Fetal and Pediatric Pathology

ISSN: 1551-3815 (Print) 1551-3823 (Online) Journal homepage: https://www.tandfonline.com/loi/ipdp20

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To cite this article: Jamshid Motaei, Arash Salmaninejad, Ebrahim Jamali, Imaneh Khorsand, Mohammad Ahmadvand, Sasan Shabani, Farshid Karimi, Mohammad Sadegh Nazari, Golsa Ketabchi & Fatemeh Naqipour (2020): Molecular Genetics of Cleidocranial Dysplasia, Fetal and Pediatric Pathology, DOI: 10.1080/15513815.2019.1710792

To link to this article: https://doi.org/10.1080/15513815.2019.1710792

Published online: 26 Jan 2020.

FETAL AND PEDIATRIC PATHOLOGY
https://doi.org/10.1080/15513815.2019.1710792

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REVIEW

Molecular Genetics of Cleidocranial Dysplasia

Jamshid Motaei[a], Arash Salmaninejad[a,b], Ebrahim Jamali[c], Imaneh Khorsand[d], Mohammad Ahmadvand[e], Sasan Shabani[f], Farshid Karimi[g], Mohammad Sadegh Nazari[h], Golsa Ketabchi[i], and Fatemeh Naqipour[i]

[a]Department of Medical Genetics, Medical Genetics Research Center, Student Research Committee, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran; [b]Halal Research Center of IRI, FDA, Tehran, Iran; [c]Department of Biology, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran; [d]Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran; [e]Hematology-Oncology and Stem Cell Transplantation Research Center, Tehran University of Medical Sciences, Tehran, Iran; [f]Department of Optometry, School of Para Medical Sciences, Mashhad University of Medical Sciences, Mashhad, Iran; [g]Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran; [h]Postgraduate Student, Department of Orthodontics, School of Dentistry, Mashhad University of Medical Science, Mashhad, Iran; [i]Postgraduate Student, Department of Oral and Maxillofacial Radiology, School of Dentistry, Mashhad University of Medical Science, Mashhad, Iran

ABSTRACT

Background: Cleidocranial dysplasia (CCD) is a genetic disorder with an autosomal dominant inheritance pattern. CCD characterized by abnormal clavicles, patent sutures and fontenelles, supernumerary teeth and short stature. Approximately 60-70% of CCD patients have mutations in the RUNX2 gene. The RUNX2 gene is an essential transcription factor for chondrocyte maturation, osteoblast differentiation and bone formation. Runx2 regulates mesenchymal cell proliferation in sutures and suture closure by inducing the signaling pathways of the genes of Fgf, Pthlh, hedgehog and Wnt. **Material and Methods:** We summarized molecular genetics aspects of CCD. **Result:** Approximately 94% of CCD patients have dental anomalies, the most common of which are supernumerary tooth. Dental anomalies are not determined solely by gene mutations of RUNX2, but are also affected by modifier genes, environmental factors, epigenetic factors and copy number variations. **Conclusion:** a definite diagnosis of CCD should include the patient's clinical history, symptoms and signs, as well as genetic analyses.

ARTICLE HISTORY
Received 29 November 2019
Accepted 16 December 2019

KEYWORDS
Cleidocranial dysplasia;
RUNX2;
supernumerary tooth

Introduction

Cleidocranial dysplasia (CCD) is a genetic disorder with an autosomal dominant pattern. It was first named in 1898 by Marie and Saintion [1]. The frequency of CCD is about one in one million people [2]. The disorder is caused by mutations in the RUNX2 gene on chromosome 6p21. The RUNX2 gene is essential for the differentiation of stem cells into osteoblasts, so mutations may cause defective bone formation. In CCD patients,

CONTACT Fatemeh Naqipour Naqipour.fn@gmail.com Postgraduate Student, Department of Oral and Maxillofacial Radiology, School of Dentistry, Mashhad University of Medical Science, Mashhad, Iran; Golsa Ketabchi Dr.gketabchi@gmail.com Postgraduate Student, Department of Oral and Maxillofacial Radiology, School of Dentistry, Mashhad University of Medical Science, Mashhad, Iran.

severe abnormalities in bone growth and development have been observed. Diagnosis of this syndrome often occurs at birth based on chest and skull anomalies, particularly aplasia or hypoplasia of clavicles and the patent fontenelles and the genetic testing of the RUNX2 gene. A multifunctional approach is needed to treat the disease, including oral and maxillofacial surgery, tooth extraction, orthodontics, prosthesis, and more [3].

