A Case of Cleidocranial Dysplasia with Peculiar Dental Features: Pathogenetic Role of the RUNX2 Mutation and Long Term Follow-Up

Michele Callea[1], Emanuele Bellacchio[2*], Mariateresa Di Stazio[3*], Fabiana Fattori[4], Enrico Bertini[4], Izzet Yavuz[5], Gabriella Clarich[1], Ayse Gunay[5]

[1]Institute for Maternal and Child Health - IRCCS "Burlo Garofolo"- Trieste, Italy. [2]Bambino Gesù Children's Hospital IRCCS, Research Laboratory, Piazza S. Onofrio 4, 00165, Rome, Italy. [3]Clinical Department of Medical, Surgical and Health Sciences, University of Trieste, Trieste, Italy. [4]Unit of Neuromuscular Disorders, Rehabilitation and Laboratory Biochemistry, Bambino Gesù Children's Research Hospital IRCCS, Rome Italy. [5]Department of Paediatric Dentistry, University of Dicle, Diyarbakir, Turkey.
*These 2 authors have equally contributed to the manuscript and should be considered both second authors.

Abstract

The report deals with a case of Cleidocranial Dysplasia (CCD) associated to a rare mutation of the RUNX2 gene and a peculiar dental phenotype, namely no supernumerary teeth. The aim consists in evaluating the long-term follow-up after treatment and discussing the pathogenetic mechanism of the mutation. We have carried out a clinical evaluation after treatment and attempted to analyze the potential pathogenetic effect of the mutation, based upon the available experimental structure of RUNX family domain and the highly conserved homology of RUNX1-3. Clinically the treatment has led to tooth development in crowns an roots, correction of cross-bite and eruption of the central maxillary incisor. The structural analysis has pointed out impairment in the DNA binding capability of the mutant protein. The described mutation, c.391C>T (p.R131C) appears to influence both structure and function of the protein by hampering the interaction of RUNX2 with DNA. The impaired function could explain the peculiar reported CCD phenotype. The dental condition of our patient has largely improved after treatment.

Key words: Cleidocranial Dysplasia (CCD), Skeletal anomalies, RUNX2, Dental anomalies

Background

Cleidocranial dysplasia (CCD; MIM 119600) is a rare autosomal dominant disorder characterized by facial, dental, and skeletal malformations. It is caused by heterozygous mutations in the runt-related transcription factor 2 gene (RUNX2), the master regulator gene of bone formation [1-3]. However, only 70% patients were found to have point mutations, 13% large/contiguous deletion but the rest of 17% remains unknown [4,5]. In CCD patients the skeletal dysplasia affects the bone ossification and results in skeletal anomalies that include facial and dental malformations characterized by delayed closure of the fontanelles, frontal bossing, hypoplastic or aplastic clavicles, short stature, supernumerary permanent teeth and other skeletal anomalies [6-8]. The diagnosis is still based on clinical and radiographic findings, confirmed by genetic analysis such as 83% of affected patients carry mutations in the RUNX2 gene.

Prenatal diagnosis is available in case of pregnancy at increased risk or if the disease-causing mutations are known, either by chorionic villi molecular genetics analysis or 3D in uterus ultrasound [9,10]. Patients affected by CCD syndrome must be followed by a multidisciplinary team, in which an important role is played by the paediatric dentist who should monitor the correct eruption and alignment of permanent teeth such as up to 94% of persons with CCD have dental anomalies and some of which are characterized by micrognathia and anomalies/defects of palate [4,11,12]. The most significant dental manifestations include anomalies of teeth eruption, anomalies of shape, multiple supernumerary teeth in the permanent dentition present most often in the mandibular premolar and maxillary incisor [13]. Supernumerary teeth have often an aberrant shape and morphology of the crown and root. The usual dental treatment approach for CCD is the extraction of the impacted teeth and the consequent orthodontic rehabilitation. At the end of growth a new intervention of maxillo-facial surgery and following orthodontic treatment is required.

