

Original Hypothesis

A Proposed Mechanism for Congenitally Missing Teeth: Basic and Clinical Evidence

Gajanan Kulkarni ^a, Reiko Nomura ^a, Ka-On Laurel Lee ^a, Seema Shah ^a

^a Department of Pediatric and Preventive Dentistry, Faculty of Dentistry, University of Toronto, Toronto, Ontario, Canada.

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Correspondence to:

Gajanan (Kiran) Kulkarni
Department of Pediatric and Preventive Dentistry, 124 Edward Street, Room 455D, Faculty of Dentistry, University of Toronto, Toronto, Ontario, Canada, M5G 1G6.

Tel: +1- 416 979-4929 ext. 4460

Fax: +1- 416 979-4753/4936

g.kulkarni@utoronto.ca

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Abstract

Introduction: Although the development of normal dentition has been explored extensively, the mechanisms underlying congenitally missing teeth are far less understood.

The hypothesis: Congenital absence of teeth occurs due to arrested development of a tooth primordium followed by involution, only at a stages preceding mineralized tissue formation.

Evaluation of the hypothesis: We compared H & E stained serial sagittal sections of wild-type and EL mice that are congenitally missing 3rd molars (3M). 3M development was followed longitudinally in both types of mice. Occurrence of apoptosis was examined using a fluorescent TUNEL assay. To determine if a similar process might account for congenital absence of human teeth, we examined serial radiographs of developing dentitions. In EL mice, congenital absence of 3M is caused, not by a failure of initiation of tooth development rather; tooth development is initiated and subsequently arrested during early cap stage. This arrested tooth primordium is subsequently removed physiologically by apoptosis. Examination of serial radiographs where missing teeth were identified lent further evidence to support this hypothesis. Follicle spaces, with no calcified tissue within them, were noted at early stages which were seen to remodel and eventually blend with adjacent bone. Permanent teeth failed to develop in those locations. Based on the animal and human data, we propose a new model for congenital absence of teeth. Validation of this model could have profound clinical implications. If the genetic mechanisms involved in this proposed mechanism can be elucidated, it might lead to non-surgical management of supernumerary teeth.

Key words: Tooth development; Congenital absence; Apoptosis; Tooth primordium; Mice; Human; Dental radiographs.

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Introduction

Although the development of normal dentition has been explored extensively, the molecular mechanisms underlying congenitally absent teeth are far less understood. Tooth development encompasses a complex series of epithelial-mesenchymal interactions and any disturbances in these processes can result in tooth agenesis (1). Tooth agenesis is the most common craniofacial malformation with 8% of the population missing one or more teeth excluding 3rd molars. Hypodontia is the congenital absence of fewer than 6 permanent teeth and oligodontia is when 6 or more permanent teeth are congenitally missing.

Hypodontia is classified as syndromic, where it is associated with other symptoms of a disease, or non-syndromic, where it is an isolated occurrence (2). There is a balance between cell division and programmed cell death (apoptosis) which regulates the development of appropriately sized teeth at the correct position. Apoptosis plays a passive and active role in tooth bud formation and morphogenesis (3). The involvement of several mutations in two transcription factor coding genes, MSX1 and PAX9, in tooth agenesis has been frequently reported (4,5). However, there are also cases of tooth agenesis in families in whom MSX1 and

PAX9 mutations have been excluded (6). Thus there must be other genes causing tooth agenesis. Accordingly, it is suggested that there may be other molecular-genetic mechanisms leading to the occurrence of non-syndromic congenitally missing teeth.

The hypothesis

The mechanism for the occurrence of congenitally missing teeth may be due to the expression or misexpression of certain genes at certain times in the development of the tooth germ. In certain cases the developing tooth germ may be initiated normally however, abnormal apoptosis leads to involution of the developing tooth germ resulting in agenesis of that tooth. We propose that certain genes promoting the progression of tooth morphogenesis are not expressed for the process to proceed. Alternatively, the genes that cause programmed cell death are inadvertently expressed causing the body to start resorbing the developing tooth germ.

