ORTHODONTICS & CRANIOFACIAL RESEARCH

ORIGINAL ARTICLE

N Suda
M Hattori
K Kosaki
A Banshodani
K Kozai
K Tanimoto
K Moriyama

Correlation between genotype and supernumerary tooth formation in cleidocranial dysplasia

Authors' affiliations:
N Suda, M Hattori, K Moriyama, Maxillofacial Orthognathics, Department of Maxillofacial Reconstruction and Function, Division of Maxillofacial/Neck Reconstruction, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan
N Suda, M Hattori, K Moriyama, Global Center of Excellence (GCOE) Program, International Research Center for Molecular Science in Tooth and Bone Diseases, Tokyo, Japan
K Kosaki, Department of Pediatrics, Keio University School of Medicine, Tokyo, Japan
A Banshodani, K Kozai, Department of Pediatric Dentistry, Division of Cervico-Gnathostomatology, Programs for Applied Biomedicine, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan
K Tanimoto, Department of Oral and Maxillofacial Radiology, Division of Medical Intelligence and Informatics, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan

Correspondence to:
Naoto Suda
Maxillofacial Orthognathics
Department of Maxillofacial Reconstruction and Function
Division of Maxillofacial/Neck Reconstruction
Graduate School of Medical and Dental Sciences
Tokyo Medical and Dental University
1-5-45 Yushima
Bunkyo-ku
Tokyo 113-8510
Japan
E-mail: n-suda.mort@tmd.ac.jp

Dates:
Accepted 21 May 2010

To cite this article:
Suda N, Hattori M, Kosaki K, Banshodani A, Kozai K, Tanimoto K, Moriyama K:
Correlation between genotype and supernumerary tooth formation in cleidocranial dysplasia
Orthod Craniofac Res 2010;**13**:197–202

Structured Abstract

Authors – Suda N, Hattori M, Kosaki K, Banshodani A, Kozai K, Tanimoto K, Moriyama K

Introduction – Cleidocranial dysplasia (CCD, MIM#119600), for which the responsible gene is *RUNX2*, is a genetic disorder characterized by hypoplasia or aplasia of the clavicles, patent fontaneles, and a short stature. Supernumerary teeth and delayed eruption and impaction of permanent teeth are frequently associated with CCD. Our previous study reported wide intrafamilial variation in supernumerary tooth formation associated with a mutation in the RUNT-domain of *RUNX2*, suggesting a low correlation between the genotype and supernumerary tooth formation. To further clarify this point, a more precise evaluation was performed.

Design – Gene mutational analysis of nine Japanese individuals with CCD was performed. Dental and skeletal characteristics were examined based on patient examinations and radiographs.

Results – Four different gene mutations, including one novel mutation in *RUNX2* gene (NM_001024630), were identified. Among them, four individuals had the R225Q mutation, three siblings had the P224S mutation, and the other two individuals had different frame-shift mutations. Wide variations in supernumerary tooth formation were observed in individuals with identical gene mutations, and discordance was seen between monozygotic twins. Asymmetric supernumerary tooth formation was noted in five out of the nine individuals.

Conclusion – Individuals with identical gene mutations showed a wide variation in the supernumerary tooth formation. Not only the genotype but also environmental factors and a complex system including epigenetics and copy number variation might regulate supernumerary tooth formation in CCD.

Key words: cleidocranial dysplasia; gene mutation; RUNX2; supernumerary tooth

Introduction

The process of tooth development is characterized by complicated and reciprocal interactions between the dental epithelia and the mesenchyme (1), and it is strictly regulated by both epithelial and mesenchymal factors (2). For example, *Bmp-2, Bmp-4, and Fgf-8* expressed by dental epithelia initiate the initial tooth development (3, 4), and *Msx-1* and *Pax-9*

expressed by mesenchyme are essential for further tooth development (5, 6). Thus, tooth development is highly under genetic control.

