

# Analysis of fused maxillary incisor dentition in *p53*-deficient exencephalic mice

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## ABSTRACT

Out of a total of 21 exencephalic *p53*-deficient embryonic and newborn mice, 6 (28.6%) possessed fused maxillary incisor teeth. On histological analysis of the 5 examples seen on day 19.5 of gestation and newborn mice, 3 varieties were observed: an example of 'simple' fusion, 3 examples of simple fusion each of which contained a 'dens in dente' ('tooth within a tooth'), and a single example in which the fused teeth were associated with a median supernumerary incisor tooth which, while deeply indenting the labial surface of the fused teeth, was in all locations a completely separate unit. 3-D reconstructions of the fused teeth demonstrated that they were all of the fusio subtotalis variety. No gross abnormalities were observed in the other dentition in these mice. It is noted that in mice fused maxillary incisor teeth are relatively commonly associated with both hypervitaminosis A-induced and trypan blue-induced exencephaly. It is believed that the presence of dens in dente within fused maxillary incisor teeth has only once been reported in mice, and the association between fused maxillary incisor teeth and a median supernumerary incisor tooth has not previously been reported in this species.

*Key words:* Exencephaly; fused maxillary incisor teeth; supernumerary incisor tooth; 3-D reconstruction.

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## INTRODUCTION

Fused midline upper incisor teeth are only very rarely encountered in mice, although they are relatively frequently observed in association with experimentally induced severe neural tube defects in this species. In one particularly large study, Knudsen (1965*a, b*) exposed pregnant mice to a high dose of vitamin A and observed some degree of fusion of the upper incisor dentition in 48% of cases (in 130 of the 272 fetuses analysed histologically). These were subdivided into 3 categories depending on the degree of fusion, from *partial* fusion (fusio partialis) in which fusion was only observed in the central parts of the tooth germs, to *subtotal* fusion (fusio subtotalis) in which both the anterior (incisal) and central parts were fused while the most posterior (or basal) parts of the 2 tooth germs (i.e. the roots) were still present as separate units, through to the third category in which *complete* fusion (fusio totalis) was present and extended from

the incisal to the basal end of the tooth germs. The incidence of the 3 types of fusion observed was 10 (7.7%), 17 (13.1%) and 103 (79.2%), respectively, and in a fourth category 'contact', but with no evidence of fusion, was observed between the 2 upper incisors in 2 additional cases.

Knudsen (1966*a*) subsequently induced exencephaly by exposing pregnant mice to trypan blue (Kalter & Warkany, 1959) in order to establish whether fused upper incisors were induced as a consequence of exposure to vitamin A, or there was an association between fused teeth and exencephaly. 47 exencephalic fetuses were induced, with only 21% displaying evidence of fusion of the upper incisor teeth: 7 displayed evidence of fusio totalis, 3 of fusio partialis and no examples were seen of fusio subtotalis. Evidence was observed of 'contact' in 2 additional cases. In man there is also a frequent occurrence of dental anomalies in the presence of a variety of brain malformations (Miles, 1954; Forrester & Miller, 1955; Via &

