A Glutamine Repeat Variant of the *RUNX2* Gene Causes Cleidocranial Dysplasia

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Key Words

Cleidocranial dysplasia · Glutamine repeat variant ·
Q/A domain · *RUNX2*

Abstract

Cleidocranial dysplasia (CCD), an autosomal dominant skeletal dysplasia characterized by hypoplastic clavicles and delayed closure of the cranial sutures, is caused by mutations of the runt-related transcription factor 2 *(RUNX2)* gene. The *RUNX2* gene consists of a glutamine and alanine repeat domain (Q/A domain, 23Q/17A), a DNA-binding Runt domain and a proline/serine/threonine-rich domain. We report on a familial case of CCD with a novel mutation within the Q/A domain of the *RUNX2* gene, which is an insertion in exon 1 (p.Q71_E72insQQQQ) representing the Q-repeat variant (27Q/17A). Functional analysis of the 27Q variant revealed abolished transactivation capacity of the mutated RUNX2 protein. This is the first case report that demonstrated a glutamine repeat variant of the *RUNX2* gene causes CCD.

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Cleidocranial dysplasia (CCD) is an autosomal dominant skeletal dysplasia characterized by hypoplastic or aplastic clavicles, delayed closure of the fontanelles and cranial sutures, delayed ossification of the pelvis, dental abnormalities such as late eruption of permanent teeth and multiple supernumerary teeth, and moderately short stature [Cooper et al., 2001]. CCD is caused by hypomorphic or haploinsufficiency of the runt-related transcription factor 2 *(RUNX2)* gene [Lee et al., 1997; Mundlos et al., 1997].

To date, the mutations occur throughout the *RUNX2* gene, but are clustered in the Runt domain in CCD. Most of the mutations within the Runt domain are missense mutations. On the other hand, nonsense mutations, insertions or deletions are predominant within the Q/A domain or the proline/serine/threonine-rich domain [Kim et al., 2006]. The Q/A domain has the capacity to mutate via strand slippage during DNA replication [Yoshida et al., 2002]. Glutamine repeat sequence expansion has been the cause of some diseases that show genetic anticipation, where severity increases in subsequent generations as the repeat length increases due to errors in replication [McMurray, 2010]. Wild-type human *RUNX2* contains a
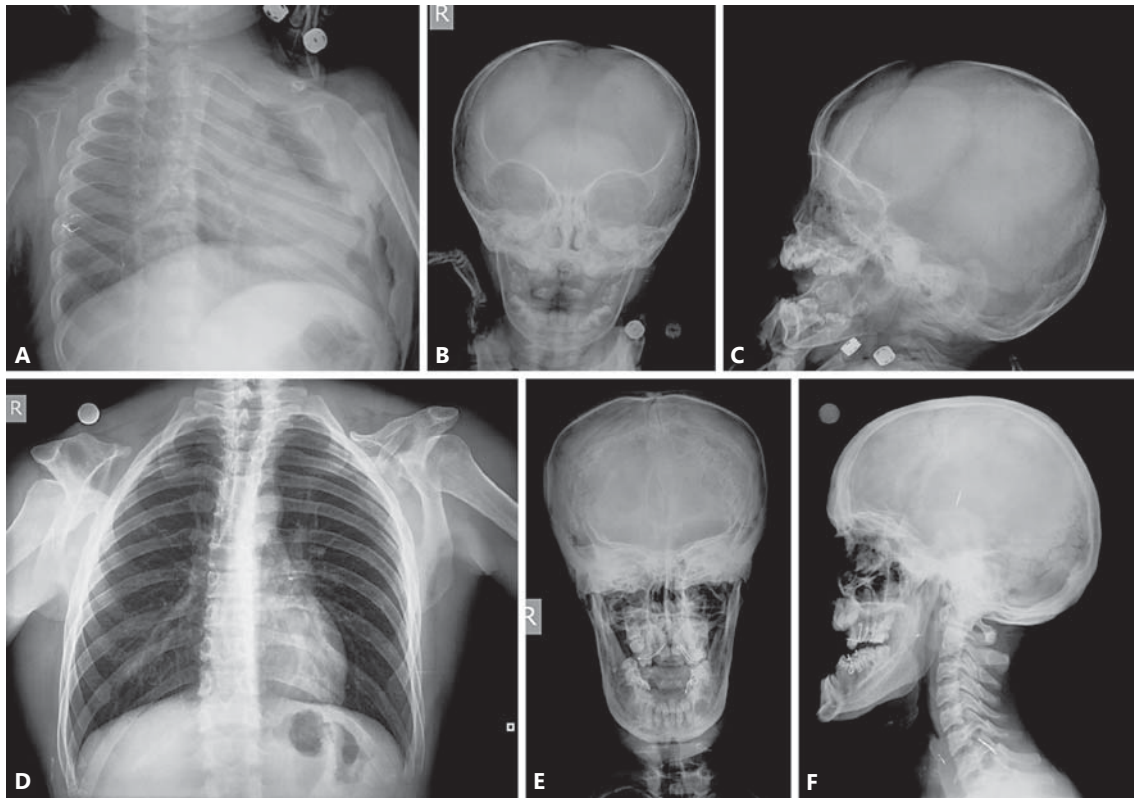
Fig. 1. Radiographs of the proband's chest and skull demonstrating the complete absence of bilateral clavicles, open large fontanelles, multiple wormian bones, supernumerary teeth, and mandibular protrusion (**A–C**). Anteroposterior radiograph of the father's chest demonstrating bilateral absence of clavicles (**D**) and radiographs of his skull demonstrating multiple wormian bones, a relatively thick skull and prognathism (**E**, **F**).