In spite of such genetic control, discordance in congenitally missing teeth has been observed between healthy monozygotic twins who have identical genomes (7–9). It has been reported that only 12.5% of healthy monozygotic twins showed concordance in congenitally missing teeth (9). These studies indicate the importance of the non-genetic regulation of tooth development.

Cleidocranial dysplasia (CCD, MIM #119600) is a genetic disorder characterized by skeletal dysplasia in patent sutures and/or fontaneles, clavicles, wormian bone formation, and a short stature (10). *RUNX2*, located in chromosome 6p21, has been identified as a gene responsible for CCD (11). Regarding dental abnormality, CCD is associated with supernumerary teeth, and the delayed eruption and impaction of permanent teeth (12–14). The position and number of supernumerary teeth vary among cases, but they are seen below the permanent teeth (incisors, canines, and bicuspids) that have replaced with the deciduous teeth. Our previous study demonstrated a variable expressivity of supernumerary tooth formation in three CCD siblings with a gene mutation in the RUNT-domain of *RUNX2* (15) (reported as P210S according to NM_004348 and represented as P224S according to NM_001024630 in this study to match with other studies). This suggests that not only healthy monozygotic twins but also members of the same family with a monogenic disease have different tooth patterns. To further examine this point, the present study performed mutational analysis and precise examination of supernumerary tooth formation in individuals with CCD.

Materials and methods

Nine Japanese individuals, clinically diagnosed as CCD by specialists and treated in the Dental Hospital of Tokyo Medical and Dental University and Hiroshima University Hospital, were examined (Table 1). They all gave informed consent for mutational and clinical analyses. A-2 was a daughter of A-1. The two individuals in family B were monozygotic twins. C-1 was a sporadic case. D-1 was also a sporadic case whose gene mutation and clinical features were reported previously (16). The three individuals in family E were siblings, as previously reported (15).

DNA was extracted from nail samples using ISOHAIR (Nippon gene, Toyama, Japan). The extracted DNA was amplified using specific primers for *RUNX2*. Primer sequences and PCR conditions are available on the website of 'Multiple Malformation Syndromes (http://www.dhplc.jp/genetics/frame.html)' provided by the Department of Pediatrics, Keio University School of Medicine. Mutations in the eight amplicons were analyzed by denaturing high-performance liquid chromatography (DHPLC), as described previously (17, 18). After DHPLC analysis, the PCR products were purified

Table 1. **Characteristics of nine individuals with cleidocranial dysplasia**

	Sex	Age	Abnormal suture	Abnormal clavicle	Stature	Gene mutation
A-1	Female	50Y	un	un	un	R225Q
A-2	Male	11Y	un	un	un	R225Q
B-1	Female	10Y	+	+	<−1 SD	R225Q
B-2	Female	10Y	+	+	<−1 SD	R225Q
C-1	Male	26Y	+	−	<−1 SD	Frame shift (1123_1124insA)
D-1	Male	16Y	+	+	<−1 SD	Frame shift (382_383insT)
E-1	Male	17Y	+	−	<−1 SD	P224S
E-2	Female	15Y	+	−	<−1 SD	P224S
E-3	Male	12Y	+	−	<−1 SD	P224S

Nine individuals of five families (Family A, B, C, D and E) are shown. Abnormal suture denotes open or delayed closure of suture. Abnormal clavicle denotes hopoplastic or aplastic clavicles. Each body height which was more than one SD shorter than the age- and sex-macthed Japanese norm (19) is highlighted as <−1 SD.
un, unknown.

using a desalting column and sequenced using the dideoxy-sequencing method (BigDye Dideoxy sequencing kit; Applied BioSystems, Foster City, CA, USA) and an automated DNA sequencer (ABI3100; Applied Biosystems) (18). All mutations were represented relative to the initial methionine of the protein encoded by NM_001024630.