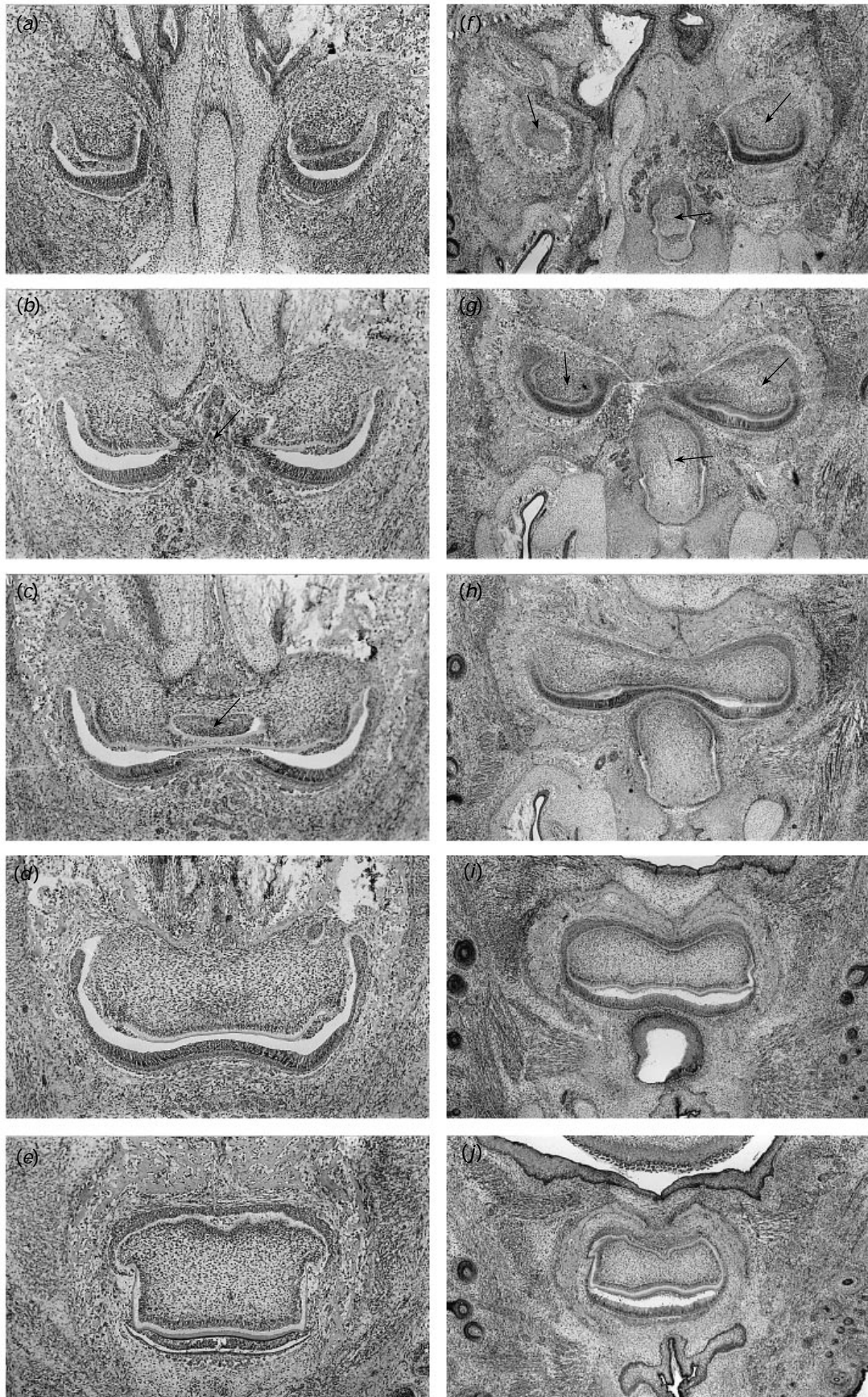


Fig. 1. For legend see opposite.

Churchill, 1957). Boyd & Miles (1951) noted that 'in many of the specimens (of cyclopic fetuses) described in the literature, although the upper incisor teeth are absent, there is a single conical tooth in the midline in the incisor region' (Ballantyne, 1904; Herbst & Apffelstaedt, 1930).

We had previously noted the presence of fused upper incisor teeth in 5 out of a total of 19 exencephalic *p53*<sup>-/-</sup> embryos and newborn mice (Armstrong et al. 1995). Homozygous animals were found to be viable but predisposed to tumours with an early onset (Donehower et al. 1992; Jacks et al. 1994; Purdie et al. 1994), and closure of the cephalic neural tube was seen to be perturbed in a significant proportion of embryos (Armstrong et al. 1995; Sah et al. 1995).

The original fetuses and newborn analysed and described previously and 2 additional newborn animals were examined in the present study. Because of the complexity of the morphology of some of the fused teeth, we decided to undertake their 3-D reconstruction using the computer-based methodology developed at the MRC Human Genetics Unit, Western General Hospital, Edinburgh, for the Mouse 3-D Atlas Project (Baldock et al. 1992; Kaufman et al. 1997). Some of the fused teeth seen in these mice appeared on histological examination to have a more complex structure than the fused teeth formerly described by Knudsen (see above).

