Phenotypic Manifestations in Cleidocranial Dysplasia with *RUNX2* Frameshift Variant

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Abstract

Cleidocranial dysplasia is a rare autosomal dominant disorder. It is mainly characterized by hypoplastic clavicles, delayed fontanelle closure, and a large foramen magnum with dental abnormalities. The condition is caused by variants in the RUNX2 gene located on chromosome 6p21.1. Currently, there remains a knowledge gap about oro-dental features in patients with cleidocranial dysplasia caused by the RUNX2 gene. The study aimed to identify the genetic variants associated with cleidocranial dysplasia and characterize the phenotypic manifestations associated with those variants. A patient with cleidocranial dysplasia was selected from the Faculty of Dentistry at Chulalongkorn University. Genomic DNA was extracted from the patient's peripheral blood leukocytes. Variant analysis was conducted using exome and Sanger sequencing. Clinical and radiographic examinations were used to characterize dental, craniofacial, and skeletal features. Medical and dental histories were acquired from the hospital record. The patient showed typical oro-dental characteristic features of cleidocranial dysplasia, like multiple unerupted teeth, including supernumerary teeth, retained deciduous teeth, caries, malocclusion, and partial edentulism. Other features included short stature, metopic depression, maxillary hypoplasia, protruding chin, wormian bones, frontal bossing, narrow thorax, and brachydactyly with brachymetatarsia on the 4th toe of the left foot. Exome sequencing revealed a heterozygous frameshift variant, c.739delA *(*p. *(*Ser247Valfs*3*))*, in exon 6 of *RUNX2 (*SCV001763563*)*. In conclusion, we can say that subjects with cleidocranial dysplasia suffer from multiple oro-dental anomalies. This can result in functional and aesthetic complications. Early diagnosis and a multidisciplinary approach are crucial to improving their quality of life.

Keywords: *Cleidocranial Dysplasia, RUNX2 Gene, Phenotypic Manifestations, Oro-dental Characteristics*

1. Introduction

Cleidocranial dysplasia (CCD; OMIM #119600) is an autosomal dominant disorder caused by pathogenic variants in the *RUNX2* gene located on chromosome 6p21.1. The medical condition was first described by Martin in 1765. Later, the condition was also termed 'cleidocranial dysostosis' by Marie and Sainton in 1897. Alternative titles for this condition include mutational dysostosis and Marie-Sainton disease (Singh et al., 2014). The term 'cleidocranial dysplasia' originates from three Greek words: 'cleido' (collar bone), 'kranion' (head), and 'dysplasia' (abnormal formation). It was thought at first that the disorder involved only the bones of intramembranous origin, like the bones of the skull, clavicles, and flat bones. However, it was established later that the bones of endochondral ossification can also be affected by this condition (Pan et al., 2017).

As the name suggests, the most obvious characteristic features are found in the head area and clavicles. Clinical features may vary from the classic CCD triad of aplastic or hypoplastic clavicles, delayed closure of the fontanelle, and dental abnormalities to isolated dental abnormalities. Cases with classic CCD spectrum disorder usually present with excessively large, wide-open fontanelles at birth and may remain open for the rest of their lives. The patients present with narrow, sloping shoulders that may get opposed at the

midline, secondary to clavicular hypoplasia (Ramalingam et al., 2023). Other features may include short ribs and/or scapula, large foramen magnum, bossing of frontal, occipital, and/or parietal bone, short stature, as well as other skeletal malformations like brachydactyly, short middle phalanges of the second and fifth fingers, cone-shaped phalangeal epiphyses, long second metacarpal, scoliosis, kyphosis, a broad femoral head with a short femoral neck, wide pubic symphysis, etc. Dental abnormalities include delayed eruption of primary and/or a permanent tooth, enamel hypoplasia, retention cysts, and supernumerary teeth. Radiographic features may include the presence of supernumerary teeth, parallel ramus, a slender pointed coronoid process, a round condylar process, a U-shaped sigmoid notch, and patent mandibular symphysis. It may also come with other systemic manifestations, such as respiratory distress in early infancy, retarded growth, syringomyelia, and deafness (Symkhampha et al., 2021). In some cases, endocrinologic features like osteopenia, osteoporosis, and vitamin D deficiency can also be found (Dinçsoy Bir et al., 2017).

