Congenital Joint Contractures and Pterygia with Multiple Fractures: A Novel Mutation in the *PLOD2* Gene

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Abstract

Arthrogryposis multiplex congenita (AMC) or arthrogryposis describes congenital joint contracture in two or more joints. The combination of fractures and pterygia with arthrogryposis is scantly reported in literature. Here we describe a case with osteogenesis imperfecta (OI)- like bone fragility in association with congenital contractures and pterygia with normal intellect. A novel mutation in the *PLOD2* gene causing the above phenotype was identified in the proband. Such cases are frequently mislabelled as OI. The aim is to alert clinicians to look for this rare syndrome in patients with an OI-like phenotype. The differentials and most recent management and therapeutic options are also discussed.

Introduction

Bruck syndrome is an autosomal recessive disorder consisting of increased bone fragility, congenital joint contractures and pterygia (Breslau-Siderius et al.,1998). It is classified according to the causative gene into types 1 and 2. Mutations in the *FKBP10* gene which are localised to chromosome 17q21, have been identified to cause Bruck syndrome type 1 [MIM#259450] and this is more common. Type 2 Bruck syndrome [MIM# 609220] is caused by mutations in the *PLOD2* gene on chromosome 3q24. Twenty seven patients with mutations in this gene causing Bruck syndrome type 2 have been described worldwide (Ha-Vinh et al., 2004). We present an additional patient with this syndrome and a novel mutation in the *PLOD2* gene. The patient has frequent fractures, congenital joint contractures, kyphoscoliosis, pterygia and pectus carinatum. The clinical and genetic features of all the previously reported cases are also reviewed.

Case Summary

The proband is an eight-year-old boy, second born to non-consanguineously married Indian parents. He was born at term by normal vaginal delivery at home after an uneventful antenatal period. Although the birth weight is not known, as per given history he was of average weight. He cried immediately after birth. Contracture of both knees and elbows were identified at birth. He had poor sucking and was never able to breastfeed. No facial dysmorphism was reported at birth. In view of normal weight gain the child did not come to medical attention till two years of age. He had the first fracture one week after birth in the humerus, and the second fracture was at two years of age and these were treated conservatively. The third fracture was at three years of age involving the ribs. His motor development was delayed and he started walking at 2 years of age. At five years of age, he sustained multiple fractures in bilateral femurs, clavicles and humeri. All these fractures occurred with trivial trauma. He also had progressive contractures involving both elbows, knees and ankle joints. He became non-ambulatory at the age of five years due to the extensive contractures and fractures. There was no history of seizures or any other prolonged illness. The family history was significant, with history of death of a similarly affected sibling at the age of 3 days due to severe pneumonia also had contractures at birth.

Examination of the proband at eight years of age revealed an alert child, responsive to surroundings. The head circumference was 49 cms (50th centile) and weight was 9.9 kg (<3rd centile). Due to severe contractures the length could not be mea-

Figure 1 A) Showing child affected with Bruck syndrome with multiple flexion contractures involving both upper and lower limbs B) Showing overcrowded malformed teeth C) Elbow with pterygium and contractures D) Radiograph of the thorax (anteroposterior view) showing severe kyphoscoliotic deformity of the dorsolumbar spine and vertebra plana at L3 E) Radiograph of the skull showing Wormian bones with a small sella and protruding mandible F) Radiograph of the left lower limb showing flexion contracture of the knee and the ankle joint.

