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Craniofacial and dental characteristics of three Japanese individuals with genetically diagnosed *SATB2*-associated syndrome

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## **CONFLICT OF INTEREST DISCLOSURE**

The authors have no conflicts of interest to declare.

## **ABSTRACT**

Craniofacial defects are one of the most frequent phenotypes in syndromic diseases. More than 30% of syndromic diseases are associated with craniofacial defects, which are important for the precise diagnosis of systemic diseases. Special AT-rich sequence-binding protein 2 (*SATB2*)-associated syndrome (SAS) is a rare syndromic disease associated with a wide variety of phenotypes, including intellectual disability and craniofacial defects. Among them, dental anomalies are the most frequently observed phenotype and thus becomes an important diagnostic criterion for SAS. In this report, we demonstrate three Japanese cases of genetically diagnosed SAS with detailed craniofacial phenotypes. The cases showed multiple dental problems, which have been previously reported to be linked to SAS, including abnormal crown morphologies and pulp stones. One case showed a characteristic enamel pearl at the root furcation. These phenotypes add new insights for differentiating SAS from other disorders.

**Key words:** *SATB2*-associated syndrome, Craniofacial defect, Tooth anomaly

## **INTRODUCTION**

Special AT-rich sequence binding protein (*SATB2*)-associated syndrome (SAS) or glass syndrome is a systemic disease that is caused by genetic mutation of *SATB2* [Glass et al., 1989; Zarate and Fish 2017]. Frequently observed phenotypes include developmental delay with severely delayed verbal development and a wide spectrum of craniofacial defects, including dental anomalies, cleft palate, and micrognathia [Yamada et al., 2019; Zarate et al., 2018]. A considerable prevalence of crowding, aberrant tooth morphology (including big incisors), dental agenesis, the delayed development of the permanent second premolars, and taurodontism are also evident in the detailed descriptions of dental abnormalities in SAS [Scott et al., 2019; Scott et al., 2018; Zarate et al., 2018]. There is also one report of a rare developing set of odontomas in a genetically diagnosed Japanese SAS patient [Kikui et al., 2018]. Therefore, the detailed documentation of craniofacial and dental phenotypes could become a critical reference for diagnosing SAS syndrome. Herein we report three unrelated Japanese cases of genetically diagnosed SAS with a special focus on craniofacial and dental characteristics. All of the cases exhibited intellectual disability, with severe retardation of verbal development and sialorrhea. One case (patient 3) exhibited isolated cleft palate, calcification of the pulp, malformation of the root, and enamel pearl at the root furcation. These phenotypes have not been described in the literature and add new insights for improving the diagnostic process of SAS and providing a better understanding of the functions of *SATB2* in tooth formation.

## **MOLECULAR ANALYSIS**

Informed consent from all of the families was obtained in accordance with each institutional review board. All patients were registered for the Initiative of Rare and Undiagnosed Disease [Adachi et al., 2017]. Whole exome sequencing was performed

on genomic DNA extracted from peripheral lymphocytes from all the patients and the families using the Sure Select Human All Exon Kit V6 (Agilent Technologies, Santa Clara, CA, USA), with sequencing on the NovaSeq 6000 platform (Illumina, San Diego, CA, USA). We checked the quality of FASTQ files using FASTQC (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) and removed low-quality reads using trimmomatic-0.36 (<http://www.usadellab.org/cms/?page=trimmomatic>). Quality-checked reads were aligned to GRCh37 using Burrows–Wheeler Aligner (<http://bio-bwa.sourceforge.net/>), and variants were called using the GATK HaplotypeCaller. These were annotated using ANNOVAR (<http://annovar.openbioinformatics.org/en/latest/>). *In silico* analyses of variants were performed using CADD (<http://cadd.gs.washington.edu/>) and PROVEAN ([http://provean.jcvi.org/seq\\_submit.php](http://provean.jcvi.org/seq_submit.php)). High frequency (minor allele frequency > 5% in the Japanese population) variants, synonymous variants, and intergenic variants were filtered out manually.

