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Original Article

A novel small deletion mutation in *RUNX2* gene in one Chinese family with cleidocranial dysplasia

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Abstract: Cleidocranial dysplasia (CCD) is a skeletal dysplasia with autosomal-dominant inheritance. The runt related transcription factor 2 (RUNX2) gene is the only gene in which mutations are known to cause CCD. We report identification of a novel small deletions mutation in the RUNX2 gene in a Chinese family with CCD. A 29-year-old female was diagnosed as proband of CCD based on the clinical findings, which show delayed closure of the fontanels, hypoplastic or aplastic clavicles and dental anomalies. Similar dental and skeletal symptoms were also observed in the other three affected individuals. We prepared genomic DNA from all four affected individuals, unaffected individual from her family members, as well as 100 unrelated healthy controls. PCR was conducted using the above genomic DNA as template and the RUNX2 gene-specific primers. The PCR product was subjected to direct sequencing and the sequence was compared to that of RUNX2 gene within the NCBI database. We detected a small deletion CCTA from nucleotide 635 to nucleotide 638 in exon 3 of RUNX2 gene of the proband. This will lead to the introduction of a translational stop codon at codon 220, resulting in a truncated RUNX2 protein, and therefore within the runt domain of the RUNX2 protein. We detected the same mutation in the the other three affected individuals, and did not detect any mutation in the unaffected family members or the 100 unrelated healthy controls, demonstrating that this is a novel missense mutation in RUNX2 gene and therefore, contributes to the molecular diagnosis of CCD.

Keywords: Cleidocranial dysplasia, RUNX2 gene, mutation

Introduction

Cleidocranial dysplasia (CCD; OMIM 119600) is a skeletal dysplasia with autosomal-dominant inheritance, which is characterized by delayed closure of the cranial sutures, hypoplastic or aplastic clavicles, and multiple dental abnormalities [1]. Jackson [2] described an extensive family with CCD. Manifestations may vary among individuals in the same family. Mutations in Runt related transcription factor 2 (RUNX2, known previously as CBFA1) results in haploinsufficiency for the protein and are associated with classic CCD. The protein, runt-related transcription factor 2 (RUNX2) is a transcription factor involved in bone and cartilage development and maintenance. RUNX2 is located at chromosome 6p21 and contains nine exons. The runt-related transcription factor 2 (RUNX2) is a 521-residue protein encoded by three RUNX2 transcripts that differ in the 5'-untranslated region (UTR). RUNX2 is essential for the osteoblast differentiation during intramembranous as well as chondrocyte maturation during endochondral ossification [3]. RUNX2 contains an N-terminal stretch of consecutive polyglutamine and polyalanine repeats known as the Q/A domain, a runt domain, and a C-terminal proline/serine/threonine-rich (PST) activation domain. The majority of RUNX2 mutations in individuals with classic CCD affect the runt domain and most mutations are predicted to abolish DNA binding [4-7]. Although additional cases of heterozygous mutation in RUNX2 gene have been identified in nearly 500 families with CCD, including familial and sporadic cases during the past ten years [7, 8], mutational screening on RUNX2 gene is still far from saturation, and more novel mutations will be identified to enrich the insights into the molecular basis for patho-

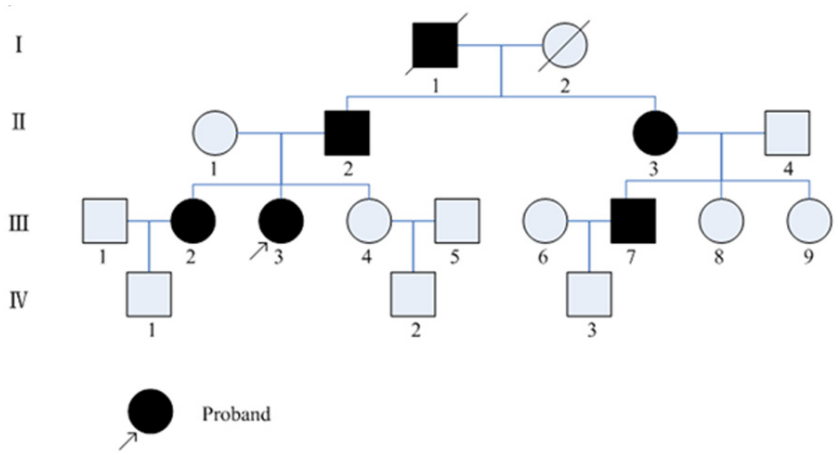

Figure 1. Pedigree of the family.

genesis of CCD. In this study, with one year collection, we obtained one family and six sporadic cases with CCD. By PCR and direct DNA sequencing, here, we report identification of a novel small deletion mutation in the RUNX2 gene in a Chinese family with CCD.