Most of the supernumerary teeth fail to erupt and this is caused by several reasons: missing of space and failure of bone resorption. Studies on RUNX2 knock out mouse suggest that haploinsufficiency of RUNX2 does not effect the femoral bone remodelling but is insufficient for the active alveolar bone resorption essential for the prompt timing of tooth eruption and also suggest the possibility that impaired recruitment of osteoclasts is one of the cellular mechanisms of delayed tooth eruption in CCD patients [14-16]. Others described that the Periodontal Ligament (PDL) cells from CCD patients express a less distinctive osteoblastic phenotype resulting in an impaired ability to support osteoclastogenesis which might, in part, account for the delayed tooth eruption that can be observed clinically [17].

In this paper we report the results of a crystallographic study of the mutant protein, c.391C>T (p.R131C), identified in a Caucasian girl, that could explain the peculiar dental phenotype observed before the treatment was started and a long-term follow-up of the dental condition [18].

Clinical Report

The proband, an 11-years-old Caucasian girl, is the first born from healthy unrelated Italian parents. The family history was negative for skeletal diseases. She was born at term after a eutocic pregnancy without perinatal problems. The Apgar index was 9/10/10 at 1°, 5°, 10° minutes. Birth weight was 3.500 Kg (50°-75° percentile), length was 50 cm (50°-75° percentile) and the head circumference was 34 cm (50° percentile). At 13 months a physical examination revealed

Corresponding author: Dr. Michele Callea, Institute for Maternal and Child Health - IRCCS "Burlo Garofolo"- Trieste, Via dell'Istria 65/1, 34100, Italy; Tel: +39 040 3785675; e-mail: mcallea@gmail.com

aplastic clavicles, mandibular hypoplasia and delayed dental eruption, brachycephaly, frontal bossing and large fontanelles. A total body Rx and an encephalic MRI were carried out which confirmed a moderate brachycephaly, frontal bossing and show a narrow chest with deformation of vertebral body and bilateral hypoplastic V finger. Moreover, the trochanteric ossification centers were not been formed and the ischiopubic symphysis was still open. Based on radiological images and clinical features a diagnosis of CCD was suggested and a genetic molecular investigation for RUNX2 mutations was performed and the mutation c.391C>T (p.R131C) was identified [18]. This mutation was described for the first time in a paper in 2010 [19]. At age 8 years the patient (*Figure 1a*) was admitted at the- Department of Paediatric Dentistry of the Institute for maternal and Child Health -IRCCS- "Burlo Garofolo" Trieste, Italy for a dental consultation. A diagnosis of unilateral left cross-bite and delayed eruption of permanent teeth was done. Surprisingly an orthopantomogram showed no supernumerary teeth and agenesis of the left mandibular lateral incisor (*Figure 1b*). The following treatment plan was designed. Correction of the left cross-bite, guidance of eruption of permanent teeth by expanding the palate with a removable orthodontic rapid expansor and a teleradiography was carried. A plan of oral hygiene was given to the patient and family to obtain a successful orthodontic treatment and to avoid dental caries and periodontal complications. Although the extraction of deciduous teeth does not promote eruption of underlying permanent teeth in CCD, we removed, under local anaesthesia, the maxillary deciduous lateral incisors to favour a normal positioning of the central maxillary permanent incisor. At control after three years and an orthopantomogram revealed tooth development in crowns and roots. The cross bite had been successfully corrected and the central maxillary incisors were close to eruption.

The dental treatment of this CCD patient has been facilitated by his good compliance and the lack of supernumerary teeth. Unfortunately the patient was lost to follow-up for three years due to family difficulties in moving from their village and reaching reference centers.

At the age of 14, three further years later, the patient could have access to our Institute, hence we could establish an orthodontic treatment aimed to force and guide the eruption of ankylosed permanent teeth. Future goals will be the replacement of the missing central mandibular incisor by prosthetic device or an implant at the end of growth.

To understand the pathogenetic effects of the c.391C>T (p.R131C) mutation in the runt-related transcription factor 2, we have examined the experimental structure available for RUNX1 representing one the three members of the family of runt-related transcription factors (RUNX1-3), which are characterized by a highly-conserved Runt domain that interacts directly with DNA and forms heterodimers with CBF-beta to strengthen the DNA binding.

