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Craniofacial abnormalities in homozygous *Small eye* (*Sey/Sey*) embryos and newborn mice

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ABSTRACT

The *Small eye* (*Sey*) gene in the mouse is lethal in the homozygous state. It is located on chromosome 2, is a mutation in the Pax-6 gene, and is genetically homologous with the human aniridia 2 (AN2) gene mutation. Numerous studies over the last few years, using genetic and molecular biological approaches, have investigated both the location of the gene as well as its possible mode of action. In the homozygous state, the primary defect appears to be limited to the failure of differentiation of the presumptive lens and nasal placodes. Such mice therefore display a characteristic phenotype; they possess neither eyes nor any nasal derivatives. Their heterozygous (*Sey*/+) and normal (+/+) littermates may be distinguished before birth only by a detailed examination of their eyes. Few detailed morphological/histological studies have been undertaken to date in the *Sey/Sey* embryos and newborn, and in the present study we describe a variety of craniofacial abnormalities that have not previously been reported. We observed, with one exception, delayed closure of the palate, and the presence in 80% of mice of an abnormal complement of upper incisor teeth, so that 35% possessed 1 supernumerary tooth while 45% possessed 2 supernumerary teeth. In these mice, a total of either 3 or 4, rather than the normal complement of 2, upper incisor teeth were present. Possibly the most unexpected finding, however, was the presence of a median cartilaginous rod-like structure which protruded between the 2 maxillae to give the Alizarin red S and Alcian blue-stained 'cleared' skulls of the newborn mice a characteristic 'unicorn-like' appearance. While this structure appeared to be a rostral extension of the chondrocranium, its exact derivation is unclear.

Key words: Small eye mutation; mice; craniofacial dysmorphism; supernumerary incisor teeth; delayed palatogenesis; cartilaginous unicorn-like horn.

INTRODUCTION

The *Small eye* (*Sey*) gene in the mouse is lethal in the homozygous state. This gene is allelic with, but not identical to Sey^{H} (Lyon et al. 1979), a radiation-induced homozygous prenatal lethal gene which has been mapped to chromosome 2, and is genetically homologous with the human aniridia 2 (AN2) gene mutation (Glaser & Housman, 1989; Glaser et al. 1990; Meer-de Jong et al. 1990; Hill et al. 1991; Ton et al. 1992). Recent evidence suggests that the *Small eye* gene is a mutation in the *Pax-6* gene. It is also allelic with *Dickie small eye* (Sey^{Dey}) (Theiler et al. 1978, 1980; Hogan et al. 1987), but not allelic with *Coloboma* (*Cm*), another mutation affecting mouse eye development which is also located on chromosome 2 (Searle, 1966; Theiler & Varnum, 1981). Although this gene mutation appeared spontaneously in Edinburgh in 1967 (Roberts, 1967), the first detailed descriptions of the *Sey/Sey* phenotype were only published about 20 years later (Hogan et al. 1986, 1988).

In these various accounts, attention is drawn to the fact that the primary defect appears to be limited to a failure of differentiation of the presumptive lens and nasal placodes. *Sey/Sey* embryos are capable of developing to term but do not possess eyes or any nasal structures, and die shortly after birth because, it

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has been suggested (Hogan et al. 1986), mice, unlike humans, cannot breathe through the mouth. While the homozygous embryos and neonates are easily recognised after 10.5 days post coitum (d p.c.) because of their characteristic phenotypic features, +/+ and *Sey*/+ littermates may also be distinguished before birth by a detailed examination of their eyes (Hill et al. 1991).

In the study by Hogan et al. (1986), 101 homozygous mutant embryos were isolated between 10.5 and 18.5 d p.c. and examined histologically, as well as a number of neonates that were isolated immediately after their delivery, before they could be eaten by the mothers. Detailed descriptions of the histological findings are, however, only provided in this account of the embryos isolated at 10.5 d p.c. In a subsequent paper (Hogan et al. 1988), the histological features of the craniofacial region of embryos isolated at 11.5 d p.c. are given, as well as an illustration of a section through the eye of a presumed *Sey*/+ embryo isolated at 15.5 d p.c. In our own series, the cephalic region of *Sey*/*Sey* embryos isolated between 12.5 and 17.5 d p.c. and newborn mice isolated shortly after their delivery were either serially sectioned or 'cleared' and bulk stained with Alizarin red S and Alcian blue in order to evaluate their craniofacial morphology.