Materials and methods

Clinical and radiographic analysis of CCD

A family and six sporadic cases of CCD were obtained in the Department of Stomatology, Nanfang Hospital, China (**Figure 1**). Patients suspicious of having CCD were determined and selected by at least two experienced dentists. Dental anomalies, delayed closure of the cranial sutures, and hypoplastic clavicles from proband, affected family members and sporadic cases were subjected to radiographic exams. Diagnosis of CCD was based on the clinical and radiographic findings.

PCR and exon sequencing

PCR was performed using above genomic DNA as a template and the RUNX2 gene-specific primers. Details of primers and PCR conditions have been published elsewhere [6]. Specifically, for amplification of exon 3 and 6, the following primer pairs were used: for exon 3, sense 5'-TCATTGCCTCCTTAGAGATGC and antisense 5'-GGACATGAAAGTGACACTAAC; for exon 6, sense 5'- CTCTGGGAAATACTAATGAGG and antisense 5'- AGTGCCATGATGTGCATTTGTAAT. The 50 μl PCR reaction system contains 100 ng of genomic DNA, 10× ThermoPol buffer: 5 μl, primer pairs (20 μM): 1 μl×2, dNTPs (10 mM): 1 μl, DNA Taq Polymerase (Takara, Japan): 0.25 μl, add ddH2O to 50 μl. The PCR conditions were: DNA Taq Polymerase activation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s and extension at 68°C for 1 min, and the final extension was 68°C for 10 min. After amplification, the PCR products were purified using Qiagen QIAquick PCR Purification Kit (Hilden, Germany), and then sequencing was performed at Life Technologies, Inc, Shanghai.

Results

Clinical and radiographic examination

All the patients diagnosed as CCD show delayed closure of the cranial sutures, hypoplastic or aplastic clavicles, and multiple dental abnormalities. She was noted to the dental anomalies including irregular forms of dentition, unerupted teeth with supernumerary permanent teeth in the maxilla and mandible, malocclusion, retention of primary teeth, and eruption failure of permanent teeth. By clinical and radiographic examinations, her dental development (**Figure 2A**) was characteristically abnormal showing supernumerary teeth that were shed spontaneously and delayed eruption of permanent teeth. She had other craniofacial and other skeletal features. Craniofacial features (**Figure 2B**, **2C**) include delayed closure of the fontanelles and sutures, wormian bones, incomplete gasification of the frontal sinus and depressed nasal bridge. Other skeletal features (**Figure 2D-G**) include delayed ossification of the pubic symphysis and pubic bones, hypoplastic iliac wings, hypoplastic or aplastic clavicles, brachydactyly and joint laxity. Typical dental and skeletal features can be seen both at the other three affected individuals and at the proband. Overall, the clinical and radiographic characteristics of the cases supported the clinical diagnosis of CCD.