Precise clinical examinations were performed in all cases. Chest and panoramic radiographs were taken. A stature was evaluated by the age- and sex-matched Japanese norm (19). Each supernumerary tooth was distinguished from the normal permanent tooth by the position, inclination, crown shape, and the root formation. If a supernumerary tooth could not be distinguished on the panoramic radiograph, intraoral radiographs were taken and evaluated.

The experimental protocol was approved by the ethical review committees of Tokyo Medical and Dental University and Hiroshima University.

Results

The clinical characteristics and *RUNX2* mutations in the nine individuals are shown in Table 1 and Fig. 1. A-1 (a 50-year-old woman) was the mother of A-2 (an 11-year-old man) (Table 1). A point mutation casing a missense mutation (R225Q) in the Runt-domain of *RUNX2* was identified (Table 1 and Fig. 1). Two members (10-year-old girls) of family B were monozygotic twins (Table 1). Both of them showed patent skull sutures and hypoplastic clavicles, and their body heights were more than one standard deviation (SD) shorter, as previously reported (20). A point mutation casing the missense mutation (R225Q) found in family A was also identified in family B (Table 1 and Fig. 1). C-1 (a 26-year-old man) was a sporadic case with patent skull sutures but lacked hypoplastic clavicles (Table 1). His body height was more than one SD shorter. A novel frame-shift mutation (1123_1124insA) in the region encoding the PST-domain was identified (Table 1 and Fig. 1). D-1 (a 16-year-old man) was also a sporadic case with patent skull sutures and hypoplastic clavicles, and his body height was more than one SD shorter (Table 1). A frame-shift mutation (382_383insT) in the region encoding the Runt-domain was identified, as previously reported (15). Family E was comprised of three siblings (E-1: a 17-year-old boy, E-2: a 15-year-old girl, E-3: a 12-year-old boy), and all members showed patent skull sutures but no apparent clavicle abnormality. Their body heights were more than one SD shorter. They had a point mutation (P224S) in the Runt-domain, as previously reported (15) (Table 1 and Fig. 1).

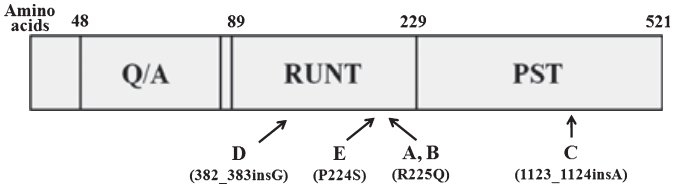

Fig. 1. Mutation in *RUNX2* in nine individuals with cleidocranial dysplasia. Q/A, 23 consecutive glutamines followed by 17 consecutive alanine residues domain; RUNT, DNA-binding and protein interaction domain; PST, proline/serine/threonine-rich transactivation and protein interaction domain. Positions of gene mutations in families A–E are shown by arrows.

All individuals showed supernumerary teeth, and the number and position are summarized in Fig. 2. Family A only had symmetrical supernumerary teeth only in the mandible (Fig. 2). A-1 had four supernumerary tooth between the cuspids and the first bicuspids, and A-2 had two between the lateral incisors and the cuspids. In contrast to family A, the monozygotic twins (B-1 and B-2) that had the same R225Q mutation as family A had three supernumerary teeth in the maxilla. Both had two supernumerary teeth similarly in the maxillary incisor region. One tooth appeared between the maxillary left cuspid and first bicuspid in B-1, and between the left second bicuspid and the first molar in B-2. The discordant phenotype of supernumerary teeth was also noted in the mandible (six and five supernumerary teeth in B-1 and B-2, respectively). The number of supernumerary teeth did not differ between B-1 and B-2 on the right side, but this was discordant on the left side of the mandible (three and two in B-1 and B-2, respectively). In total, B-1 and B-2 had nine and eight supernumerary teeth, respectively.

C-1 showed asymmetric supernumerary tooth formation (Fig. 2). In the maxilla, two and three supernumerary teeth were on the right and left, respectively. One was seen in the left maxillary molar region, while the others were around the lateral dentition. In the mandible, two supernumerary teeth were observed symmetrically in the incisor region. One and four supernumerary teeth were observed in the right and left lateral dentitions, respectively. In total, twelve supernumerary teeth were counted.