Over the last few years, numerous studies (indicated above) have been undertaken to determine, using genetic and molecular biological approaches, both the location and possible mode of action of the gene involved in the *Small eye* mutation in the mouse, and establishing its homology with human aniridia 2 (AN2). Similarly, a mutation in the *Pax-6* gene in the rat has also recently been described, and the findings suggest that the *Small eye* mutation, at least in this species, may induce impaired migration of midbrain neural crest cells (Matsuo et al. 1993). In the human, recent evidence suggests that a variety of anterior segment abnormalities of the eye may be associated with *Pax-6* mutations when present in heterozygous individuals (Hanson et al. 1994). Studies of the distribution of *Pax-6* gene expression during the development of the zebrafish (Krauss et al. 1991; Püeschel et al. 1992) and mouse (Walther & Gruss, 1991) indicate that in addition to being expressed in the lens and olfactory placodes, it is also expressed in defined regions of the forebrain, optic cup, hindbrain and spinal cord (for review, see Li et al. 1994).

No detailed morphological/histological studies have, however, been undertaken since the earlier reports of Hogan et al. (1986, 1988). We have therefore taken this opportunity of studying the craniofacial region in *Sey*/*Sey* mice in greater detail.

MATERIALS AND METHODS

The mice used in the present study are from the colony of *Small eye* mice established by Dr Ruth Clayton, formerly of the Department of Genetics and subsequently in the Division of Biological Sciences, Institute of Cell, Animal and Population Biology, The King's Buildings, University of Edinburgh. The breeding colony has been maintained by brother × sister matings of heterozygous male and female mice, and represent the progeny of the *Small eye* mice originally described by Roberts (1967). These mice have provided the mutant gene with which all subsequent studies involving the 'Edinburgh-derived *Small eye*' mice have been used. The morning of finding a vaginal plug was designated 0.5 d p.c. In the present study, the pregnant females were killed by cervical dislocation and the embryos were isolated on either 12.5, 13.5, 14.5, 15.5, 16.5 or 17.5 d p.c. The litters were then examined under the low power magnification of a Wild M5 dissecting microscope.

Analysis of the craniofacial region revealed that the embryos could readily be divided into two principal groups, namely those that did and those that did not possess eyes. It was also clearly apparent that those in the second category possessed no nasal derivatives. Furthermore, their facial appearance was grossly abnormal, principally because of the abnormal morphogenesis of the frontonasal region (see Fig. 1). With regard to the first group of littermates, after an initial period of uncertainty, it became apparent that these could readily be separated into two subgroups according to the gross morphological appearance of their eyes. In the case of the normal (+/+) embryos, the shape of the pigmented iris, which bounds the pupil, was approximately circular, though in some of the embryos the pupil was almost square but with rounded angles. The shape of the pupil in the heterozygous littermates, by contrast, was quite characteristic and slit-like. The external appearance of the ocular region in +/+ and *Sey*/+ littermates is shown in Figure 2. Because of the heavy degree of pigmentation of the iris, it was also possible to distinguish easily between +/+ and *Sey*/+ littermates, even in the developmentally more advanced embryos in which the eyelids had closed some time previously. At the time that the autopsies were carried out in the present study, we were unaware that this observation had previously been reported by Hill et al. (1991).

