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Diversity of supernumerary tooth formation in siblings with cleidocranial dysplasia having identical mutation in *RUNX2*: possible involvement of non-genetic or epigenetic regulation

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Structured Abstract

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Introduction – Cleidocranial dysplasia (CCD, MIM #119600) is an autosomal-dominant disorder characterized by hypoplasia or aplasia of clavicles, patent fontanelles and short stature. The responsible gene has been identified as *RUNX2*. CCD is also accompanied by characteristic dental abnormalities, e.g. supernumerary teeth, delayed eruption and impaction of permanent teeth. Intrafamilial variations of skeletal abnormalities are reported but those of dental abnormalities are obscure. To clarify this point, a precise examination of the dental features of CCD siblings having identical mutation was performed.

Design – Gene mutational analysis of three Japanese CCD siblings and their father was performed. Skeletal and dental characteristics were examined by the inquiry and radiographs.

Results – Three siblings uniformly showed patent fontanelles and short stature. They and their father had a novel missense mutation in the RUNT-domain (P210S) of *RUNX2*. The siblings were completely discordant for the dental characteristics with the position and number of supernumerary teeth being completely different. The youngest, a 12-year-old boy, had six supernumerary teeth, which appeared symmetrically around the maxillary canines and mandibular premolars. The second, a 15-year-old girl, had four supernumerary teeth which appeared around the mandibular incisors. The oldest, a 17-year-old boy, had 11 supernumerary teeth, which were symmetrically around the mandibular lateral dentition and asymmetrically around the maxillary incisors and premolars.

Conclusion – The present study suggests the involvement of non-genetic or epigenetic regulation in supernumerary tooth formation in CCD.

Key words: cleidocranial dysplasia; RUNX2; supernumerary tooth; tooth development

Introduction

Cleidocranial dysplasia (CCD, MIM #119600) is an autosomal-dominant disorder characterized by skeletal dysplasia in clavicles, patent sutures and fontanelles, formation of Wormain bones and short stature (1). In

addition to skeletal dysplasia, patients with CCD are known to exhibit dental abnormalities, e.g. supernumerary teeth, delayed eruption and impaction of the permanent teeth (2, 3). It is reported that intrafamilial variations of skeletal abnormalities show wide variety, but those of dental abnormalities are obscure (4). Baumert et al. (4) reported variations in the shape of the nasal bones, missing mastoid and frontal sinus pneumatization, marked Wormian bones, and deformation of the sphenoid bones in family members with identical gene mutations. Intrafamilial variations of dental abnormalities were not clear in this study, as the numbers of supernumerary teeth and delayed tooth eruption were roughly scored and precise information was missing (4). Yoshida et al. (5) reported a marked difference in the severity of short stature in patients with identical gene mutations, however, there was no record of the age of patients, which has a profound influence on the evaluation of supernumerary teeth.

RUNX2 is mapped to chromosome 6p21 and identified as the responsible gene for CCD (6). This gene encodes a transcription factor which has DNA binding ability in the Runt-domain (7). Runt-domain is also responsible for the dimerization of α and β subunits and many CCD patients are reported to have mutations in this domain (8, 9). In order to examine the intrafamilial variations of dental abnormalities in CCD patients with an identical mutation in *RUNX2*, we performed a precise examination of dental characteristics in one affected family. Three Japanese CCD siblings had a novel mutation in Runt-domain (P210S), but had completely discordant dental features.

Materials and methods

The three siblings and their father gave informed consent for mutational analysis. DNA was extracted from nail samples using ISOHAIR (Nippon Gene CO., Ltd, Tokyo, Japan). Extracted DNA was amplified using specific primers for *RUNX2*. Primer sequences and PCR conditions are 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. Mutation in the amplified eight amplicons was analyzed by denaturing high-performance liquid chromatography (DHPLC), as described previously (10, 11). After DHPLC analysis, PCR products were purified using a desalting column, and were sequenced using a dideoxy-sequencing method (BigDye Dideoxy sequencing kit; Applied BioSystems, Foster City, CA, USA) and an automated sequencer (ABI3100; Applied Biosystems) (11).

Precise inquiry and inspection of the three siblings and their father were performed. Chest and panoramic radiographs were taken from three siblings.

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

Results

In the three siblings (III:1, 17-year-old male; III:2, 15-year-old female; III:3, 12-year-old male), characteristic patent sutures and fontanelles, and Wormain bone formation were noted in their skull. They exhibited short stature but no apparent lack of clavicles was seen. Detailed clinical examination could not be performed on their father (II:2, 52-year-old) but he was said to have abnormalities in the numbers and eruption of the permanent teeth (Fig. 1). No other family members (I:1, I:2, I:3, I:4, II:1 and II:3) were affected with CCD.