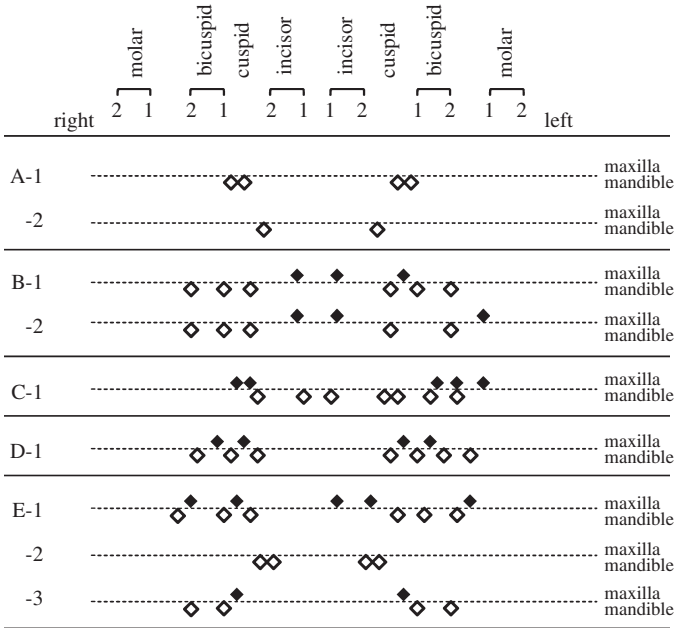

Fig. 2. The appearance of supernumerary teeth in nine individuals with cleidocranial dysplasia. Black and white rhombuses denote teeth in the maxilla and mandible, respectively.

In D-1, a total of eleven supernumerary teeth (four in the maxilla and seven in the mandible) were found in the cuspid and bicuspid regions (Fig. 2), as reported previously (16). Symmetric supernumerary tooth formation was noted in the maxilla but not in the mandible. Three and four teeth were found in the right and left side of the mandible, respectively.

In family E, the position and number of supernumerary teeth were completely different (15) (Fig. 2). E-1 showed a total of 11 supernumerary teeth. They appeared symmetrically around the mandibular lateral dentition (three on each side). In the maxilla, an asymmetric appearance was noted around the maxillary incisors (two on the left side) and bicuspids (one and two on the left and right sides, respectively). E-2 showed four supernumerary teeth symmetrically around the mandibular incisors and cuspids (two on each side). E-3 showed six supernumerary teeth symmetrically around the maxillary cuspids (one on each side) and mandibular bicuspids (two on each side).

Discussion

The present study demonstrated a wide variation of supernumerary tooth formation in individuals with CCD having identical gene mutations (families A and B with R225Q; family E with P224S). It is known that the number of supernumerary teeth increases with age in CCD (16). Thus, the variation in the supernumerary tooth number in present individuals might be related to age. However, the monozygotic twins (B-1 and B-2) showed discordance in supernumerary tooth number and position (Fig. 2). In addition, the youngest E-3 showed more supernumerary teeth than the older E-2 in Family E.

Generally, teeth develop symmetrically without showing a large difference between the left and right sides. However, there was asymmetrical supernumerary tooth formation in five (B-1, B-2, C-1, D-1, and E-1) out of nine individuals. This suggests that gene mutation in *RUNX2* did not result in symmetrical supernumerary tooth development and that determination of the supernumerary tooth number and position is not solely governed by *RUNX2* mutation.

Eight out of the nine individuals had mutations in the Runt-domain (Fig. 2). This domain is known to have an important function in DNA binding and the dimerization of α and β subunits (11). Many missense mutations reportedly abolish DNA binding and alter transactivation activity (21, 22). D-1 showed a novel gene mutation (1123_1124insA) in the PST-domain. The PST-domain is rich in proline/serine/ threonine and is involved in transactivation and protein interaction (10). Many kinds of mutations, including insertion, deletion, nonsense, and missense mutations, have also been reported in this domain.