Although learning disabilities and cognitive impairment are not common findings in cases with CCD, they can still be found in some cases (Cano-Pérez et al., 2022). Speech and language problems are also common findings. Abnormalities with articulatory placement may be found due to the presence of malocclusion, an anterior open bite, and a high-arched palate. Hypernasality can also be observed as a result of midface hypoplasia (Jirapinyo et al., 2020).

There are no definite guidelines for the dental management of CCD patients. The treatment plan varies based on the patient's age, craniofacial and oral characteristics, and social and economic circumstances. A multidisciplinary approach is needed to treat these patients, including pediatric dentists, oral and maxillofacial surgeons, periodontists, orthodontists, and prosthodontists (Inchingolo et al., 2021). Treatment options may include extraction of supernumerary and retained deciduous teeth, orthodontic traction of impacted permanent teeth (Morikava et al., 2016), orthodontic alignment, and fixed or removable prostheses (Patel et al., 2017).

Although many previous studies have reported cases of CCD, research specifically focused on oro-dental anomalies and the characteristics of unerupted teeth is scarce (Impellizzeri et al., 2018; Mabrouk et al., 2020; Shih-Wei Cheng et al., 2022). The current study is intended to fill this knowledge gap.

2. Objectives

1) To identify the genetic variants associated with CCD.
2) To characterize the phenotypic manifestations associated with that genetic variant.

3. Materials and Methods

A patient with CCD was recruited from the Faculty of Dentistry at Chulalongkorn University. Written informed consent was obtained from the participant. The study was approved by the Human Research Ethics Committee (HREC-DCU 2021-030, date of approval: July 9, 2021). The clinical criteria for diagnosis of CCD included a) aplasia or hypoplasia of the clavicles, b) prominent forehead, delayed closure of the anterior fontanelle or enlargement of the fontanelles, and/or c) prolonged retention of deciduous teeth, failure of eruption of permanent teeth, and presence of supernumerary teeth.

Three mL of peripheral blood leukocytes were collected from the patient to extract genomic DNA. Variant analysis was conducted using exome sequencing, followed by Sanger sequencing. Exome sequencing involved capturing genomic DNA with a SureSelect Human All Exon version 4 kit (Agilent Technologies, Santa Clara, CA, United States) and sequencing on a Hiseq 2000 Sequencer (Macrogen, Seoul, South Korea). The resulting sequence reads were aligned to the human genome reference sequence (University of California Santa Cruz (UCSC) hg19) with the help of the Burrows-Wheeler Alignment software (http://bio-bwa.sourceforge.net/). Downstream processing utilized SAMtools (http://samtools.sourceforge.net/) and

annotations via the dbSNP and 1000 Genomes. Afterwards, the variants were filtered based on a frequency threshold of 1% in the 1000 Genomes Project, the Genome Aggregation Database (gnomAD), and a local database of 2,166 Thai exomes. Causal variants were confirmed through Sanger sequencing. Prediction software, PolyPhen-2, SIFT, and MutationTaster, were employed to evaluate the possible pathogenicity of the variants based on the possible impact structure and function of the protein. The variants were classified following the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMGAMP) guidelines (Richards et al., 2015).

Radiographic examinations, including OPGs and CT scans, were used to identify the number and characteristics of the unerupted teeth. Other than clinical examination, radiographs such as skull anteroposterior (AP) view, lateral skull view, lateral cephalometry (CO position), and posteroanterior (PA) cephalometry were used to characterize craniofacial features. Radiographs taken from thoracolumbar spine AP view, pelvis AP view, and AP view of the feet and ankles were also examined to detect skeletal abnormalities. Medical and dental histories were obtained from the hospital records.