Mutational analysis was performed on the three siblings and their father. A heterozygous point mutation (C to T) was identified in the nucleotide sequence at the position of 628 (from the translation site of NM_004348) in II:2, III:1, III:2 and III:3 (Fig. 2A). This nucleotide conversion was resulted in the replacement of Pro by Ser at the amino acid position 210 (P210S) in the Runt-domain (Fig. 2B). This mutation was not reported elsewhere (8, 9) and is regarded as a novel mutation for CCD.

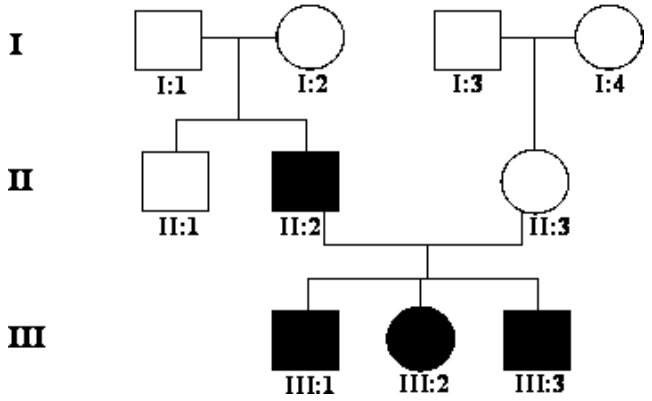

Fig. 1. Pedigree of the present family. Filled squares and circle denote the affection with cleidocranial dysplasia.

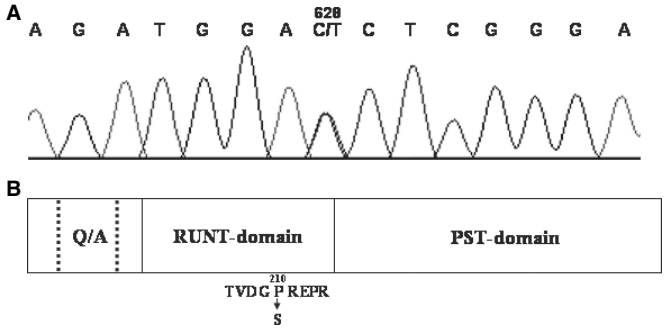

Fig. 2. Mutation in *RUNX2* in the present three siblings and their father. (A) A heterozygous point mutation (C to T) was identified in the nucleotide sequence at position 628 (from the translation site of NM_004348) in II:2, III:1, III:2 and III:3. (B) Conversion of Pro to Ser in the Runt-domain (at position 210).

In addition to these uniform skeletal abnormalities, delayed eruption and impaction of permanent teeth, and supernumerary teeth were noted in the panoramic X-ray of the three siblings (Fig. 3). The position and number of supernumerary teeth were completely different among the siblings. The oldest, III:1, had 11 supernumerary teeth, which appeared symmetrically around the mandibular lateral dentition (three on each side), and asymmetrically around the maxillary incisors (two on the left side) and premolars (one and two on the left and right sides, respectively) (Fig. 3A). III:2 had four supernumerary teeth, which appeared around the mandibular incisors (two on each side) (Fig. 3B and C). The youngest, III:1, had six supernumerary teeth symmetrically around the maxillary canines (one on each side) and mandibular premolars (two on each side) (Fig. 3D).

Regarding the delayed eruption of permanent molars, it was apparently seen in both mandibular second molars of III:1, but not in any maxillary or mandibular molar of III:2 (Fig. 3). III:3 was 12 years old but the maxillary and mandibular molars were far from the occlusal plane.