It is difficult to completely exclude the possibility that the diversity in supernumerary formation was related to a genetic factor other than *RUNX2*. The phenotype of CCD has (in most cases) an autosomal dominant inheritance pattern and is caused by haploinsufficiency of *RUNX2*. Phenotypes caused by haploinsufficiency of a gene product are known to be sensitive to modulation by modifier genes (23). In addition, it is reasonable to speculate that non-genetic or epigenetic regulations are involved in the variable expressivity. Non-genetic environmental factors include teratogens, the fetal position, nutrition, trauma, and exposure to radiation. Epigenetic regulation comprises DNA methylation and histone modifications, and they are heritable regulators of gene function that cannot be explained by changes in the DNA sequence (24).

Furthermore, copy number variation could also be a factor responsible for the diversity between families.

Copy number variation is known as human genetic variation and includes large insertions, deletions, and inversions of genes (25). It was reported that 1695 structural variations were found in eight healthy individuals (26). It is possible to speculate that structural variation might be present between families A and B and that this accounts for the different number and position of supernumerary teeth.

Conclusion

Wide variation and asymmetric appearance of the supernumerary tooth formation were seen among individuals with CCD with identical gene mutations in *RUNX2*. It is likely that environmental factors and a complex system including epigenetics and copy number variation might be involved in the supernumerary tooth formation in CCD.

Clinical relevance

CCD is a genetic disorder characterized by hypoplasia or aplasia of the clavicles, patent fontaneles, and a short stature. As a dental feature, supernumerary teeth, and delayed eruption and impaction of permanent teeth are frequently observed in CCD. Supernumerary teeth cause serious problems in occlusion, dentition, and mastication, and prediction of supernumerary teeth formation has a great clinical significance for dental treatment. As *RUNX2* was identified as the gene responsible for CCD, we anticipated that the genetic analysis of *RUNX2* of affected individuals would help the prediction and diagnosis of the appearance and position of supernumerary teeth.

Acknowledgements: This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (19659547, 21390546). Part of this study was presented at the Annual Meeting of the Japanese Orthodontic Society (September, 2007).

