Expanding the phenotype of *SPARC*-related Osteogenesis Imperfecta: Clinical findings in two patients with pathogenic variants in *SPARC* and literature review

Short title: *SPARC*-related OI

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Abstract:

Background

Secreted protein, acidic, cysteine rich *(SPARC*-related Osteogenesis Imperfecta (OI), also referred to as OI type XVII was first described in 2015, since then there has been only one further report of this form of OI. *SPARC* is located on chromosome 5 between bands q31 and q33. The encoded protein is necessary for calcification of the collagen in bone, synthesis of extracellular matrix and the promotion of changes to cell shape.

Methods

We describe a further two patients with previously unreported homozygous *SPARC* variants with OI: one splice site; one nonsense pathogenic variant. We present detailed information on the clinical and radiological phenotype and correlate this with their genotype. There are only two previous reports by Mendozo-Londono *et al.,* 2015 and Hayat *et al.,* 2020 with clinical descriptions of patients with *SPARC* variants.

Results

From the data we have obtained, common clinical features in individuals with OI type XVII caused by *SPARC* variants include scoliosis (5/5), vertebral compression fractures (5/5), multiple long bone fractures (5/5) and delayed motor development (3/3). Interestingly, 2/4 patients also had abnormal brain magnetic resonance imaging (MRI), including high sub-cortical white matter changes, abnormal fluid attenuated inversion in the para-atrial white

matter and a large spinal canal from T10-L1. Of significance, both patients reported here presented with significant neuromuscular weakness prompting early work-up.

Conclusion

Common phenotypic expressions include delayed motor development with neuromuscular weakness, scoliosis and multiple fractures. The data presented here broadens the phenotypic spectrum establishing similar patterns of neuromuscular presentation with a presumed diagnosis of 'myopathy'.

Introduction:

Osteogenesis imperfecta (OI) is the most common heritable connective tissue disorder characterised by extreme phenotypic variability.[1] OI is characterised by bone fragility, fractures and low bone mass with clinical symptoms ranging from mild to severe with a variable prognosis. [2] Extra-skeletal features such as hearing loss, dentinogenesis imperfecta and muscle weakness may also be present. The introduction of bisphosphonates as a treatment is not a cure but helps increase bone mineral mass, alongside physiotherapy, orthopaedic treatment and rehabilitation to improve the quality of life for individuals with OI. [3] Over 85% of OI is attributed to a premature stop codon in *COL1A1* or glycine substitutions in *COL1A1* or *COL1A2*, the two genes that encode for type 1 collagen.[4] Several recessive variants in genes involved in production and/or processing of type 1 collagen have been associated with rarer forms of OI.[5]

In this manuscript, we discuss the *SPARC* gene which has been linked to OI type XVII, with Mendozo-Londono *et al.,* 2015, publishing the first paper identifying pathogenic variants in

SPARC in 2 affected individuals and Hayat *et al.,* 2020 recently describing two further affected individuals.[6,7]

SPARC gene is located on chromosome 5 between bands q31 and q33. The encoded protein – also called "osteonectin" - is necessary for calcification of the collagen in bone, synthesis of extracellular matrix and the promotion of changes to cell shape. It is found mainly in the extracellular compartment but has also been described as being localised both to the cell nucleus and the cytoplasm. SPARC has been observed in different cell types including active osteoblasts, bone marrow progenitor cells, odontoblasts, endothelial cells, fibroblasts, pericytes, astrocytes and macrophages.[8] Pathogenic variants within this gene have been implicated with several different phenotypes including OI type XVII and Ehlers Danlos syndromes.[6, 9]

In this study, we describe the clinical data of a further two patients with previously unreported *SPARC* variants with a focus on clinical manifestations of OI. We present information on the clinical phenotype and genotype of these two patients under the OI national service, building on the data for *SPARC*-related OI previously reported by Mendoza-Londono et al., 2015.[6]

Materials and methods:

Clinical Information and DNA extraction

Two patients cared for within the Highly Specialised OI service in our centres were included in this study. Clinical information was obtained from the patients' medical records and a

timeline established for their radiological phenotype. The patients and their parents provided informed consent.

DNA Extraction.

Total genomic DNA was isolated from 2 to 5 ml peripheral blood taken from patients and parents using standard extraction methods. DNA from whole blood samples was extracted using the QIAamp DNA Blood Midi kit (Qiagen, Venlo, The Netherlands).