## RESULTS

### Patient 1

Patient 1 was a 9-year-old girl. Medical management started due to her speech and developmental delay at the age of 1 year and 6 months. The score of IQ using Tanaka–Binet Intelligence Scale (Japanese version of Stanford–Binet Intelligence Scale) at 10 years old was very low as 23 [Tanaka Research Institute for Educational Research 2003]. A retrognathic facial profile with sialorrhea could be seen (Figures 1A and 1B). Tooth eruption showed delay, with unerupted first permanent molars at the age of 9 years. The permanent incisors showed shoveled shapes and diastema (Figure 1C). High-arched palate could be seen (Figure 1C). Occlusion showed an anterior open bite (Figure 1D). Periapical radiographs showed a pulp stone in a mandibular deciduous canine and large

dental pulp spaces in mandibular deciduous molars (Figure 1E). Accessory cusp could be detected in a permanent maxillary first premolar (Figure 1F). The first and second deciduous molars showed macrodontia and accessory cusps (Figures 1G and 1H) with severe abrasion on the occlusal surface. We could not detect the maxillary permanent second premolar in the same periapical radiographs, which suggested tooth agenesis. The exome was done on the proband and both unaffected parents, which revealed a missense pathogenic variant in *SATB2* (NM\_015265.4):c.1165C > T (p.Arg389Cys) in this patient, and the patient was thus diagnosed with SAS.

### **Patient 2**

Patient 2 was a 4-year-old boy. He exhibited sialorrhea and delayed speech development (Figure 1I). The same missense pathogenic variant detected in *SATB2* in Patient 1 (*SATB2* (NM\_015265.4):c.1165C > T (p.Arg389Cys)) was detected by the exome on the proband and both unaffected parents. It was difficult for him to cooperate in taking intraoral records; therefore, we could not describe the detailed dental phenotypes for this patient.

### **Patient 3**

Patient 3 was a 10-year-old girl born with a secondary cleft palate. Some of the phenotypes overlapped with those of patient 1, including developmental delay, evident linguistic problems, sialorrhea, retrognathic facial profile, shovel-shaped maxillary permanent incisors, and occlusal abrasion of deciduous molars (Figures 2A–D). She had a history of trauma on a maxillary incisor. Her maxilla showed high-arched palate with constriction caused by scarring following surgical closure of the secondary palate cleft (Figure 2D). Lateral cephalogram showed a skeletal class 2 relationship with a small and clockwise-rotated mandible (Figures 2E and 2F). An enlarged adenoid was also evident from the lateral cephalogram (Figure 2E). From the initial panoramic X-ray, the second premolars were all missing (Figure 2G). Pulp stone formation could be detected in

multiple teeth with taurodontism in the upper first molars (Figures 2G and 2H). Ectopic enamel formation could be detected at the position of the developing root, which eventually developed an enamel pearl on furcation (Figures 2G and 2H). The Exome on the proband, unaffected parents and one sibling revealed a *de novo* heterozygous missense pathogenic variant in this patient (*SATB2*(NM\_015265.4):c.1175G > A (p.Gly392Glu)).

## **DISCUSSION**

Syndromic craniofacial defects are involved in around 30% of systemic diseases [Trainor and Andrews 2013]. Representative craniofacial defects include orofacial cleft and tooth abnormalities, which can occur in both syndromic and sporadic manners. Therefore, the accurate evaluation of craniofacial defects provides valuable information for the precise diagnosis of certain syndromic diseases. Interestingly, a wide range of craniofacial defects can be observed in SAS, including dental anomalies, cleft palate, and micrognathia [Zarate et al., 2018]. However, patients with SAS are sometimes left undiagnosed due to its rarity. Two recent studies have estimated the frequency of SAS in large cohorts of individuals with undiagnosed intellectual disability / developmental delay as 0.24%–0.3% [Bengani et al., 2017; Zarate et al., 2018]. For this reason, describing the detailed dental and craniofacial phenotypes of SAS is important for improving the diagnostic rate of SAS among patients with neurodevelopmental phenotypes. In this case series, no obvious familial history could be detected in any family, which reflects the fact that most SAS cases occur in a sporadic manner. Facial dysmorphism is detected in most cases of SAS [Zarate et al., 2017]. The features include wide variety of features, with micrognathia being one of the most frequent findings [Zarate et al., 2017]. Two out of the three cases (patients 1 and 3) showed a convex-type facial profile, and one patient showed a small mandible in cephalometric analysis. A small