References

1. Nanci A. Physiologic tooth movement. Eruption and shedding. In: Nanci A, editor. *Ten Cate*'s Oral Histology – Development, Structure, and Function, 7th edn. St. Louis, MO, USA: Mosby Elsevier; 2007. pp. 268–89.
2. Thesleff I, Sharpe P. Signalling networks regulating dental development. *Mech Dev* 1997;67:111–23.
3. Vainio S, Karavanova I, Jowett A, Thesleff I. Identification of BMP-4 as a signal mediating secondary induction between epithelial and mesenchymal tissues during early tooth development. *Cell* 1993;75:45–58.
4. Heikinheimo M, Lawshé A, Shackleford GM, Wilson DB, MacArthur CA. Fgf-8 expression in the post-gastrulation mouse suggests roles in the development of the face, limbs and central nervous system. *Mech Dev* 1994;48:129–38.
5. Satokata I, Maas R. Msx1 deficient mice exhibit cleft palate and abnormalities of craniofacial and tooth development. *Nat Genet* 1994;6:348–56.
6. Peters H, Neubüser A, Kratochwil K, Balling R. Pax9-deficient mice lack pharyngeal pouch derivatives and teeth and exhibit craniofacial and limb abnormalities. *Genes Dev* 1998;12:2735–47.
7. Gravely JF, Johnson DB. Variation in the expression of hypodontia in monozygotic twins. *Dent Pract Dent Rec* 1971;21:212–20.
8. Kotsomitis N, Dunne MP, Freer TJ. A genetic aetiology for some common dental anomalies: a pilot twin study. *Aust Orthod J* 1996;14:172–8.
9. Townsend GC, Richards L, Hughes T, Pinkerton S, Schwerdt W. Epigenetic influences may explain dental differences in monozygotic twin pairs. *Aust Dent J* 2005;50:95–100.
10. Lee B, Zhou G. RUNX2 and cleidocranial dysplasia. In: Jones KL, editor. *Smith*'s Recognizable Patterns of Human Malformation, 6th edn. Philadelphia, USA: WB Saunders; 2005. pp. 331–9.
11. 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;89:773–9.
12. Jensen BL, Kreiborg S. Development of the dentition in cleidocranial dysplasia. *J Oral Pathol Med* 1990;19:89–93.
13. Kreiborg S, Jensen BL, Larsen P, Schleidt DT, Darvann T. Anomalies of craniofacial skeleton and teeth in cleidocranial dysplasia. *J Craniofac Genet Dev Biol* 1999;19:75–9.
14. Golan I, Preising M, Wagener H, Baumert U, Niederdellmann H, Lorenz B et al. A novel missense mutation of the CBFA1 gene in a family with cleidocranial dysplasia (CCD) and variable expressivity. *J Craniofac Genet Dev Biol* 2000;20:113–20.
15. Suda N, Hamada T, Hattori M, Torii C, Kosaki K, Moriyama K. Diversity of supernumerary tooth formation in siblings with cleidocranial dysplasia having identical mutation in RUNX2 : possible involvement of non-genetic or epigenetic regulation. *Orthod Craniofac Res* 2007;10:222–5.
16. Yokozeki M, Ohyama K, Tsuji M, Goseki-Sone M, Oida S, Orimo H et al. A case of Japanese cleidocranial dysplasia with a CBFA1 frameshift mutation. *J Craniofac Genet Dev Biol* 2000;20:121–6.
17. Kosaki K, Udaka T, Okuyama T. DHPLC in clinical molecular diagnostic services. *Mol Genet Metab* 2005;86:117–23.
18. Udaka T, Samejima H, Kosaki R, Kurosawa K, Okamoto N, Mizuno S et al. Comprehensive screening of CREB-binding protein gene mutations among patients with Rubinstein-Taybi syn-

drome using denaturing high-performance liquid chromatography. *Congenit Anom* 2005;45:125–31.
19. Physical development of student 2006. Ministry of Education, Culture, Sports, Science and Technology.
20. Banshodani A, Kaihara Y, Nakae H, Suzuki J, Kozai K. A case report of fraternal twin sisters who were diagnosed with cleidocranial dysostosis with delayed eruption of the permanent teeth. *Jpn J Pediatr Dent* 2007;45:109–17.
21. Lee B, Thirunavukkarasu K, Zhou L, Pastore L, Baldini A, Hecht J et al. Missense mutations abolishing DNA binding of the osteoblast-specific transcription factor OSF2/CBFA1 in cleidocranial dysplasia. *Nat Genet* 1997;16:307–10.
22. Zhou G, Chen Y, Zhou L, Thirunavukkarasu K, Hecht J, Chitayat D et al. CBFA1 mutation analysis and functional correlation with phenotypic variability in cleidocranial dysplasia. *Hum Mol Genet* 1999;8:2311–6.
23. Bourdeau A, Faughnan ME, McDonald ML, Paterson AD, Wanless IR, Letarte M. Potential role of modifier genes influencing transforming growth factor-beta1 levels in the development of vascular defects in endoglin heterozygous mice with hereditary hemorrhagic telangiectasia. *Am J Pathol* 2001;158:2011–20.
24. Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat Genet* 2003;33:245–54.
25. Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, Andrews TD et al. Global variation in copy number in the human genome. *Nature* 2006;444:444–54.
26. Kidd JM, Cooper GM, Donahue WF, Hayden HS, Sampas N, Graves T et al. Mapping and sequencing of structural variation from eight human genomes. *Nature* 2008;453:56–64.