Once this difference in the appearance of the ocular region between +/+ and *Sey*/+ littermates had been noted, it was then possible to divide the litters

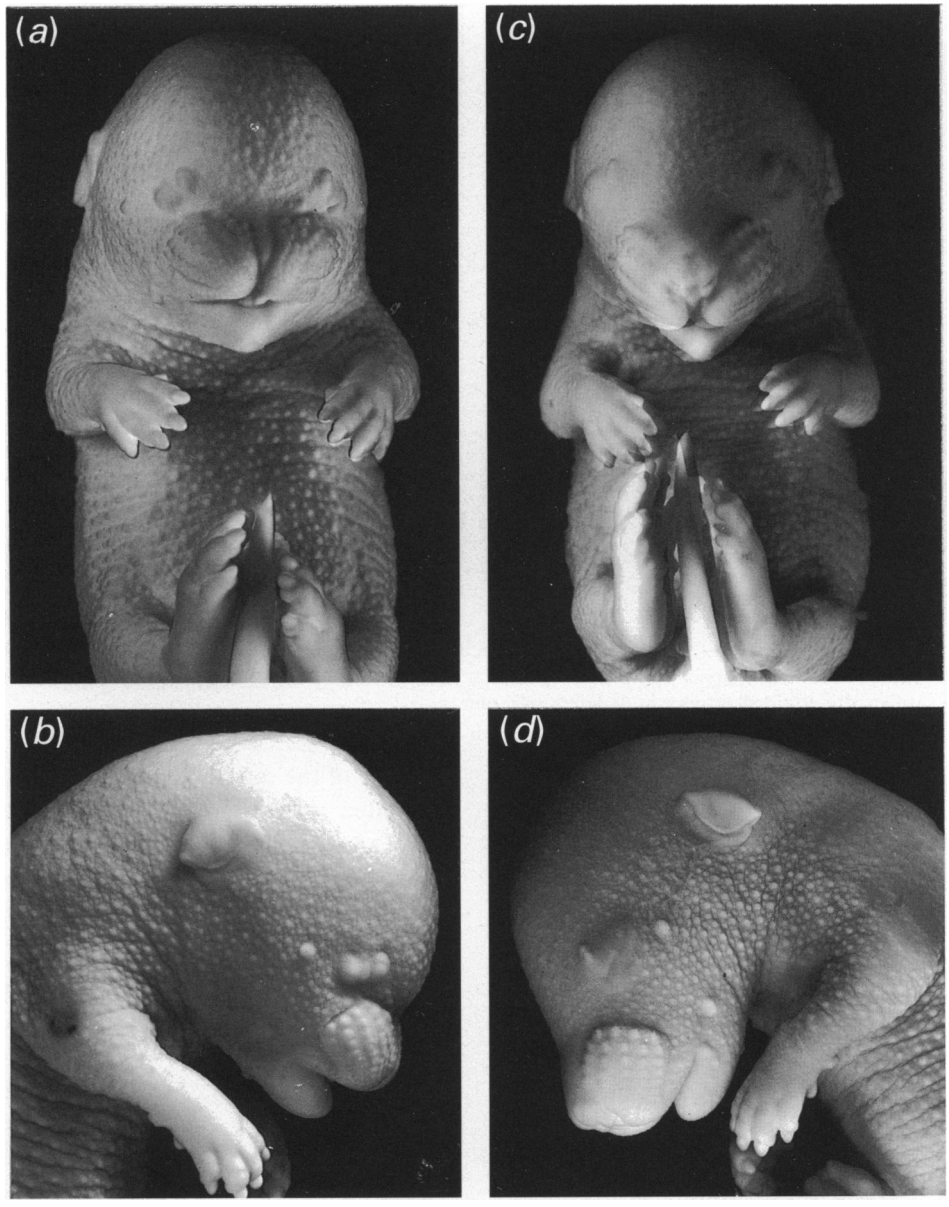

Fig. 1. Frontal and lateral views of the craniofacial region of a 16.5 d p.c. homozygous *Small eye* (*Sey*/*Sey*) embryo (*a*, *b*), and a heterozygous *Small eye* (*Sey*/+) littermate (*c*, *d*). Note the presence of diminutive eyelids in the *Sey*/*Sey* embryo, and that its frontonasal region is broader and flatter than in the *Sey*/+ embryo which has a normal facial appearance. The postcranial features of the *Sey*/*Sey* embryo are normal.

into *Sey*/*Sey*, *Sey*/+ and +/+ groups for subsequent detailed histological analysis.