Discussion

The present case was diagnosed with CCD at 13 months of age on the basis of clinical and radio- logical features. The diagnosis was subsequently molecularly confirmed by the identification of the missense mutation in the RUNX2 gene, c.391C>T (p.R131C), when she was 3 years old. To the best of our knowledge was the first case of CCD with no supernumerary teeth and agenesis of one tooth [18]. The absence of supernumerary teeth has largely facilitated part of the treatment. Unfortunately the discontinuation of the follow-up for the above reasons has created inconvenience and complications that have forced us to modify the strategy or to post-pone interventions. Due to the rarity of the peculiar dental phenotype associated with the mutation c.391C>T (p.R131C) of the RUNX2 gene, we have studied the conformation of the mutant protein by analysis of available experimental structures to understand the pathogenetic effect and provide insight for a better understanding of phenotype determining mechanisms. We have examined the crystal structure available for RUNX1 who presents high homology of Runt domain with RUNX2 proteins sequence. In particular, as it can be observed in the ternary complex formed with CBF-beta and DNA (Protein Data Bank structure 1H9D) Arg-80 of the runt-related transcription factor 1, which corresponds to Arg131 of the runt-related transcription factor 2, is engaged in interactions with the DNA molecule (*Figure 2*). The high amino acid conservation shared by the Runt domains of Runx1-3 suggests that they exploit same DNA binding mode, and on the base of this analogy we can infer that the pathogenic effects of the c.391C>T (p.R131C) mutation detected in the runt-related transcription factor 2 might result from an impairment in the DNA binding capability of the protein. This is particularly true considering that arginine

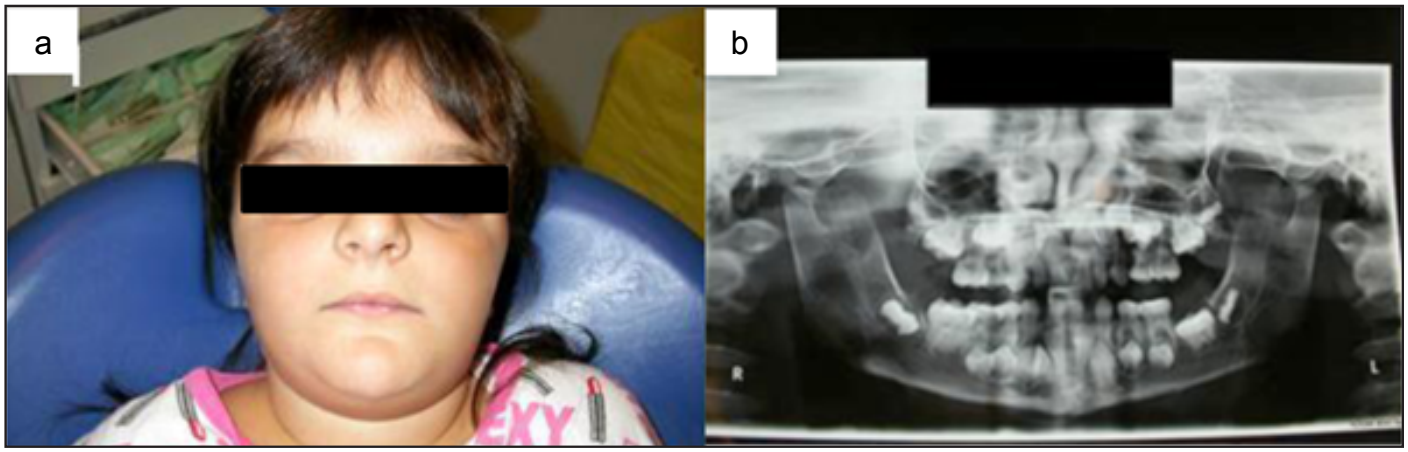

Figure 1. a. *Face of the patient affected by CCD.* ***b.*** *Orthopanthomography showing no supernumerary teeth and agenesis of the left mandibular lateral permanent incisor.*