## MATERIALS AND METHODS

### *Generation of p53-deficient mice*

The introduction of an inactivating deletion of exons 2–6 of *p53* into mouse embryonal stem cells and the production of germ-line chimeras has been described previously (Clarke et al. 1993). The embryos and newborn mice analysed here were derived from *p53*<sup>-/-</sup> × *p53*<sup>-/-</sup> matings (Armstrong et al. 1995). In brief, female mice at specific stages of pregnancy were autopsied, and their embryos were isolated from within their extraembryonic membranes.

The additional 2 newborn mice were homozygous for *p53* and heterozygous for either *Rb-1* (reference number 5/1) or *Apc* (reference number 9/2). The former (5/1) was derived from a cross between *p53*<sup>-/-</sup> animals and mice carrying a disrupted copy of the retinoblastoma (*Rb-1*) tumour suppressor gene (Clarke et al. 1992), while the latter (9/2) was obtained from a colony in which animals that lacked *p53* had been crossed to mice which were heterozygous for a nonsense mutation of the adenomatous polyposis coli (*Apc*) tumour suppressor gene (Su et al. 1992). Genotyping from yolk sac DNA was carried out by the polymerase chain reaction using primers for *p53* (Malcolmson et al. 1997), *Rb-1* (J. F. Armstrong et al. unpublished observations) and those reported previously for *Apc* (Luongo et al. 1994).

A total of 15 exencephalic embryos between day (d) 14.5 of gestation and full-term, and 6 newborn exencephalic *p53*<sup>-/-</sup> mice were isolated, fixed in buffered formalin, embedded in paraffin wax, and serially sectioned in the transverse plane, cut at a nominal thickness of ~5 µm then stained with haematoxylin and eosin. The tissues were not decalcified during processing.

While a detailed description of the craniofacial features of *p53*-deficient mice has been given in an earlier report, the dental findings were only briefly alluded to (Armstrong et al. 1995). Attention should, however, be drawn to the fact that in this study, in a significant proportion of the female embryos (23%), the normal process of neural tube closure in the cephalic region failed to occur, giving rise to exencephaly in midgestation and subsequently to anencephaly in the late gestation and newborn mice.

### *3-D reconstruction of the fused teeth*

The grey-level voxel (3-D array of grey values) image of the teeth of each embryo was reconstructed from images of the individual sections using software and techniques developed for the Mouse Atlas and Gene-Expression Database Project (Ringwald et al. 1994; Guest & Baldock, 1995). A digital image of each

Fig. 1. (a–e) Intermittent transverse serial sections through the maxillary region of a *p53*-deficient exencephalic newborn mouse with an example of fused maxillary incisor teeth associated with a dens in dente (reference number 4/1). Note the presence of 2 discrete basal ends (a, b) which merge together to form a single incisal end (d, e). At the base of the site of union a region of disorganised tissue (b, arrow) gives rise to an involuted element which has the form of a 'tooth within a tooth' (c, arrow). Both the 'crown' of the latter and the labial aspect of the crown of the fused teeth are covered by a very thin layer of preameloblast. A thin layer of dentine is present with an artefactual space separating it from the overlying layer of preameloblast. Definitive enamel has yet to form. The bottom of the sections is directed towards the anterior (or labial) surface of the face, while the top is directed towards the oral cavity. (f–j). Intermittent serial sections through the maxillary region of a *p53*-deficient newborn mouse with an example of fused maxillary incisor teeth associated on their labial aspect with a median supernumerary 'peg-like' tooth (reference number 9/2). Note the presence of 3 distinct basal ends (f, g, arrows) 2 of which merge to form a single crown (h–j). While the supernumerary tooth indents the labial surface of the fused teeth (h), it remains a distinct entity throughout its length. The orientation of these sections is similar to those in the previous series (a–e). Both series stained with haematoxylin and eosin. a–e, ×63; f–j, ×40.