4. Results and Discussion

4.1 Results

The 28-year-old female patient came for dental treatment with the chief complaints of missing teeth and having chewing difficulty. The patient had a short stature and narrow, down-sloping shoulders. Her craniofacial features included metopic depression, hypertelorism, prominent orbital ridges, and a protruding chin (Figure 1A). From radiographic examinations, brachycephalic skull with widened cranial sutures, wormian bones, frontal and parietal bossing, concave frontonasal suture, hypoplastic nasal bone, zygomatic arch with downward bend, hypoplasia of the maxilla and maxillary sinuses, and mandibular prognathism with a slender pointed coronoid process and a narrow ascending ramus with nearly parallel anterior and posterior borders were also observed. Furthermore, calcification of the styloid ligament at both sides with elongated styloid processes was also detected.

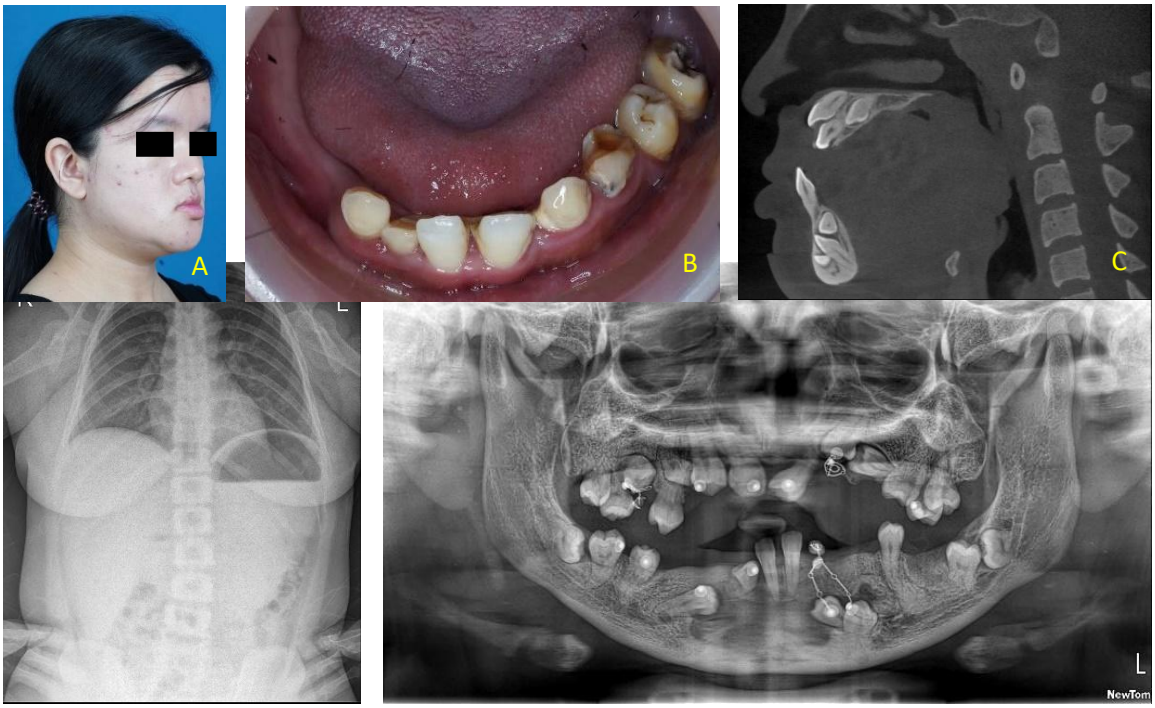

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Figure 1 Extra-oral photo showing hypertelorism, frontal bossing, hypoplastic maxilla, and mandibular prognathism (A) ; intra-oral photos showing hypoplastic mandibular edentulous ridge with multiple caries and retained deciduous teeth (B) ; CT scan with multiple unerupted teeth (C) ; chest x-ray showing hypoplastic clavicle, narrow thorax (D) ; and OPG after extractions and placement of orthodontic buttons (E)

