrophic muscles were present. Skin changes of DSS, such as red rashes, were present. Oral candidiasis was detected although serum immunoglobulin levels, immune phenotyping, nitroblue tetrazolium (NBT) testing, metabolic screening (including phosphoethanolamine for hypophosphatasia), blood gases, and lead level were normal. Adenoidectomy was performed at age 3.5 years. Scoliosis...
Mutation analyses

Mutation analyses were performed for patients 1, 3, and 4 in our research laboratory at Washington University School of Medicine, St. Louis, MO, USA. Written consent was obtained as approved by the Marmara University Medical Faculty Research Ethics Committee (MAR-Y-09.2021-623) Istanbul, Turkey in accordance with the 1964 Helsinki declaration and its later amendments. Coding exons and exon-intron boundaries of SLC29A3 were Sanger sequenced using methods we had developed.[4,5] When no SLC29A3 mutation was identified (patients 3 and 4), Ion Torrent (Thermo Fisher Scientific) next-generation sequencing (NGS) examined 35 genes that: (i) cause osteosclerotic disorders, (ii) condition skeletal remodeling, or (iii) reflect mouse models featuring elevated bone mass: i.e., TNFRSF11A (RANK), TNFRSF11B (OPG), TNFRSF11 (RANKL), VCP, SQSTM1, TGFBI, IFITM5, MAFB, CSF1, CSF1R, TRAF6, RELA, RELB, REL, NFKB1, NFKB2, TFEB, CA2, CLCN7, CTSK (CATHESPIN K), OSTM1, PLEKHM1, TCIIRG1, SOST, SLC29A3, LRP4, LRPS, LRPS, SNX10, FAM20C, FAM123B (AMER1), TYROBP, LEMD3, DLX3, and PTSS1.[4,5] Variants were then verified by Sanger sequencing. For patient 4, LRRK1 was Sanger sequenced using primers we designed (sequences available on request). At least 10 bases of intronic DNA sequence at each exon/intron boundary were sequenced to identify potential messenger RNA (mRNA) splice site mutations. Patient 2 was studied at Marmara University School of Medicine where clinical exome sequencing was performed using SOPHIA Clinical Exome Solution V2 via Illumina Nextseq 550 platform (Illumina, San Diego, CA, USA). Twenty-seven genes: TNFRSF11A (RANK), TNFRSF11B (OPG), VCP, SQSTM1, TGFBI, IFITM5, MAFB, CSF1, CSF1R, TRAF6, NFKB2, CA2, CLCN7, CTSK (CATHESPIN K), OSTM1, TCIIRG1, SOST, SLC29A3, LRPS, LRPS, SNX10, FAM20C, FAM123B (AMER1), TYROBP, LEMD3, and DLX3 were filtered. SOPHIA DDM-V4 analyzed the data. For segregation analysis, we used the Illumina MiSeq platform.

Results

Mutational analyses

Patient 1

Sanger sequencing of SLC29A3 revealed a novel homozygous 18-basepair (bp) duplication in exon 3 (c.303_320dup, p.102_107dupYFESYL) predicting in-frame tandem duplication of six amino acid residues (Fig. S1). Her consanguineous parents and one of two healthy sisters were heterozygous for this mutation.
Patient 2

NGS of SLC29A3 revealed a novel homozygous nonsense mutation (c.1284C>G, p.Tyr428*) that was heterozygous in her consanguineous parents (Fig. S2).

Patient 3

All exons and adjacent mRNA splice sites of SLC29A3 were intact. In 2017, our NGS revealed a novel homozygous splice donor site variant (c.616+3A>G) in intron 6 of TNFRSF11A (Fig. S3). This variant was heterozygous in her mother; the father was not available for study. The following year, Guo and colleagues (2019) confirmed this mutation and proposed it caused DSS (see Discussion).

Patient 4

Our mutation analysis was negative for SLC29A3, TNFRSF11A, TCIRG1, LRRK1, and CSF1R and the OPT-associated genes CLCN7, TNFSF11, CA2, OSTM1, PLEKH1, and SNX10. This included sequencing all coding exons and at least 10 bases of intronic DNA at exon/intron junctions while recognizing that Guo and colleagues in 2019 had reported deep intronic mutations in CSF1R causing DSS, resulting in “abnormal inclusion of intron sequences in the mRNA.” Additional methodologies to identify this type of mutation (e.g., reverse transcription-polymerase chain reaction using patient-derived RNA) could not be undertaken for this patient.