In this article, we describe the clinical features of CCD with the study of the structure and function of RUNX2 gene in bone formation and supernumerary teeth. In addition, the correlation between phenotype-genotype of RUNX2 mutations in CCD and its association with other syndromes has been investigated.

Clinical and radiological features

Cleidocranial dysplasia (CCD) is associated with severe abnormalities in bone development. The main symptoms of the disease include hypoplasia, aplasia or lack of clavicles, sloping shoulders, open fontenelles, delayed bone formation of the skull, short stature and supernumerary teeth. Afflicted people with a lack of clavicle can put their shoulders close together [4]. Dental disorders include supernumerary teeth, maxillary hypoplasia, follicular cysts in the jaw, delayed tooth eruption, delay in the development of the root of permanent teeth and delay in the absorption of the roots of deciduous teeth [5,6]. Other characteristics of CCD are hypertelorism, hypoplasia the anterior part of the face, a short nasal bridge, and narrow thorax. Patients' intelligence is usually normal, but in some cases, deafness has been mentioned [7,8]. The presence of anomalies in the clavicle bones has been considered in several studies as a clinical finding of this syndrome. Complete recognition of clinical symptoms and differential diagnosis of CCD from other diseases with similar clinical symptoms is necessary [9]. When the clinician suspects CCD, a skeletal survey and genetic analysis should be obtained. There are several therapeutic options for CCD. To address dental problems, extensive and comprehensive dental treatments such as extraction of teeth, surgery, and orthodontics are necessary to maintain proper mastication [10].

If bone density is less than normal, calcium treatment and vitamin D supplementation should be initiated for early prevention of osteoporosis in young people. If necessary, an invasive treatment for recurrent infections of the middle ear and sinus may be needed. If the skull has a significant structural defect, then risk of injury to the skull is high when hit and can be protected by surgery and a protective helmet. Clinical manifestations and radiographic findings are commonly used to diagnose the disease. If the final diagnosis is not possible based on clinical manifestations and radiographic findings, genetic analysis is required [8].

RUNX genes

RUNX proteins are important transcription factors that contribute to a wide range of biologic processes. The RUNX1, RUNX2, and RUNX3 heterodimeric proteins are all three family members of the Runt-related transcription factors that have a DNA-binding α subunit and a non-DNA-binding β subunit. These proteins have a domain Runt with 128 amino acids. Each RUNX gene has two isoforms of the N-terminal. In general, invertebrates have a RUNX gene that is similar to the RUNX3 gene in the vertebrate, but insects like Drosophila melanogaster have four RUNX genes. The vertebrates have three genes [11].

Thirty percent of patients with acute myeloid leukemia (AML) and ten percent of patients with myelodysplasia (MDS) have mutations in the RUNX1 gene [12]. RUNX1 plays an important role in hematopoietic cells. Hereditary mutation in RUNX1 causes familial platelet disorder with predisposition to myeloid malignancy (FPD/AML) with autosomal dominate inheritance pattern [13]. RUNX2 plays an important role in the development of the skeletal system and the morphogenesis of other organs, such as thyroid and breast. The role of RUNX2 is increasingly recognized in various cancers, including thyroid, prostate, lung and breast cancer. Many studies have shown that the deregulation of RUNX2 is associated with the progression and metastasis of various tumors [14–17]. RUNX3 is a tumor suppressor gene that plays a role in various biological processes, including development of the cranial and dorsal root ganglia, gastrointestinal tract and T-cell differentiation. Mutations in RUNX3 have been reported in various diseases including colon and gastric cancers, glioma, melanoma, prostate cancer, renal cell carcinoma and neural disorders [18].