Upon oral examination, 15 erupted teeth were found, among which 9 were permanent teeth (# 16, 14, 25, 26, 27, 36, 35, 31, 41) and six were retained deciduous teeth (# 52, 53, 73, 74, 82, 83). Caries was found on 10 teeth (# 16O, 14O, 53La, 25O, 26OM, 27O, 35O, 36OB, 74MDB, 73D), erosion was present on 3 teeth (# 53I, 52I, 74O), and 2 of the deciduous teeth showed mobility (# 63, 82). Other oral abnormalities included class III malocclusion, crossbite, partial edentulism with hypoplastic mandibular edentulous ridge, gingival inflammation, torus palatinus, macroglossia with scalloped tongue, and tongue tie (Figure 1B).

Panoramic radiographs revealed 30 unerupted teeth, including multiple supernumerary teeth. Most of these unerupted teeth were located in the anterior region and mainly in the mandible. Two teeth also showed dilaceration. CT scans were used to understand the orientation of the unerupted teeth (Figure 1C). Among the unerupted teeth, 13 were present in the maxillary arch, and 17 were in the mandibular arch. All of them were of similar anatomical shape as regular teeth, although some of them were smaller than normal. 21 of them were tilted mesially or distally, 3 were inverted, 1 was transversely oriented, and the rest were in normal orientation in the coronal plane. In the sagittal plane, buccal/labial and palatal/ lingual tilt were present in 21 and 3 of them, respectively; 1 of them was positioned mesio-distally, and 5 teeth had a normal orientation. 13 of them, were on the left side of the midline, and 17 of them were on the right side. None of them showed retention cysts.

Skeletal features included brachydactyly with brachymetatarsia on the 4th toe of the left foot. Upon radiographic examination, both clavicles showed hypoplasia on the lateral part. Moreover, spina bifida in the lower cervical spine and narrow thorax were found as well (Figure 1D). No other systemic conditions like pulmonary, cardiac, renal, gastrointestinal, hepatic, thyroid, or hematologic diseases were identified.

It was revealed from the exome sequencing that there was a heterozygous frameshift variant, c.739delA (p. (Ser247Valfs*3)), in exon 6 of *RUNX2* (SCV001763563). All the phenotypes present in the patient are summarized in Tables 1 and 2.

Table 1 Summary of the oral, craniofacial, and skeletal phenotypes

Features	Findings
Oral features	
Dentition	Permanent
Total number of teeth in the mouth	45
Number of unerupted teeth	30
Number of erupted teeth	15 (9 permanent, 6 retained deciduous)
Supernumerary tooth	Present
Caries	10 teeth
Erosion	5 teeth
Dilaceration	2 teeth
Malocclusion	Class III
Crossbite	Present
Gingival inflammation	Present
Macroglossia	Present
Tongue tie	Present
Torus palatinus	Present
Craniofacial features	
Metopic depression	Present
Hypertelorism	Present

Prominent orbital ridges	Present
Protruding chin	Present
Wormian bones	Present
Frontal bossing	Present
Maxillary hypoplasia	Present
Mandibular prognathism	Present
Styloid processes	Elongated
Skeletal features	
Hands	Brachydactyly
Feet	Brachymetatarsia on the 4th toe of the left foot
Clavicular hypoplasia	Present on the lateral part.
Spine	Spina bifida in the lower cervical spine
Other systemic conditions	none