DNA Sequencing

Next generation sequencing was performed using Osteogenesis Imperfecta (OI) autosomal dominant, autosomal recessive and X-linked panels containing:

NM_052854.3 *(CREB3L1);* NM_006371.4 (*CRTAP*); NM_001025295.1 (*IFITM5);* NM_022356.3 (*P3H1*); NM_000942.4 (*PPIB*); NM_021939.3 (*FKBP10*); NM_152860.1 (*SP7*); NM_002615.4 (*SERPINF1*); NM_001235.2 (*SERPINH1*), NM_182943.2 (*PLOD2*); NM_006129.4 and NM_001199.3 (*BMP1*); NM_ 018112.1 (*TMEM38B*); NM_005430.3 (*WNT1*), NM_000918.2 *(P4HB);* NM_005032.6 *(PLS3);* NM_014822.2 *(SEC24D);* NM_003118.3 *(SPARC);* NM_153365.2 *(TAPT1);* NM_022167.3 *(XYLT2).*

Library Preparation

The shearing of genomic DNA was performed using the Covaris E220 sonicator. End repair, A tailing and ligation of adaptors was performed using the SureSelectXT library system (Agilent Technologies). Target enrichment was performed by SureSelect target enrichment (Agilent Technologies) using custom in house designed probes. Sequencing was performed on the Illumina HiSeq using the HiSeq Rapid SBS Kit v2 performing 2 x108 base pair paired end reads.

Data Analysis

This was based on the open source 'Best Practices' workflow by the Broad Institute (for additional information see http://www.broadinstitute.org/gatk/guide/best-practices). Reads were mapped to the GRCH37/hg19 human reference sequence using BWA alignment. A minimum threshold of 30-fold read depth was set for exonic sequences and intronic sequences up to and including 5 bp from the ends of each exon. A minimum threshold of 18-fold read depth was set for intronic sequences from 6 bp to 25 bp from the ends of each exon. Variants were identified using HaplotypeCaller (Broad Institute). Variants were filtered against the in-house polymorphism list.

Variant Reporting

A standard panel with a standard 21 number of genes as described above was used providing uniform coverage of genes. The variants identified in the *SPARC* gene were compared to cDNA reference sequences NM_003118.3 and assessed using Alamut Visual version 2.11 QT v5.5.1 (Interactive Biosoftware, Rouen, France). All sequence variants are classified using practice guidelines for variant interpretation: ACMG/AMP and ACGS Best Practice Guidelines for variant classification 2020 (https://www.acgs.uk.com/quality/best-practice-guidelines/).[10] Confirmation of clinically significant sequence variants was performed by Sanger sequencing as necessary.

Clinical Presentation

Patient 1:

Patient one is a 19-year-old female born to non-consanguineous Slovakian parents with no significant family history. She is the second child, and her older sister is fit and well. The pregnancy was complicated by reduced foetal movements but antenatal scans were reportedly normal. The patient was born with upturned feet and no evident fractures or joint dislocations. She was induced 2 weeks post-term weighing 2.7 kilograms (9th centile) and was initially thought to have sustained a brachial plexus injury during delivery.

Given that early medical care was in Slovakia and parents have limited English, it was difficult to determine the early clinical course in this patient. She was thought to have hypotonia in the first year of life and started having fractures at around 1 year of age. She was advised bed rest by doctors in Slovakia and as a result had reduced mobility. Her development, especially motor milestones, was delayed although she was thought to have normal intellectual development early on and a presumed diagnosis of 'myopathy' was made. Her fracture history was initially attributed to her immobilisation and lack of calcium intake with advice to be on calcium supplementation.

By the age of 9 years, she had sustained about 20 long bone fractures in total, including spontaneous fractures of her femurs several times over, humerus and forearm fractures and right tibia and fibula and assuming a lying posture by default. She was treatment naïve and was subsequently seen by a specialist in Prague who diagnosed her with OI.

This patient's family relocated to UK from Slovakia when she was 8 years of age and she was referred to the Highly Specialised OI Service in view of her prior diagnosis. She was commenced on bisphosphonates; 10 years on treatment with 3-monthly regimen of pamidronate 1.5mg/kilogram on each of 2 successive days. She has never had any seizures

but has bilateral symmetrical high signal sub-cortical white matter changes noted on magnetic resonance (MR) brain imaging performed during an episode of abdominal sepsis for which no specific cause was identified.

