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Atypical Osteogenesis Imperfecta caused by a 17q21.33 deletion involving *COL1A1*

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ABSTRACT

The majority of patients with osteogenesis imperfecta (OI) have a mutation in *COL1A1* or *COL1A2*. Whole gene deletions appear to be an infrequent cause of OI.

Here we describe a 8-year-old female with OI, intellectual disability and behavioural problems caused by a 2.3Mb deletion of chromosome 17q21.33 containing *COL1A1*. This deletion was detected using array comparative genomic hybridization (aCGH), performed to identify a cause for her intellectual disability. *DLX3, DLX4, CA10*, *CACNA1G, ITGA3* and *XYLT2* were also deleted and likely to be contributing to her phenotype.

This case provides further evidence that aCGH is an important test for children with OI when it is associated with additional features, such as intellectual or behavioural disability. With greater use of aCGH, the proportion of patients with atypical OI due to contiguous gene deletions or copy number variation elsewhere in the genome is likely to become clearer. This case also provides evidence that deletions in *COL1A1* resulting in haploinsuffiency are pathogenic and that a contiguous gene deletion may modify the patient's phenotype.

KEYWORDS: Osteogenesis imperfecta; atypical; *COL1A1* deletion**;** Array comparative genomic hydridization (aCGH)

INTRODUCTION

Osteogenesis imperfecta (OI) is an inherited cause of bone fragility. The majority of OI is dominantly inherited and in 85% of cases due to mutations in the *COL1A1* or *COL1A2* genes [Forlino and Marini 2016]. There have been reports of patients with 'atypical' presentations, with features that cannot be explained by OI, such as intellectual disability [Balasubramanian and others 2016]. Use of array comparative genomic hybridization (aCGH) technology in these patients has identified copy number variants, which explain the additional features, alongside pathogenic mutations in type 1 collagen [Balasubramanian and others 2016].

There are reports of chromosomal deletions involving *COL1A1* causing OI associated with intellectual and/or behavioural difficulties (Table 1). These deletions have been detected using gene sequencing [Bardai and others 2016] and multiplex ligation-dependent probe amplification (MLPA) [van Dijk and others 2010], with confirmation provided by aCGH. There have also been case reports of deletions causing OI identified primarily using aCGH technology in adulthood [Harbuz and others 2013; Mannstadt and others 2014].

Here we describe a child with OI associated with features not typically seen in cases of type 1 OI, who had aCGH to investigate her intellectual disability. This identified a deletion on chromosome 17q21.33, including *COL1A1 DLX3, DLX4, CA10*, *CACNA1G, ITGA3* and *XYLT2*.

MATERIALS AND METHODS

Clinical phenotype:

The female proband was born to non-consanguineous parents, White European parents. There was no family history of non-traumatic or multiple fractures. Intra-uterine growth retardation was observed on scans in pregnancy, but the child was born at term with a birth weight of 3.29kg (25-50th centile). Following birth, her oral intake was poor; aged 10 months, her weight had dropped to the 0.4th centile.

Aged 17 months, the patient fell from a low bed and suffered a spiral fracture of her right tibia. Radiographs showed that her bones were osteopenic. On examination, she had dark blue sclerae, brachycephaly, ligamentous laxity, an umbilical hernia and barrel-shaped chest. There was clinical (and, later, radiographic) evidence of dentinogenesis imperfecta. Lateral spine radiograph showed minor changes in 2-3 vertebrae shape, but no definite wedging or flattening.

A clinical diagnosis of osteogenesis imperfecta (OI) was made and she remained under follow-up. She suffered fractures of her left clavicle aged 2 years, of her left thumb aged 4 years, right middle finger aged 6 years but no further fractures of major long bones. Aged 4 years, significant wedging (>25% loss of anterior height) of T6 vertebra was identified on routine screening and bisphosphonate treatment started. She was started on a standard dose of pamidronate, (12mg/kg/year in 4 divided doses). Medical treatment led to noticeable increase in her energy and activity levels. Pamidronate treatment was ongoing at her last clinical review, aged 8 years, although the dose has been halved following stepwise reductions since 6 years of age.

Developmentally, her gross motor skills were delayed: rolling at 13 months, sitting without support at 18 months and walking at 3 years of age. She still requires ankle-foot orthoses and supportive footwear. She has delayed speech development

and feeding difficulties, requiring speech therapy. She had astigmatism and hypermetropia associated with a squint. Mild loss of hearing was identified on testing from the third year of life. She underwent an adeno-tonsillectomy aged 5 years with improved hearing. An MRI-scan undertaken prior to 18 months of age showed mild ventricular enlargement and sulcal widening.