Table 2 Characteristics of unerupted teeth

Characteristics	Number of unerupted teeth
Arch	
Maxilla	13
Mandible	17
Morphology	
Conical	0
Tuberculate	0
Supplemental	30
Positional variation in the coronal plane	
Normal	5
Oblique (Mesial/Distal)	21
Inverted	3
Transverse	1
Positional variation in the sagittal plane	
Normal	5
Buccal/ Labial	21
Palatal/ Lingual	3
Mesio-distal	1
Position related to the mid-sagittal plane	
Left	13
Right	17
Midline	0

The patient was treated with the extraction and surgical removal of 18 embedded teeth and the extraction of all the retained deciduous teeth, followed by the artificial eruption of 12 teeth using orthodontic buttons. The extraction of tooth number # 36 was also done later to create space for orthodontic treatment. All the surgical procedures were performed either under local anesthesia or under general anesthesia (Figure 1E).

4.2 Discussion

CCD is a rare genetic condition that is seen in 1/1,000,000 cases. It can be familial or sporadic without gender predilection. The responsible *RUNX2* gene is associated with the differentiation of osteoblasts

as well as with the signaling networks that regulate tooth initiation. Loss of function mutations of this gene end up causing delayed intra-membranous and endochondral ossification, which results in abnormal bone and cartilage formation (Shih-Wei Cheng et al., 2022).

In this case, the patient presented with short stature, metopic depression, prominent orbital ridges, hypertelorism, wormian bones, frontal bossing, maxillary hypoplasia, underdeveloped maxillary sines, narrow down-sloping shoulders, hypoplastic clavicles, and brachydactyly, consistent with previously reported cases, confirming these as the typical features of CCD (Adhikari et al., 2022; Xue et al., 2021). The characteristic dental features of CCD include multiple supernumerary teeth and retained deciduous teeth, both of which are seen in this case. The prolonged retention of deciduous teeth, multiple embedded teeth, malocclusions, and partial edentulism explain the cause of the chief complaint of difficulty chewing (Agarwal et al., 2023).

In cases of CCD, the dental lamina for the deciduous and permanent dentition remains normal. However, the succeeding dental lamina and the lamina for the permanent molars do not completely undergo apoptosis at the anticipated time. As a result, the remnants of the dental lamina become activated again and form the supernumerary teeth. Delayed or arrested eruption of the permanent teeth, even in regions with no supernumerary teeth, can be caused primarily by decreased bone resorption and reduced resorption of the deciduous tooth roots, and secondarily due to the presence of multiple supernumerary teeth in that region. The ectopic location of the permanent teeth may result either from migration due to an arrested eruption or interference with supernumerary teeth in the eruption pathway (Kreiborg, & Jensen, 2018).

Management of a case with CCD requires multidisciplinary approaches (Shekhawat, & Gulati, 2020). The clinical features of CCD can be identified in early childhood. Furthermore, with the help of ultrasound investigations, the identification of CCD can be done even in the prenatal period (Gong et al., 2022). Diagnosing CCD at an early stage is important, as combined orthodontic-surgical management can ensure the proper development of the maxilla (Li et al., 2016). In this patient, the artificial eruption of some of the permanent teeth was done, as it is well established that only extraction of primary teeth may not ensure the eruption of permanent teeth (Dhobley et al., 2023).

The most significant part of this study is that it is the first study in our knowledge to describe the characteristics of unerupted teeth in a patient with CCD. All the unerupted teeth found in this case were supplementary. Furthermore, almost all the unerupted teeth showed some degree of tiltation, irrespective of direction. However, the study was conducted with only one patient. Further studies with larger sample sizes should be done to establish these features as the characteristic features of the unerupted teeth in CCD.

5. Conclusion

CCD manifests its characteristic features at an early age. They are often prone to malocclusion, retained deciduous teeth, and impaction and, as a result, develop caries, periodontal diseases, and other functional and aesthetic complications. In order to take effective preventive and interceptive measures, it is crucial to diagnose CCD at an early stage. Providing multidisciplinary treatment to patients can improve their function, aesthetics, and quality of life.

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