This patient was first seen in the specialist metabolic clinic in our centre at 8 years of age and history noted. She had no learning difficulties, normal intelligence and was not thought to be dysmorphic. On examination, she was found to have blue sclerae, serrated teeth with mild translucency; flat occiput, marked pectus carinatum with marked long bone bowing. She has normal hearing. She was clinically diagnosed with a severe OI and commenced on treatment with pamidronate as described above. Imaging demonstrated multiple vertebral crush fractures; popcorn calcification of her long bone epiphyses, gracile, osteopenic and bowed long bones; an S-shaped scoliosis with a thoracolumbar Cobb angle of 60 degrees, a thoracic kyphosis and turribrachycephaly (Figure 1a and b).

Bone mineral density measurements using dual-energy X-ray absorptiometry (DXA) scanning showed L2-L4 bone mineral density (BMD) Z score of -2.6 and total body less head (TBLH) BMD Z score of -3.4; her lateral spine radiograph showed 11 vertebral bodies with loss of height >25% and her lateral skull radiograph showed her odontoid peg to be 1.5 mm below McGregor's line with a basilar angle of 132 degrees at 12 years of age. Her scoliosis has continued to progress and at recent review at 18.5 years of age, it was noted that her spine curvature (i.e. Cobb angle) was 90 degrees and unlikely to be amenable to surgical correction. Her growth continued to remain below the 0.4[th] centile with short stature, complicated by the significant scoliosis.

This patient had been transitioned to the adult metabolic bone services with a plan to continue monitoring her scoliosis and treatment with pamidronate 1.5 mg/ kilogram in one day infusion dosing. Her last fracture was three years earlier and her last dose of bisphosphonates was at 19 years of age as she had completed growth and there were no recent fractures. Her BMD has been difficult to monitor currently due to difficult positioning making data obtained unreliable. She was seen in the Genetics clinic at 18.5 years when she was referred in view of need to establish a genetic diagnosis to provide recurrence risk information. Genetic testing was undertaken through targeted whole exome sequencing with application of extended OI panel testing as described above, which identified homozygous nonsense variants in *SPARC*.

Patient 2

Patient two is an 11 year-old male born to consanguineous, first cousin parents from Kurdistan with no significant family history. He is the second child of this union, his older 16-year-old brother being entirely well. He was born at 38 weeks gestation by elective lower segment Caesarean section with a birthweight of 3.25 kg (>25th centile). Antenatal scans were normal with normal liquor volume, although mother reported reduced foetal movements. At birth, no resuscitation was required but he was noted to be floppy and was admitted to the special care baby unit because of poor feeding requiring nasogastric tube feeding. By discharge, he was taking bottle and breast feeds.

Aged 3 months, he was seen by the Neuromuscular team because of continuing hypotonia (axial more than peripheral) and muscle weakness (affecting trunk and legs more than arms with sub-gravity power, apart from left upper limb); he was not dysmorphic; there were normal eye movements; no facial weakness; no limb contractures; mild scoliosis; normal

chest wall shape and breathing pattern; preserved deep tendon reflexes with brisk knee jerks. Electromyography (EMG) showed myopathic changes with similar findings on a repeat study 3 years later.

Several investigations were performed to look for an underlying neuromuscular disorder, including metabolic work-up, muscle and skin biopsies. MRI-brain imaging was normal. He had a normal creatinine kinase; muscle biopsy showed prominent linear mitochondria, excess lipid staining and mild variation in fibre size. Muscle respiratory chain enzymology showed borderline-low complex IV activity (cytochrome oxidase 0.012, reference range 0.014-0.034) but normal levels of cytochrome oxidase activity on skin fibroblast culture.

Aged 8 months, the patient presented with an acute spiral fracture of the right proximal femoral diaphysis (Figure 2b) whilst sitting on his mother's lap, and her undertaking some gentle physiotherapy of his legs. Subsequent skeletal imaging confirmed an osteopenic skeleton with multilevel vertebral compression fractures, involving essentially all vertebrae by 2 years of age; a thin calvarium with a large fontanelle and a few Wormian bones, not considered an abnormal pattern but overall skeletal features that could represent a form of osteogenesis imperfecta (OI).

