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Case Report

Bilateral Pseudoarthrosis of the Clavicles without *Cbfa1/Runx2* Gene Mutation: A Case Report

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Abstract

We have identified an individual with bilateral pseudoarthrosis of the clavicle, presumed to be congenital, with no family history of the condition, no evidence of postnatal trauma to the clavicles and no other overt skeletal abnormalities. The genetic basis of pseudoarthrosis of the clavicle has not been identified, although Cleidocranial Dysplasia (CCD), a congenital condition characterized by skeletal anomalies that include hypoplastic and/or aplastic clavicles, has been mapped to chromosome 6p21 and shown to result from mutations in the *Cbfa1/runx2* gene. We therefore investigated the possible involvement of *Cbfa1/runx2* mutation by sequencing all 7 exons of the gene. No changes were found in the coding sequence of *Cbfa1/runx2* in this case of pseudoarthrosis of the clavicles and the genetic basis for congenital pseudoarthrosis of the clavicles remains to be determined.

Keywords: Clavicle; Bilateral Pseudoarthrosis; *Cbfa1* gene

Case Presentation

We report a case of bilateral pseudoarthrosis of the clavicles in a 21 year-old white male, who was otherwise skeletally normal and showed no adverse effects from his condition. His parents and a younger female sibling were unaffected. Since visualisation of the clavicle is suboptimal using standard radiographs, a high resolution shoulder-to-neck CT, with 0.5mm slice thickness, was performed. 3D reconstruction of the CT was performed using True Life Anatomy software (TLA Generator, Rubamas, Adelaide; www.rubamas.com) to create a surface rendered model of the upper thorax showing skin, clavicles and lungs as separate objects. Figure 1 demonstrates clearly the complete discontinuity at the midshaft of both clavicles. The overall alignment was reasonable, although there was inferior displacement of the lateral fragment with respect to the medial shaft. The appearance was symmetrical, and the clavicles were well aligned with the thorax. There was no significant tendency for medialisation of the shoulder girdle. There was no particular overriding of the clavicles, which was explained after further manipulation of the 3D images, using different image thresholds, revealed a cartilage anlage joining the ends of the clavicles together (not shown). This would explain the relatively stable position of the shoulder girdle, despite the apparent discontinuity, as distinct from the normal overriding following acute clavicular fracture.

To identify mutations in coding regions of the *Cbfa1/runx2* gene, peripheral blood was taken from the subject and white blood cells were harvested to prepare chromosomal DNA, using standard procedures. The *Cbfa1/runx2* exons were amplified using intron- and exon-specific primers shown in Figure 2 [1]. No mutation in any coding region or splice-donor/acceptor sites in the *Cbfa1/runx2* gene was identified.

Discussion

Congenital pseudoarthrosis of the clavicle is a rare condition, whose aetiology and true prevalence are unknown. The condition is usually benign and rarely produces any functional impairment, although occasionally a progressive swollen deformity [2], or venous thoracic outlet syndrome [3], can occur. Surgical repair is sometimes undertaken when symptoms limit activities of daily living or engender aesthetic concerns [4,5]. Most commonly, there is a defect in the shaft of one clavicle and, in a large series, these were overwhelmingly on the right side [2]. Rarely, the defect occurs bilaterally [6,7]. The origins of congenital pseudoarthrosis may be in early embryogenesis, since the clavicle is the first of all bones to ossify in mammals [8]. The clavicle appears in the seventh week of gestation as two centres of ossification, which spread during the eighth week towards the sternum and towards the acromium, ossification being preceded by a formation of true cartilage [8]. Arrest or interruption of this process may prevent the union of the two ossific centres of the clavicle.

No convincing genetic pattern has been identified and no gene has been implicated in congenital pseudoarthrosis of the clavicle. There is a rare variant of bilateral congenital pseudoarthrosis reported to accompany pycnodysostosis, due to cathepsin K deficiency, but this syndrome has generalised skeletal features, including short stature and altered bone quality [9], which did not match the case we describe here. Our rationale for investigating *Cbfa1/runx2* as a candidate gene was that *Cbfa1/runx2* mutations, in both mice and humans, are associated with skeletal abnormalities that include hypoplastic clavicles [10,11]. Specifically, Cleidocranial Dysplasia (CCD) is an autosomal dominant disorder characterized by skeletal anomalies that may include some or all of, patent fontanelles, persistently open or delayed closure of cranial sutures, dental abnormalities, hypoplastic and/or aplastic clavicles, and short stature [12,13]. The locus for CCD has been mapped to chromosome 6p21 [14], and shown to result from mutations in the *Cbfa1/runx2* gene [12]. *Cbfa1/runx2* is a member of the runt family of transcription factors and its expression is restricted to bone and cartilage [15]. Mice with a homozygous mutation in *Cbfa1/runx2* died just after birth due to respiratory difficulties and