The embryos isolated on 12.5–15.5 d p.c. were fixed in Bouin's solution, embedded in paraffin wax, sectioned serially in the transverse plane and cut at a nominal thickness of 7 μm, while the embryos isolated on 16.5–17.5 d p.c. and the newborn mice were first decapitated and the heads mostly serially sectioned in the coronal plane. Two additional 17.5 d p.c. embryos were sectioned in the sagittal plane. The sections were stained with haematoxylin and eosin.

During the period when the size of the breeding colony was being increased, all newborn mice were examined within, at most, a few hours of delivery. In this way, a considerable number of liveborn *Sey*/*Sey* mice were recognised because of their characteristic craniofacial features and isolated. These mice were subsequently killed by deep ether anaesthesia and then either fixed in Bouin's solution for subsequent histological analysis, or fixed in 80% ethanol. The newborn that were fixed in ethanol were subsequently decapitated and the 'cleared' skulls bulk-stained with Alizarin red S and Alcian blue in order to stain the ossification centres and cartilage, respectively (for methodology, see Kaufman, 1992, this being a modification of the technique of Meyer & O'Rahilly, 1958). All newborn mice that were examined histologically were decapitated following fixation, then serially sectioned in the coronal plane, and the sections subsequently stained with haematoxylin and eosin.

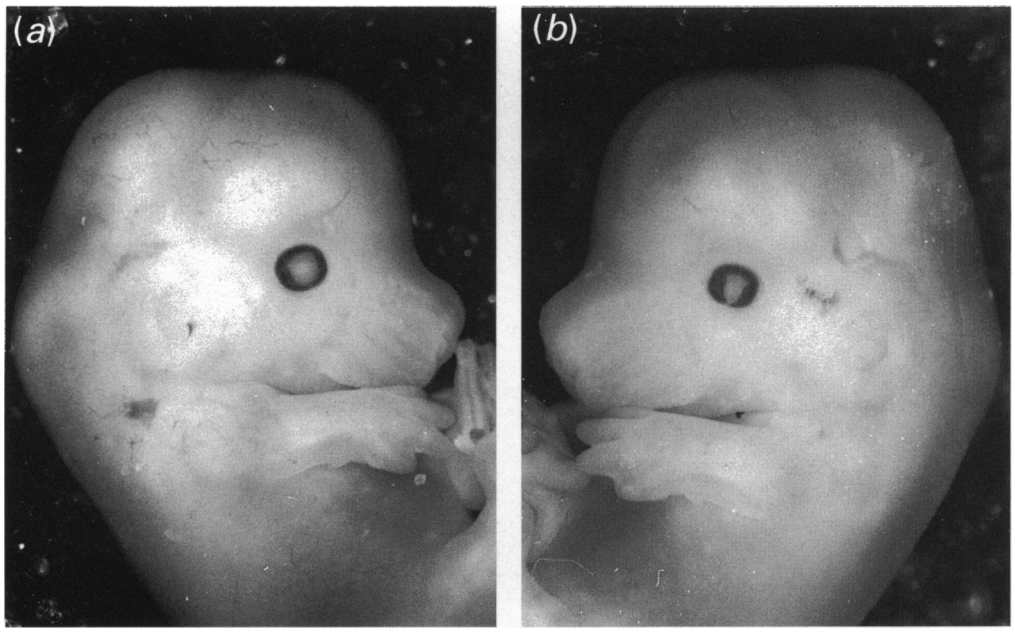

Fig. 2. Lateral views of a 14.5 d p.c. normal (+/+) embryo (*a*) and a heterozygous mutant *Small eye* (*Sey*/+) embryo (*b*). While the shape of the pigmented iris which bounds the pupil is approximately circular in the +/+ embryo, that in the *Sey*/+ embryo has a characteristic appearance, being slit-like, due to the deficiency in its inferior margin.