Given the multilevel vertebral compression fractures, osteopenia and pathological fracture, he was seen in the metabolic bone clinic and commenced on pamidronate therapy at the age of 1 year, 7 months, administered via central venous port in a dose regimen of 0.5 mg/kg per dose per 3 days every 6 weeks; subsequent modifications to dose regimen were made between 2 and 3 years of age and from 3 years of age, dose regimen of pamidronate continued as 1

mg/kg per dose per 3 days every 3 months with good response. Currently, the patient continues on bisphosphonate therapy as 6-monthly zoledronic acid infusions.

The patient was referred to the Highly Specialised Osteogenesis Imperfecta Service at aged 2 years, 2 months by which time he had sustained 3 long bone fractures following minimal trauma. Other notable supportive clinical features for OI included pale grey-blue sclerae (different from parents); marked distal joint laxity with soft skin; tendency to be warm and sweaty, including at night-time; longstanding history of constipation; and small stature. Hearing was normal. Cognitive development appeared normal. He had short stature with height less than 0.1 percentile, complicated by the significant scoliosis and his weight was tracking along the second centile.

There was no dentinogenesis imperfecta, confirmed by the specialist dental team. However, the patient had caries in his primary teeth, requiring preformed metal crowns on his primary molars under general anaesthesia at 2 years of age, and further extraction of primary teeth under local anaesthesia. At his last review at age 10, it was noted the patient had delayed dental development, and several dental anomalies were noted; infraocclusion of the upper second primary molars, taurodontism of the upper first permanent molars, failure of eruption of the lower right second primary molar, hypomineralisation of the upper right central permanent incisor and severe crowding.

At 2 years of age, he was making some progress with his muscle strength and function - he was attempting to roll from supine to prone and to sit unsupported for brief periods, otherwise needing full support. Clinically, there was slim muscle bulk throughout with relative wasting of deltoids bilaterally and thinness of lower legs; there were antigravity

movements across all joints and muscles but not through all the available range; there was palpable mild lateral bowing of the left femur; positional calcaneo-valgus of feet; marked kyphoscoliosis of the spine with associated rotation and right upper thoracic posterior rib hump, not fully correctable; asymmetry of anterior chest wall with prominent pectus carinatum; increased work-of-breathing at rest with tachypnoea, expiratory grunt and mild subcostal recession; and resting tachycardia, otherwise normal cardiovascular system.

Nutrition and growth were sub-optimal, requiring a period of gastrostomy overnight feeding until aged 5 years when the gastrostomy was removed and subsequently oral nutrition has been maintained. Bilateral orchidopexies for undescended testes and dysplastic scrotum were undertaken with circumcision at 4 years of age.

Sleep studies have shown mild sleep-disordered breathing, not requiring intervention with any additional respiratory support and over time respiratory function has improved. The patient has had multiple long bone fractures over time with a propensity to femur and arm fractures with residual bony deformities in upper limbs. Orthopaedic management has included bilateral Fassier-Duval intramedullary rodding of both femurs. No evidence of basilar invagination or other skull base deformity has been shown on interval lateral foramen magnum-centred skull radiographs, or MR brain and spine imaging throughout his disease course.

The early onset and rapidly progressive right convexity thoracic kyphoscoliosis (Figure 2a) progression of Cobb angle from 47 degrees aged 14 months to 84 degrees aged 4 years was a major part of this patient's phenotype with complexities around decision-making for management of his spine. The Cobb angle (prior to spinal surgery) = right convex thoracic,

Cobb angle = 90 degrees; Left convex lumbar Cobb angle = 84 degrees was noted in this patient.

A bracing programme was initially instituted but only tolerated for short periods of time due to respiratory compromise. There were multidisciplinary team discussions both in-house and nationally as regards management of his scoliosis and the optimal timing and nature of surgical intervention. He was considered not to be a candidate for growth modulation surgery and at aged 9 years, underwent instrumented posterior spinal fusion and costoplasty. Recovery was slow but since surgical correction, his scoliosis has remained stable and metalwork intact. Figures 2a and b summarise radiological findings in patient 2.