DSS phenotype of our patients

Our four patients’ clinical, radiographic, and genetic findings indicating DSS (Table 1) and assessments of mineral and skeletal metabolism (Table S1) were then compared with published reports of DSS.

Literature evaluation

The genotype/phenotype spectrum of DSS was assessed from the literature using Medline, Embase, Scopus, Web of Science, bone abstracts and Mendeley and was then summarized (Table 2).
Discussion

From its first reporting in 1934 until evidence of genetic heterogeneity in 2013, DSS seemed a distinctive Mendelian OPT. In 2010, we described in a girl with classic features of DSS absence of OCs and unresorbed calcified primary spongiosa emblematic of OPT. In 2012, we reported that she and a similarly affected girl manifested impaired osteoclastogenesis and OC action and discovered they harbored biallelic SLC29A3 mutations. In 2015, The International Skeletal Dysplasia Registry regarded DSS as an OPT-like disorder. Although osteosclerosis and impaired bone modeling (ie, “undertubulation”) characterize the OPTs including DSS, the platyspondyly and spontaneous resolution of the osteosclerosis of DSS do not. Hence, DSS has a particularly complex and enigmatic pathogenesis. The changing skeletal phenotype can go undocumented or unappreciated and therefore undiagnosed. By 2022, among 23 individuals with mutational analysis from 45 with the DSS phenotype, six, four, two, and 11 had defects of SLC29A3, TNFRSF11A, TCIRG1, or CSF1R, respectively. The heterogeneity among the clinical and radiological presentations, complications, and prognoses for each etiology is as follows.

Our patients’ DSS

Patient 1, our oldest at 23 years old, provided almost a 20-year follow-up, whereas patient 2 is the youngest reported with DSS (Tables 1 and 2). Short stature was invariable whereas arm length was unaffected. Disproportionate dwarfism featuring a short trunk became a constant finding by adulthood despite recurrent femoral fracturing. The body proportions of patient 3 changed from age 10 to 17 years, highlighting her progressive trunk shortening. However, platyspondyly with a shortened trunk was not evident early on in our youngest patients 2 and 4. Patient 3’s fractures began relatively late; ie, in adolescence.

In DSS, discoloration and delayed eruption of teeth, dentition embedded into the gum, frequent caries, and mandibular osteomyelitis have been reported. Patients 1 and 2 had delayed shedding of deciduous teeth followed by delayed eruption of permanent teeth, or were too young for teething, respectively. Patient 3 suffered tooth decay. Patient 4 had discolored teeth. Only patient 3, homozygous for a TNFRSF11A mutation, had nystagmus due to optic atrophy from narrowed optic canals related to the osteosclerosis of her cranial bones.

Fig. 4. Patient 4. Radiographic findings in early childhood. (A) At age 2 years, the skull has slightly increased vertical diameter. (B) There is marked metadiaphyseal osteosclerosis and widening of the proximal humeri, marked osteosclerosis and some widening of the ribs, and osteosclerosis of the spine, clavicles, and scapulae. (C.1,C.2) Between ages 2-1/3 and 4-1/3 years, osteosclerosis has decreased in the metaphyses and carpals in the hands and wrists, and metadiaphyses in the radius and ulna. Metadiaphyseal widening has increased. (D.1,D.2,E.1,E.2) Osteosclerosis has decreased in the pelvis and lateral spine and metadiaphyses of the femurs and proximal tibias and fibulas. Metadiaphyseal expansion and cortical thinning are developing in the tubular bones.
Herein, we found metaphyseal osteosclerosis in early childhood. However, patient 2 demonstrated it might not be present neonatally, yet appear by age 2 months. For patient 1, followed to age 22 years, metaphyseal osteosclerosis extended as she grew, but mid-diaphyseal radiolucency persisted up to age 11 years. Then, patchy osteosclerosis with sparse radiolucency involved the diaphyses. At older ages, radiolucent areas predominated, especially after physeal fusion and cessation of growth. Axial osteosclerosis, including of the ribs, pelvis, and vertebral in patients 1, 3, and 4 was a more constant finding. However, in patient 1 it was decreased during adulthood. In patient 3, osteosclerosis increased steadily with better demarcated focal osteosclerosis, especially mid-diaphyseal, and fracture-prone dense bone within osteopenic long bones. Perhaps absence of apparent metaphyseal sclerosis together with unaffected hand and forearm bones indicates DSS due to TNFRSF11A mutation. Patient 4’s metaphyseal and marginal osteosclerosis faded by age 4 years, leaving severely osteopenic long bones. Resolution of osteosclerosis can occur in the carbonic anhydrase II deficiency form of OPT (OMIM type 3). Perhaps because its unique metabolic acidosis leaks mineral from bone. Patient 4 manifested unexplained marked hypercalcemia once, but was without a CA2 mutation or acidosis. Although no fractures had been detected, perhaps his hypercalcemia reflected his immobility. Hypercalcemia is not a feature of the OPTs unless following restoration of OC action by marrow cell transplantation. In our initial patient harboring SLC29A3 mutations, Serum ionized calcium was high-normal at approximately 1 year old, but decreased after excessive dietary calcium was corrected. In DSS, low serum PTH levels can occur, suggesting mineral homeostasis is impacted. Hypocalcemia from decreased bone resorption and diminished gastrointestinal absorption of calcium underlies “osteoporotics” from TCIRG1 deactivation.

