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Oro-dental phenotype in patients with RUNX2 duplication

Laure Merametdjian[a,b,c,1], Tony Prud'Homme[b,d,e,1], Cédric Le Caignec[f,g], Bertrand Isidor[f,g], Serena Lopez-Cazaux[b,d,e,*]

[a] Département d'Odontologie Conservatrice et Endodontie, UFR Odontologie, Université de Nantes, France
[b] Service d'Odontologie Conservatrice et Pédiatrique, Centre de compétences Malformations orales et dentaires rares, CHU de Nantes, France
[c] INSERM, U1229, RMeS, Nantes, France
[d] Département D'Odontologie Pédiatrique, UFR Odontologie, Université de Nantes, France
[e] Unité d'Investigation Clinique Odontologie (UIC), France
[f] CHU Nantes, Service de Génétique Médicale, Centre de compétences Malformations orales et dentaires rares, CHU de Nantes, France
[g] INSERM, UMR 1238, Bone Sarcoma and Remodeling of Calcified Tissue, Nantes, France

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ABSTRACT

Runt-related transcription factor 2 (RUNX2) is well-known for its role in bone development and tooth morphogenesis. Most *RUNX2* mutations described in the literature result in loss-of-function mutations of RUNX2 responsible for cleidocranial dysplasia, an autosomal dominant disorder. We describe here the oro-dental phenotype of four patients of a unique family with a 285 kb duplication including the entire sequence of *RUNX2*, likely responsible for three functional copies of the gene, leading to an increased RUNX2 dosage. Several dental anomalies of number (hypodontia or oligodontia), morphology (microdontia, radiculomegaly, taurodontism or dens invaginatus) and tooth position (rotation) were found in these patients.

1. Introduction

Runt-related transcription factor 2 (*RUNX2*) belongs to the Runt-related transcription factor. (*RUNX*) family of genes, also called core binding factor-α (CBFα). RUNX proteins form a heterodimeric complex with CBFβ which confers increased DNA binding and stability to the complex. It includes RUNX1, also known as acute myeloid leukemia 1 protein (AML1) which is a transcription factor that regulates the differentiation of hematopoietic stem cells into mature blood cells (Okuda et al., 2001), and RUNX3 which is supposed to act as a tumor suppressor gene (Cohen, 2009). RUNX2, also known as Cbfa1, is a transcription factor known for its regulatory role in bone and tooth formation (Ryoo and Wang, 2006), but also in cell migration, and vascular invasion of bone; with a potential regulatory role in tumorigenesis (Cohen, 2009). RUNX2 is expressed in osteoblasts, odontoblasts, vascular endothelial cells, breast and prostate cancer cells, adult bone marrow, thymus and peripheral lymphoid organs. Transcription factor RUNX2 is necessary for bone and dentin – forming cells differentiation (Camilleri and McDonald. 2006; Bruderer et al., 2014; Vimalraj et al., 2015) and regulates many bone- and tooth-related gene expressions such as osteocalcin, collagen type I, bone sialoprotein or ameloblastin (Cohen, 2009). RUNX2 determines the lineage of osteoblasts (Komori et al., 1997; Ducy et al., 1997) and odontoblasts from mesenchymal cells (Komori, 2017). The temporal-spatial RUNX2 expression pattern during bone and tooth formation has been described (Jiang et al., 1999; Bronckers et al., 2001; Yamashiro et al., 2002). Though its importance in odontogenesis has long been evidenced since loss-of-function mutations of this gene and deletions result in humans in cleido-cranial dysplasia (CCD; OMIM 119600) characterized by delayed closure or non-closure of the fontanels, aplastic and/or hypoplastic clavicles, moderate short stature, ectopic and delayed eruption of teeth, supernumerary teeth and other skeletal abnormalities (Lee et al. 2008, 2013; Ryoo et al., 2010; Chen et al., 2013, Singh et al., 2015), few patients with gain-of-function mutations have been described. Recently, copy number variants (deletions, duplications) have been identified as an important source of mutation contributing to abnormal phenotypes such as for RUNX2 metaphyseal dysplasia with maxillary hypoplasia and brachydactyly (Halal et al., 1982; Moffatt et al., 2013; Avela et al., 2014) or craniosynostosis and oligodontia (Mefford et al., 2010; Greives et al., 2013).

We describe here the oro-dental phenotype in four patients of a unique family with a 285 kb duplication including the entire sequence of *RUNX2* and the 5' half of *SUPT3H* identified by array comparative genomic hybridization (aCGH) on chromosome 6 (arr[hg19]

* Corresponding author. Faculté de Chirurgie-Dentaire, 1 place Alexis Ricordeau, BP84215, 44042, Nantes, France.
E-mail address: serena.lopez-cazaux@univ-nantes.fr (S. Lopez-Cazaux).
[1] Both author equally contributed to the manuscript.