Bone mineral density (BMD) measurements are reported here consistently as L2-4 BMD. A dual-energy X-ray absorptiometry (DXA) scan aged 2 years showed ~~L2-L4 bone mineral density~~ a BMD of 0.301g/cm^2 (no normative values available for children under 5 years of age). Repeat DXA measurements post-bisphosphonate treatment at 3 years of age (12-months since commencing treatment) showed a ~~L2-L4~~ BMD improvement of 0.628 g/cm^2. Aged 5 years, DXA showed BMD Z-score of -2.5 ~~L2-L4~~ with consistent improvement to a BMD Z-score of ~~L2-L4~~ -0.6 aged 6 years with a spinal BMAD of -0.5, however, this was difficult to measure due to the severity of the scoliosis and spinal surgery.

Despite the predominant neuromuscular phenotypic presentation with hypotonia and significant muscle weakness, there has been an overall course of gradual improvement. At peak functional mobility prior to spinal surgery, the patient was able to walk with the assistance of a Kaye walker and ankle-foot-orthoses (AFOs); mobilise on the floor through bottom shuffling and maintain independent sitting. Following spinal surgery, due to

increasing tendo-achilles contractures, he has been unable to undertake assisted standing or walking but this is the goal for his mobility.

Initial differential diagnosis based on his phenotypic expression included mitochondrial/ lipid storage myopathies, structural and other congenital myopathies. It was only with an increasing fracture history and the move away from a possible metabolic/ mitochondrial disorder, that a diagnosis of OI was considered and further investigations undertaken to exclude this as a unifying diagnosis. Genetic testing was undertaken through targeted whole exome sequencing with application of extended OI panel testing as described above which identified homozygous splice site variants in *SPARC*.

Molecular Genetic Testing

Patient 1

Patient 1 was found to have homozygous *SPARC* c.145C>T,p.Gln49* variant. Testing for a panel of genes associated with autosomal dominant OI was negative; subsequent analyses of sequence data for an extended autosomal recessive panel identified that this patient was homozygous for a pathogenic c.145C>T variant in exon 4 of the *SPARC* gene. This likely pathogenic variant is predicted to replace the glutamine at position 49 with a premature termination codon. c.145C>T would result in a premature stop at codon 49 (exon 4). This result in a significantly shortened product which would be expected to undergo nonsense mediated decay, but this has not been demonstrated experimentally. Loss-of-function is thought to be a mechanism of disease for *SPARC*, based on the literature described above and an LOF Z-Score = 3.24, however, there are no other truncating mutations described in

HGMD, ClinVar or LOVD. We would speculate that the c.145C>T pathogenic variant may be a founder mutation, assuming each parent is a carrier and given that they are from same restricted geographic region and may share common ancestry without being consanguineous, however, further study of the population would be needed to confirm this.

Patient 2

Patient two was found to have a pathogenic homozygous *SPARC* c.57+1G>T splice site variant in intron 2. His parents were confirmed carriers for the *SPARC* variant. c.57+1G>T is predicted to result in the loss of the native donor splice site, which is predicted to result in the skipping of exon 2. Exon 2 is the first coding exon of the gene, containing the translation initiation codon. Therefore, skipping of this exon would be expected to result in an entirely untranslated RNA, or if an alternative initiation codon is used, a significantly foreshortened protein product that would be expected to undergo nonsense mediated decay. Skipping of exon 2 would be in frame, the next ATG is not until p.92 in exon 5.

An alternative change at this base, G>C, is also predicted to result in the loss of the native donor site. It has been described previously.[11,12] Maddirevula *et al*., 2020 described RNA studies in Mendelian disorders, this *SPARC* G>C variant is described in this excel document (https://static-content.springer.com/esm/art%3A10.1186%2Fs13059-020-02053-9/MediaObjects/13059_2020_2053_MOESM2_ESM.xlsx), line 228.[12] In this instance, the authors performed RTPCR using cDNA-specific primers and RNA from blood (lymphoblastoid cell lines). The result of this RTPCR was exon skipping – the authors here provide the r. nomenclature as r.1_57del, and the p. as p.?. Although there are no similar studies on a G>T change at this base, it is likely to have a similar impact to the G>C change.

Table-1 below shows the *SPARC* variant interpretation for Patients 1 and 2.