23Q/17A repeat: 23 consecutive glutamine residues followed by 17 alanine residues. An insertion of the polyalanine tract (23Q/27A) was previously observed in only one CCD patient [Mundlos et al., 1997].

Here, we describe a familial case of CCD with a novel mutation within the Q/A domain, which is an insertion of the polyglutamine tract (27Q/17A). In vitro functional analysis was performed to assay the transactivation capacity of the mutant RUNX2 protein.

Case Report

A family with the clinical diagnosis of CCD from the Erciyes University, Turkey, was examined in this study. The proband, a 2-year-old boy, is the only child of an affected father (27 years old) and a healthy mother (23 years old). Radiographs of the proband showed a large defect of the parietal and occipital bones, supernumerary teeth, sclerosis of the cranial base, multiple wormian bones, and bilateral absence of clavicles (fig. 1A–C). The last 2 radio-

graphic manifestations (multiple wormian bones and absent clavicles) were also observed in the boy's father (fig. 1D, E), although he had a relatively thick skull and prognathism (fig. 1F). The craniofacial manifestations, including frontal bossing, midface hypoplasia and a small face, were shared in both the proband and his father.

Methods

After informed consent was obtained from all family members, genomic DNA was extracted from peripheral blood leukocytes. The exons (0–7) and their flanking intronic regions of the *RUNX2* gene were amplified by PCR using sets of primers. Direct sequence analysis of the affected patients' DNA from this family demonstrated a novel heterozygous mutation within the Q/A domain, c.213_214insCAGCAGCAGCAG (p.Q71_E72insQQQQ).

For in vitro functional studies of the mutant RUNX2 protein identified in this family, the entire cDNA of p.Q71_E72insQQQQ (27Q) was constructed as follows. We confirmed that the mutation was located between 2 PstI sites (181 bp) in exon 1 and obtained the oligonucleotide duplex containing the mutation (Integrated
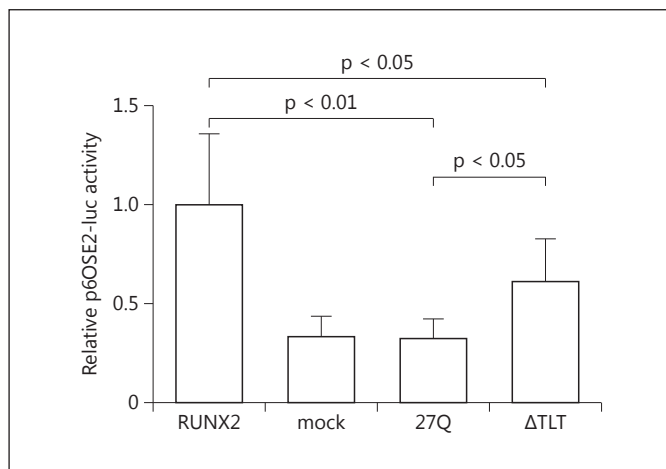
Fig. 2. Transactivation ability of the wild-type and mutant RUNX2 proteins. COS7 cells were transfected with p6OSE2-luc as a reporter plasmid, full-length, wild-type or mutant RUNX2 as effector plasmids, and phRL-TK as an internal control of transfection efficiency. Data are presented as fold activation relative to the activity obtained with wild-type RUNX2 vector plasmid. Bars represent the average ratios of luciferase to Renilla activity. Standard deviations are represented by error bars. Both the 27Q variant and ΔTLT198_200 mutants showed significantly reduced transactivation ability compared to the wild type. Moreover, transactivation of the 27Q variant was significantly lower than that of the ΔTLT198_200 mutant.