sured. Contractures were present in all four limbs, bilateral knee joints, elbow joints, wrist joints and fingers (Figure 1A). There was a flexion deformity at the right hip joint. Pterygia were present at both elbow and knee joints (Figure 1C). He was not ambulatory in view of these deformities. There was no facial dysmorphism. He had white sclera, overcrowded malformed teeth (Figure 1B), normal hearing, pectus carinatum, severe kyphoscoliosis, mild bilateral clubfoot, and overriding of second and third digits of the right foot. His intellect was normal. There was no organomegaly and other systemic examination was normal. The radiological evaluation showed evidence of old fractures in bones of lower and upper limbs. Wormian bones were present and a small sella turcica was seen (Figure 1E). The mandible was prominent. Both lower limbs were flexed at the knee joints with very thin diaphysis and osteoporosis (Figure 1F). Both upper limbs also had flexion deformities, diffuse osteopenia and thin bones. Pterygia were present at all major joints. There was severe kyphoscoliotic deformity of the dorsolumbar spine and vertebra plana of L3 (Figure 1D). Serum calcium (10.2 mg/dl) and phosphorus (4.8 mg/dl) were normal and alkaline phosphatase (320 IU/ml) was mildly elevated. A phenotype of arthrogryposis with pterygia and fractures was consistent with a clinical diagnosis of Bruck syndrome. Other differential diagnoses considered for this child were osteogenesis imperfecta (OI), multiple pterygium syndrome and arthrogryposis multiplex congenita. Molecular analysis by next generation sequencing to confirm the clinical diagnosis was performed for the proband after informed consent. A novel, missense, homozygous variant in the *PLOD2* gene was identified: NM_182943.2: c.797 G>T, p.Gly266Val. This variant has not been described in the general population databases (gnomAD and 1000 Genome databases). In silico tools (AlignGVGD, SIFT, MutationTaster, PolyPhen2) predicted it to be deleterious. The variant is conserved across species and was confirmed to be present in heterozygous form in both the mother and the father by Sanger sequencing (Figures 2A, 2B, 2C). The variant is likely pathogenic according to the American College of Medical Genetics and Genomics (ACMG) criteria.

Discussion

We describe a patient with a rare autosomal recessive disorder, Bruck syndrome that has few cases reported worldwide. Our patient had the characteristic phenotype of multiple joint contractures, recurrent fragility fractures, pterygia and progressive scoliosis. Bruck syndrome type 1

with mutations in *FKBP10* gene and type 2 due to mutations in *PLOD2* gene (Van der et al., 2003; Ha-Vin et al., 2004) cannot be distinguished on clinical and radiological features and testing for both genes, as performed in this case, is required. In these patients, hydroxylation of lysine residues in the telopeptides of skeletal type I collagen is reduced, whereas that in the triple helix of collagen I is normal. (Bank et al.,1999 and Schwarze et al., 2013). *PLOD2* gene codes for lysyl hydroxylase 2 (LH2), which is the enzyme responsible for hydroxylation of type-I collagen telopeptide lysine residues. This is a key step in collagen biosynthesis as telopeptide hydroxylysines are the precursors of a series of biochemical reactions, known as the hydroxyallysine route, which finally form intermolecular lysylpyridinoline and hydroxylysylpyridinoline cross-links within collagen fibrils. Collagen crosslinks provide stability and tensile properties to collagen fibrils. In keeping with this, BS patients have reduced levels of hydroxyallysine-derived cross-links in type-I collagen from bone (Bank et al.,1999).

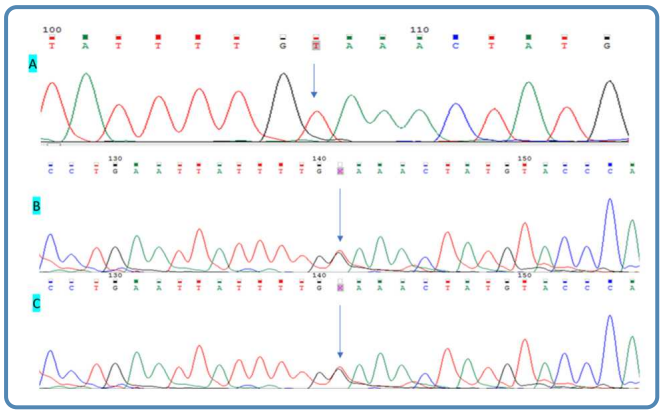

Figure 2 A) Chromatograph of *PLOD2* gene in the index child shows homozygous mutation present in the child having Bruck syndrome. The arrow head indicates the position of the mutation. B) and C) The chromatograph shows heterozygous mutation in both parents in the *PLOD2* gene. The arrow head indicates the position of the mutation.