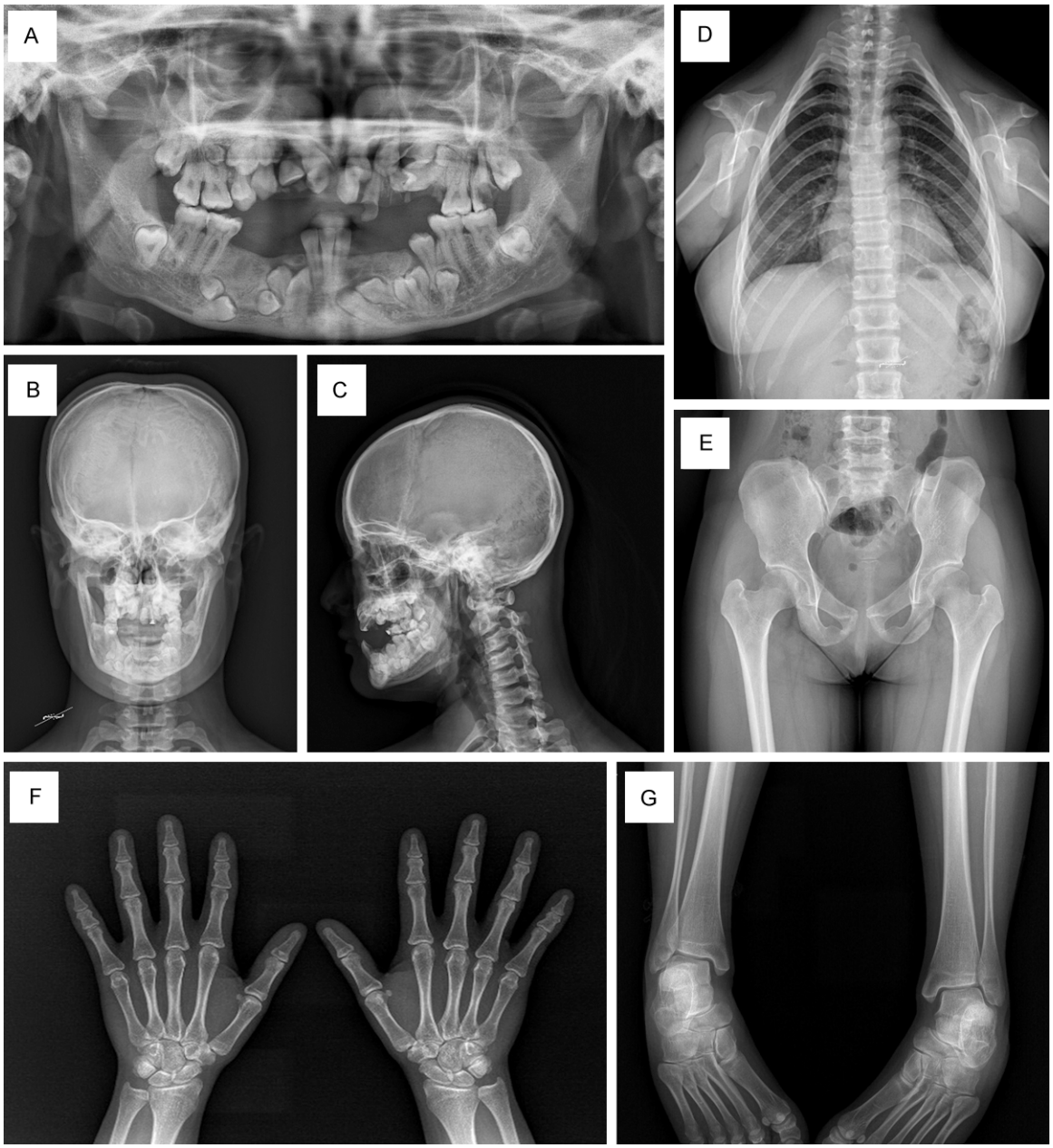

Figure 2. Radiological findings in the proband. (A) Panoramic view showing supernumerary teeth that were shed spontaneously and delayed eruption of permanent teeth. (B, C) Cephalometric radiograph showing delayed closure of the fontanelles and sutures, wormian bones, incomplete gasification of the frontal sinus and depressed nasal bridge. (D-G) Radiograph showing delayed ossification of the pubic symphysis and pubic bones, hypoplastic iliac wings, hypoplastic or aplastic clavicles, brachydactyly and joint laxity.