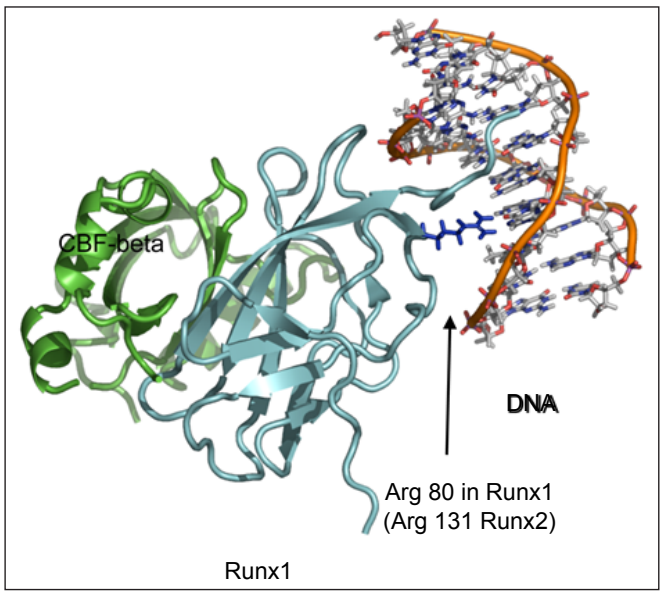

Figure 2. *Crystal structure of the runt-related transcription factor 1/CBF-beta/DNA ternary complex. The Runt domain of Runx1 is represented as cyan ribbons, and the arginine residue corresponding to Arg 131 of Runx2 (site of the R131C mutation described in the text) is shown as blue sticks. It can be observed that this arginine residue is involved in interactions with the DNA double stranded molecule (nucleotide atoms are shown with sticks and the phosphate atoms are highlighted by orange ribbons). CBF-beta is shown as green ribbons.*

is a positively charged amino acid, and, as visible from its position in the crystal structure, it provides both hydrogen bonding interactions with nucleotide bases and electrostatic interactions with the negatively charged DNA sugar-phosphate moiety. These interactions cannot be reproduced in the c.391C>T (p.R131C) mutant RUNX2 protein where Arg-131 is replaced by a cysteine residue. Of interest is also the observation that this arginine does not appear to participate in the stabilization of the Runt domain structure, thus if the mutant protein is expressed, localized and folded correctly, the replacement with a cysteine may be expected to have an impact on the interaction with the DNA only rather than on the structure of the protein. This could explain why c.391C>T (p.R131C) mutation of RUNX2 presents a phenotype that differs from that of other mutations in the same domain of this protein that might cause structural destabilization.

Conclusion

In this report the authors describe a CCD phenotype characterized by peculiar dental phenotype, namely no supernumerary teeth and agenesis of one tooth. A missense mutation was identified in RUNX2 gene, c.391C>T (p.R131C), and by a protein crystallography study, based upon the available experimental structure of RUNX domain and the highly conserved homology of RUNX1-3, they describe for the first time the pathogenetic mutation effect. The described mutation, c.391C>T (p.R131C) appears to influence both structure and function of the protein by hampering the interaction of RUNX2 with DNA. The impaired function could explain the peculiar reported CCD phenotype. Enlightening the genotype/phenotype spectrum with 3D protein structure could be useful in developing orphan drugs in times where orphan diseases claim attention. In the absence of reliable clinical trial the benefit of an early diagnosis allows a better treatment [10]. The patient's dental condition after the first approach with a rapid maxillary expansor and a careful hygiene plan has largely improved in terms of occlusion and aesthetics.

References

1. Mundlos S. Cleidocranial dysplasia: clinical and molecular genetics. *Journal of Medical Genetics* 1999; **36,** 177–182.

2. Mundlos S, Otto F, Mundlos C, Mulliken JB, Aylsworth AS, Albright S, et al. Mutations involving the transcription factor CBFA1 cause cleidocranial dysplasia. *Cell*. 1997; **89:** 773–779.

3. Otto F, Thornell AP, Crompton T, Denzel A, Gilmour KC, Rosewell IR, et al. Cbfa1, a candidate gene for cleidocranial dysplasia syndrome, is essential for osteoblast differentiation and bone development. *Cell*. 1997; **89,** 765–771.

4. Lee MTM, Tsai AC-H, Chou C-H, Sun F-M, Huang L-C, Yen P, et al. Intragenic microdeletion of RUNX2 is a novel mechanism for cleidocranial dysplasia. *Genome Medicine.* 2008; **2:** 45–49.

