Case Report

Novel mutation of RUNX2 gene in a patient with cleidocranial dysplasia

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Abstract: Background: Cleidocranial dysplasia is a rare hereditary skeletal disorder due to heterozygous loss of function mutations in the RUNX2 gene that encodes runt-related transcription factor 2 (RUNX2). Here we report a 52 year-old woman with cleidocranial dysplasia due to a novel RUNX2 mutation. Case description: A 52 year-old Han Chinese woman presented with short stature and skeletal dysplasia that was first noted during early childhood. She was 153 cm in height and 40 kg in weight. Her skull was deformed with hypertelorism, midface hypoplasia, protrusion of chin, and dental abnormalities. Radiological examination revealed shortened clavicles and depressed skull bone and that were consistent with the clinical diagnosis of cleidocranial dysplasia. There was no family history of a similar skeletal disorder. We sequenced the RUNX2 gene and discovered a novel heterozygous mutation in exon 3 (c.476 del G, p.G159fs175X) that is predicted to cause a frameshift and premature termination that leads to the loss of the final 347 amino acid residues. This severely truncated protein is expected to be inactive. Literature review: RUNX2 gene controls osteoblast differentiation and chondrocyte maturation. Around 90 RUNX2 mutations have been discovered in patients with cleidocranial dysplasia. Clinical relevance: We identified a case of cleidocranial dysplasia due to a novel mutation of RUNX2 gene at exon 3 (c.476 del G).

Keywords: Cleidocranial dysplasia, RUNX2 gene, mutation

Introduction

Cleidocranial dysplasia (CCD) (MIM 119600), also known as cleidocranial dystosis, is a rare hereditary skeletal disorder. In most cases the disorder is inherited as an autosomal dominant trait, but in some cases the disorder appears sporadic. The main clinical features of CCD are recognized during early childhood and include proportionate short stature, delayed closure of fontanelles, prominent forehead, drooping shoulders, and abnormal dental development. The distinctive radiological features are shortened or absent clavicles, delayed ossification of the skull bones, and delayed ossification of pelvic bones [1].

Heterozygous mutations in the RUNX2 gene (OMIM 600211) that encodes runt-related transcription factor 2 (RUNX2), also termed core-binding factor alpha1 (CBFA1), at chromosome 6p21 are the principal cause of CCD [2, 3]. The human RUNX2 gene encodes a 521 amino-acid length protein (GenBank: CAI19639.1) that contains a highly conserved 128-amino-acid region termed the “Runt domain” [4]. In addition, the RUNX2 protein contains an N-terminal stretch of glutamine/alanine repeats (Q/A domain) and a C-terminal proline-serine/threonine-rich (PST) domain [5, 6]. The RUNX-binding site is the element binding to the DNA sequence and may regulate several bone-related genes [7].

RUNX2 is the master gene of osteoblast differentiation and also controls chondrocyte maturation [8]. The RUNX2 protein is essential for osteoblastic differentiation and skeletal morphogenesis. RUNX2 binds DNA both as a monomer or, with more affinity, as a subunit of a heterodimeric complex, and behaves as a
scaffold for nucleic acids and regulatory factors involved in skeletal gene expression. Variant transcripts that encode different protein isoforms result from the use of alternate promoters as well as alternate splicing [7]. RUNX2 binds to the core site, 5'-PYGPYGTT-3', that is present in the promoter regions of a number of genes, including osteocalcin, osteopontin, bone sialoprotein, and alpha 1(I) collagen. RUNX2 participates in both intramembranous and endochondral ossification. Endochondral ossification is characterized by formation of cartilage model that is later replaced by bone, and accounts for most skeletal development [9]. By contrast, intramembranous bone develops directly from osteoblastic action and is limited to the cranial bones, some facial bones, and parts of the mandible and clavicle [10]. The two mechanisms of bone formation, intramembranous and endochondral ossification, are necessary to form the clavicular anlagen in the clavicle [11]. Hence, RUNX2 haploinsufficiency accounts for the distinctive bone dysplasia that is limited to the cranium and clavicle.

To date, fewer than 90 RUNX2 mutations have been described in subjects with CCD, including insertions, deletions, nonsense, and missense mutations, and CCD has been reported in people of Mongoloid ethnicity including Japanese, Korean and the Chinese living in China and Taiwan [12-33]. In most cases mutations occur in the runt domain [3, 5]. Here we report a patient with a typical clinical manifestation of CCD with a novel mutation in the RUNX2 gene that leads to a truncated RUNX2 protein.