Evaluation of the hypothesis

Histological evidence in the EL mouse model

We have documented evidence for support of this hypothesis from histological examination of the Epilepsy-like (EL) mice that exhibit 97% incidence of congenital

absence of third molars (3M) (7). The EL mouse was established as an animal model for the study of complex partial seizures in humans (8). Sagittal sections of the maxilla and mandible in the third molar

region of EL mice were examined and compared to normal mice. There were no signs of maxillary or mandibular third molars in adult EL mice (Figure 1).

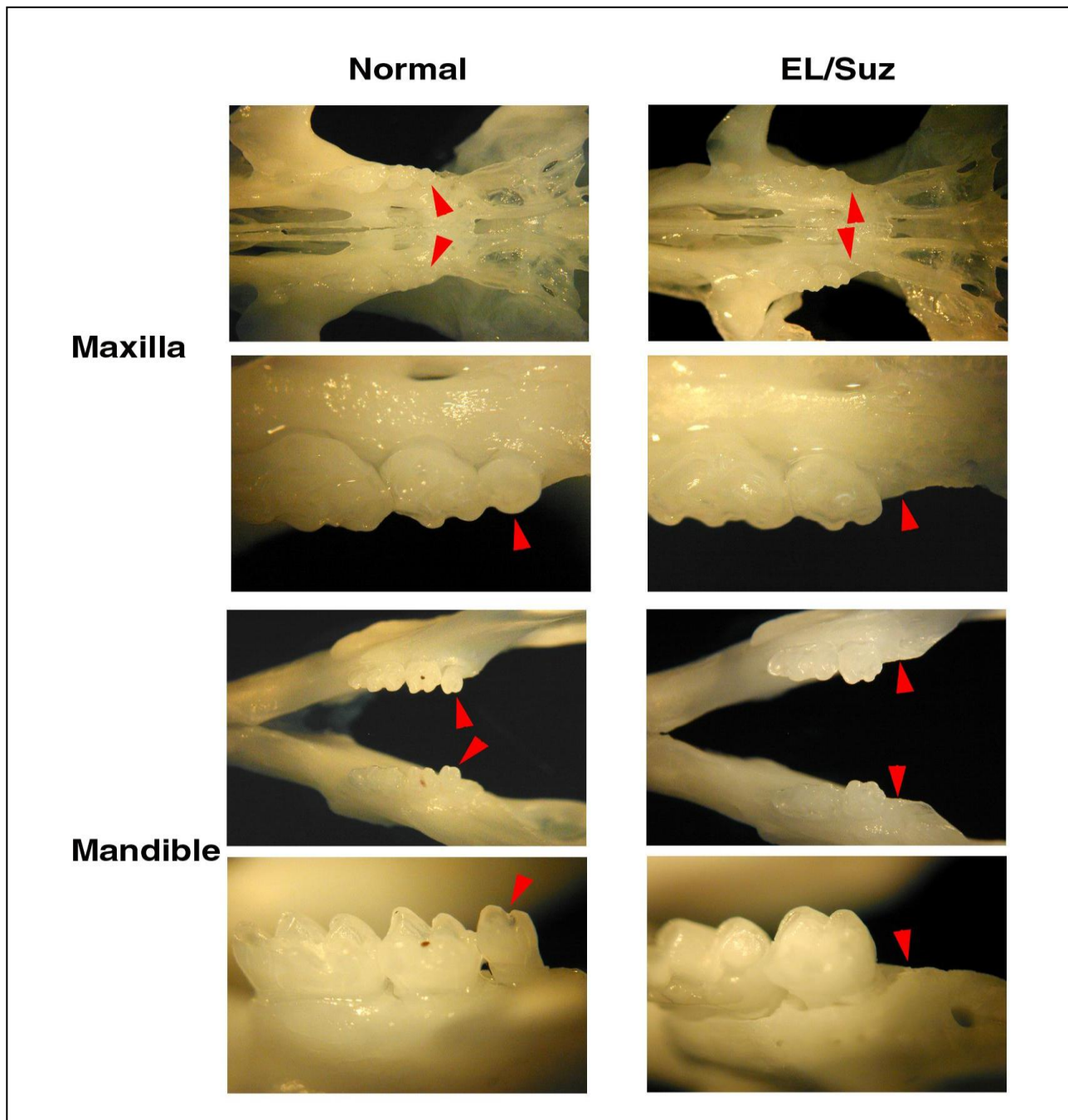


Figure 1. Maxillae and mandibles from adult wild-type and EL mice. Arrowheads point to third molars in wild-type and missing third molars in EL mice.

Alveolar bone in the missing third molar region was significantly underdeveloped. Developing mandibular third molars in normal mice progressed to bud stage by

postnatal (P) day 0 (P0), progressing to cap stage by P3 and bell stage by P5. At P7, the tooth was in differentiation stage (Figure 2a).

