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Case Report

Novel mutation of RUNX2 gene in a patient with cleidocranial dysplasia

Ya-Wun Guo[1,2], Chih-Yang Chiu[3], Chien-Lin Liu[4,5], Tjin-Shing Jap[2,5], Liang-Yu Lin[2,5]

[1]Department of Internal Medicine, Taipei City Hospital, Zhongxing Branch, Taipei, Taiwan; [2]Division of Endocrinology and Metabolism, Department of Medicine, Taipei Veterans General Hospital-Taipei, ROC. 112, Taiwan; [3]Department of Pathology and Laboratory Medicine, Taipei Veterans General Hospital-Taipei, Taiwan; [4]Department of Orthopaedics and Traumatology, Taipei Veterans General Hospital, Taipei, Taiwan; [5]Faculty of Medicine, National Yang-Ming University School of Medicine, ROC. 112, Taiwan

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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 are as a monomer or, with more affinity, as a subunit of a heterodimeric complex, and behaves as a

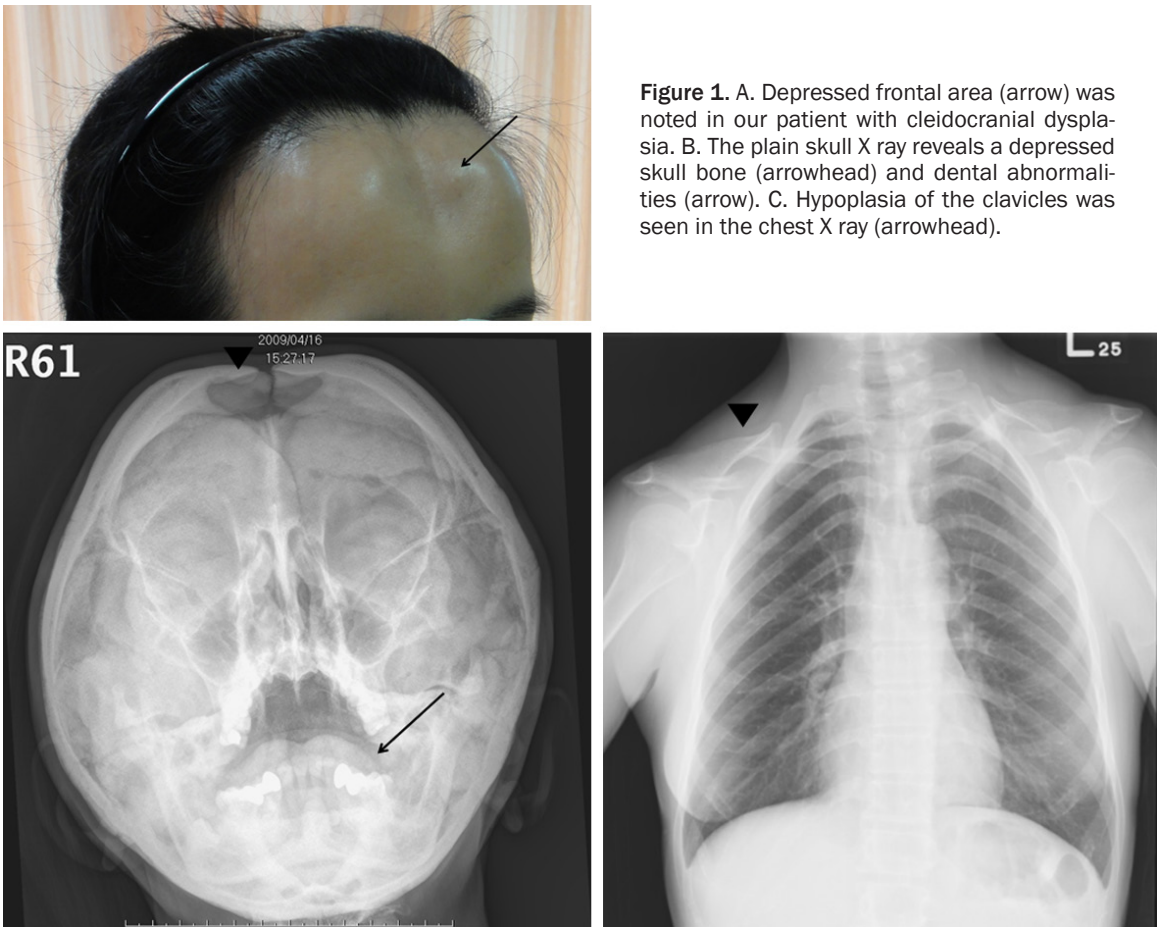

Figure 1. A. Depressed frontal area (arrow) was noted in our patient with cleidocranial dysplasia. B. The plain skull X ray reveals a depressed skull bone (arrowhead) and dental abnormalities (arrow). C. Hypoplasia of the clavicles was seen in the chest X ray (arrowhead).