Slow fracture healing can occur in DSS, as in other OPTs. Indeed, quiescent bone remodeling was suggested in our four patients by low serum OCN levels and low-normal ALP activity. Patients 1 and 2 had elevated urinary DPD, a marker of bone resorption, whereas serum CTX levels were normal. However, BTMs were inconsistent among our four patients, perhaps reflecting their genetic heterogeneity for DSS as well as evolving skeletal phenotype.

SLC29A3-associated DSS

The two unrelated girls we reported in 2012 with DSS were compound heterozygous (c.607T>C, p.Ser203Pro; c.1157G>A, p.Arg386Gln) and homozygous (c.1346C>G, p.Thr449Arg) for SLC29A3 mutation. The former had Turkish heritage. Their few OCs formed from peripheral blood weakly demineralized a crystalline calcium-phosphate surface. Low serum tartrate-resistant acid phosphatase (TRAP) matched a paucity of OCs. Therefore, OC formation and function in DSS can become impaired and increase bone mass, but then apparently recover sufficiently to resorb calcified primary spongiosa and osteosclerosis. Currently, however, OMIM does not mention our 2010 report of OC-poor OPT in DSS and considers the two patients examples of “H syndrome” or “histiocytosis-lymphadenopathy plus syndrome” (602782), which features biallelic SLC29A3 mutation causing short stature (but not fracturing), histiocytosis, and lymphadenopathy with or without cutaneous, cardiac, and/or endocrine features (insulin-dependent diabetes mellitus, hypogonadism), joint contractures, and/or deafness. Now, six individuals have been reported to have DSS from SLC29A3 mutation.

TNFRSF11A-associated DSS

Beginning in 2008, homozygous mutation of TNFRSF11A encoding RANK was reported to cause “OC-poor” OPT. About 20 examples are published. However, in 2018 Guo and colleagues specified that our patient 3 had DSS. Now, they attribute the DSS phenotype to four individuals with biallelic loss-of-function defects in TNFRSF11A. (11) Splice site mutations of TNFRSF11A, exemplified by our patient 3, that truncate or extend the encoded RANK protein can cause DSS, whereas “DSS” from TNFRSF11A missense mutation p.R129C featured diffuse osteosclerosis and extramedullary hematopoiesis consistent with a severe OPT. TNFRSF11A mutations that alter N-terminal folding of the encoded RANK and compromise its interaction with RANKL cause OPT, whereas some functional RANK seems to cause DSS. Nevertheless, the skeletal phenotype of the severe OPT, can also improve with aging. The eldest person (age 59 years) reported with biallelic TNFRSF11A mutations had severely osteopenic long bones.

TCIRG1-associated DSS

In 2018, in two siblings, compound heterozygosity of TCIRG1 (c.117+4A>C with c.2380_2381delCT, p.A796fs*34) reportedly caused metaphyseal sclerosis typical of DSS. (21) TCIRG1 encodes a component of the vacuolar proton (H+) pump, and biallelic mutations thereby cause “osteopetrocritics” from an abundance of nonfunctional OCs together with impaired gastrointestinal absorption of calcium due to hypochlorhydria. The OPT from biallelic TCIRG1 defects is “OC-rich” and treatable by hematopoietic stem cell transplantation that generates functional OCs. The TCIRG1 splice site mutation at the beginning of this paragraph (c.117+4A>C), is homologous to splice site mutation c.117+4A>T reported in 2000 to cause OPT in a Turkish patient. When this mutation accompanied p.A796fs*34 above, the phenotype was milder.