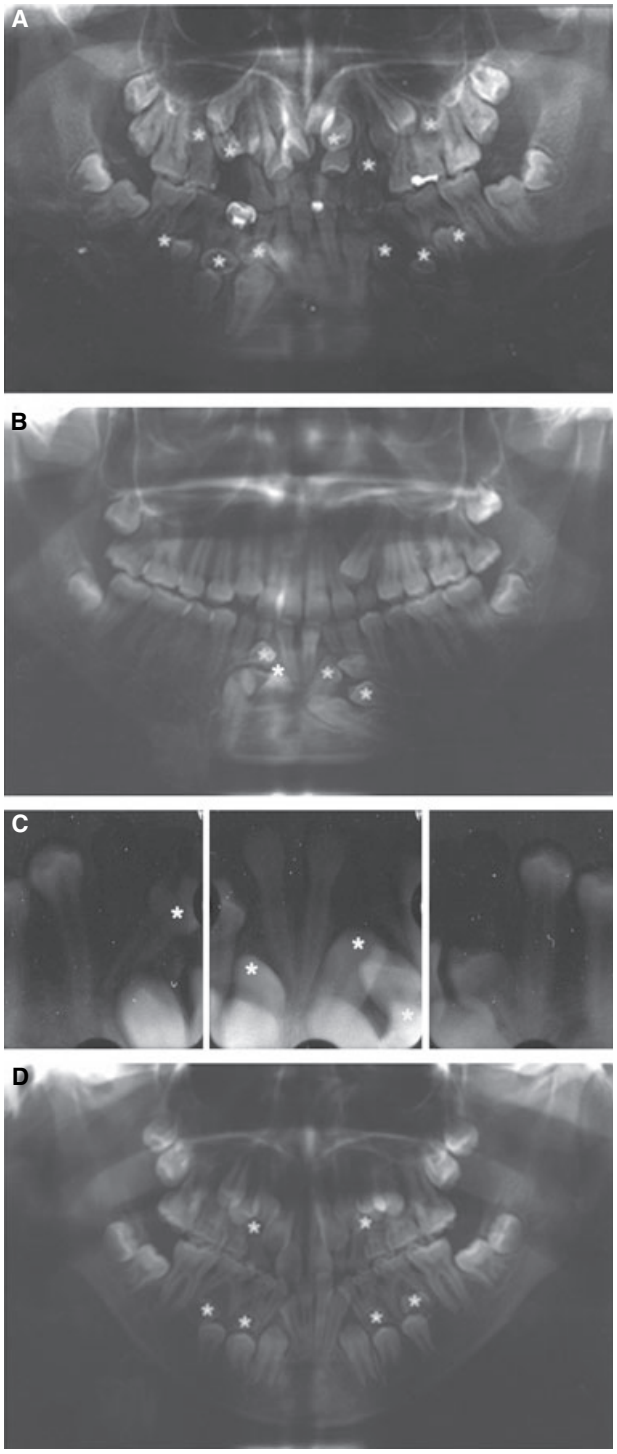

Fig. 3. Panoramic X-rays of III:1 (A: 17-year-old male), III:2 (B: 15-year-old female) and III:3 (D: 12-year-old male). Dental X-rays of the mandibular incisal regions are shown to highlight the exact numbers of supernumerary teeth in III:2 (C). Note the different position and number of supernumerary teeth among the three siblings. Each asterisk denotes a supernumerary tooth.

Discussion

Tooth formation and morphogenesis are known to be strictly governed by the genetic control regulating signal pathways and cellular communications (12). Among numerous genes, *RUNX2*, and its mouse homolog, Runx2, are reported to be crucial molecules (13, 14). In CCD patients, there are wide intrafamilial variations of the skeletal characteristics, but intrafamilial variations of the dental characteristics are obscure (4). Interestingly, the present siblings were discordant for the position and number of

supernumerary teeth, in spite of similar skeletal features (Fig. 3). There was also a clear difference in the delayed molar eruption between III:1 and III:2. There might be an increase in the number of supernumerary teeth with age, as reported previously (15), but the youngest, III:3, had more supernumerary teeth than the older III:2. Moreover, asymmetrical supernumerary tooth formation was seen in the maxilla of III:1. These observations demonstrate that these siblings had completely different patterns of supernumerary tooth formation, even though they share an identical gene mutation.

A possible explanation for the diversity of supernumerary tooth formation in the present siblings is non-genetic or epigenetic regulation of tooth formation. Non-genetic regulation would include environmental factors, e.g. fetal position, nutrition, trauma and exposure to X-ray. Epigenetic regulation is defined as the heritable regulation of gene function that can not be explained by changes in the DNA sequence (16). DNA methylation and histone modifications are known as two molecular mechanisms mediating epigenesis (16).

The present siblings had a mutation in Runt-domain (P210S) (Fig. 2B), which fulfils an important function for DNA binding and dimerization of α and β subunits (6). Many missense mutations are reported to abolish the DNA binding and alter transactivation activity (17, 18). A particularly interesting point is whether the present novel mutation (P210S) alters the DNA-binding and/or transactivation activity of *RUNX2*.

Conclusion

A novel missense mutation in the RUNT-domain (P210S) of *RUNX2* was identified in three Japanese CCD siblings. Intrafamilial variations in the dental characteristics are reported to be obscure in CCD patients; however, the present siblings showed a wide variation in the number and position of supernumerary teeth. These observations suggest the involvement of non-genetic or epigenetic regulation in the formation of supernumerary tooth in CCD patients.

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