	Patient 1	Patient 2
c.DNA change/protein change	c.145C>T, p.(Gln49*)	c.57+1G>T
Zygosity	Homozygous	Homozygous
Inheritance	Biparental	Biparental
In silico analysis summary[a]	Nonsense mutation, premature termination introduced in exon 4 (out of 10 exons)	Loss of exon 2 donor splice site predicted
Frequency	Not seen in GnomAD, ExAC or ESP normal populations	Not seen in GnomAD, ExAC or ESP normal populations
Segregation analysis		
Supporting Literature/Database records	Abstract from Canada (likely relatives)	c.57G>C previously reported as pathogenic and shown to result in exon skipping.[11,12]
Patient's phenotype	Phenotype consistent with a *SPARC* pathogenic variant	Phenotype consistent with a *SPARC* pathogenic variant
Variant Classification	PVS1_Very Strong, PM2, PM3_supporting, PP4_supporting =Pathogenic	PVS1_Very Strong, PM2, PM3_supporting, PP4_supporting =Pathogenic

Discussion:

SPARC encodes for SPARC (Secreted Protein Acidic and Cysteine Rich) and is a matricellular glycoprotein that has been evolutionary conserved and is involved in a large number of biological processes. These include tissue remodelling, wound repair, morphogenesis, cell differentiation, proliferation, migration and angiogenesis.[13] SPARC has been further shown to regulate the activity of matrix metalloproteinases which are a family of enzymes that are able to break down proteins such as collagen.

Osteogenesis imperfecta is most commonly associated with pathogenic variants in *COL1A1* and *COL1A2* which are responsible for the production of collagen type I alpha 1 and 2

chains. Commonly OI presents with a skeletal phenotype on a spectrum from low bone mass to increased incidence of fractures. Extra-skeletal phenotypes include abnormal tooth development, joint hypermobility, blue sclerae and lung abnormality.[14]

Osteogenesis imperfecta has been linked to *SPARC* and referred to as OI type XVII.[6,7] Previous studies have found that those with osteogenesis imperfecta have a lower expression of SPARC in osteoblasts.[15] *SPARC* mutations have also been linked to osteoporosis with mice studies showing that SPARC-null mice had a lower number of osteoblasts and osteoclasts therefore, showing a decreased bone turnover and presenting with an osteoporosis phenotype affecting the trabecular bone in particular.[16]] Bisphosphonate therapy in Patient 1 was stopped at 19 years of age as her last fracture was 3 years prior, whilst Patient 2 remains on bisphosphonate therapy. Hayat *et al*., 2020 and Mendoza-Londono *et al*., 2015 did not specifically address the response to bisphosphonate therapy of their patients. It is likely that bisphosphonates are effective in *SPARC*-related OI through inhibiting osteoclast-mediated bone resorption on the endosteal surface of bone by binding to hydroxyapatite. As a result, unopposed osteoblastic new bone formation on the periosteal surface results in an increase in cortical thickness, following the same principles of response in OI caused by type 1 collagen defects. Further research to delineate the specific response to bisphosphonates in *SPARC*-related OI is required to ascertain if this could be more specific to the genotype.

There are only 4 pathogenic *SPARC* variants reported on HGMD (https://my.qiagendigitalinsights.com/bbp/view/hgmd/pro/all.php). However, cellular studies do support a role for SPARC in the development of osteoporosis. The SPARC protein is expressed in cells synthesising extracellular matrix, such as osteoblasts in bone.[17] SPARC has been demonstrated to bind to collagen type I.[18] SPARC-null mice develop progressive

osteoporosis, due to a defect in bone formation.[16] SPARC synthesis is low in primary osteoblasts from children with OI and SPARC content is low in OI bone.[19] The homozygous nonsense pathogenic variant in Patient 1 is absent from control populations and has not previously been reported in association with disease. Although, there are no nonsense *SPARC* variants reported, missense variants have been reported before.[6] In this report, two individuals homozygous for missense variants in *SPARC* had scoliosis, kyphosis, vertebral compression fractures and femoral bowing. These missense variants were shown to affect functioning by abolishing the affinity of *SPARC* to collagen I. Therefore, the variants that are described here i.e., the splice site and nonsense variants are an addition to the missense variants that have been previously reported. More recently Hayat *et al*., 2020 has reported the same pathogenic variant as Mendonzo-Londono *et al*., 2015 with an autosomal recessive, homozygous c.497G>A,p.Arg166His.[6,7]

Both patients reported here had an initial diagnosis of myopathy due to significant hypotonia and muscle weakness with EMG studies being performed for patient 2. Both patients have normal cognitive development but delayed motor development milestones. As the age of the patients increased so did the number of fractures and both patients suffered vertebral compression fractures and severe scoliosis. The two patients described by Mendozo-Londono *et al.,* 2015 also presented with vertebral fractures and scoliosis with spinal fusion surgery performed at 6.7 years in one patient.[6] Patient 2 in this paper also underwent spinal fusion surgery at 9 years of age. Further the muscle mass of both patients was reduced as was seen in the patients reported in this paper showing that this may be a frequent phenotypic expression in those with OI due to *SPARC* variants.