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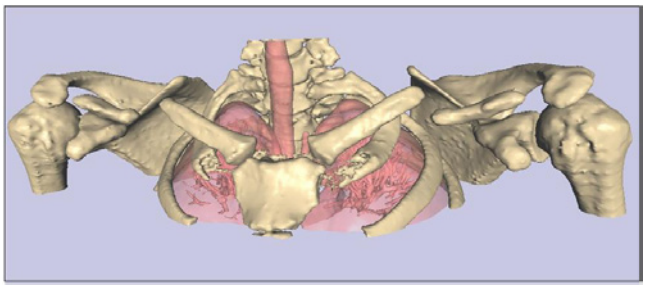

Figure 1: 3D reconstruction of a CT of the shoulders, showing bilateral disruption of the clavicles. Note that using different CT image thresholds, a cartilage anlage, not shown here, was visualised joining the midsection of the clavicles together.

EXON	PRIMER SEQUENCES SENSE (S), ANTISENSE (AS)	
A	S	GCT ATT TGG AAA AGC TAG CAG
	AS	ATG GTT AAT CTC CGC AGG TCA
B	S	CCC GGC CAC TTC GCT AAC TTG
	AS	TCC GCC GCT CCC GGC CGG
1	S	AAC ACT AAG TCC TGA TAA GAC
	AS	GAAGGT GCT GAT TTG TAT ACA '
2	S	AAT TTA GAA GAA GGA GTC CTG
	AS	AAA TAT ATG CAG ATA GCA AAG
3	S	ATT CCT TGG CTT AAA CTC CCA G
	AS	GCC GCT TCA CAG CTC CAG G
4	S	TAAGGCTGCAATGGTTGCTAT
	AS	GTCACTGTGAGCATGGATGAG
5	S	CTC TGG GAA ATA CTA ATG AGG GA
	AS	AGT GCC ATG ATG TGC ATT TGT AAT
6	S	TGT GGC TTG CTG TTC CTT TAT G
	AS	GAT ACC ACT GGG CCA CTG CT

Figure 2: Primers for the PCR amplification of *Cbfa1/runx2*.

examination of their skeletons showed a complete lack of ossification [16]. Both intramembranous and endochondral ossification were completely blocked, owing to the maturational arrest of osteoblasts in the mutant mice, demonstrating that *Cbfa1/runx2* plays an essential role in osteogenesis. Interestingly, heterozygous *Cbfa1/runx2* mutant mice display symptoms very similar to human CCD, including hypoplastic clavicles [10,11]. Subsequently, it was found that another transcription factor, osterix (Osx), acts downstream of *Cbfa1/runx2* and is required for osteoblast differentiation and bone formation [17]. *Cbfa1/runx2* null mice do not express Osx. A third transcription factor, CbfB is expressed in developing bone and interacts functionally with *Cbfa1/runx2*. Disruption of the CbfB gene resulted in a delay in endochondral and intramembranous ossification as well as in chondrocyte differentiation, similar to but less severe than delays observed in Runx2(-/-) mice [18].

Sequencing of the coding sequence of the *Cbfa1/runx2* gene in the proband did not reveal any mutations. It remains possible that causative mutations exist in non-coding regions of the gene, including introns or regulatory domains. It is also possible that mutations in proteins that regulate the levels of *Cbfa1/runx2*, or participate in transcription complexes with it, or lie downstream from it, could lead to abnormal bone development. *Cbfa1/runx2* activity is modulated by a large number of proteins, including Cbfb, TAZ, MOZ/MORF, STAT1, MSX2, DLX5 and HES1 [23,24]. Indeed, tetranucleotide duplication in the MSX2 homeobox gene (505-508dupATTG) has been reported to segregate with a condition known as Parietal Foramina with Cleidocranial Dysplasia (PFMCCD), which also displays deficient ossification of the clavicles [25]. It is not known how mutations in *Cbfa1/runx2* and other interacting molecules can lead to differential effects across the skeleton or to different penetrance in different individuals. However, the early and distinct development of the clavicles [8] could render these bones particularly sensitive to mutations in certain genetic backgrounds or to environmental influences in early foetal life. An additional possibility is that the current case was caused by epigenetic and/or environmental factors.

Conclusion

An otherwise healthy young man was identified with bilateral pseudoarthrosis of the clavicles, and no other skeletal abnormalities. Because of its involvement in Cleidocranial Dysplasia (CCD), manifestations of which can include hypoplastic and/or aplastic clavicles, we investigated the possible involvement of *Cbfa1/runx2*. However, no mutations were found in the coding regions of the *Cbfa1/runx2* gene and the genetic basis for this condition remains to be determined. It is likely that identification of the gene or genes associated with pseudoarthrosis of the clavicles will provide fresh insights into skeletal development in general, and into that of the unique and interesting clavicular bone in particular.

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