The structure and function of the RUNX2 gene

The CDD disease is caused by mutations in a central binding sequence factor (CBFα1) gene, which was later renamed RUNX2. This gene is a transcription factor and an essential regulator of osteoblast differentiation and bone formation. Mutations may result in defective bone formation. Studies have shown that bone formation does not occur correctly if there is homozygous deletion of this gene in animals. As the knockout mice for the $RUNX2^{-/-}$ gene lack osteoblasts, and thus completely lack bone, heterozygous mice ($RUNX2^{+/-}$) showed abnormalities similar to CCD. In humans, heterozygous mutations in the RUNX2 gene lead cleidocranial dysplasia [19,20].

The RUNX2 gene on 6p21 has 8 exons spanning 222.76 kb and contains two distinct domains. The exons 1, 2, and 3 encode a DNA-binding domain called runt and exons 4,5,6,7 encode the activation and repression domains for transcription. All mutations which lead to the loss of function (haploinsufficiency) in the gene RUNX2 cause cleidocranial dysplasia (OMIM #119600) [11,14].

Transcription of the Runx2 gene is regulated by two promoters P1 (distal promoter) and P2 (proximal promoter), which result in two isoforms of mRNAs that differ in region 5′. Type I RUNX2 isoforms start are encoded by P2 promoter and type 2 RUNX2 isoform are encoded by P1 promoter [21]. Type I and II RUNX2 isoforms, starting with the N-terminal amino acid sequence MRIPVD and MASNSL respectively. The most abundant isoform of RUNX2 gene in osteoblasts is the type II isoform. Type I Runx2 is expressed extensively in T cells. The expression of this isoform of RUNX2 gene is also observed in osteoblasts and chondrocytes [22]. Type I and type II isoforms differ only in a small number of amino acids in the N-terminal. The enhancer sequence upstream of the P1 promoter regulates the expression of the RUNX2 gene in chondrocytes and osteoblasts, but both P2 promoter and enhancers regulate RUNX2 expression in thyroid and breast cancers [17,23].

Function of P1 and P2 promoters results in the production of RUNX2 mRNA with two untranslated regions (5′ -UTR1 and 5′ -UTR2). Both UTR1 and UTR2 are long and have a complex secondary structure which can potentially inhibit the translation based