On recent review at 8 years of age, her height was 122.2cm, weight 21.1kg and OFC 51cm (all growth parameters at the 2nd-9th centile). Her sclerae were blueish grey in colour, she had thick eyebrows, down-slanting palpebral fissures, high forehead and bilaterally low set ears (Figure 1). She was hypermobile with generalised muscle weakness. Her behavioural difficulties were more apparent at this age with food aversions, tantrums, poor sleep and disruptive behaviour. She was resistant to change, would scream and become highly agitated when asked to do something she did not want to. Her mother felt her behaviour was extreme; it was hard to reason with her and she did not respond well to standard behaviour management strategies. She was on melatonin for sleep. She had limited visual motor skills and motor coordination. Her perceptive and cognitive skills were also limited. Special schooling was being considered. She exhibited age-appropriate bathing but required support with toileting. She struggled with coordination and balance. A WISC-IV completed by the Clinical Psychologist indicated her Full Scale IQ fell below the 0.1^{st} centile confirming the diagnosis of 'Intellectual Disability'.

Genetic analyses:

Mutation analysis was performed at the age of 7 years by sequencing a custom designed panel of genes associated with OI (*COL1A1, COL1A2, CRTAP,*

LEPRE1, PPIB, FKBP10, SP7, SERPINF1, SERPINH1, PLOD2, BMP1, TMEM38B, WNT1, IFITM5 c.-14C>T). This testing did not identify any causal variants of interest.

Array comparative genomic hydridization (aCGH) was performed on genomic DNA using the GeneChip System 3000 platform and Affymetrix Cytoscan 750K array. The array consists of 550,000 non-polymorphic probes and 200,000 single nucleotide polymorphisms. The result was confirmed using fluorescent in situ hybridization (FISH) using a probe for *COL1A1*. Analysis was based on NCBI Build 37.

RESULTS

Array CGH showed a 2.3Mb loss of chromosome 17q21.33 (47,777,193-50,153,165), confirmed using FISH. This area includes 8 OMIM morbid genes including *COL1A1.* Deletion of *COL1A1* would be consistent with the clinical diagnosis of OI [van Dijk and others 2010]. This patient also had a 361Kb gain at 7q36.2 (154,624,073-154,984,631), which was not thought to be significant. Analysis of the mother's chromosomes did not reveal any array abnormalities or rearrangements. The father's sample was unavailable for testing.

DISCUSSION

This patient demonstrates how a genetic investigation for intellectual difficulties can identify a cause for a disorder that is typically monogenic. The diagnosis of OI was made early in life on clinical grounds, but mutation testing was normal. Array CGH was subsequently performed in relation to developmental delay and behavioural difficulties, which are not features of OI. This identified both a cause for her OI and additional features.

Clinically, the patient had type 1 OI, which is usually caused by nonsense or frameshift mutations in *COL1A1* leading to haploinsufficiency of the collagen type 1 alpha 1 chain [Marini and Blissett 2013]. The deletion contained *COL1A1* resulting in haploinsufficiency of type 1 collagen.

Deletions of the entirety of *COL1A1* appear to be an uncommon cause of classical forms of OI, with Bardei and colleagues identifying deletions in 3% of their series of 161 families [Bardai and others 2016]. Deletions detected by aCGH have been identified in adult patients with bone fragility, intellectual disability and other features [Harbuz and others 2013; Mannstadt and others 2014]. This report describes a case of OI caused by a unique deletion identified in childhood. Table 1 compares the deletions identified to-date.

A number of other OMIM morbid genes were deleted in our patient and may contribute to her phenotype, specifically *DLX3, DLX4, CA10*, *CACNA1G, ITGA3* and *XYLT2*.

DLX3 and *DLX4* are involved in craniofacial development [Takechi and others 2013]. Mutations in *DLX3* have been found in patients with Tricho-dento-osseous syndrome, a condition with variable features affecting hair, teeth and bones [Price and others 1998] [Wright and others 2008] and amelogenesis imperfecta, associated with tooth enamel defects [Dong and others 2005; Kim and others 2016]. A common mutation in tricho-dento-osseous syndrome appears to have a dominant negative effect, interfering with normal *DLX3* causing reduced activation of enamel matrix protein genes during tooth development [Zhang and others 2015]. There is also evidence that single nucleotide polymorphisms in *DLX3* are associated with molar-incisor hypomineralization [Jeremias and others 2016].