LRRK1-associated osteosclerotic metaphyseal dysplasia

The eight individuals reported to date with “osteosclerotic metaphyseal dysplasia” (OMIM % 615198) harbored homozygous defects of LRRK1. Their clinical and radiographic features of DSS resemble those attributable to SLC29A3 or TCIRG1 mutation, including fractures without severe extramedullary hematopoiesis or short stature. Radiological features include metaphyseal and vertebral sclerosis with mildly under-modeled long bones that may evolve, including diminishing metaphyseal sclerosis. However, there is no prominent platyspondyly.

CSF1R-associated DSS-Pyle disease spectrum

The “DSS-Pyle disease spectrum” features generalized osteosclerosis and irregular dysplastic metaphyses associated with neonatal and infant lethality from leukoencephalopathy, intracranial calcification, Dandy-Walker malformation, cystic dilation of the posterior fossa and ventricles, and agenesis of the corpus callosum. In 2017, in first-cousin carrier parents, homozygous mutation (p.Y540*) of CSF1R was detected. Biallelic CSF1R mutations were identified by Guo and colleagues and Kinds in 2019 and 2021, respectively. The 2019 report concerned a homozygous splice acceptor site
mutation (c.1754→G>C), published back-to-back by Guo and colleagues. However, these two reports describe an osteosclerotic phenotype atypical for DSS, in fact, biallelic CSF1R defects do not always have a bone phenotype; eg, the second case, harboring p.H643Q, reported in 2019 by Oosterhof and colleagues, and two siblings with the p.T833M mutation. Monoallelic CSF1R mutation underlies hereditary diffuse leukoencephalopathy with spheroids (HDLS)—a rapidly progressive lethal neurodegenerative disease of adults that features cerebral white matter, behavioral, cognitive, and motor changes as well as dementia. However, no bone changes were reported in 2021 by Guo and Ikegawa from a relatively large experience.

Typical HDLS has occurred with “sclerosing skeletal dysplasia,” with undertubulation and flaring of the metaphyses of most of the tubular long bones and fish-shaped vertebral arches. An osteosclerotic adolescent boy with HDLS reported in 2020 by Breeningstall and Asis was heterozygous for CSF1R. His radiographs, kindly provided to us by Dr. G.N. Breeningstall (unpublished), showed platyspondyly and osteopenic long bones with Erlenmeyer flask deformity and focal osteosclerosis.

His CSF1R defect was compound heterozygous in a patient with “brain abnormalities, neurodegeneration, and dysosteosclerosis” (BANDDOS; OMIM # 618476). The father, who was heterozygous, had mild cortical hyperostosis and normal cranial tomography, yet short-term memory loss from age 70 years, and parenchymal calcification were detected in the 76-year-old paternal grandfather, who was also heterozygous.

Our patient 4, lacking identification of a causal gene, uniquely suffered severe mental/motor retardation, infections, and one episode of severe unexplained hypercalcemia. In DSS, infections have been frequent without any detectable immune defect. Neurodevelopmental delay, macrocephaly, seizures, intracranial calcification, and delayed myelination are features of the DSS-Pyle disease spectrum. His cranial MRI showed a myelination defect, but no leukoencephaly or findings of Pyle disease. Microcephaly, not macrocephaly, was present.

DSS from SLC29A3 versus TNFRSF11A mutation

The DSS phenotype associated with SLC29A3 and TNFRSF11A mutations features short stature. However, fracturing sometimes with slow healing (eg, patient 1), begins earlier and seems more common with SLC29A3 mutations (eg, patients 1 and 2). Metaphyseal sclerosis characteristic of DSS was not observed at different ages in three of four patients harboring TNFRSF11A mutations. The exception differed from DSS because diffuse diaphyseal sclerosis ended with a small area of osteopenia and continued with the osteosclerotic metaphyses. TNFRSF11A-associated DSS features particularly severe osteosclerosis of the axial skeleton together with optic atrophy, craniosynostosis, and extramedullary hematopoiesis. Contrary to DSS from defective SLC29A3 and TCIRG1, mild TNFRSF11A mutations likely leave hand bones unaltered (eg, patient 3), whereas severely compromised RANK protein can cause marked osteosclerosis of the entire skeleton including hand bones.

Conclusions

Our experience improves understanding of the clinical, radiographic, and genetic heterogeneity of the DSS phenotype. The changing radiographic hallmarks can be important to suspect and diagnose DSS, now associated with mutations of SLC29A3, TNFRSF11A, TCIRG1, LRRK1, and CSF1R. Further genetic heterogeneity, including an X-linked recessive form seems likely. In clinical practice, establishing the genetic basis for DSS will help understand the complications, prognosis, and treatment.

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Author Contributions

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Conflicts of Interest

None.

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All data and material will be available upon request.

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