figure 2 (control 93238)

a

b

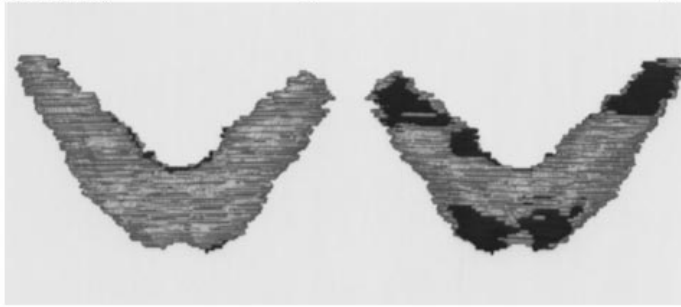


figure 3 (59/1)

a

b



figure 4 (4/1)

a

b

c

d

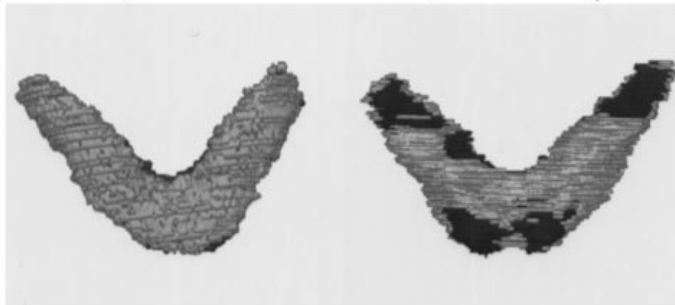


figure 5 (5/1)

a

b

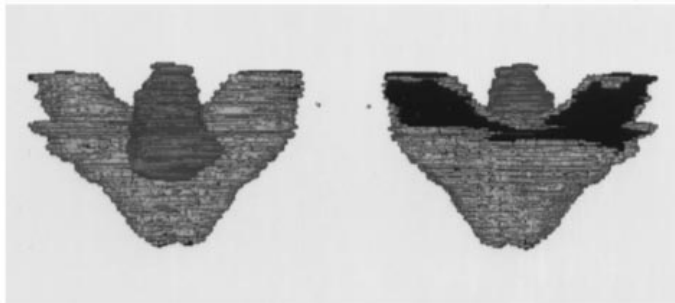


figure 6 (9/2)

a

b

Figs 2–6. 3-D reconstructions of labial and oral surfaces, respectively, of a normal pair of maxillary incisor teeth from a control newborn mouse (2a, b, reference number 93238) and a selection of fused maxillary incisor teeth (3–6). In 1 example (3a, b, reference number 59/1), ‘simple’ fusion between 2 distinct basal ends is observed with the formation of a single crown. In 2 examples (4a–d, reference number 4/1;

section was acquired using a Zeiss Axioplan microscope fitted with a  $\times 2.5$  Neofluor objective lens and a Xillix Technologies Microimager-1400 12bit CCD camera connected to a Sun Microsystems Sparc 10 Unix workstation. The digital image of each section was aligned using as guides anatomical landmarks such as the margins of the nasal septum, olfactory cavity and secondary palate, and warped (Guest & Baldock, 1995) to register the images and remove some of the sectioning artefacts.

The teeth in the resulting 3-D image were delineated using the MRC proprietary software 'paint' (personal communication, Baldock, 1996; further details of this software are available from Dr R. A. Baldock, MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK, or from R. Baldock@hgu.mrc.ac.uk.) which allows segmentation of arbitrary structures at arbitrary section orientations within the 3-D volume defined by the voxel image. This software was used to define the volumes occupied by the dentine and enamel for subsequent visualisation using the commercial 3-D rendering software AVS (AVS/Uniras Ltd.). The surfaces of each of the identified structures were displayed in 3-D using the isosurface and geometry-viewer modules on a Silicon Graphics Indigo 2 workstation.

## RESULTS

A total of 15 exencephalic embryos and 6 newborn *p53*<sup>-/-</sup> mice were examined. Of these, 1 d 14.5 p.c. and 1 d 19.5 p.c., and 4 newborn mice possessed maxillary incisor teeth with some evidence of fusion, giving an overall incidence of 6/21 (28.6%) of the exencephalics analysed in this study. On re-evaluation of one of the d 14.5 embryos previously described as having fused teeth (Armstrong et al. 1995), it was difficult to confirm this observation. Accordingly, this embryo has not been included in those with fused teeth in this analysis.