Table 1 summarizes the characteristics of 29 patients from 16 families reported to have pathogenic variants in *PLOD2* and we compare the findings with those seen in our patient. All patients presented with fractures at birth or in early childhood and had normal intellect. None of the these have independent ambulation after the age of 7 years. Kyphoscoliosis was found in 18/27 (68%) patients. with onset childhood adolescence and rapid progression. Wormian bones were observed in 92%. Almost all patients with *PLOD2* gene mutations had low bone mineral density (BMD), although kyphoscoliosis and proximal femur deformity made it difficult to measure BMD accurately.

In our patient the phenotype was more severe compared to previously reported cases. Our patient had more number of fractures and significant joint contractures affecting all major joints, with onset of kyphoscoliosis since birth. He also exhibited camptodactyly and loss of ambulation at the age of five years. As the novel mutation identified in our patient in exon 8 is away from the reported hotspot region on exon 17 (Puig-Hervas et al., 2012), it could be inferred that mutations away from the N terminal domain may be associated with a more severe phenotype. However, this needs further validation.

Bisphosphonates have been widely used to treat bone fragility in children with OI (Trejo et al., 2016). Only eleven patients with *PLOD2* mutations received treatment with bisphosphonates (Ha-Vinh et al., 2004; Bank et al.,1999). Zoledronic acid was shown to reduce fracture incidence and increase the BMD with good tolerance. In a study by Lv et al. (2017) the children received a relatively large dose of zoledronic acid infusion and a significant increase in BMD after 6 months of treatment was noted. This indicated that zoledronic acid could increase BMD through effectively inhibiting bone resorption, though further studies are required to validate this. Release of contractures has been tried to improve ambulation, but with little benefit (Leal et al., 2018). Bisphosphonate therapy was started for this child and he is on follow up.

In summary, we report the first case of Bruck syndrome in India confirmed by molecular studies. This case report aims to alert clinicians to consider Bruck syndrome in presence of recurrent fractures with contractures and other abnormalities as a close differential diagnosis of osteogenesis imperfecta. Bisphosphonate therapy can improve the outcome with decrease in fractures and improvement of bone density. It is important to suspect and confirm the diagnosis of Bruck syndrome to appropriately counsel families for management and recurrence risks. Once the mutation is identified, prenatal diagnosis is possible to prevent future recurrences.

Table 1 Phenotypic features of 29 reported patients with Bruck syndrome due to mutations in the *PLOD2* gene.

Family Number	Age at onset	Intellect	Contractures [K, A, H, E]	Fractures	Sclerae	Scoliosis [S] / Kyphosis [KY]	Other abnormal physical findings	Radiological Features	Treatment	Variant found	Protein change	Ex, In	Reference
One	B	N	Congenital K, A, H	+++	White	NA	-	-	NA	c.1886C>T	p.Thr629Ile	Ex18	Van der et al., 2003
Two	B	N	Congenital K, A	+++	Blue	Present	-	WB	NA	c.1865G>T	p.Gly622Val	Ex18	Breslau-Siderius et al., 1998 and Van der et al., 2003
Three	B	N	Congenital K, A	+++	White	Absent	Clubfoot	WB	Pamidronate	c.1856G>A	p.Arg619His	Ex17	Ha-Vinet al.,2004
Four	B	N	Congenital K, A, H	+++	White	Present	Camptodactyly Clubfoot	WB	Four patients received Zoledronic acid	c.1856G>A	p.Arg619His	Ex17	Puig-Hervás et al., 2012
Five	B	N	Congenital K, H	Numerous +++	White	Present	-	WB	-	c.1856G>A	p.Arg619His	Ex17	Puig-Hervás et al., 2012
Six	B	N	Congenital K	Numerous +++	White	Present	Camptodactyly Clubfoot	WB	-	c.1559dupC	p.Val523Cysfs	Ex14	Puig-Hervás et al., 2012
Seven	B	N	Congenital K, A	Numerous +++	White	Present	Camptodactyly	WB	-	c.1559dupC	p.Val523Cysfs	Ex14	Puig-Hervás et al., 2012
Eight	-	N	H, A	+++	White	Present	-	WB	-	c.1358+5 G>A	-	In12	Puig-Hervás et al., 2012
Nine	-	N	K, A	+++	Blue	Present	-	WB	Pamidronate	C.1864G>T c.2122-2A>G	p.Gly622Cys	Ex18, In19	Puig-Hervás et al., 2012
Ten	B	N	K, H	+++	White	Present	Clubfoot	WB	Alendronate	C.1624DELT	p.Trp54Thrfs*25	Ex15	Lv et al., 2017
Eleven	B	N	K, A	+++	No to Greyish Blue	Present	-	-	NA	c.1828T>C	p.Trp589Arg	Ex17	Caparros-Martin et al., 2017
Twelve	B	N	K	+++	Grayish blue	-	-	WB	NA	C.1358+5 G>A	-	In12	Lv et al.,2017
Thirteen	B	N	Congenital K, A, H	+++	White	Present	Camptodactyly	WB	Zoledronic acid	C.503-2A>G C.1138C>T	p.Arg380Cys	In4, Ex11	Lv et al., 2017
Fourteen	B	N	Congenital	+++	Grayish blue	Present	Clubfoot	WB	Zoledronic acid	C.1153T>C C.1928G>A	p.Cys385Arg p.Gly661Asp	Ex11, Ex18	Lv et al., 2017
Fifteen	B	N	Congenital	+++	White	-	Camptodactyly Clubfoot	WB	Zoledronic acid	C.1138C>T C.2038C>T	p.Arg380Cys p.Arg680X	Ex11, Ex19	Lv et al., 2017
Sixteen	NA	N	K, H, A, E	+++	NA	+	-	WB	Pamidronate	C.2050A>G	p.His687Arg	Ex20	Leal et al., 2018
Seventeen	B	N	K, A, E, H	+++++	Light Blue	Present	Camptodactyly Clubfoot	WB	Started therapy on oral zoledronate	c.797G>T	p. Gly266Val	Ex8	This Study