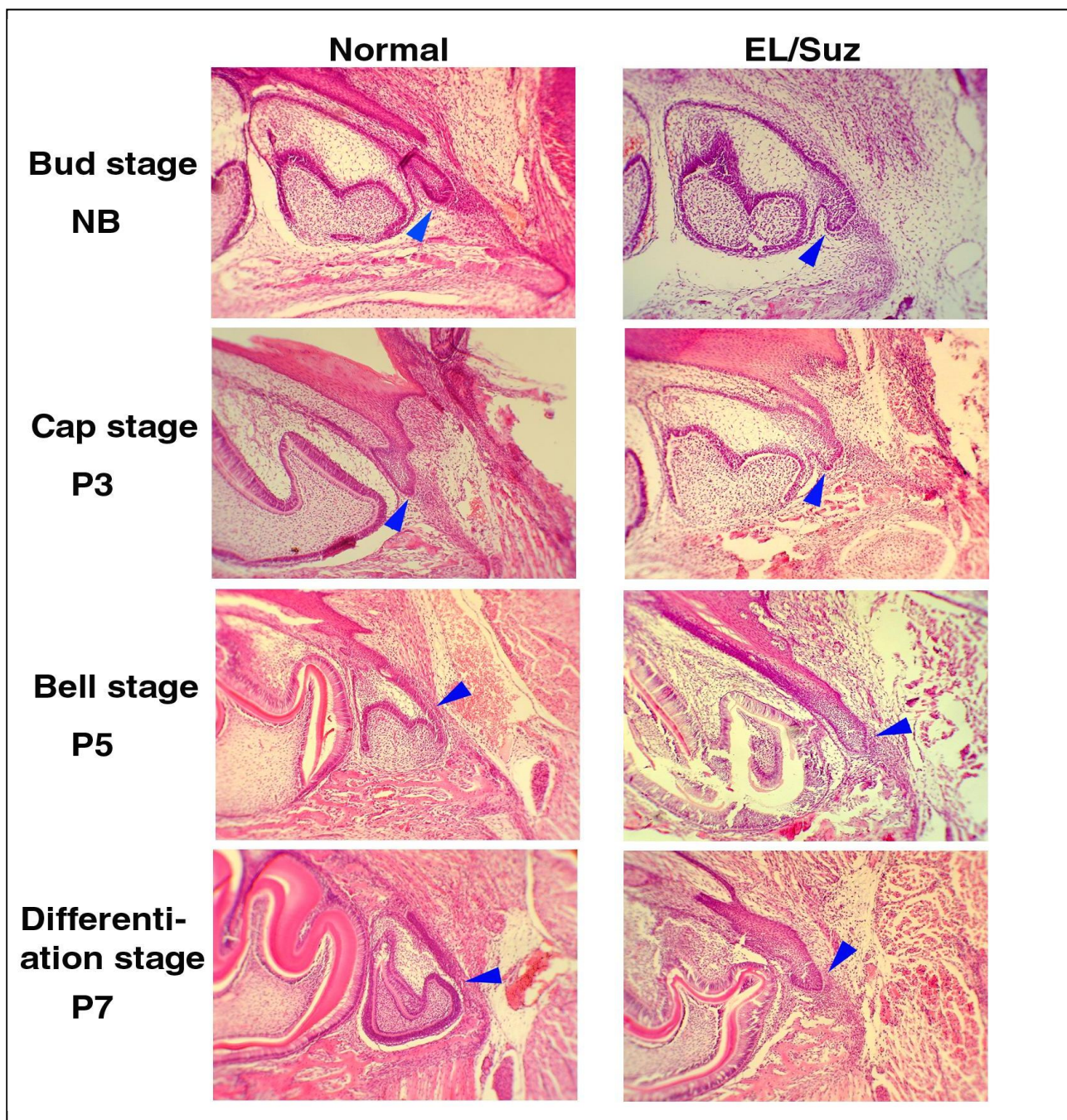


Figure 2a. Third molar development in wild-type and EL mice. Arrowheads point to third molars in wild-type and missing third molars in EL mice.

Compared to normal mice, mandibular 3M development in EL mice progressed from bud to early cap from P0 to P3, but did not progress beyond the cap stage (Fig. 2a). The developing tooth gradually

regressed, and finally at P15 it completely disappeared (Fig. 2b). In young adult EL mice at P21, attrition of the alveolar bone accompanied regression of the third molar (Figure 2b).