The fused teeth were of 3 varieties: 1 example displayed (1) simple fusion (reference number 59/1); 3 examples of a second class (2) were observed which were grossly similar to the latter example but each contained a small involuted element located at the proximal side of the site of fusion which contained a

central core of tissue covered by a thin layer of dentine which was itself covered by a very thin layer of preameloblast [reference numbers 1/1 (the d 19.5 gestation mouse), 4/1 and 5/1], representing examples of dens in dente (also termed dens invaginatus or dilated composite odontome) and a single example (3) in which the fused teeth were associated with an additional *discrete* midline incisor tooth element (reference number 9/2) which, while deeply indenting the labial surface of the fused teeth, was in all locations a completely separate entity.

Representative sections are provided to illustrate the histological appearance observed at various levels between the incisal and basal ends of 2 of these complex tooth germs. In Figure 1*a–e*, intermittent sections through one of the fused teeth associated with a dens in dente are illustrated (reference number 4/1). The involuted element arose from a region of apparently disorganised tissue at the site of union of the 2 tooth germs, and consisted of a central core of tissue covered by a thin layer of dentine which was itself covered on its incisal surface by a very thin layer of preameloblast, giving it the appearance of a 'tooth within a tooth'. The sections of the other 2 examples of this group, (reference numbers 1/1 and 5/1), were similar to those illustrated in Figure 1*a–e*. In Figure 1*f–j*, intermittent sections through the fused teeth associated with the median supernumerary tooth are illustrated (reference number 9/2). While the supernumerary tooth is closely apposed to the labial aspect of the fused teeth at their site of union, it is in no location adherent to them.

In order to provide baseline information, a normal pair of maxillary incisor teeth was reconstructed in 3-D, and these are illustrated in Figure 2*a, b*. At their anterior (incisal) parts, a moderate distance separates the 2 upper incisor teeth. This contrasts with the situation observed in the 4 reconstructed fused teeth. 3-D reconstructions of the following representative examples of these 3 classes are illustrated: class (1) Figure 3*a, b* (59/1), class (2) Figure 4*a–d* (4/1) and Figure 5*a, b* (5/1). In all cases, 2 distinct basal elements are seen, but the incisal ends of adjacent teeth merge to form a single unit. The exceptional dental unit belonging to class (3) is illustrated in Figure 6*a, b* (9/2). While the root of the midline tooth

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and 5*a, b*, reference number 5/1), the fused teeth were associated with an involuted element, giving rise in each case to a dens in dente. This is not shown in these reconstructions. An example of fused teeth associated with a supernumerary median 'peg-like' tooth is shown (6*a, b*, reference number 9/2). These reconstructions confirm that in each of the cases of fused teeth, these are of the fusio subtotalis variety. In all the examples illustrated in Figures 2–6, the core of the tooth is covered by a thin layer of dentine (shown in black) which is itself covered over most of its surfaces by a very thin layer of preameloblast (shown in pale grey). In the case of 1 of these teeth (Fig. 4), there are considerable deficiencies in the covering of preameloblast on the outer aspects of the fused teeth (Fig. 4*c, d*, right and left lateral views, respectively). In Figure 6, only the very thin covering of dentine of the supernumerary tooth is shown (in dark grey).

extended slightly more posteriorly than the roots of the fused teeth, its incisal extent barely extended beyond the middle of the fused element.

In all the fused teeth, their very thin covering of preameloblast was mostly complete, although it tended to be deficient on the oral and medial sides of their basal ends. In one example, symmetric deficiencies were also observed on the lateral sides of the proximal part of the fused element (see Figure 4*c, d*). 3-D reconstruction of the fused teeth confirmed that in all cases, the fusion observed was of the fusio subtotalis variety, being similar to those illustrated by Knudsen (1965*b*, 1966*a*) using the wax plate reconstruction technique.

While the other components of the upper and lower dentition were examined, no gross abnormalities were observed.

#### DISCUSSION

According to Atkins & Mourino (1986), fusion is defined as the union by dentine of 2 adjacent, normally separate tooth germs. This union may be complete, with the formation of a single large tooth, or incomplete, with union at either the crown or the root. The terminology in this area is complex, as *fusion* has to be distinguished from other similar but probably unrelated conditions such as gemination, twinning and conrescence. The other conditions are defined in Stedman's Medical Dictionary (1995) as follows: *gemination* – the gemination of a single tooth germ results in 2 partially or completely separated crowns in a single root; *twinning* – the production of equivalent structures by division, or the tendency of divided parts to assume symmetric relations; *conrescence* – the union of the roots of 2 adjacent teeth by cementum.