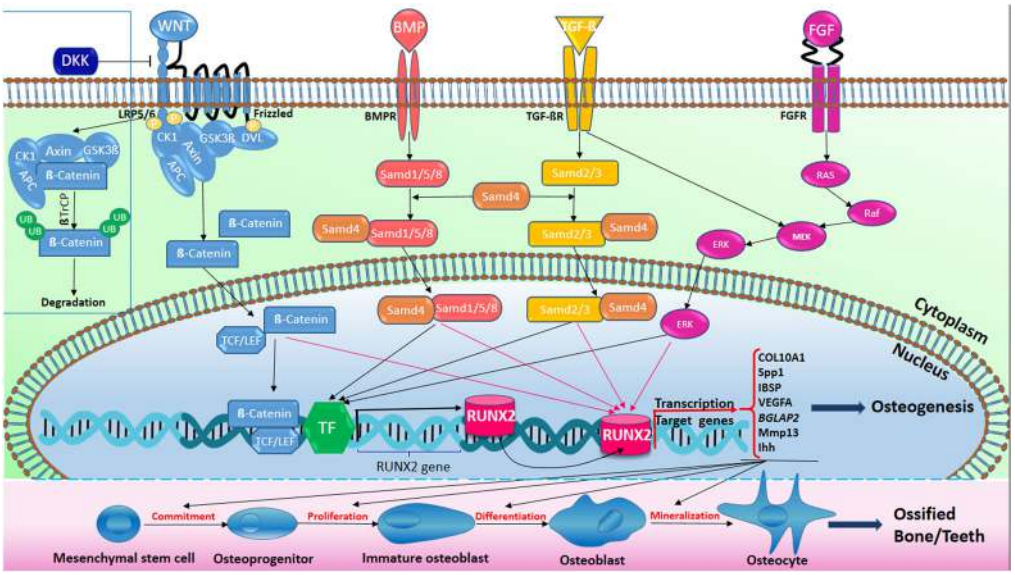

Figure 1. There are many signaling pathways involved in the development of skeletal bone formation. Several morphogenic growth factors such as transforming growth factor-beta (TGFβs), bone morphogenetic proteins (BMPs), fibroblast growth factors (FGFs), and Wnt ligand can induce these signaling pathways,modulate transcription factors and gene expression. Runx2 is a pivotal mediator of a variety of these signal pathways and is one of the most important transcription factors in the osteogenic process. Runx2, directly or indirectly, regulates the expression of a set of genes whose transcription induces osteoblast and chondrocyte differentiation during osteogenesis [27]. Some of these genes include, COL10A1, SPP1, Ihh, IBSP, MMP13, BGLAP2, and VEGFA, each may be expressed during distinct stages of differentiation. The Wnt pathway is one of major regulatory pathways involved in the regulation of Runx2 and active Runx2 via the stabilization and accumulation of β-catenin, which then moves to the nucleus and induces gene expression [28]. Besides the skeleton, Runx2 may play critical roles during tooth initiation, morphogenesis, and dental cell differentiation. Runx2 regulates the expression of molecules in mesenchymal tissues as well; acting reciprocally on the dental epithelium to control its growth and differentiation, partly explaining the dental abnormalities found in patients with cleidocranial dysplasia [29].

on "CAP". However, both regions contain IRES elements that regulate expression of RUNX2 in different cellular conditions. For example, IRES elements in the UTR1 and UTR2 regions increase RUNX2 translation under "Genotoxic" stress caused by mitomycin C during osteoblastic maturation [21]. The most important induction modification after translating is by phosphorylation. This phosphorylation is essential for RUNX2 activity that can be stimulated by several signaling pathways [24].

There are different signaling pathways that can activate the Runx2 transcription factor. Runx2 regulates various stages of mesenchymal stem cells (MSC) development, including commitment, proliferation, differentiation and mineralization, transforming MSC cells into mature osteocytes. These pathways, after binding their ligands, activate the mediators, which subsequently enhance the expression or activation of Runx2 and ultimately regulate the expression of genes associated with osteogenesis processes (Figure 1). Therefore, Runx2 plays a pivotal role in the formation, development and proper growth of bones and teeth. The mutation in this gene disrupts these signaling pathways, which can explain the cause of skeletal and dental anomalies observed in

patients with CCD [25–27]. Despite the numerous molecular pathways for the development and differentiation of mesenchymal cells into osteocytes, the Wnt/β-catenin signaling pathway plays an important role in this developmental process. The activation of the Wnt/β-catenin signaling pathway is carried out by Wnt proteins, which act as ligands for receptor Frizzled (Fzd) and co-receptor LRP5/6. In the absence of Wnt proteins or binding of inhibitors of this pathway such as DKK1, the β-catenin protein is phosphorylated by the GSK3 and CK1 enzymes of the APC, Axin, CK1 and GSK3 complexes. This phosphorylation leads to the recognition of β-catenin by β-Trcp and E3 ubiquitin ligase subunit and ultimately causes ubiquitination and proteinaseomal degradation of β-catenin protein. In the absence of phosphorylation, the stability and concentration of β-catenin in the cytoplasm increases, which can then migrate to the nucleus, and form a complex with TCF/LEF transcription factor family members. This complex leads to the expression of target genes, including RUNX2 [28,29].