PCR and sequence analysis of RUNX2 gene

All the coding exons of RUNX2 gene were successfully amplified by PCR using corresponding exon-specific primers. Sequence comparison was conducted according to the NCBI database. We did not detect any mutation in exon 3 of healthy controls (**Figure 3E**), while a novel small deletion mutation in exon 3 (c.635-638delCCTA) was detected in a 29-year-old female proband (**Figure 3A**). This mutation will result in an amino acid change at codon 212 (P.Thr212Iso.), which is from an arginine codon (ACC) to a glycine codon (ATC). The small deletion/frameshift in the posterior of the exon 3 leads to the introduction of a translational stop

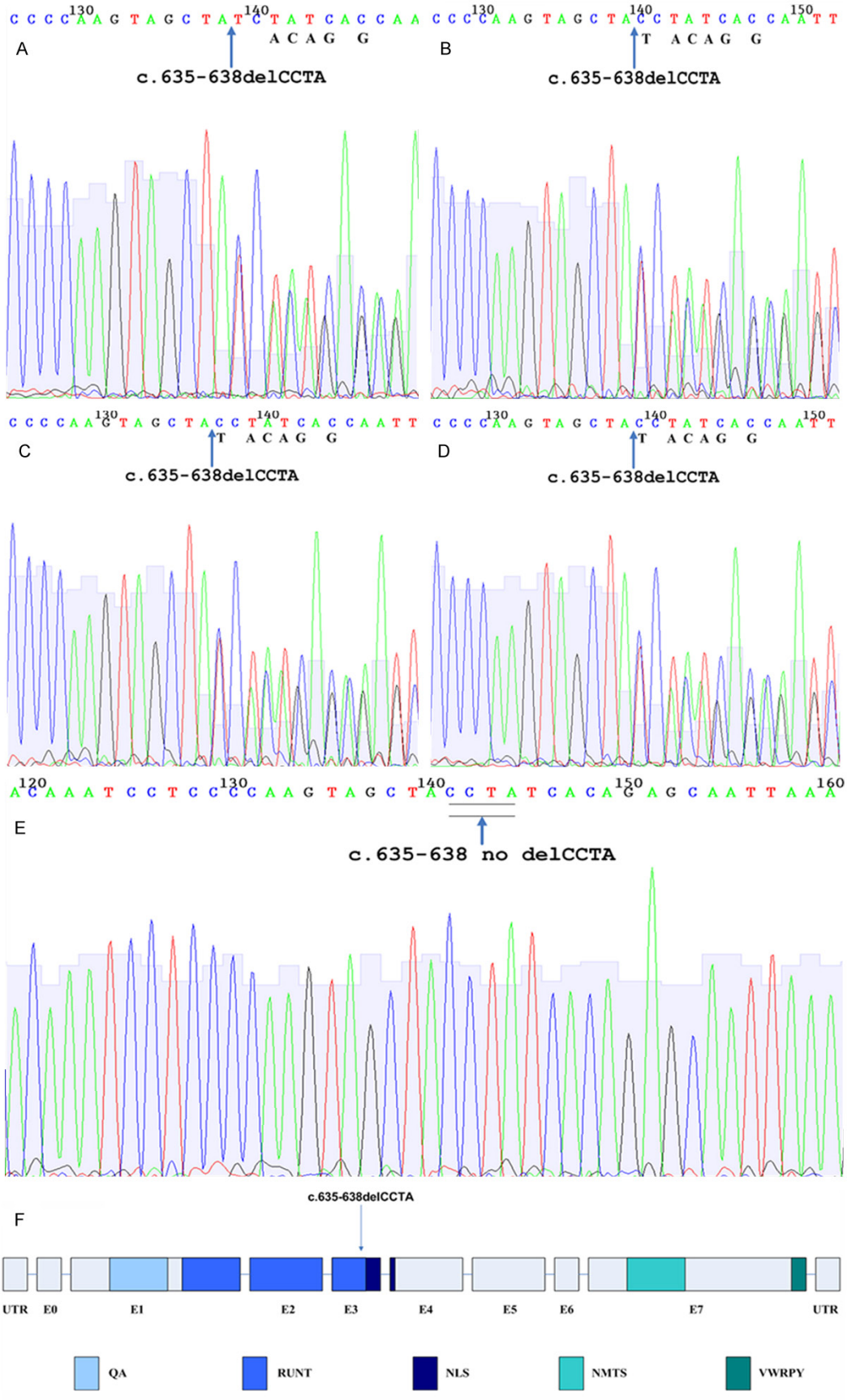
A
c.635-638delCCTA
B
c.635-638delCCTA
C
c.635-638delCCTA
D
c.635-638delCCTA
E
c.635-638 no delCCTA
F
c.635-638delCCTA
UTR
E0
E1
E2
E3
E4
E5
E6
E7
UTR
QA
RUNT
NLS
NMTS
VWRPY