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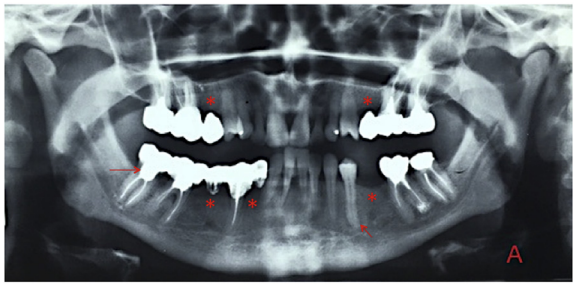

Fig. 1. Patient 1: A: Panoramic radiography at 33 years old.
The patient presented agenesis of the 4 s premolar and the right mandibular canine (red stars). Radiculomegaly was noticed on the first left mandibular premolar, taurodontism on the second right mandibular molar and rotation on the first left maxillary premolar (red arrows).(For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

6p21.1(45, 128, 523 X 2, 45, 233, 225–45, 518, 790 X 3, 45, 595, 945 X 2) and confirmed by real-time quantitative PCR (Molin et al., 2015).

2. Clinical report

2.1. Patient 1

The first patient is a 41- year's old woman, mother of the three other patients. As previously described in Molin et al. (2015), the patient was 150 cm tall (−2 SD) and weighed 55.5 kg (within the normal range). She wasn't referred with brachydactyly.

She presented hypodontia (agenesis of the 4 s premolar and the right mandibular canine). She related teeth missing in her father and paternal grandmother. Radiculomegaly was also noticed on the first left mandibular premolar, and taurodontism on the second right mandibular molar. The first left maxillary premolar was in rotation. Four of the five missing teeth were compensated by fixed partial denture (Fig. 1).

No dysmorphic features was identified.

2.2. Patient 2

The second patient is an 18-year old boy. As previously described in Molin et al. (2015), the patient had, at birth, a weight of 2760 g (< 10th centile), a height of 51 cm (within the normal range) and an Occipitofrontal Circumference (OFC) of 35 cm (within the normal range). Craniosynostosis was initially suspected because of mild turricephaly, but favorable outcome was observed without surgical care. At the age 17 years 9 months, his height was 163 cm (−2 SD), his weight 49 kg (−2 SD) and his OFC 55 cm (−1 SD). The patient wasn't referred with brachydactyly.

At the first consultation, following the orthodontic treatment, the presence of carious lesions on seven teeth was recorded (Fig. 2).

Clinical examination showed hypodontia (agenesis of the two maxillary lateral incisors, the second left maxillary premolar and the second right mandibular premolar). Radiculomegaly is also noticed on the second right maxillary molar, the first left maxillary premolar, the first right mandibular premolar, and the second left mandibular molar. Finally, we observed that the third left maxillary molar had an atrophied form (Fig. 2).

He presented micrognathia and other dysmorphic features were noticed: large eyebrows, synophrys, facial asymmetry, malar hypoplasia and low set ears with posterior angulation.

2.3. Patient 3

The third patient is a 15-year old boy, diagnosed with Asperger syndrome. As previously described in Molin et al. (2015), the patient birth weight was 1960 g (25th centile), his birth height 42 cm (10th centile) and his OFC 31 cm (30th centile). At the age 8 years 8 months, his height was 121 cm (−1 SD), his weight 19.4 kg (−2.5 SD), and his OFC 52 cm (−1.5 SD). The patient wasn't referred with brachydactyly.

At the first consultation, following the orthodontic treatment, the presence of carious lesions on eight teeth was recorded (Fig. 3).

Clinical examination revealed two missing teeth: the left maxillary canine and the left mandibular lateral incisor. We also observed microdontia on the two maxillary lateral incisors, a dens-in-dente (tooth invagination) was noticed on the lateral left maxillary incisor and taurodontism on the 4 s molars (Fig. 3).