RUNX2 gene plays an important role in osteoblast differentiation. The expression of Indian hedgehog (Ihh) in chondrocytes induces expression of RUNX2 in mesenchymal stem cells during the development of the endochondral bone. Then, Runx2, by inhibiting the differentiation of the mesenchymal stem cells into chondrocytes and adipocytes, induces them to osteoblast progenitors. In knockout mice for $Ihh^{-/-}$, osteoblasts and expression of Runx2 in perichondrium are completely absent [30]. Sp7, Runx2 and canonical Wnt signaling cause osteoblast progenitors differentiation into immature osteoblasts. Expression of Sp7 is regulated by Runx2. Osteoblast progenitors have the ability to differentiate into chondrocytes that are inhibited by canonical Wnt signaling and Sp7 (27). Notch signaling inhibits Runx2 through the Hes and Hey transcriptional inhibitors, as a result, with the proliferation of mesenchymal cells, their differentiation into osteoblasts are inhibited [31]. Runx2 expression decreases during osteoblasts maturation [32]. In the process of endochondral ossification, Runx2 plays an important role in the chondrocytes maturation. Sox5, Sox6 and Sox9 control the differentiation of mesenchymal cells into immature chondrocytes [27]. Overexpression of RUNX2 in transgenic mice increased the chondrocyte maturation and endochondral bone formation. While the expression of dominant-negative Runx2 in mice inhibited chondrocyte maturation and delayed endochondral ossification [33]. Therefore, Runx2 plays an important role in the development of chondrocytes from immature chondrocytes.

Animal models

In 1997, two researchers generated a mouse model for the mutated locus of RUNX2 (Cbfa1). Mice with homozygous mutations in RUNX2 died after a birth without breathing. The examination of their skeletal system revealed a complete absence of osteogenesis. Although osteogenesis was completely blocked throughout the body, but the development of the cartilage was almost normal. Therefore, RUNX2 is essential for maturation of osteoblasts in both endochondral and intramembranous ossification. Heterozygous mice ($Cbfa1^{+/-}$) showed skeletal abnormalities similar to cleidocranial dysplasia in humans. The heterozygote embryos of the mice had open fontenelles,

hypoplasia of clavicles and nasal bones, and delayed ossification of nasal bones and calvarial bones. Therefore, the two researchers suggested that the RUNX2 gene plays an important role in osteogenesis and osteoblast differentiation [19,20].

Reducing Runx2 by 30% in mice causes a defect similar to CCD in the development of the clavicles and calvaria [34]. It is unclear what mechanism Runx2 regulates suture closure and calvarial development. In one study, the posterior frontal (PF) and sagittal (SAG) sutures were compared between wild type and $Runx2^{+/-}$ mice [35]. $Runx2^{+/-}$ mice lacked cartilage formation in PF suture. The suture closure in PF and SAG was completely disrupted. In this study, the decrease in mesenchymal cell proliferation and the expression of signaling pathways genes of Fgf, Pthlh, hedgehog and Wnt in $Runx2^{+/-}$ sutures were observed in comparison with the wild type. The expression of these genes is directly regulated by Runx2. The ligand or agonist of these genes in $Runx2^{+/-}$ calvariae mice increased the proliferation of mesenchymal cells in sutures and bone formation. By contrast, the use of their antagonists inhibited the proliferation of mesenchymal cells in sutures and bone formation. Thus, Runx2 regulates mesenchymal cell proliferation in sutures and suture closure by inducing the signaling pathways of the genes of Fgf, Pthlh, hedgehog and Wnt.

Supernumerary teeth in cleidocranial dysplasia are related to permanent teeth. Probably because the mice are monophyodont, they did not have extra teeth in the CCD heterozygote model [36]. In a study in 2005, Runx2 knockout mouse, the development of molars in the late stage of the bud, especially for lower molars, was inhibited. The expression of the genes of the enamel knot marker, including P21, FGF4, EDAR, and BMP4, decreased in the lower molars but were normal in the upper molars. In Runx2 knockout mice, the expression of sonic hedgehog (Shh) was completely absent in the lower molars, while poor expression was observed in the upper molars. Therefore, RUNX2 is essential for shh signaling in the lower but not upper molars [37].