Mendozo-Londono *et al*., 2015 had suggested an overlap with type VI which is caused due to loss-of-function in *SERPINF1*.[6] This was due to the absence of skeletal deformities at birth but subsequent clinical course with severe bone fragility. However, the lack of similar histological appearance to type VI OI and normal serum pigment-epithelium derived factor (PEDF) suggests that *SPARC*-related OI is distinct from type VI OI caused due to pathogenic variants in *SERPINF1*. Of interest, Patient 2 was found to carry a c.242C>G variant in exon 3 of *SERPINF1* variant of uncertain significance (VUS) and no second variant was identified; this along with confirmation of type XVII OI due to *SPARC* variants in him meant this VUS was not pursued further.

Mendoza-Londono *et al.,* 2015 and Hayat *et al.,* 2020 in total reported four individuals with recessive osteogenesis imperfecta caused by missense pathogenic variants in *SPARC*.[6,7] Table 2 summarises the reported clinical features, in the Hayat *et al*., 2020 study Family C, one patient died at the age of 22 months (IV-4) and further clinical features were not reported; however, individual IV-2 had some clinical phenotype mentioned which has been expanded in Table 2. [7] All variants reported were homozygous. Consistent clinical features in this cohort in comparison to reported patients include, delayed motor development showing an increase in likelihood to present with motor difficulties as opposed to cognitive difficulties. Neuromuscular weakness with significant generalised hypotonia seems to be an initial presentation in the entire cohort which along with a combination of low-trauma fractures and early-onset scoliosis should alert clinicians to the possibility of a *SPARC*-related OI. Interestingly, out of the four patients with MR brain imaging, 2/4 were abnormal, both abnormalities were within the white matter of the brain, including white matter changes, abnormal fluid signals in the para-atrial white matter and large spinal canal.

A study by Forlino and Marini (2016) found that skull base abnormalities such as platybasia, basilar invagination are found in patients that have a Z score of less than -3 indicating the more severe the disease, the increase in likelihood of cranial symptoms.[20] Basilar invagination is particularly serious as it can cause compression of the brain stem which we did not find in this study.[20] None of our patients presented with seizures but Mendoza-Londono *et al.* (2015) reported one patient who presented with right-sided focal seizure due to an intra-ventricular haemorrhage.[6] The average age of the first fracture was 29 months for all four patients with a range in months from 12-60. All patients presented with scoliosis and vertebral fractures showing a link between a *SPARC* variant and a pathological neuromuscular presentation.

Table 2: Summary of individuals reported with pathogenic *SPARC* variants

	Individual 1 (this paper)	Individual 2 (this paper)	Individual 3[6]	Individual 4[6]	Individual 5[7]
Family history	Nil	Nil	Nil	Nil	Nil
Ethnicity	Slovakian	Kurdish	North African	Indian	Pakistani
Consanguinity	No	Yes	No	Yes	Yes
Pregnancy	Normal	Normal	Normal	Normal	Normal
Gestation	Term +2 weeks	38 weeks	37 weeks	34 weeks	NA
Birth weight	2700g	3250g	3000g	2000g	NA
Age at first presentation	Birth	Birth	Birth	10 weeks	Birth
Age at first fracture	<12 months	8 months	15 months	5 years	<1 month
Treatment with BP	9 years	19 months	30 months	6 years	
Genotype	c.145C>T,	c.57+1G>T	c.497G>A	c.787G>A	c.497G>A
Protein Change	p.Gln49*		p.Arg166His	p.Glu263Lys	p.Arg166His
Hetero/homozygous	Homozygous	Homozygous	Homozygous	Homozygous	Homozygous
Cognitive development	Normal	Normal	Speech delay	No	Data unavailable
Motor development	Delayed	Delayed[+]	N/A	Delayed	Data unavailable
Motor skills	Walking short distances; wheel chair use for longer distance	Peak–assisted therapeutic standing and walking with Kaye-walker and AFOs	No independent walking; wheelchair for mobility	Walking independently at 3 years	NR
MR brain abnormality	Bilateral symmetrical high signal sub-cortical white matter changes	No	No	Non-specific abnormal fluid-attenuated inversion recovery signal in the para-atrial white matter, a large spinal canal	N/A