mandible may produce a large overjet and make the incisors prone to trauma [Batista et al., 2018]. Indeed, patient 3 had a history of incisor trauma. These results suggest that orthodontic treatment should be considered to reduce the overjet in SAS patients. A cleft palate such as the Robin sequence might also result from the development of a small mandible during embryogenesis [Godbout et al., 2014]. It is possible that this small mandible is related to the etiology of the cleft palate because majority of facial cleft of SAS result in an isolated cleft palate without cleft lip [Zarate and Fish 2017; Zarate et al., 2017; Zarate et al., 2018]. High-arch palate was reported to be one of the phenotypes of SAS [Docker et al., 2014]. High-arch palate was observed in patient 1 and 3, which indicated that it is a characteristic of the syndrome. Hypotonia is a common phenotype in SAS [Zarate et al., 2018]. Hypotonia in orofacial muscle can cause insufficient lip seal and result in flared out incisors and anterior open bite that could be seen in patients 1 and 3 [Kiliaridis and Katsaros 1998].

More than 90% of SAS patients carry at least one dental anomaly, such as macrodontia, malformed crowns, or tooth agenesis [Scott et al., 2018]. Especially for patients 1 and 3, crown malformation with pulp stone could be observed. Large dental pulp space could be seen in mandibular deciduous molars, which indicate defective dentin formation.

Macrodontia could result in a lack of space for tooth alignment and cause dental crowding, another typical characteristic in SAS. Conversely, tooth agenesis, which is also frequently observed in the mandibular second premolars in SAS, can cause extra space, which sometimes requires dental management. Animal experiments have revealed that the loss of *Satb2* in mice results in small mandibles by inhibiting osteoblast differentiation [Britanova et al., 2006; Dobрева et al., 2006]. Additionally, the absence of *Satb2* results in alterations in the expression of transcription factors that are important for tooth development in developing jaws, such as *Msx1* and *Pax9*, which underlies the molecular etiology of tooth malformation [Britanova et al., 2006].

The calcification of the dental pulp in patients with SAS has been reported in the literature [Scott et al., 2018]. Other disorders, including FAM20A-associated Enamel-Renal-Gingival and Ehlers-Danlos Syndromes, have been linked to pulp stone development [Kantaputra et al., 2017; Kapferer-Seebacher et al., 2020; Wang et al., 2013]. In a research on animals, *Satb2* has demonstrated its ability to act as a regulator of genes crucial for the calcification of bone and dentin, including osteocalcin [Dobrevva et al., 2006]. Additionally, *Fam20a* stimulates *Fam20c*, a kinase that phosphorylates proteins in the secretory pathway to control biomineralization, and its disruption may potentially result in the pulp stone development [Vogel et al., 2012]. These findings suggest that a disturbed biomineralization process caused by mutations in *SATB2* and *FAM20A* shares a common mechanism for generating the pulp stones. On the other hand, the process of producing the pulp stones in the Ehlers-Danlos syndrome is thought to link with disturbed pulp homeostasis caused by the mutations in *COL5A1* or *COL5A2* [Kapferer-Seebacher et al., 2020].

The detailed molecular and cellular etiology of enamel pearls is still elusive. The most widely accepted theory for enamel pearl formation is that a tissue known as epithelial cell rests of Malassez (ERM), which is a remnant of the Hertwig's epithelial root sheath, differentiate into ameloblasts to deposit an ectopic enamel matrix on the surface of the tooth root [Cavanha 1965; Pinho and Cavanha 1945; Zengin et al., 2022]. A signaling pathway may be inhibited by *SATB2*, and this could induce ERMs to differentiate into ameloblasts. This enamel pearl phenotype has not been mentioned much in the literature. However, enamel pearls on the molars can be seen in some of the published cases [Kikuiiri et al., 2018, Figure 2(e) and (f)]. Enamel pearls sometimes preclude normal gingival attachment and thus induce deeper periodontal pockets, which requires periodontal management [Risnes et al., 2000].