Wnt/β-catenin signaling plays an important role in the onset and development of the tooth and the differentiation of dental cells. Wnt ligands in the early morphogenesis of the teeth are expressed in the epithelium of the tooth [38]. The mandatory activation of Wnt signaling in the oral epithelium has led to the formation of extra teeth in the embryonic stage and maturation of the mice. WHile the high expression of Dkk1 (an inhibitor of Wnt) in the oral epithelium has led to the inhibition of the development of the tooth in the early bud stage [39,40]. During the development of the tooth, the Runx2 function is regulated by Fgfs, however, the bone development study showed that Wnt/β-catenin signaling positively regulates Runx2 [41,42]. In a 2018 study by Järvinen E et al., the effect of Wnt/β-catenin signal modulation on mouse models and ex vivo cultures were investigated on sequential formation of molar teeth. They indicated that increasing the activity of Wnt/β-catenin in dental mesenchyme inhibited the development of posterior molars and sequential tooth formation, whereas the reduction of mesenchymal Wnt/β-catenin signaling was associated with continuous tooth development. In addition, their results showed that heterozygous mutations in the AXIN2 and RUNX2 genes, which cause hypodontia and hyperdontia respectively, resulted from the modulation of the Wnt/β-catenin signal in the mesenchyme of the tooth. Runx2 transcription factor inhibits mesenchyme of Wnt/β-catenin inhibitors, including Dkk1 and Axin2 (29).

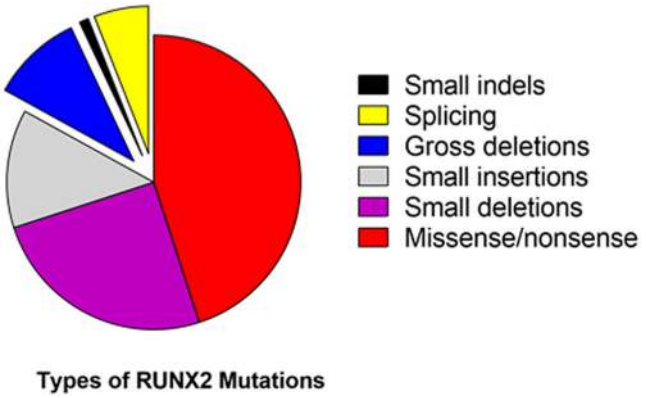

Figure 2. The percentage of different types of mutations in the RUNX2 gene in CCD: About 45% of the RUNX2 mutations form Missense /nonsense mutations. Other mutations in this gene are small deletions (25%), small insertions (13%), gross deletions (10%), splicing (6%), and indels (1%).

RUNX2 mutations and genotype–phenotype correlation

Approximately 60-70% of CCD patients have missense, nonsense, deletions and insertions mutations in the RUNX2 gene. Most of them occur in the Runt domain, which prevent the binding of this transcription factor (Runx2) to DNA [43,44]. To date, more than 184 mutations have been reported for RUNX2 on the human gene mutation database (HGMD), the most common of these mutations are missense/nonsense (Figure 2).

Due to the widely variable expressivity in CCD patients, there is a weak correlation between genotype and phenotype. For instance, Dan Ma [43] reviewed 183 CCD patients with missense and 25 CCD patients with nonsense mutations in the Runt domain of RUNX2, and compared five phenotypic categories, including hypoplastic clavicles, short stature, delayed closure of sutures or wormian bones, supernumerary teeth, and mid-face hypoplasia or mandibular hyperplasia. There was no significant difference between these phenotypes based on missense and nonsense mutations. Then, by comparing the clinical manifestations in 239 CCD patients based on position of nonsense and missense mutations in the Runt domain and non-Runt domain regions, there was a significant mutation rate for supernumerary teeth and mid-face hypoplasia or mandibular hyperplasia in the Runt domain but not the non-Runt domain regions. Therefore, they suggested the importance of Runt domain in craniofacial and dental development [43]. In another study, correlation between Runt domain mutations and developmental teeth abnormalities was observed in CCD patients [45]. Therefore, there was no significant relationship between phenotype type and mutation type in CCD patients, whereas there was correlation between phenotype type with mutation locations in RUNX2 gene. On the whole, diagnosis of CCD based on clinical and radiographic findings is not a challenge. However, among members of an affected family, they may show variable manifestations, such as dental abnormalities and craniofacial dysplasia [43].