DNA Technologies MBL, Japan). PCR fragments of the oligonucleotide duplex were double-digested with PstI. This insert was cloned into the human full-length *RUNX2* cDNA (Ori-Gene Technologies, Rockville, Md., USA) at the PstI sites. On the other hand, p.T198_T200del (ΔTLT), which was previously identified in a patient with CCD, was constructed by using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, Calif., USA) [Matsushita et al., 2014]. Transient transfection experiments in COS7 cells were performed using FuGENE 6 (Roche, Indianapolis, Ind., USA). Aliquots of 400 ng expression plasmid containing either wild-type or mutagenized *RUNX2* were cotransfected with 400 ng of a reporter plasmid p6OSE2-luc (kindly provided by T. Komori, Nagasaki University, Japan) [Harada et al., 1999]. All transfection experiments were done 8 times. The transactivation study showed that the 27Q variant and ΔTLT mutants had significantly lower transcription activities (32 and 61% of the wild type, respectively) (fig. 2).

Discussion

Clinical and radiographic manifestations of the present cases seemed to be typical for CCD, including complete absence of bilateral clavicles, multiple wormian bones and supernumerary teeth. Mutation analysis of this family showed a Q-repeat variant within the Q/A domain, which resulted in a significant reduction of transactivation of the RUNX2 protein.

Q-repeat variants within the *RUNX2* gene were identified in an Australian fracture cohort (15Q, 16Q, 24Q, and 30Q) [Vaughan et al., 2002], a randomly selected population from Aberdeen (16Q) [Vaughan et al., 2004], and a Spanish population study (16Q, 18Q and 30Q) [Pineda et al., 2010]. A 30Q variant of the *RUNX2* gene has never been reported to be associated with CCD phenotypes. On the other hand, a novel 27Q variant caused CCD by downregulating the transactivation activity of the RUNX2 protein. Generally, triplet repeat expansion disorders accelerate their phenotypes according to the repeat length. Huntington's disease, for example, is one of the polyQ-repeat disorders, and its severity is usually associated with the length of the polyQ tracts. It has been suggested that aggregation of the polyQ fibers is pathogenic of the disease. Perutz [1996] reported that Huntington's disease has not been observed in individuals with <37 repeats, and absence of disease has never been found in those with >41 repeats. This indicated that polyQ expansion beyond the pathological threshold of 36–40 repeats leads to a clinical manifestation. According to the model of Perutz et al. [2002], polyQ fibers are composed of nanotubes with 20 residues per turn, and a minimum of 2 turns (40 repeats) is necessary for pathogenic polyQ aggregates. It is possible that the 27Q variant is pathogenic, while the 30Q variant is benign, since the repeat length is not necessarily related to the severity of the disease when it is <40 repeats.

Sears et al. [2007] showed that Q/A tandem repeat ratio correlated to *RUNX2* transcriptional activity. Morrison et al. [2012] demonstrated that transactivation activity was reduced by the *RUNX2* Q-repeat variants, but rescued by PEBP2β, which is the partner subunit for heterodimerization with the Runt domain. In a study on dogs, Fondon and Garner [2004] demonstrated that the length of the Q repeat is significantly associated with midface length and nose curvature. We previously reported a CCD patient with the in-frame deletion (ΔTLT) who showed a milder phenotype than the present cases, including mild short stature (–1.75 SD), delayed fontanelle, midface hypoplasia, pseudoarthrosis of the right clavicle, and hypoplasia of the left clavicle [Matsushita et al., 2014]. ΔTLT mutation decreased the transactivation activity of the RUNX2 protein by abolishing the heterodimerization of the RUNX2 protein with the PEBP2β. Significantly lower transactivation activity of the 27Q variant than that of the ΔTLT mutant may reflect the phenotypic severity of the disease.

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