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'-PYGPYGGT-3', that is present in the promoter regions of a number 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* haploin sufficiency 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 proportionate 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

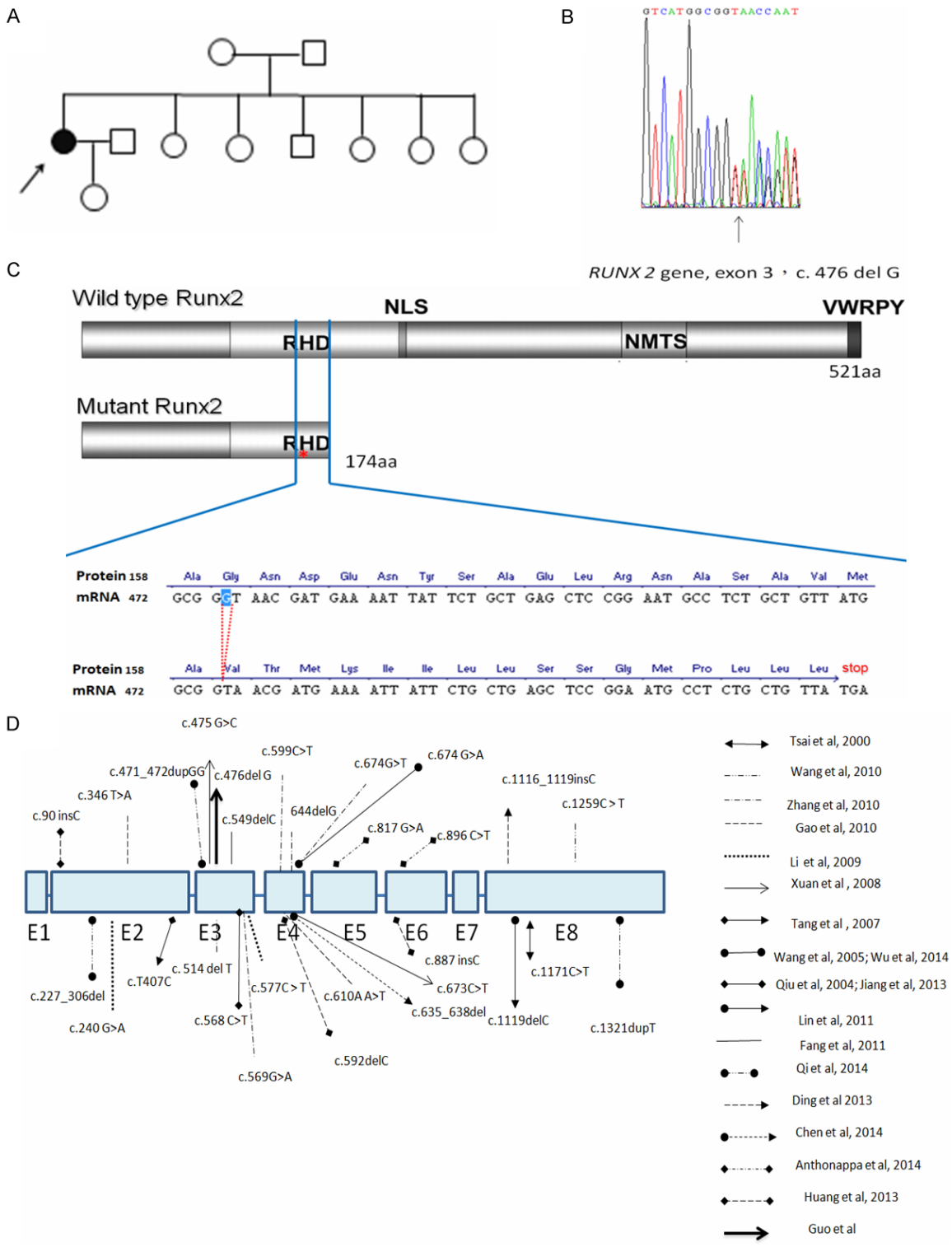

Figure 2. A. There was no similar skeletal disorder in the family. B. Partial sequence chromatograms of the *RUNX2* gene revealed a single base deletion at nucleotide position 476 in exon 3 (c.476 del G, p.G159fs175X). C. Schematic representation of wild-type and mutant alleles, including mature mRNA 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.

Address correspondence to: Dr. Liang-Yu Lin, Division of Endocrinology and Metabolism, Department of Medicine, Taipei Veterans General Hospital, Taipei, 112, Taiwan. E-mail: linly@vghtpe.gov.tw

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