There are various types and positions of mutation in *SATB2* that are reported to be

responsible for SAS. Several aspects show genotype–phenotype correlation, such as growth retardation and sialorrhea, which correlate significantly with large deletions and missense mutations in *SATB2*, respectively [Zarate et al., 2018]. The microdeletion of the 2q33.1 locus, incorporating the genomic locus of *SATB2*, has demonstrated a clinical resemblance to the dental anomalies seen in SAS [Balasubramanian et al., 2011]. Hence, even in cases where the chromosome has a microdeletion encompassing genes close to *SATB2*, the haploinsufficiency of *SATB2* is most likely the main underlying cause of the SAS phenotype (Supplemental Table1).

In this case series, all three patients exhibited missense mutations and two patients (patients 1 and 2) had identical mutations. All of the patients in this study exhibited sialorrhea. However, there is still a lack of evidence for genotype–phenotype correlations for dental findings in SAS. From these results, it is important to continuously report craniofacial and dental characteristics of SAS for precise diagnosis and dental treatment planning. In this study, we have reported three unrelated Japanese genetically diagnosed SAS cases and revealed new dental phenotypes, including enamel pearls, adding to the diagnosis criteria for SAS.

#### **AUTHOR CONTRIBUTION**

Hiroshi Kurosaka was responsible for the conception and design of the study. Sayuri Yamamoto, Kyoko Hirasawa, Tomoe Yanagishita, Kaoru Fujioka, Namiki Nagata, Takayuki Tsujimoto, Toshihiro Inubushi were clinically involved in the patients. Miho Nagata, Yasuki Ishihara, Ayumi Yonei and Yoshihiro Asano were responsible for acquisition and analysis of whole exome sequencing data. Hideaki Yagasaki, Toshiyuki Yamamoto, Norio Sakai and Takashi Yamashiro supervised the study. All authors have read and approved the final article.

## REFERENCES

- Adachi T, Kawamura K, Furusawa Y, Nishizaki Y, Imanishi N, Umehara S, Izumi K, Suematsu M. 2017. Japan's initiative on rare and undiagnosed diseases (IRUD): towards an end to the diagnostic odyssey. *Eur J Hum Genet* 25(9):1025-1028.
- Batista KB, Thiruvengkatachari B, Harrison JE, O'Brien KD. 2018. Orthodontic treatment for prominent upper front teeth (Class II malocclusion) in children and adolescents. *Cochrane Database Syst Rev* 3:CD003452.
- Balasubramanian M, Smith K, Basel-Vanagaite L, Feingold MF, Brock P, Gowans GC, Vasudevan PC, Cresswell L, Taylor EJ, Harris CJ, Friedman N, Moran R, Feret H, Zackai EH, Theisen A, Rosenfeld JA, Parker MJ. 2011. Case series: 2q33.1 microdeletion syndrome--further delineation of the phenotype. *J Med Genet* 48(5):290-298.
- Bengani H, Handley M, Alvi M, Ibitoye R, Lees M, Lynch SA, Lam W, Fannemel M, Nordgren A, Malmgren H, Kvarnung M, Mehta S, McKee S, Whiteford M, Stewart F, Connell F, Clayton-Smith J, Mansour S, Mohammed S, Fryer A, Morton J, Consortium UK, Grozeva D, Asam T, Moore D, Sifrim A, McRae J, Hurles ME, Firth HV, Raymond FL, Kini U, Nellaker C, Ddd S, FitzPatrick DR. 2017. Clinical and molecular consequences of disease-associated de novo mutations in SATB2. *Genet Med* 19(8):900-908.
- Britanova O, Depew MJ, Schwark M, Thomas BL, Miletich I, Sharpe P, Tarabykin V. 2006. Satb2 haploinsufficiency phenocopies 2q32-q33 deletions, whereas loss suggests a fundamental role in the coordination of jaw development. *Am J Hum Genet* 79(4):668-678.
- Cavanha AO. 1965. Enamel Pearls. *Oral Surg Oral Med Oral Pathol* 19:373-382.
- Dobrev G, Chahrour M, Dautzenberg M, Chirivella L, Kanzler B, Farinas I, Karsenty G, Grosschedl R. 2006. SATB2 is a multifunctional determinant of craniofacial patterning and osteoblast differentiation. *Cell* 125(5):971-986.
- Docker D, Schubach M, Menzel M, Munz M, Spaich C, Biskup S, Bartholdi D. 2014. Further delineation of the SATB2 phenotype. *Eur J Hum Genet* 22(8):1034-1039.
- Glass IA, Swindlehurst CA, Aitken DA, McCrea W, Boyd E. 1989. Interstitial deletion of the long arm of chromosome 2 with normal levels of isocitrate dehydrogenase. *J Med Genet* 26(2):127-130.
- Godbout A, Leclerc JE, Arteau-Gauthier I, Leclerc LD. 2014. Isolated versus pierre robin sequence cleft palates: are they different? *Cleft Palate Craniofac J* 51(4):406-411.
- Kantaputra PN, Bongkochwilawan C, Lubinsky M, Pata S, Kaewgahya M, Tong HJ,