By our usage of the term 'fused teeth', we have implied that at an appropriate early stage of development, we would expect to encounter 2 separate tooth germs (1 on each side of the midline) which enlarge and subsequently merge across the midline. We cannot, however, exclude the possibility that the fused tooth initially arises from a single abnormal tooth germ in the midline which subsequently develops 2 basal elements.

In man, the prevalence of fused maxillary incisors, other than across the midline, that is, involving the central and lateral incisors is relatively commonly encountered; it is said to be seen in less than 1% in Caucasian populations (Clayton, 1956; Brook & Winter, 1970; Buenviaje & Rapp, 1984), although a

higher prevalence has been reported in Japanese (Niswander & Sujaku, 1963) and in American Indians (Curzon & Curzon, 1967). Dental fusion may occur elsewhere, and cases have been described involving the upper molars and canines and members of the mandibular dentition, and appear to be frequently associated with a missing tooth in the same area (Levitas, 1965; O'Reilly, 1989), or the fused tooth may be fused to a supernumerary tooth (Miles, 1954; Blank et al. 1985). This anomaly is more frequent in the deciduous dentition, but there is no difference in incidence between the sexes (Brook & Winter, 1970). It is believed that physical forces acting to induce compression bring the tooth germs into contact with resultant fusion.

In man, the association between abnormal maxillary incisor dentition and a variety of brain malformations has been noted, although the presence of a single midline enlarged upper incisor tooth without associated brain malformations has also occasionally been observed, but is extremely rare (Axrup et al. 1966; Bazan, 1983). The association between fused maxillary incisor teeth and exencephaly in the mouse may be a manifestation of a species difference, in that the mouse has a relatively narrow premaxilla and only normally possesses 2 maxillary incisors, whereas the human has a wider premaxilla and normally possesses 4 such teeth.

Knudsen (1966*b, c*) also reported other dental anomalies in association with hypervitaminosis A-induced exencephaly, involving the incisor germs of the lower jaw and the molar teeth of both the upper and lower jaws, though fusion of the maxillary incisors was the most frequent malformation observed; in the present study, no abnormalities involving members of the other dentition were observed. In the small series of fused teeth analysed in the present study, it was particularly surprising that 3 of the 5 examples displayed evidence of dens in dente, a finding only reported once previously (to our knowledge) in mice (see Robins & Rowlett, 1971).

In man, dens in dente occurs most commonly in maxillary permanent incisors (Shafer et al. 1983; Soames & Southam, 1985), often bilaterally (Swanson & McCarthy, 1947; Augsburger & Brandebura, 1978; Burton et al. 1980), and is usually recognised because of its characteristic radiographic appearance. This condition is said not to occur in the deciduous dentition (Beynon, 1982). In the most extreme form, as seen here, the condition resembles a 'tooth within a tooth' (Vajrabhaya, 1989). This developmental anomaly is believed to be the result of an infolding of the enamel organ into the dental papilla beginning at

the crown and sometimes extending into the root before calcification occurs (Beynon, 1982; Soames & Southam, 1985).

The small number of fused teeth observed in this study covered a broad spectrum of forms, ranging from the 'simple' fusio subtotalis variety, to those with the same gross morphology, but associated with an extreme form of dens in dente, to the third form in which the fused tooth was associated with a discrete median supernumerary incisor tooth. Whatever its underlying aetiology, this latter configuration must represent an extremely rare if not unique finding. None of the dental abnormalities described in this study have previously been reported in either the *Rb-1* or *Apc* colonies.

3-D reconstructions, while helpful in providing an overview of the gross morphology of the fused teeth, are only of limited value, particularly in relation to visualising the morphology of the examples of dens in dente observed in 3 of the fused teeth seen in this study, and it is for this reason that it is essential that serial histological sections through these aberrant teeth are available for detailed analysis.