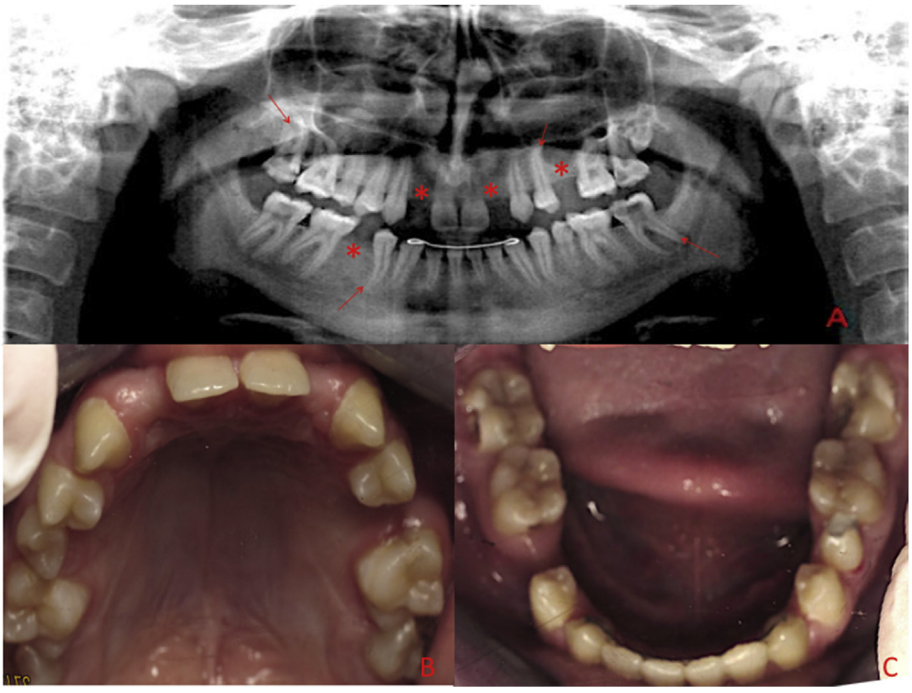

Fig. 2. Patient 2 at 18 years old: A: Panoramic radiography; B: Maxillary clinical view; C: Mandibular clinical view.
The patient presented agenesis of the two maxillary lateral incisors, the first left maxillary premolar and the second left mandibular premolar (red stars). Radiculomegaly was noticed on the second right maxillary molar, the first right maxillary premolar, the first left mandibular premolar, and the second right mandibular molar (red arrows). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

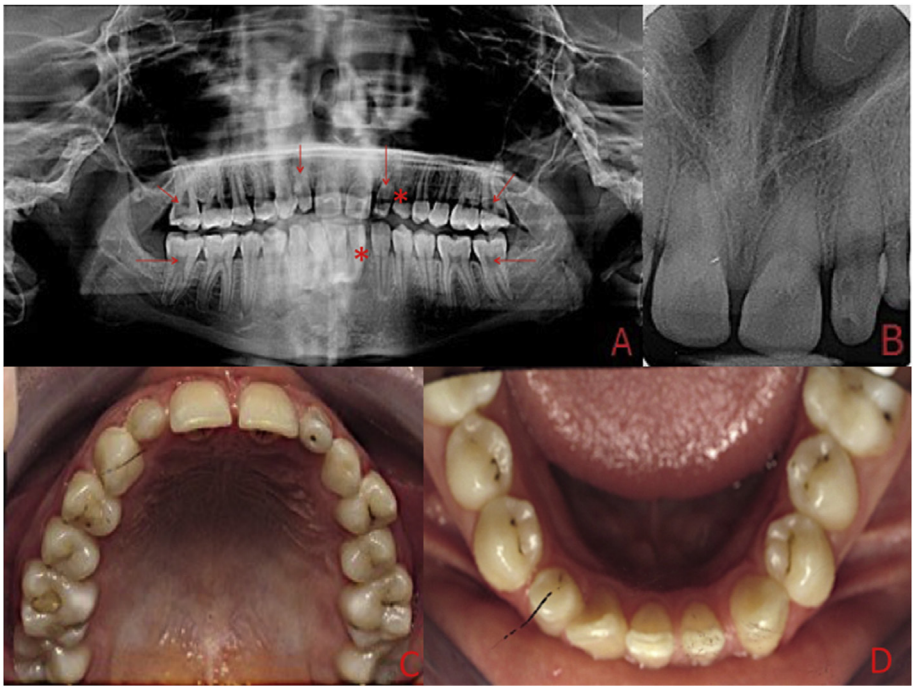

Fig. 3. Patient 3 at 15 years old. A: Panoramic radiography,B: Radiography of the second left maxillary incisor for patient 3 at 15 years old; C: Maxillary clinical view; D: Mandibular clinical view. The patient presented agenesis of the left maxillary canine and the left mandibular lateral incisor (red stars). Microdontia was noticed on the two maxillary lateral incisors, a tooth invagination was noticed on the lateral left maxillary incisor and taurodontism on the four first molars (red arrows).(For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