- Ketudat Cairns JR, Guven Y, Chairisookumporn N. 2017. Periodontal disease and FAM20A mutations. *J Hum Genet* 62(7):679-686.
- Kapferer-Seebacher I, Schnabl D, Zschocke J, Pope FM. 2020. Dental manifestations of Ehlers-Danlos syndromes: a systematic review. *Acta Derm Venereol* 100(7):adv00092.
- Kikuri T, Mishima H, Imura H, Suzuki S, Matsuzawa Y, Nakamura T, Fukumoto S, Yoshimura Y, Watanabe S, Kinoshita A, Yamada T, Shindoh M, Sugita Y, Maeda H, Yawaka Y, Mikoya T, Natsume N, Yoshiura KI. 2018. Patients with SATB2-associated syndrome exhibiting multiple odontomas. *Am J Med Genet A* 176(12):2614-2622.
- Kiliaridis S, Katsaros C. 1998. The effects of myotonic dystrophy and Duchenne muscular dystrophy on the orofacial muscles and dentofacial morphology. *Acta Odontol Scand* 56(6):369-374.
- Pinho A, Cavanha AO. 1945. Study on the dental health pattern of Brazilian workers and breeding methods. *Rev Bras Odontol* 3:74-77.
- Tanaka Research Institute for Educational Research. 2003. Tanaka Binet Chinou Kensa V [Tanaka-Binet Intelligence Scale V]. Taken Publishing.
- Risnes S, Segura JJ, Casado A, Jimenez-Rubio A. 2000. Enamel pearls and cervical enamel projections on 2 maxillary molars with localized periodontal disease: case report and histologic study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 89(4):493-497.
- Scott J, Adams C, Beetstra S, Zarate YA. 2019. SATB2-associated syndrome (SAS) and associated dental findings. *Spec Care Dentist* 39(2):220-224.
- Scott J, Adams C, Simmons K, Feather A, Jones J, Hartzell L, Wesley L, Johnson A, Fish J, Bosanko K, Beetstra S, Zarate YA. 2018. Dental radiographic findings in 18 individuals with SATB2-associated syndrome. *Clin Oral Investig* 22(8):2947-2951.
- Trainor PA, Andrews BT. 2013. Facial dysostoses: Etiology, pathogenesis and management. *Am J Med Genet C Semin Med Genet* 163C(4):283-294.
- Vogel P, Hansen GM, Read RW, Vance RB, Thiel M, Liu J, Wronski TJ, Smith DD, Jeter-Jones S, Brommage R. 2012. Amelogenesis imperfecta and other biomineralization defects in *Fam20a* and *Fam20c* null mice. *Vet Pathol* 49(6):998-1017.
- Wang SK, Aref P, Hu Y, Milkovich RN, Simmer JP, El-Khateeb M, Daggag H, Baqain ZH, Hu JC. 2013. FAM20A mutations can cause enamel-renal syndrome (ERS). *PLoS Genet* 9(2):e1003302.
- Yamada M, Uehara T, Suzuki H, Takenouchi T, Yoshihashi H, Suzumura H, Mizuno S,