RESULTS

Incidence of Sey/Sey, Sey/+ *and* +/+ *in prenatal material studied*

Sixteen pregnant *Sey*/+ female mice previously mated to *Sey*/+ males were autopsied on either 12.5 (3), 13.5 (3), 14.5 (3), 15.5 (2), 16.5 (1) or 17.5 (4) d p.c., where the number of females at each age is given in parentheses; 144 implantation sites were examined, and yielded a total of 130 viable embryos and 14 resorptions. 29 (22.3%) of the viable embryos were found to be *Sey*/*Sey*, and were readily recognised because of their characteristic craniofacial features.

In the 8 litters in which the *Sey*/+ and +/+ littermates were readily distinguished by the difference in their ocular features (see Fig. 2), these two groups of embryos were found to be present in the ratio of 35 *Sey*/+ : 14+/+.

Isolation of Sey/Sey *newborn*

When all litters from the matings of *Sey*/+ females to *Sey*/+ males were examined, a small proportion of *Sey*/*Sey* mice were recognised because they possessed the characteristic morphological features associated with the *Sey*/*Sey* genotype. In 10 out of the 12 instances, the *Sey*/*Sey* newborn mice were alive at the time of their retrieval from the litter. In the case of the 2 dead *Sey*/*Sey* newborn mice, these had been partly eaten by the mothers, though in both instances the head region was still intact. The findings from the analysis of the serially coronally sectioned material is presented in sections (*a*) and (*b*) below. The heads from an additional 6 *Sey*/*Sey* newborn mice were cleared and bulk stained with Alizarin red S and Alcian blue. The findings from this study are presented in section (*c*) below.

(*a*) *Histological analysis of the palatal, maxillary and premaxillary region in* Sey/Sey *mice.* The earliest stage at which palatal shelf closure was present was observed in 1 embryo out of 3 *Sey*/*Sey* embryos examined on 14.5 d p.c.; in most strains of mice palatal shelf closure occurs between 14.5 and 15.5 d p.c. (Kaufman, 1992). In this embryo, the 2 palatal shelves had fused completely in the midline. Only 1 embryo was analysed on 15.5 d p.c., and this displayed no evidence of palatal shelf elevation or fusion, the 2 palatal shelves being vertically directed and located on either side of the tongue mass. The single *Sey*/*Sey* embryo that was isolated on 16.5 d p.c. displayed normal closure of the palate. In all 12 newborn *Sey*/*Sey* mice, the palates were fused completely in the midline.

Histological analysis of 9 *Sey*/+ embryos isolated on 13.5 (3), 14.5 (3) and 17.5 (3) d p.c., 4 newborn *Sey*/+ mice, and 3+/+ littermates isolated on 14.5 (2) and 16.5 (1) d p.c., revealed that while palatal shelf elevation and closure had occurred by 16.5 d p.c., evidence of palatal shelf elevation had yet to occur in the embryos analysed on 14.5 d p.c.

Almost the entire maxillary area of the face rostral to the ethmoid/sphenoid region in *Sey*/*Sey* mice