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#### REFERENCES

- ARMSTRONG JF, KAUFMAN MH, HARRISON DJ, CLARKE AR (1995) High-frequency developmental abnormalities in p53-deficient mice. *Current Biology* **5**, 931–936.
- ATKINS CO, MOURINO AP (1986) Management of a supernumerary tooth fused to a permanent maxillary central incisor. *Oral Surgery* **61**, 146–148.
- AUGSBURGER RA, BRANDEBURA J (1978) Bilateral dens invaginatus with associated radicular cysts. A case report. *Oral Surgery, Oral Medicine, Oral Pathology* **46**, 260–264.
- AXRUP K, D'AVIGNON M, HELLGREN K, HENRIKSON CO, JUHLIN IM, LARSSON KS et al. (1966) Children with thalidomide embryopathy: odontological observations and aspects. *Acta Odontologica Scandinavica* **24**, 3–21.
- BALDOCK R, BARD J, KAUFMAN MH, DAVIDSON D (1992) A real mouse for your computer. *BioEssays* **14**, 501–502.
- BALLANTYNE JW (1904) *Antenatal Pathology and Hygiene. The Embryo*, p. 398. Edinburgh: William Green & Sons.
- BAZAN MT (1983) Fusion of maxillary incisors across the midline: clinical report. *Pediatric Dentistry* **5**, 220–221.
- BEYNON AD (1982) Developing dens invaginatus (dens in dente). *British Dental Journal* **153**, 255–260.
- BLANK BS, OGG RR, LEVY AR (1985) A fused central incisor. Periodontal considerations in comprehensive treatment. *Journal of Periodontology* **56**, 21–24.
- BOYD JD, MILES AEW (1951) An erupted tooth in a cyclops foetus. *British Dental Journal* **91**, 173–181.
- BROOK AH, WINTER GB (1970) Double teeth. A retrospective study of 'geminated' and 'fused' teeth in children. *British Dental Journal* **129**, 123–130.
- BUENVIAJE TM, RAPP R (1984) Dental anomalies in children: a clinical and radiographic survey. *Journal of Dentistry for Children* **51**, 42–46.
- BURTON JD, SAFFOS RO, SCHEFFER RB (1980) Multiple bilateral dens in dente as a factor in the etiology of multiple periapical lesions. *Oral Surgery, Oral Medicine, Oral Pathology* **49**, 496–499.
- CLARKE AR, MAANDAG ER, VAN ROON M, VAN DER LUGT NMT, VAN DER VALK M, HOOPER ML et al. (1992) Requirement for a functional *Rb-1* gene in murine development. *Nature* **359**, 328–330.
- CLARKE AR, PURDIE CA, HARRISON DJ, MORRIS RG, BIRD CC, HOOPER ML et al. (1993) Thymocyte apoptosis induced by p53-dependent and independent pathways. *Nature* **362**, 849–852.
- CLAYTON JM (1956) Congenital dental anomalies occurring in 3,557 children. *Journal of Dentistry for Children* **23**, 206–208.
- CURZON JA, CURZON MEJ (1967) Congenital dental anomalies in a group of British Columbia children. *Canadian Dental Association Journal* **33**, 554–558.
- DONEHOWER LA, HARVEY M, SLAGLE BL, McARTHUR MJ, MONTGOMERY CA, BUTEL JS et al. (1992) Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature* **356**, 215–221.
- FORRESTER RM, MILLER J (1955) The dental changes associated with kernicterus. *Archives of Disease in Childhood* **30**, 224–231.
- GUEST E, BALDOCK RA (1995) Automatic reconstruction of serial sections using the finite element method. *Bioimaging* **3**, 154–167.
- HERBST E, APFFELSTAEDT M (1930) *Malformations of the Jaws and Teeth*, pp. 306–312. London: Oxford University Press.
- JACKS T, REMINGTON L, WILLIAMS BO, SCHMITT EM, HALACHMI S, BRONSON RT et al. (1994) Tumor spectrum analysis in p53-mutant mice. *Current Biology* **4**, 1–7.
- KALTER H, WARKANY J (1959) Experimental production of congenital malformations in strains of inbred mice by maternal treatment with hypervitaminosis A. *American Journal of Pathology* **38**, 1–21.
- KAUFMAN MH, BRUNE RM, BALDOCK RA, BARD JBL, DAVIDSON D (1997) Computer-aided 3-D reconstruction of serially sectioned mouse embryos: its use in integrating anatomical organization. *International Journal of Developmental Biology*, in press.
- KNUDSEN PA (1965a) Congenital malformations of upper incisors in exencephalic mouse embryos, induced by hypervitaminosis A. I. Types and frequency. *Acta Odontologica Scandinavica* **23**, 71–90.
- KNUDSEN PA (1965b) Congenital malformations of upper incisors in exencephalic mouse embryos, induced by hypervitaminosis A. II. Morphology of fused upper incisors. *Acta Odontologica Scandinavica* **23**, 391–410.
- KNUDSEN PA (1966a) Malformations of upper incisors in mouse embryos with exencephaly, induced by trypan blue. *Acta Odontologica Scandinavica* **24**, 647–676.
- KNUDSEN PA (1966b) Congenital malformations of lower incisors and molars in exencephalic mouse embryos, induced by hypervitaminosis A. *Acta Odontologica Scandinavica* **24**, 55–90.
- KNUDSEN PA (1966c) Congenital malformations of the jaws and related structures in exencephalic mouse embryos with anomalous molar germs, induced by hypervitaminosis A. *Acta Odontologica Scandinavica* **24**, 677–708.
- LEVITAS TC (1965) Geminatio, fusion, twinning and condescence. *Journal of Dentistry for Children* **32**, 93–100.
- LUONGO C, MOSER AR, GLEDHILL S, DOVE WF (1994) Loss of *Apc*<sup>+</sup> in intestinal adenomas from Min mice. *Cancer Research* **54**, 5947–5952.
- MALCOMSON RDG, CLARKE AR, PETER A, COUTTS SB, HOWIE