Our patient had evidence of dentinogenesis imperfecta. As previously suggested by Bardei and colleagues, this may be related to loss of *DLX3* as opposed to *COL1A1*, as dentinogenesis imperfecta is not usually seen with haploinsufficiency of *COL1A1* [Bardai and others 2016; Ben Amor and others 2013]. Reduced expression of *DLX3* may have similar effects to the dominant negative common mutation in trichodentoosseous syndrome. This hypothesis remains speculative however and is not supported by the observation that deletion of *DLX3* is not consistently associated with dentinogenesis imperfecta in published cases (Table 1) [Harbuz and others 2013; Mannstadt and others 2014; van Dijk and others 2010].

XYLT2 was also deleted. Recessive frameshift and nonsense mutations in this gene are associated with spondylo-ocular syndrome, associated with bone fragility, hearing impairment, cataracts, cardiac septal defects and intellectual difficulties [Munns and others 2015; Taylan and others 2016]. Potentially, haploinsufficiency of this gene may have a similar effect to loss-of-function mutations, contributing to both the skeletal and cognitive features of our patient.

Other genes that could contribute to our patient's intellectual and behavioural difficulties include *CACNA1G, CA10, ITGA3* which have been associated with brain development, neurological processes and glia-neuron interactions [Anton and others 1999; Preiksaitiene and others 2012; Schmid and Anton 2003], but the direct consequences of a deletion on intellect and behaviour is not apparent.

This patient also had duplication at 7q36.2, the breakpoint of which may disrupt *DPP6*. Deletions and mutations in *DPP6* have been associated with intellectual disability and microcephaly [Liao and others 2013]. Our patient was not

microcephalic and so it is unlikely that involvement of this gene is contributing to her intellectual difficulties.

Increasing use of aCGH with improved phenotyping of children with intellectual and behavioural difficulties are likely to clarify the proportion of such children with OI who have a deletion involving *COL1A1*. Children with these deletions may already have the clinical diagnosis of OI, as in this case, but deletions may be incidentally identified. Based on the literature to-date, these deletions are pathogenic and we would suggest these children are investigated for bone fragility and managed as a patient with OI. Conversely, in patients with OI associated with developmental delay or intellectual disability, aCGH may identify contiguous gene deletion syndromes involving *COL1A1* or copy number variation elsewhere in the genome [Balasubramanian and others 2016]. Further research is required to clarify how deletion of additional genes may modify the phenotype of these patients.

FIGURE LEGENDS

Figure 1: Facial features of the patient (a) Front-on photo (b) Profile: The patients sclerae were blueish grey in colour, she had thick eyebrows, down-slanting palpebral fissures, a high forehead and bilateral low-set ears.

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TABLES

Table 1: Previous reported deletions involving *COL1A1*. Features of the proband in each reported family are listed.

Deletion						Phenotypic information						
Initial method of detection	**Start**	**End**	**Min size**	**Max size**	**Number of genes deleted**	**Age of last clinical review (years)**	**Bone fragility**	**Sclera**	**DI**	**ID**	**BD**	**Reference**
aCGH[1]	47777193	50153165	2.3Mb	2.4Mb	30	8.6	+	Blue	+	+	+	Current report
NGS	47332514	49565743	2.2Mb	2.3Mb	47	9.8	+	Blue	+	+	NR	[Bardai and others 2016]
	48099388	49348322	1.3Mb	1.3Mb	34	17.6	+	Blue	-	+	NR	
	48242601	48379926	137Kb	171Kb	4	2.7	+	Blue	-	-	NR	
	48211636	48289249	78Kb	99Kb	4	15.0	+	Blue	-	-	NR	
	48261783	48280296	19Kb	37Kb	1	3.1	+	Blue	-	-	NR	
MLPA			MLPA only			10	+	Blue	-	NR	NR	[van Dijk and others 2010]
	NR	NR	182Kb	217Kb	7	44	+	Blue	-	NR	NR	
	NR	NR	353Kb	512Kb	13	37	+	Blue	+	NR	NR	
	NR	NR	451Kb	481Kb	14	40	+	Blue	-	NR	NR	
aCGH	NR	NR	1.0Mb	NR	28	27	+	Blue	-	+	NR	[Mannstadt and others 2014]
aCGH[2]	44909862	48312957	3.4Mb	NR	39	22	+	Blue	+	+	NR	[Harbuz and others 2013][1]

NR not recorded, DI dentinogenesis imperfecta, ID intellectual difficulties, BD behavioural difficulties

1. Also duplication at 7q36.2.
2. Also duplication at 7q32.3-q33.