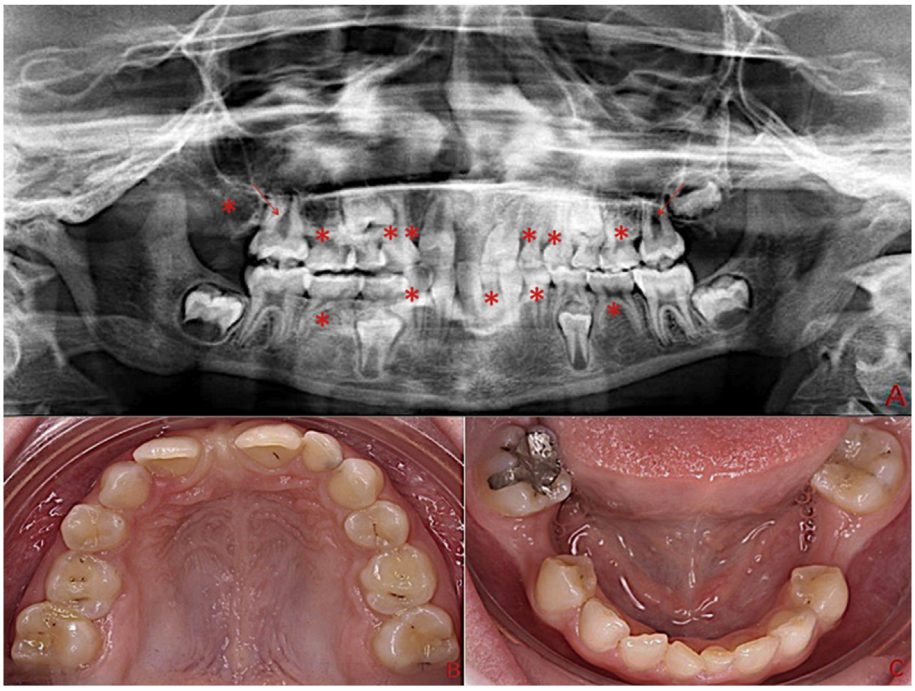

Fig. 4. Patient 4 at 8 years old. A: Panoramic radiography; B: Maxillary clinical view; C: Mandibular clinical view.
The patient presented agenesis of the two maxillary lateral incisors, the four canines, the 4 s premolar, the lateral right mandibular incisor and the second right maxillary molar (red stars). Taurodontism was reported on the two first maxillary molar (red arrows).(For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

He also presented micrognathia and other dysmorphic features, similar to what was observed in his older brother, were noticed: large eyebrows, malar hypoplasia and low set ears with posterior rotation.

2.4. Patient 4

The fourth patient is an 11-year old girl. As previously described in Molin et al. (2015), the patient presented, at birth, low growth parameters: birth weight 1950 g (4th centile), height 44 cm (12th centile) and OFC 29 cm (below 1st centile). Craniosynostosis was initially suspected because of mild turricephaly, but favorable outcome was observed without surgical care. At the age of 11 years 7 months, her height was 143 cm (within the normal range), her weight 28 kg (−2 SD), and her OFC 51 cm (−1 SD). The patient wasn't referred with brachydactyly.

The presence of carious lesions was recorded at the first consultation (Fig. 4).

She presented oligodontia (agenesis of the two maxillary lateral incisors, the four canines, the 4 s premolar, the central left mandibular incisor and the second right maxillary molar) and taurodontism on the two first maxillary molar (Fig. 4).

She also presented micrognathia and other dysmorphic features already observed in her two older brothers: large eyebrows, malar hypoplasia and low set ears with posterior rotation.

This family several oro-dental anomalies were summarized in Table 1.

Table 1
Summary of oro-dental findings on the four patients.

	Age	Numerical anomalies		Morphological anomalies				Positional anomalies	Jaw anomalies
		Hypodontia	Oligodontia	Microdontia	Radiculomegaly	Taurodontism	Dens invaginatus		Micrognathia
Patient 1	41	15, 25, 35, 43, 45	/	/	34	47	/	Rotation	/
Patient 2	18	12, 22, 25, 45	/	28	17, 24, 37, 44	/	/	/	X
Patient 3	15	23, 32	/	12, 22	/	17, 27, 37, 47	22	/	X
Patient 4	11	/	12, 13, 15, 17, 22, 23, 25, 31, 33, 35, 43, 45	/	/	16, 26	/	/	X
Patient 1007 and 1019 (Mefford et al., 2010)	/	X	/	/	/	/	/	/	/
Patient from Greives et al., 2013	10	24, 33, 43	/	/	/	16, 26	/	13, 14, 15, 35	/
5 affected patient (Moffatt et al., 2013)	/	/	/	The authors report dysplastic and yellowish teeth.				/	/
Patient from Avela et al., 2014	20	/	/	The authors report that roots of some permanent teeth are short and thin, some of them were resorbed.				/	/

/ : not affected; X: affected. IDF numeration of teeth.