Relationship between RUNX2 and others diseases

Although RUNX2 is mainly expressed in osteoblasts, a few studies have shown that RUNX2 is also expressed in non-skeletal systems [46,47]. Complete recognition of

clinical symptoms and differential diagnosis of CCD from other diseases with similar clinical symptoms is necessary. CCD predominantly affects the skeletal and dental system. In one study described a patient with late-onset limb girdle myopathy, an uncommon phenotype in CCD, due to a heterozygous missense mutation (c.G674A, p.R225Q) in the RUNX2 gene [48]. Therefore, patients with myopathy who have skeletal or dental abnormalities and facial dysmorphism, in order to avoid misdiagnosis and inappropriate treatment, in addition to the patient's clinical history, signs and symptoms, genetic analysis should be performed. In another study, three CCD patients in three families for more than three generations were misdiagnosed as rickets. No mutation was detected for the RUNX2 gene with sanger sequencing, but a reexamination with qPCR and multiplex ligation-dependent probe amplification (MLPA) revealed a novel deletion in exons 1-3 of the RUNX2 gene. Because of the similar physical appearance and very low frequency of CCD, all three patients were reported as rickets and had been treated with vitamin D for many years [49]. Aplasia or hypoplasia of clavicles and the patent fontenelles are not specific sign to the CCD and may be seen in other syndromes or disorders. Reducing bone mineralization may cause patent sutures and open fontenelles in disorders like rickets, hypophosphatasia, and osteogenesis imperfecta [4].

However, the abnormal arm and shoulder mobility commonly observed in CCD can be a symptom of other conditions, particularly Ehlers-Danlos syndrome hypermobility type (EDS-HT). EDS-HT is characterized by joint laxity with musculoskeletal complaints, soft tissue overuse injury and often semitransparent skin [50], but does not have additional specific clinical features and cannot be diagnosed through laboratory tests. Unfortunately, in the patient with EDS-HT no genetic defect has been found [50]. There are no additional specific clinical features or laboratory tests to diagnose EDS-HT. Bedeschi et al, initially misdiagnosed a child as EDS-HT, while a precise diagnosis indicated that the child had CCD. CCD was confirmed several years later with genetic findings. They performed DNA sequencing to confirm this diagnosis, but no point mutations were detected in RUNX2. They identified a new heterozygous deletion mutation in gene RUNX2 using a MLPA test [51]. Therefore, a definite diagnosis of CCD should include the patient's clinical history, symptoms and signs, as well as genetic analyses.

Supernumerary teeth in cleidocranial dysplasia

Human have two teeth series, 20 teeth in the deciduous dentition and 32 teeth in the permanent dentition. Supernumerary teeth (ST), or hyperdontia are defined as additional teeth to the normal dental components. Supernumerary teeth (ST) are one of the most common human anomalies [52]. They may occur anywhere in the mandible or in the maxilla, singly or in multiples, unilaterally or bilaterally, erupted or unerupted. The etiology of ST is still unknown. They may be associated with a syndrome or non-syndrome patients. The prevalence of ST in the general population is 0.2% to 0.8% in the deciduous dentition and 0.5% to 5.3% in the permanent dentition [52] . The presence of one, two and multiple ST for non-syndromic cases has been reported to be 76–86%, 12–23%, and 1%, respectively [53]. Multiple supernumerary teeth have been reported in normal individuals, but most are associated with other disorders or syndromes [52, 54].