- Kosaki K. 2019. SATB2-associated syndrome in patients from Japan: Linguistic profiles. *Am J Med Genet A* 179(6):896-899.
- Zarate YA, Fish JL. 2017. SATB2-associated syndrome: Mechanisms, phenotype, and practical recommendations. *Am J Med Genet A* 173(2):327-337.
- Zarate YA, Kalsner L, Basinger A, Jones JR, Li C, Szybowska M, Xu ZL, Vergano S, Caffrey AR, Gonzalez CV, Dubbs H, Zackai E, Millan F, Telegrafi A, Baskin B, Person R, Fish JL, Everman DB. 2017. Genotype and phenotype in 12 additional individuals with SATB2-associated syndrome. *Clin Genet* 92(4):423-429.
- Zarate YA, Smith-Hicks CL, Greene C, Abbott MA, Siu VM, Calhoun A, Pandya A, Li C, Sellars EA, Kaylor J, Bosanko K, Kalsner L, Basinger A, Slavotinek AM, Perry H, Saenz M, Szybowska M, Wilson LC, Kumar A, Brain C, Balasubramanian M, Dubbs H, Ortiz-Gonzalez XR, Zackai E, Stein Q, Powell CM, Schrier Vergano S, Britt A, Sun A, Smith W, Bebin EM, Picker J, Kirby A, Pinz H, Bombei H, Mahida S, Cohen JS, Fatemi A, Vernon HJ, McClellan R, Fleming LR, Knyszek B, Steinraths M, Velasco Gonzalez C, Beck AE, Golden-Grant KL, Egense A, Parikh A, Raimondi C, Angle B, Allen W, Schott S, Algrabli A, Robin NH, Ray JW, Everman DB, Gambello MJ, Chung WK. 2018. Natural history and genotype-phenotype correlations in 72 individuals with SATB2-associated syndrome. *Am J Med Genet A* 176(4):925-935.
- Zengin AZ, Sumer AP, Ozturk G, Noujeim M. 2022. Imaging characteristics of enamel pearls on CBCT and their co-relation with supernumerary tooth. *Oral Radiol* 38(3):370-377.