				with syrinx from T10 to L1	
Facial features	Not dysmorphic	Not dysmorphic	Strabismus	Normal	Normal
Seizures	No	No	Right-sided focal seizure due to an intra-ventricular haemorrhage	No	No
Sclerae	Blue	Grey-blue	White	Slightly grey	White
Teeth	Serrated teeth with mild translucency	Normal; no DI	Normal	Normal	Normal
Hearing	Normal	Normal	Normal	Normal	Normal
Vision	Normal	Normal	Strabismus	Normal	Data unavailable
Scoliosis	+++	+++	++	+	++
Vertebral compression fractures	Yes	Yes	Yes	Yes	Yes
Muscle weakness	++	+++	++	++	+
Age-matched DXA Z-score (pre-bisphosphonates)	-5.2 (aged 8 years and 3 months)	0.301g/cm^2 (aged 2 years, no Z-score available)	N/A	-4.0	N/A
Age-matched DXA Z-score (post-bisphosphonates)	-3.6 (aged 17 years and 2 months)	-0.8 (maximum)	-3.1	N/A	N/A
Spinal fusion	NDB	9 years	6.7 years	NR	NR

NR: Not reported; NDB: Not deemed to be beneficial; N/A: Not available

Conclusion:

In summary, we present two individuals with *SPARC*-related OI caused due to nonsense and splice site novel variants. This is a rare form of OI with very little published literature. Common phenotypic expressions include delayed motor development with neuromuscular weakness, scoliosis and multiple fractures with increasing need for active management of scoliosis given the rapid nature of progression of the spinal curve. The data presented here broadens the phenotypic spectrum establishing similar patterns of neuromuscular presentation with a presumed diagnosis of 'myopathy'. It is important for clinicians to be aware of this as a differential diagnosis for neuromuscular weakness when presenting in the neonatal period/ early infancy, especially in the context of long bone fractures following minimal trauma and scoliosis. Further expansion of the clinical phenotype and extended natural history data collection for *SPARC*-related OI will allow more informed decision-making regarding timing of surgical intervention and medical management in this specific form of OI.

Figure Legends:

Figure 1a: Patient 1 aged 8 years. Anteroposterior chest radiograph (a), anteroposterior thoracolumbar spine radiograph aged 11 years (b) and coronal reformat of CT chest and abdomen aged 15 years, demonstrating progressive left convexity thoracolumbar scoliosis

Figure 1b: Patient 1 aged 8 years. Anteroposterior radiographs of left humerus (a), left femur (b) and left tibia and fibula (c) and lateral base of skull radiograph (d) showing extremely gracile and osteopenic bones with bowing, healing fractures and popcorn epiphyses of the

knee. There is turribrachycephaly and platybasia, but there are no Wormian bones. Note the lower limb muscle wasting (b) and (c).

Figure 2a: Patient 2 Anteroposterior thoracic and lumbar spine radiographs aged 20 weeks (a), 3 years (b) 5 years (c), demonstrating early onset and progressive right convexity thoracic scoliosis (d) AP spinal radiograph aged 9 years, 3 months following posterior instrumented fusion.

Figure 2b: (a) Pelvic radiograph in patient 2 at aged 35 weeks. There is generalised osteopenia, with an acute spiral fracture of the right proximal femoral diaphysis (black arrow). (b) AP skull radiograph at aged 42 weeks. A small number of Wormian bones are visible in the lambdoid sutures. This appearance is within normal limits. The cranial vault is thin. Coronal (c) and sagittal (d) reformats of thoracic CT performed at aged 19 months shows widespread vertebral body collapse due to insufficiency fractures. (e) Radiograph of right humerus at aged 5 years demonstrates and acute transverse fracture of the right humeral diaphysis. The radial head is laterally dislocated (white arrow).

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