**Case report**

We evaluated a 52 year-old female with a history of childhood onset disproportionate short stature and skeletal dysplasia. She was 153 cm in height and 40 kg in weight. Her face was unusual and showed features of hypertelorism, depressed frontal area, protrusion of chin and supernumary teeth. Radiological examination revealed marked shortening of clavicles and depressed skull bone (Figure 1A-C). There were no other family members with similar clinical
Figure 2. A. There was no similar skeletal disorder in the family. B. Partial sequence chromatograms of the RUNX2 gene revealed a single base deletion at nucleotide position 476 in exon 3 (c.476 del G, p.G159fsX175). C. Schematic representation of wild-type and mutant alleles, including mature mRNA sequences and predicted proteins, were shown. The mutant allele with single base deletion at nucleotide position 476 generated an abnormal 5’ splice site at codon 175, leading to early termination and protein truncation with loss of 347 amino acid residues. D. Summary of mutation spectrum of RUNX2 gene mutations among Han-Chinese with cleidocranial dysplasia was displayed.
characteristics (Figure 2A). The clinical evaluation was most consistent with CCD, and thus we analyzed her RUNX2 gene. The study protocol was approved by institutional review board of the hospital, and informed consent was obtained from the patient.

Genomic DNA was extracted from peripheral whole blood. Genomic DNA was used to amplify exons 1 through 8 and the flanking intronic sequences of the RUNX gene using eight pairs of PCR primers that were designed as previously described [6]. Purified PCR products were sequenced in both directions at our on-site biochemistry sequencing facility using Big Dye Version 3.1 and a 3730 XL sequencer (Applied Biosystems, Foster City, CA).

The patient was found to be heterozygous for single-base deletion (c.476 del G, p.G159fs17-5X) in exon 3 of RUNX2, which predicts a termination site at the 159th codon and leads to a truncation in the runt domain of RUNX2 protein (Figure 2B, 2C).

**Discussion**

Several lines of evidence indicate that the deletion G in position 476 is related causally to CCD in this patient. 1) c.476 del G residue in the exon 3 is evolutionally conserved in human, rat and mouse RUNX2 genes; 2) the c. 476 del G mutation, results in a frame shift and premature termination at amino acid 175, which is predicted to lead to a markedly truncated protein that lacks the NLS and the PST domain. Other missense or truncated mutations in the carboxyl end of RUNX2, such as T420I, can cause CCD [6]. Hence, a truncated protein that lacks 347 amino acid residues and would certainly be expected to be inactive.

**RUNX2** mutations are scattered throughout the entire gene and include deletions, insertions, or missense mutations. However, most mutations occur in the runt domain and missense mutations are the most common. Loss of the runt domain is expected to abolish ability of the protein to bind DNA [3]. The runt domain has the ability to mediate DNA binding and protein heterodimerization [26]. The C-terminal PST domain is suggested to be the transcription activation domain and is involved in functional interactions with various other transcription factor. Besides, a nuclear-localization signal (NLS) is located at the junction of the runt and PST domains and is a short basic stretch with nine amino acids [amino acids 221-229 in RUNX2]. It is necessary for nuclear localization of the protein [7, 26, 34].

Mundlos et al demonstrated that RUNX2 mutations segregate with the CCD phenotype and found that heterozygous loss of function is sufficient to induce the characteristic clinical findings [11]. However, there is a wide spectrum of phenotypic variability, from primary dental anomalies to complete CCD. Lou et al. suggested that there is a critical gene dosage requirement for the formation of intramembranous bone formation during embryogenesis and a decrease to 70% of wild-type Runx2 levels will result the CCD syndrome. There is a strong relationship between the phenotype of CCD and quantitative reduction in the functional activity of RUNX2 [35]. The RUNX2 mutation that we identified in this study is novel and differs from the mutations that have been previously described in Chinese patients with CCD [14, 17-33] (Figure 2D), indicating that there is significant variability in RUNX2 mutations in this population.

In conclusion, the symptoms in this patient are due to c. 476 del G mutation of RUNX2 gene. To our knowledge, it is a novel mutation which was not reported before.

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**Disclosure of conflict of interest**

None.

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RUNX2 gene and cleidocranial dysplasia