## FIGURES

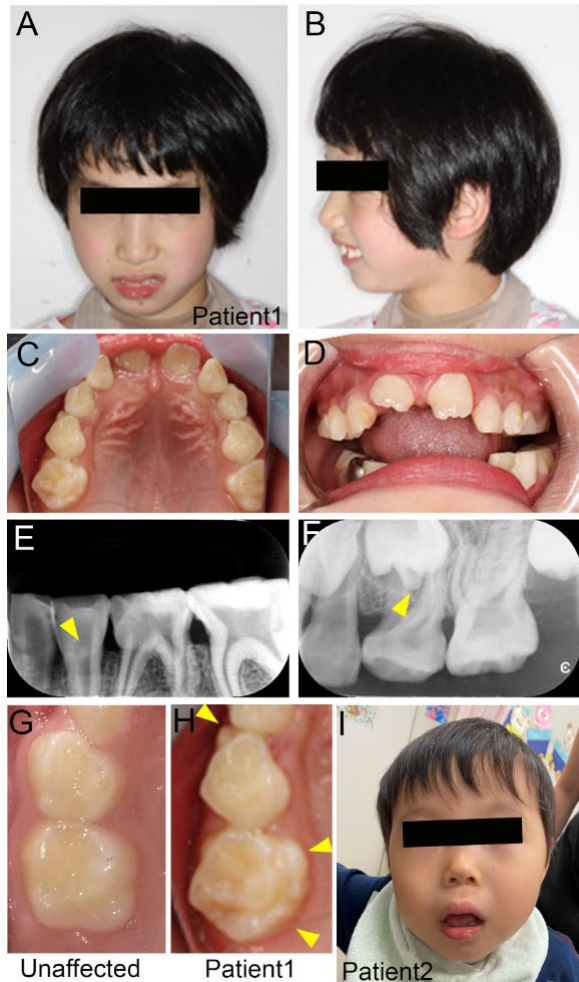


Figure 1

**Figure 1. Facial and dental features of patients 1 and 2.** Facial photograph of the 9 year-old patient (patient 1) (A and B). Intraoral photograph of patient 1 (C and D). Patient 1 showed high-arched palate (C) and anterior open bite (D). Periapical radiograph of patient 1 at mandible right molar area (E) and maxillary left molar area (F). Large dental pulp space could be detected (E). Yellow arrowheads in E and F indicate pulp stone and ectopic cusp, respectively. Magnified occlusal view of maxillary right deciduous molar area of unaffected individual (G) and patient 1 (H). Yellow arrowheads in H indicate ectopic cusps. Facial photograph of patient 2 (I).

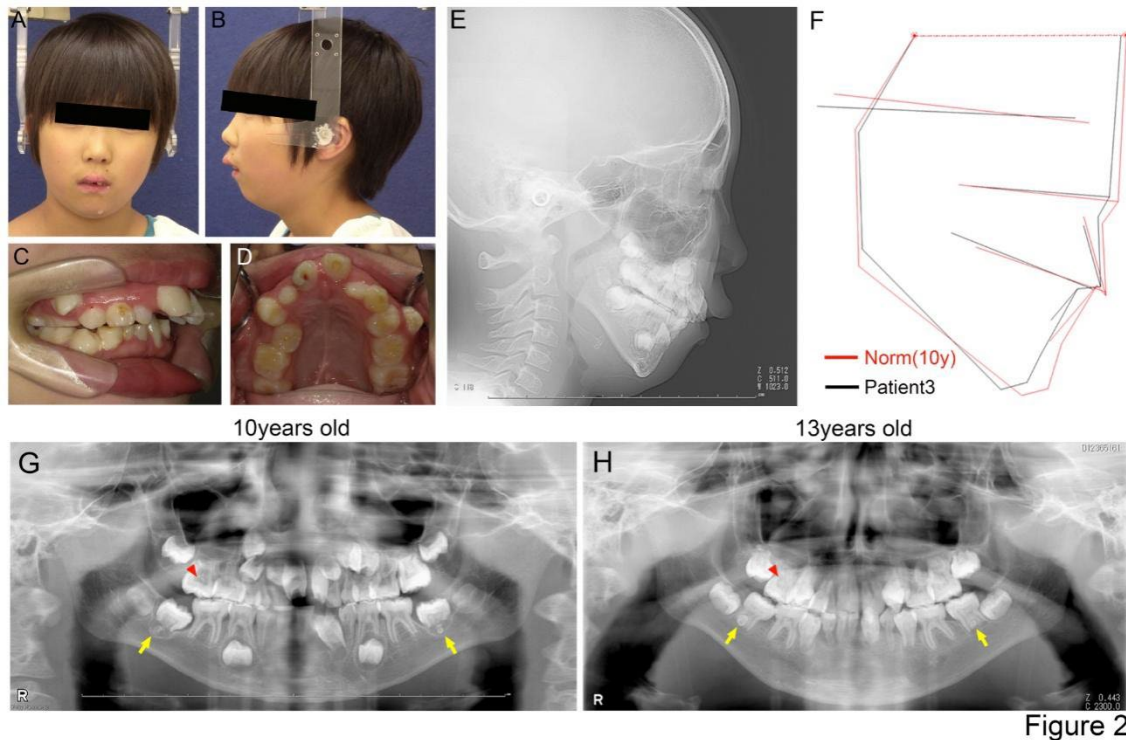


Figure 2

**Figure 2. Facial and dental features of patient 3.** Facial photograph of Patient 3 (A and B). Intraoral photo of patient 3 (C and D). Occlusal view of maxilla showed high-arched palate (D). Lateral cephalogram (E) and profilogram (F) of patient 3. Sequential orthopantomogram of patient 3 at 10 years old (G) and 13 years old (H). Red arrowheads in G and H indicate progressive pulp stones and taurodontism. Yellow arrowheads indicate developing enamel pearls in the area of furcation and delayed eruption of mandibular first molars.