B- since birth, N- normal, K- knee, A- ankle, H- hip, E- elbow, K- kyphosis, Ks- Kyphoscoliosis, WB- Wormian bones, Ex- exon, In- intron, NA- not applicable

References

1. 1. Bank RA, et al. Defective collagen crosslinking in bone, but not in ligament or cartilage, in Bruck syndrome: indications for a bone-specific telopeptide lysyl hydroxylase on chromosome 17. Proc Natl Acad Sci USA 1999; 96: 1054-1058.
2. Breslau-Siderius EJ, et al. Bruck syndrome: a rare combination of bone fragility and multiple congenital joint contractures. J Pediatr Orthop Part B 1998; 7: 35-8.
3. Caparros-Martin JA, et al. Molecular spectrum and differential diagnosis in patients referred with sporadic or autosomal recessive osteogenesis imperfecta. Mol Genet Genomic Med 2017; 5: 28-39.
4. Ha-Vinh R, et al. Phenotypic and molecular characterization of Bruck syndrome (osteogenesis imperfecta with contractures of the large joints caused by a recessive mutation in PLOD2. Am J Med Genet A 2004; 131: 115-120.
5. Leal GF, at al. Expanding the Clinical Spectrum of Phenotypes Caused by Pathogenic Variants inPLOD2. J Bone Miner Res 2018; 33: 753-760.
6. Lv F, et al. Novel Mutations in PLOD2 Cause Rare Bruck Syndrome. Calcif Tissue Int 2017; 102: 296-309.
7. Puig-Hervás MT, et al. Mutations in PLOD2 cause autosomal-recessive connective tissue disorders within the Bruck syndrome–osteogenesis imperfecta phenotypic spectrum. Hum Mutat 2012; 33: 1444-1449.
8. Schwarze U, et al. Mutations in FKBP10, which result in Bruck syndrome and recessive forms of osteogenesis imperfecta, inhibit the hydroxylation of telopeptide lysines in bone collagen. Hum Mol Genet 2013; 22: 1-17.
9. Trejo P, et al. Osteogenesis imperfecta in children and adolescents-new developments in diagnosis and treatment. Osteoporosis Int 2016; 27: 3427-3437.
10. Van der Slot AJ, et al. Identification of PLOD2 as telopeptide lysyl hydroxylase, an important enzyme in fibrosis. J Biol Chem 2003; 278: 40967-40972.