Figure 3. Mutational analysis of exon 3 of *RUNX2*. (A-D) The sequences are from the proband (A), the father (B), the aunt (C) and the cousin (D) which show a new mutation (c.635-638delCCTA) in the runt domain. (E) The sequence is from the proband's normal sister. (F) The schematic shows functional domains and the mutation site (c.635-638delCCTA) identified in the present study. QA, glutamine and alanine repeats; RUNT, Runt domain; NLS, nuclear localization signal; NMTS, nuclear matrix targeting signal.

codon at codon 220, resulting in a truncated RUNX2 protein, and therefore within the runt domain of the RUNX2 protein. The same mutation was detected in the other three affected individuals in this family (**Figure 3B**, **3D**). No mutations were detected in non-affected family members and the one hundred healthy controls.

Discussion

Cleidocranial dysplasia was originally described as dento-osseous dysplasia affecting several individuals in a large pedigree. The term cleidocranial dysostosis has been used; however, given that RUNX2 has important functions both during skeletal formation and in bone maintenance, the disease is more correctly considered a dysplasia [9]. Multiple cases with Cleidocranial dysplasia have now been reported with most as sporadic cases. Cleidocranial dysplasia is inherited in an autosomal dominant manner. Jackson [2] described an extensive family with CCD. Posteriorly, other familial cases with Cleidocranial dysplasia suggesting autosomal dominant inheritance pattern were described in the literature [10-16]. These authors reported familial occurrence of Cleidocranial dysplasia in which father or mother and his or her son and daughter were affected, suggesting autosomal dominant inheritance. CCD affects most prominently those bones derived from intramembranous ossification, such as the cranium and the clavicles, although bones formed through endochondral ossification can also be affected. Our case, including six affected individuals in three generations of a family, presents typical dento-osseous appearance and clinical findings of CCD. The locus for CCD has been mapped to chromosome 6p21 where the responsible RUNX2 has been located [5]. At the genomic level, the longest RUNX2 transcript variant (NM_001024630.3) contains nine exons. Transcript variants that encode different protein isoforms [17] result from the use of alternate promoters as well as alternate splicing. Mutations in RUNX2 result in haploinsufficiency for the protein and are associated with classic CCD. RUNX2 contains a Q/A domain, a runt domain, and a C-terminal PST activation domain. The runt domain is a 128-amino-acid polypeptide motif originally described in the Drosophila runt gene that has the unique ability to independently mediate DNA binding and protein heterodimerization [18].

In this study, we performed a direct DNA sequencing of RUNX2 gene in one familial and 4 sporadic cases of DSAP and one hundred unrelated healthy controls. We found a novel small deletion mutation (c.635-638delCCTA) in the affected individuals, while the normal sister did not carry the same mutation. The small deletion/frameshift in exon3 leads to the introduction of a translational stop codon at codon 220, resulting in a truncated RUNX2 protein at a length of 220 amino acids. The frameshift mutation caused the deletion of the nuclear-localization signal (NLS). RUNX proteins, in general, have an NLS at the C-terminal border of the Runt domain. Since NLS is necessary for accumulation of RUNX2 protein in the nucleus, it was supposed that mutations that deleted NLS or located in the NLS might affect the subcellular distribution of RUNX2 [19]. The mutation is located in the carboxy terminus of the runt domain of RUNX2 protein, which has been frequently identified in CCD patients [7, 8, 20].

In summary, we detected a novel small deletion mutation in exon 3 of RUNX2 gene of the proband. This will result in an amino acid change at codon 212 (P.Thr212Iso.), and the introduction of a translational stop codon at codon 220, resulting in a truncated RUNX2 protein at a length of 220 amino acids, which will further our understanding of the pathogenesis of CCD and help with the molecular diagnosis of CCD.

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Disclosure of conflict of interest

None.

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