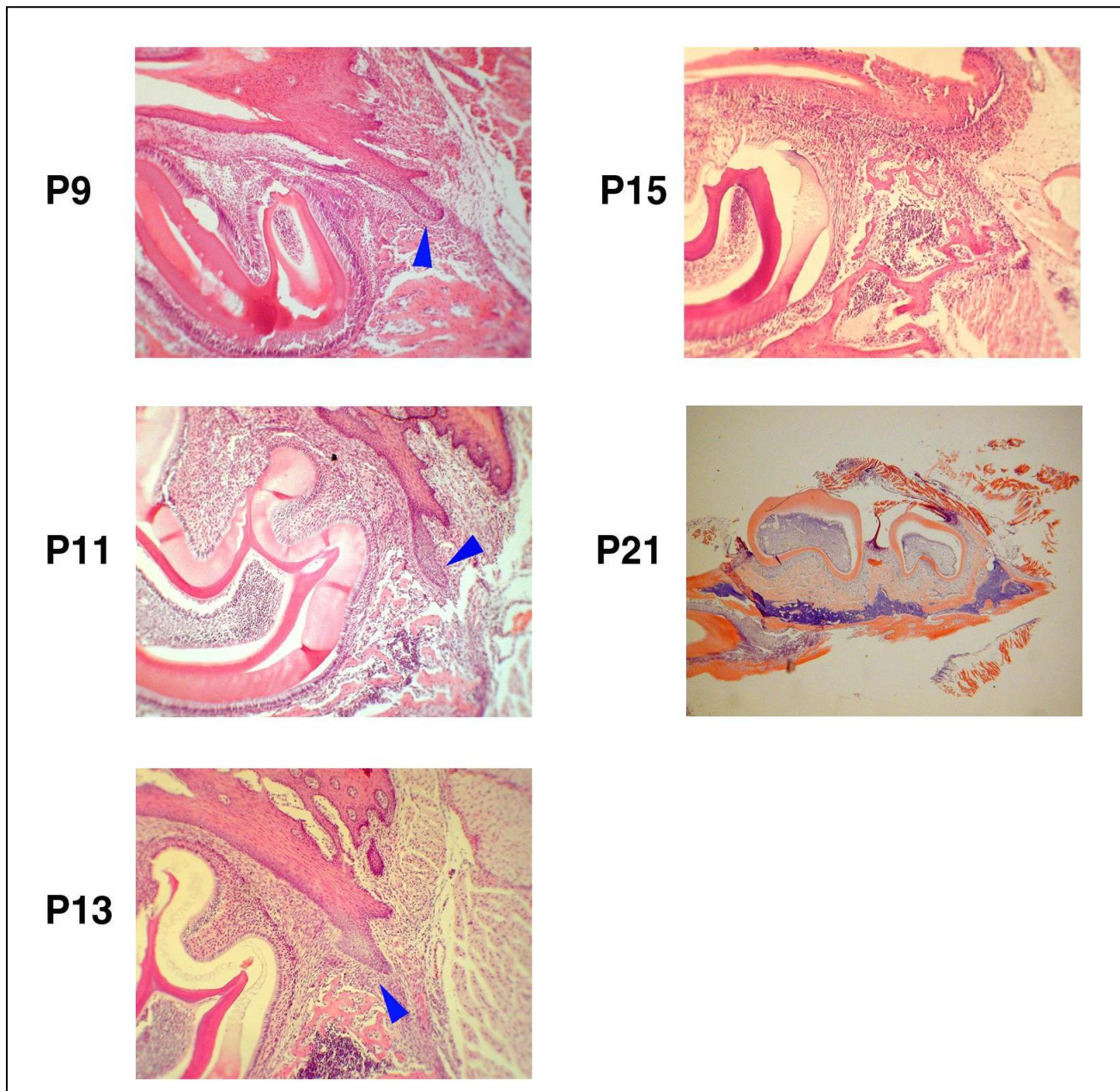


Figure 2b. Third molar development in EL mice from post natal days 9 to 21 showing progressive involution of 3M primordium (arrowheads) to complete elimination by P21.

Apoptotic cells (Figure 3) appeared from NB to P5 and were seen mainly in mesenchymal tissues including the dental follicle. At P7 and P9, apoptotic cell death was clearly detected in epithelial tissues and only some apoptotic cells were noted in mesenchymal tissues. Pronounced cell death again moved to mesenchymal tissues, such as the dental follicle, at P11 and P13. No apoptosis was noted beyond P13 consistent with our histological finding of complete disappearance of the tooth by P15. The apoptosis was detected by the TUNEL assay (Figure 3), performed using

an in situ cell death detection kit (TMR red and Fluorescein; Roche Molecular Biochemicals, Germany). Sagittal sections were examined by epifluorescence microscopy. The green excitation wavelength range (520-560 nm) and the red detection range (570-620 nm) were used for TMR red labelled samples. The blue excitation wavelength range (570-620 nm) and green detection range (450-500 nm) was used for fluorescein labelled samples. For positive and negative controls, osteoblastic cells treated with and without DNase I were used.



Figure 3. In situ detection of apoptosis in third molars of EL mice. Arrowheads point to apoptosing cells.

Clinical evidence for the hypothesis

Figure 4 is a radiograph showing the corticated border around the follicle of the developing tooth germs of both lower permanent 2nd premolar teeth.

In this radiograph there is no evidence of calcification in either of these lower 2nd premolar follicles. On the right there is no evidence of the corticated border of the developing bicuspid as indicated by the green arrow. On the left, there is some residual cortication with disappearance of the inferior half along with bony infill as indicated by the red arrow. The yellow arrows show early mineralization of the

cusps tips of the lower 3rd molars. This is sometimes referred to as the 'snow-cap' stage due to its typical radiographic appearance.

Figure 5 shows that the lower right permanent 2nd premolar is developing normally however the lower left permanent 2nd premolar has involuted. The corticated border of the follicle can still be seen however bone infill is also evident as indicated by the red arrow.

Figure 6 shows the initial stages of mineralization of the cusps tips of the lower left 2nd premolar indicated by the red arrow.



Figure 4. Radiograph showing congenital absence of lower 2nd premolars and the normal early stage of calcification of the cusps tips of the 3rd molars.



Figure 5. Radiograph showing a normally developing lower right 2nd premolar and congenital absence of lower left 2nd premolar.



