Case Report

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

Thomas D¹, Sandow MJ², Evdokiou A¹ and Findlay DM*¹

¹Department of Medicine, University of Melbourne at St Vincent’s Hospital, Fitzroy, Australia
²Department of Orthopaedics and Trauma, Royal Adelaide Hospital, Adelaide, Australia
³Discipline of Orthopaedics and Trauma, University of Adelaide, Adelaide, Australia

*Corresponding author: David M Findlay, Discipline of Orthopaedics and Trauma, Adelaide Health and Medical Sciences Building, University of Adelaide, North Terrace, Adelaide, South Australia 5000, Australia

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 skeletal 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 acromion, 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...
3D reconstruction of a CT of the shoulders, showing bilateral 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 and/or aplastic clavicles [10,11]. Subsequently, it was found that another transcription factor, osterix (Osx), acts downstream of Cbfa1/runx2 [10,11]. 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 pseudarthrosis 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 pseudarthrosis 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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