Most ST are isolated cases, but some may be hereditary and associated with some syndromes. In a review article, Lubinsky et al, reports about 8 genetic syndromes with strong evidence for ST [52]. Syndromes that show supernumerary teeth include cleidocranial dysplasia, familial adenomatous polyposis, trichorhinophalangeal syndrome type I, Rubinstein–Taybi syndrome, Nance–Horan syndrome, Opitz BBB/G syndrome, oculofaciocardiodental syndrome and autosomal dominant Robinow syndrome. Several members of a single kindred in the Kreiborg–Pakistani syndrome (OMIM 614188) and insulin resistant diabetes mellitus with acanthosis nigricans (OMIM 610549) have been reported with ST [52,55,56]. Some Mendelian disorder have ST: Fabry disease, Ellis–van Creveld syndrome, Apert and Crouzon syndromes, Hallermann–Streiff syndrome, Zimmermann–Laband syndrome and Ehlers–Danlos syndrome [52].

Supernumerary teeth are usually associated with genetic syndromes. Several genes have been identified, including RUNX2, TRPS1, NHS, APC, and EVC [43]. However, the etiology of ST is not clear. The most common syndrome associated with supernumerary tooth is CCD [52], approximately 94% of CCD patients have dental anomalies, including ST. Dental abnormalities in CCD include delayed or failure of eruption of teeth, especially the permanent teeth, retention of primary teeth, crown and root abnormalities, high arched palate, underdevelopment of maxilla, delayed or no mandibular symphysis ossification, multiple impacted permanent teeth and also multiple supernumerary teeth [4,57]. Dentin formation (primary and permanent teeth) is normal, although there are problems in shedding of deciduous teeth and eruption of permanent teeth [44]. The reasons for delayed or failure eruption of permanent teeth in CCD are absence of cellular cementum at the root apex, decreased alkaline phosphatase levels and mechanical obstruction [57,58]. The presence of multiple ST in CCD may cause mechanical obstruction and may be a major factor for the impaction of permanent teeth [59].

Most studies indicate that correlation between the genotype-ST is very low. Quack et al examined unrelated patients with CCD of different ethnic backgrounds, but they were not able to find significant correlation between genotype-phenotype, although clavicular anomalies were inevitably present but the number and presence of supernumerary teeth was highly variable and did not correlate with other phenotypes or the type of mutation [60]. On the other hand, Yoshida et al were able to find a significant correlation between short stature and the number of supernumerary teeth [61]. Suda et al reported intrafamilial variations in position and number of supernumerary teeth among the three siblings in CCD patients with an identical mutation in the RUNT-domain of RUNX2 (P210S) [62]. Therefore, position and number of supernumerary teeth are not determined solely by gene mutations RUNX2, but also determined by epigenetic factors, copy number variations and the non-genetic factors, such as environmental factors [44,62].

Conclusion

Approximately 60-70% of CCD patients have loss of function or haploinsufficiency mutations in the RUNX2 gene. The RUNX2 gene is an essential transcription factor for chondrocyte maturation, osteoblast differentiation and bone formation. Due to the widely variable expressivity in CCD patients, there is a weak correlation between genotype and phenotype. It is unclear how Runx2 regulates suture closure and calvarial

development. In Runx2$^{+/-}$ mice with impaired PF and SAG, there was a decrease in the proliferation of mesenchymal cells and the expression of signaling pathways genes of Fgf, Pthlh, hedgehog and Wnt compared to normal mice. Runx2, by inducing the expression of these signaling pathways genes, regulates the proliferation of mesenchymal cells in sutures and suture closure. Most supernumerary tooth are isolated cases, but some may be hereditary or syndromic. Most common syndrome with supernumerary tooth is CCD. Wnt/β-catenin signaling plays an important role in the development and differentiation of teeth and also positively regulated Runx2. Runx2 transcription factor inhibits the expression of Wnt/β-catenin inhibitors in dental mesenchymal cells. Dental abnormalities may vary among members of a family with the same mutation in RUNX2. Therefore, dental abnormalities are not determined solely by gene mutations RUNX2, but also determined by epigenetic factors, copy number variations and the non-genetic factors, such as environmental factors.

Disclosure statement

All the authors have approved the manuscript and declared no potential conflicts of interest with respect to the authorship and/or publication of this article.

ORCID

Arash Salmaninejad http://orcid.org/0000-0001-6141-3915
Fatemeh Naqipour http://orcid.org/0000-0002-0556-250X

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