3. Discussion

The present survey details the oro-dental phenotypes of 4 patients from a single family with *RUNX2* duplication (supplementary figure) comprising the whole coding sequence of *RUNX2* and its promoting region localized on chromosome 6 (arr[hg19] 6p21.1(45, 128, 523 X 2, 45, 233, 225–45, 518, 790 X 3, 45, 595, 945 X 2).

These four patients presented also various oro-dental manifestations: agenesis, radiculomegaly, microdontia, taurodontism, micrognathia and other dysmorphic features. Most *RUNX2* mutations described in the literature result in loss-of-function mutations and are responsible for cleidocranial dysplasia, an autosomal dominant disorder characterized by delayed closure of cranial sutures, aplastic or hypoplastic clavicles, moderate short stature and supernumerary teeth. Few duplications involving *RUNX2* have been described. Few duplications involving RUNX2 have been described. The first was detected by qPCR in a patient presenting with CCD (Ott et al., 2010). Here the oro dental phenotype described likely results in an increased RUNX2 dosage (Molin et al., 2015). The first confirmed *RUNX2* duplication was described in two affected cousins presenting with metopic craniosynostosis and hypodontia. A similar clinical phenotype of syndromic craniosynostosis with a quadruplication was reported by Greives et al., (2013) in a case study of a boy. The phenotype described in a family of French Canadian origin by Moffatt et al. (2013) was called maxillary hypoplasia and brachydactyly (MDMHB). The patients presented metaphyseal dysplasia with associating short stature, long bone, spinal and dental abnormalities A second patient with MDMHB, not of French Canadian origin, has been reported by Avela et al., in 2014 with a supposedly similar mutation to the previous case.

The features described herein included similarities with MDMHB for the three children but not for the mother, but also with the phenotypes described by Mefford et al., in 2010 and Greives et al., in 2013. Interestingly the same mutation in this family led to various phenotypes differing from the patient: isolated agenesis for the mother, oligo and hypodontia for the children associated with facial dysmorphic features, taurodontism, micrognathia, radiculomegaly, microdontia. This point a wider effect of runt-related transcription factor 2 that previously described in human physiopathology and relate to its specific role in odontogenesis as in odontogenesis.

RUNX2 was described not only to be involved in the early signaling networks regulating tooth initiation and early morphogenesis but also to regulate key epithelial-mesenchymal interactions that control advancing morphogenesis and histodifferentiation of the dental organ (D'Souza et al., 1999). *Runx2*$^{-/-}$ mouse molars show arrested development at the bud stage, whereas incisors, which develop earlier, progress to the bell stage and show dentine formation, although odontoblasts are abnormal and no enamel is formed (D'Souza et al., 1999; Aberg et al., 2004). Furthermore Li et al. (2011) showed in a transgenic mouse model that overexpression of Runx2 in odontoblasts interfere with the maturation of these cells and leads to disordered tooth structure: odontoblasts lost their tall columnar shape and polarization and dentinal tubules were absent, dental pulp chamber was dramatically enlarged and the dentin in transgenic mice was thinner, osteoblast-like cells were seen instead of normal odontoblasts and were embedded in a bone-like matrix, indicating that dentin formation was replaced with bone, predentin was disorganized possessing lacunae that contained odontoblasts. Interestingly Moffatt et al. (2013) reported that MDMHB patients teeth were yellowish and dystrophic, leading to total dental extraction before the end of the second decade in the majority of affected individuals. While there was no major enamel abnormalities, we reported few dyschromic and dystrophic zones of lacteal enamel. These four patients presented though a perfectible oral hygiene leading to a high carious risk. Thus it is difficult to point out the cause of their high carious prevalence, we raise the question, in line with animal models studies and previous reports, of dental tissues abnormalities in this family caused by *RUNX2* duplication. Interesting perspective would be a

histological analysis of these patients teeth to identify clearly the effects of this *RUNX2* duplication on dental tissues structure and mineralization.

4. Conclusion

This case report described a family with an affected mother and three affected children carrying a duplication including the entire sequence of RUNX2. The duplication of *RUNX2* is likely responsible of oro-dental anomalies: teeth agenesis, teeth morphological anomalies and maxillary anomalies. The relationship between this mutation and structural teeth anomalies need further investigations.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmg.2018.05.019.

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