Figure 6. Periapical radiograph showing the snow-cap stage of early mineralization of the cusp tips of lower left 2nd premolar.

Discussion

The serial sagittal sections of EL mice suggest that the congenital absence of the 3M was not caused by a failure of initiation of tooth development. Rather, tooth development is initiated and subsequently arrested during the early cap stage. The remaining tooth primordium is removed physiologically.

The phenomenon of spontaneous abortion and disappearance of the follicle does not seem to occur if any mineralized tissue is visible radiographically. In other words, this phenomenon seems to be possible only in the initial stages of tooth development, namely, initiation, proliferation and morphodifferentiation stages but prior to mineralization. This might suggest the differential expression sensitivity of different genes and the robustness of others. In figure 4 the partial loss of the corticated border and bony infill of the follicle of the lower left 2nd premolar suggests that this developing follicle is undergoing apoptosis and the tooth will be congenitally missing. The developing 3rd molars however will now not undergo involution as they have passed the point of no return, that is, after the first evidence of calcification the tooth will continue through the normal stages of development. This snow-cap stage is clearly shown in Figure 6 in the case of a lower left 2nd premolar which will now continue through the normal

stages of dental development. Figure 5 also suggests involution of the lower left 2nd premolar tooth germ. This can occur at any time before calcification is seen within the follicle, resulting in tooth agenesis.

In the radiographic cases presented no reference to chronological or dental age is made as these described phenomena can occur regardless of age. A very significant delay in tooth development can be mistaken or incorrectly diagnosed as congenital absence. However the longitudinal radiographic follow up of the tooth with criteria described above can help distinguish between the two. It is important to remember that the time when a tooth erupts can vary significantly from the norms established for different races, ethnicities and sexes (9). A permanent tooth should erupt no later than 6 months after natural exfoliation of its predecessor. This is considered normal, however to be conservative most practitioners will only consider eruption to be delayed after 1 year. In the case of second premolars, eruption can be significantly delayed for several years. No reference to chronological or dental age is made as these described phenomena can occur regardless of age. If practitioners follow up the delayed eruption with longitudinal radiographs then a delay in tooth development would still show normal radiographic stages of development,

namely, cortication of the follicle followed by mineralisation of the developing tooth. As soon as there is the first radiographic evidence of calcification of the developing tooth it can be assumed that this tooth is going to develop completely, although it might be significantly delayed. To definitively label a tooth as congenitally absent, the following radiographic signs should be considered: significant delay compared to the contralateral side, involution of the corticated border with bone infill and no evidence of calcification of cusp tips. This is important for the management of the deciduous predecessor and spacing in the arch according the long-term treatment plan.

If the genes which cause the inadvertent involution of the developing teeth can be identified then artificial intra-osseous injection of these genes into developing tooth follicles could prevent the formation of unwanted 3rd molars or supernumerary teeth avoiding later surgical extraction. On the flip side, if expression or misexpression factors could be identified which prevent the involution of the developing tooth then these could be artificially injected into developing follicles to rescue those teeth in patients who have a family history of congenitally missing teeth or in the early stages of mineralisation if there is any evidence of involution starting to occur. There need to be addi-

tional suitable animal models to study these phenomena and to identify which genes are involved in the involution of the developing teeth. The concept of “congenital” absence of teeth might have to be revisited and possibly revised if genetic evidence can be correlated to longitudinal radiographic findings.

List of abbreviations

- H & E: Haematoxylin and Eosin.
- 3M: Third molar.
- TUNEL: Terminal deoxynucleotidyl transferase dUTP nick end labeling.
- EL: Epilepsy-like.
- P: Postnatal day.

Conflicts of interests

The authors declare that they have no competing interest. GK has editorial involvement with *Dental Hypotheses*.

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Authors' contributions

- *Main idea:* by GK.
- *Literature search:* by RN, KL, SS.
- *Data interpretation:* by GK, RN, KL.
- *Manuscript preparation:* by all authors.
- *Data collection:* by RN, GK.
- *Funds Collection:* GK.

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