5. Lo Muzio L, Tetè S, Mastrangelo F, Cazzolla AP, Lacaita MG, Margaglione M, et al. A novel mutation of gene CBFA1/RUNX2 in cleidocranial dysplasia. *Annals of Clinical & Laboratory Science.* 2007; **37:** 115–120.

6. Karagüzel G, Aktürk FA, Okur E, Gümele HR, Gedik Y, Okten A. Cleidocranial dysplasia: a case report. *Journal of Clinical Research in Pediatric Endocrinology.* 2010; **2:** 134–136.

7. Wang GX, Sun RP, Song FL. A novel RUNX2 mutation (T420I) in Chinese patients with cleidocranial dysplasia. *Genetics and Molecular Research.* 2010; **9:** 41–47.

8. Zhang C, Zheng S, Wang Y, Zhao Y, Zhu J, Ge L. Mutational analysis of RUNX2 gene in Chinese patients with cleidocranial dysplasia. *Mutagenesis. 2010;* **25:** 589–594.

9. Hermann N V, Hove HD, Jørgensen C, Larsen P, Darvann TA, Kreiborg S, et al. Prenatal 3D ultrasound diagnostics in cleidocranial dysplasia. *Fetal Diagnosis and Therapy.* 2009; **25:** 36–39.

10. Mendoza-Londono R, Lee B. Cleniocradial Dysplasia. 2006 Jan 3 [Updated 2013 Aug 29] In: Pagon RA, Adam MP, Ardinger HH, Bird TD, Dolan CR, Fong CT, Smith RJH, Stephens K (Editors) GeneReviews®. Seattle (WA): University of Washington, Seattle; 1993-2014. Accessed at: http://www.ncbi.nlm.nih.gov/books/NBK1513/

11. D'Alessandro G, Tagariello T, Piana G. Craniofacial changes and treatment of the stomatognathic system in subjects with Cleidocranial dysplasia. *European Journal of Paediatric Dentistry.* 2010; **11:** 39–43.

12. Angle AD, Rebellato J. Dental team management for a patient with cleidocranial dysostosis. *American Journal of Orthodontics and Dentofacial Orthopedics.* 2005; **128:** 110–117.

13. Bufalino A, Paranaíba LMR, Gouvêa AF, Gueiros LA, Martelli-Júnior H, Junior JJ, et al. Cleidocranial dysplasia: oral features and genetic analysis of 11 patients. *Oral Diseases.* 2012; **18:** 184–190.

14. Faienza MF, Ventura A, Piacente L, Ciccarelli M, Gigante M, Gesualdo L, et al. Osteoclastogenic potential of peripheral blood mononuclear cells in cleidocranial dysplasia. *International Journal of Medical Sciences.* 2014; **11:** 356–364.

15. Yoda S, Suda N, Kitahara Y, Komori T, Ohyama K. Delayed tooth eruption and suppressed osteoclast number in the eruption pathway of heterozygous Runx2/Cbfa1 knockout mice. *Archives of Oral Biology.* 2004; **49:** 435–442.

16. Gao YH, Shinki T, Yuasa T, Kataoka-Enomoto H, Komori T, Suda T, et al. Potential role of cbfa1, an essential transcriptional factor for osteoblast differentiation, in osteoclastogenesis: regulation of mRNA expression of osteoclast differentiation factor (ODF). *Biochemical and Biophysical Research Communications.* 1998; **252:** 697–702.

17. Lossdörfer S, Abou Jamra B, Rath-Deschner B, Götz W, Abou Jamra R, Braumann B, et al. The role of periodontal ligament cells in delayed tooth eruption in patients with cleidocranial dysostosis. *Journal of Orofacial Orthopedics.* 2009; **70:** 495–510.

18. Callea M, Fattori F, Yavuz I, Bertini E. A new phenotypic variant in cleidocranial dysplasia (CCD) associated with mutation c.391C>T of the RUNX2 gene. *BMJ Case Reports.* **2012;** 2012: bcr1220115422.

19. Ott CE, Leschik G, Trotier F, Brueton L, Brunner HG, Brussel W, et al. Deletions of the RUNX2 gene are present in about 10% of individuals with cleidocranial dysplasia. *Human Mutation.* 2010; **31:** E1587–E1593.