HYPOTHESIS

Expansion of polyalanine tracts in the QA domain may play a critical role in the clavicular development of cleidocranial dysplasia

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Cleidocranial dysplasia (CCD) is an autosomal-dominant inheritable disease which is secondary to haploinsufficiency of the transcription factor runt-related transcription factor 2 (RUNX2). During the past 10 years, additional cases of RUNX2 mutation have been identified in nearly 500 families with CCD, including familial and sporadic cases (Otto *et al.* 2002). Some degree of clavicular hypoplasia is the most consistent feature of the disease and is often opposed at the mid line (Tang *et al.* 2007), while complete absence is rare and complicated to explain the specific mechanism.

RUNX2, which is mapped to chromosome 6p21, was identified as the CCD causative gene in 1997 (Mundlos *et al.* 1997). This gene is the key transcription factor for osteoblastic differentiation, chondrocyte maturation and skeletal morphogenesis (Komori *et al.* 1997). Moreover, stability of RUNX2 protein, regulated by CBFb, is required for skeletal development by regulating chondrocyte differentiation, proliferation and osteoblast differentiation (Qin *et al.* 2014). RUNX2-deficient mice displayed impaired bone development manifestation as wide sutures, decreased bone ossification and hypoplastic clavicles (Kundu *et al.* 2002).

RUNX2 consists of eight coding exons and spans a genomic region of 130 kb. According to the analysis of the protein structure, RUNX2 has several functional domains: QA domain, runt domain, nuclear localization signal (NSL), PST domain, VWRPY and nuclear matrix targeting sequence (NMTS). The QA domain, a stretch of 23 polyglutamine and 17 polyalanine tracts, is located in the N-terminus of RUNX2. An *in vitro* mutagenesis study showed that the QA domain prevented CBFb from binding to RUNX2. Deletionof the QA domain resulted in a four-fold decrease in the transactivation ability of proteins, indicating that the QA domain plays a key role in cleidocranial dysplasia (Thirunavukkarasu *et al.* 1998). Polymorphic changes within the QA domain are also associated with low bone mineral density and the risk of fracture (Vaughan *et al.* 2002), which suggests that QA domain may affect the sketetal development.

It has been demonstrated that the single amino acid repeat-containing proteins (SARPs) are involved in functions that require formation of multiprotein complexes (Siwach *et al.* 2006). An analysis of 10,000 human genes showed that 20% have at least one homopolymer tract, of which 16% is polyalanine and results in a total of 300 polyalanine containing genes (Karlin *et al.* 2002). Polyalanine tracts are generally stable and have a strong conservation of size and position between species, suggesting a functional and structural evolutionary pressure for their appearance (Lavoie *et al.* 2003). In addition, expansions of polyalanine repeat in transcription factors result in misfolding, degradation and cytoplasmic aggregation of the mutant proteins (Albrecht *et al.* 2004). In addition, the evolution of repeat tracts in the proteome appears to be selected for or against by the cellular toxicity (Siwach *et al.* 2009). All these results indicate that any change in polyalanine tracts may result in deleterious effects.

Expanded polyalanine tracts in transcription factor genes, such as RUNX2, PABPN1, SOX3 and FOXL2, have been shown to cause several inheritable human diseases or malformations (Brown and Brown 2004). Mutational analysis in CCD patients suggests that the length of polyalanine tracts in the QA domain of RUNX2 influences the transcriptional activity of proteins, and the phenotypes of the patients are different from the classical CCD phenotypes (Mundlos *et al.* 1997). Homopolymeric glutamine or alanine repeats form CC structures that can trigger protein aggregation and

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toxicity upon expansion in human genetic diseases (Pelassa *et al.* 2014). Deletion of the polyalanine tracts in PABPN1 affects the cellular aggregation and localization and leads to great cellular toxicity (Klein *et al.* 2008). An *in vitro* research showed that deletions of polyalanine tracts, associated with hypopituitarism, in SOX3 have functional consequences (Alatzoglou *et al.* 2011). Moreover, the deletion of polyalanine tract in FOXL2 may be responsible for premature ovarian failure with blepharophimosis, ptosis, epicanthus inversus syndrome (Gersak *et al.* 2004), and a complete deletion of the polyalanine tracts in FOXL2 induces a significant intranuclear aggregation (Moumne *et al.* 2005). In addition, the change of the polyalanine tracts is closely related to the GC content of the genomewide genes, which is positively correlated with animal evolution (Du *et al.* 2010). These researchers suggest that the length of the polyalanine tracts might result in multiple loss of normal function.

After analysing the literatures, we evaluated relationship between polyalanine tracts in the QA domain and the clavicular development. Since majority of the species have polyalanine tracts in the QA domain and are warm blooded mammals, we identified the RUNX2 coding sequences of warm blooded mammals from http://www.ensembl.org/index.html, then aligned the sequences by MEGA6.06. As shown in table 1, the species which have clavicle, such as *Homo sapiens, Pan troglodytes, Macaca mulatta* and *Rattus norvegicus*, all their polyalanine tracts in the QA domain are 17. Species, such as *Mus musculus*, has more polyalanine tracts than 17, it still shows intact clavicle. As for the species having a shortened or no polyalanine tracts in the QA domain, such as birds (*Haliaeetus leucocephalus* and *Gallus gallus*) and fast-running animals (*Equus caballus* and *Canis lupus familiaris*), they suffer the absence of the clavicle (Romer and Parsons 1977) Our analysis indicates that the polyalanine tracts in the QA domain of RUNX2 may play a critical role in the clavicular development.

Table 1. The relationship between polyalanine tracts in the QA domain and the clavicular development.

Species	Polyalanine tracts	Clavicle
Homo sapiens	17	+
Pan troglodytes	17	+
Macaca mulatta	17	+
Rattus norvegicus	17	+
Mus musculus	18	+
Bos taurus	16	−
Equus caballus	11	−
Capra hircus	0	−
Camelus dromedarius	0	−
Sus scrofa	0	−
Cavia porcellus	4	−
Canis lupus familiaris	0	−
Odobenus rosmarus	15	−
Pygoscelis adeliae	0	−
Felis catus	0	−
Haliaeetus leucocephalus	0	−
Gallus gallus	2	−

+, specie has clavicle; −, specie has no clavicle.

According to the literature review and evolutionary analysis, we propose that polyalanine tracts in the QA domain contribute to the clavicular development of CCD in warm-blooded mammals. This viewpoint will lead to a better understanding of the clavicular development of cleidocranial dysplasia and provide a novel method for diagnosing cleidocranial dysplasia with the absent of clavicles. To assess whether polyalanine tracts in the QA domain plays a critical role in the clavicular development of cleidocranial dysplasia patients, further studies on the correlation between clavicular development and the polyalanine tracts in the QA domain need to be conducted.

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