- SEM, HARRISON DJ (1997) Apoptosis induced by  $\gamma$ -irradiation, but not CD4 ligation, of peripheral T lymphocytes *in vivo* is *p53*-dependent. *Journal of Pathology*, in press.
- MILES AEW (1954) Malformations of the teeth. *Proceedings of the Royal Society of Medicine* **47**, 817–826.
- NISWANDER JD, SUJAKU C (1963) Congenital anomalies of teeth in Japanese children. *American Journal of Physical Anthropology* **21**, 569–574.
- O'REILLY PMR (1989) A structural and ultrastructural study of a fused tooth. *Journal of Endodontics* **15**, 442–446.
- PURDIE CA, HARRISON DJ, PETER A, DOBBIE L, WHITE S, HOWIE SEM et al. (1994) Tumour incidence, spectrum and ploidy in mice with a large deletion in the *p53* gene. *Oncogene* **9**, 603–609.
- RINGWALD M, BALDOCK R, BARD J, KAUFMAN M, EPPIG JT, RICHARDSON JE et al. (1994) A database for mouse development. *Science* **265**, 2033–2034.
- ROBINS MW, ROWLATT C (1971) Dental abnormalities in aged mice. *Gerontologia* **17**, 261–272.
- SAH VP, ATTARDI LD, MULLIGAN GJ, WILLIAMS BO, BRONSON RT, JACKS T (1995) A subset of *p53*-deficient embryos exhibit exencephaly. *Nature Genetics* **10**, 175–180.
- SHAFFER WJ, HINE MK, LEVY BM (1983) *A Textbook of Oral Pathology*, 4th edn, pp. 41–42. Philadelphia: W. B. Saunders.
- SOAMES JV, SOUTHAM JC (1985) *Oral Pathology*, pp. 207–209. Oxford: Oxford University Press.
- STEDMAN TL (1995) *Stedman's Medical Dictionary*, 26th edn. Baltimore: Williams and Wilkins.
- SU LK, KINZLER KW, VOGELSTEIN B, PREISINGER AC, MOSER AR, LUONGO C et al. (1992) Multiple intestinal neoplasia caused by a mutation in the murine homolog of the APC gene. *Science* **256**, 668–670.
- SWANSON WF, MCCARTHY FM (1947) Bilateral dens in dente. *Journal of Dental Research* **26**, 167–171.
- VAJRABHAYA L (1989) Nonsurgical endodontic treatment of a tooth with double dens in dente. *Journal of Endodontics* **15**, 323–325.
- VIA WF, CHURCHILL JA (1957) Relationships of cerebral disorder to faults in dental enamel. *A.M.A. Journal of Diseases of Children* **94** 137–142.