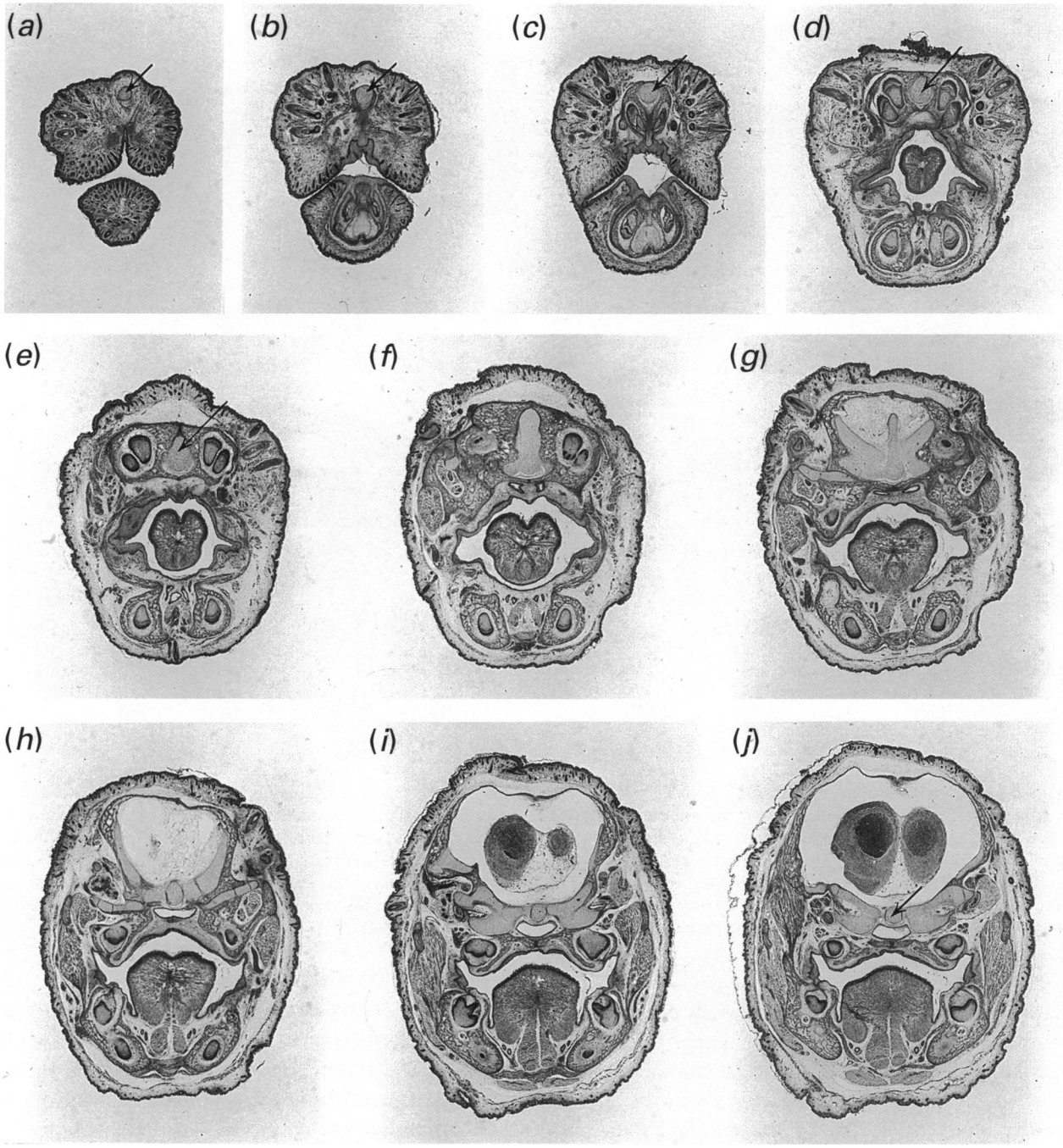

Fig. 3. Intermittent serial coronal sections through the facial region of a d 1 postnatal *Sey/Sey* mouse. The cartilaginous rod-like structure (arrowed in sections *a–e*) is seen to protrude almost to the tip of the snout. More posteriorly, in sections *e–g*, the rod-like structure, which is possibly of ethmoidal origin, has a dorsal crest, and is seen to be continuous with the basisphenoid (arrow in *j*). Sections through the frontal lobes of the brain are seen in sections *i* and *j*. All sections × 16. The presence of 4 upper incisor teeth is clearly seen in sections *d* and *e*.

appeared to be filled with cancellous bone in which, most posteriorly, the molars were embedded. Posterolateral to this region, the orbits were recognisable and, in the absence of eyes and optic nerves which were only present as short stumps beyond the chiasma, were largely filled with an excess of glandular tissue (harderian) (Payne, 1994). Connective tissue and disorganised muscle of extrinsic ocular origin were also found in the orbit. The contents of the 2 orbits, however, sometimes varied quite considerably, so that while one may have contained a large volume of glandular tissue, the other had a much smaller overall volume and was often completely devoid of glandular tissue. Despite the absence of eyes, diminutive eyelids differentiated and subsequently closed at about the normal time.

The soft tissues of the premaxillary/maxillary region were characterised by the presence of a midline

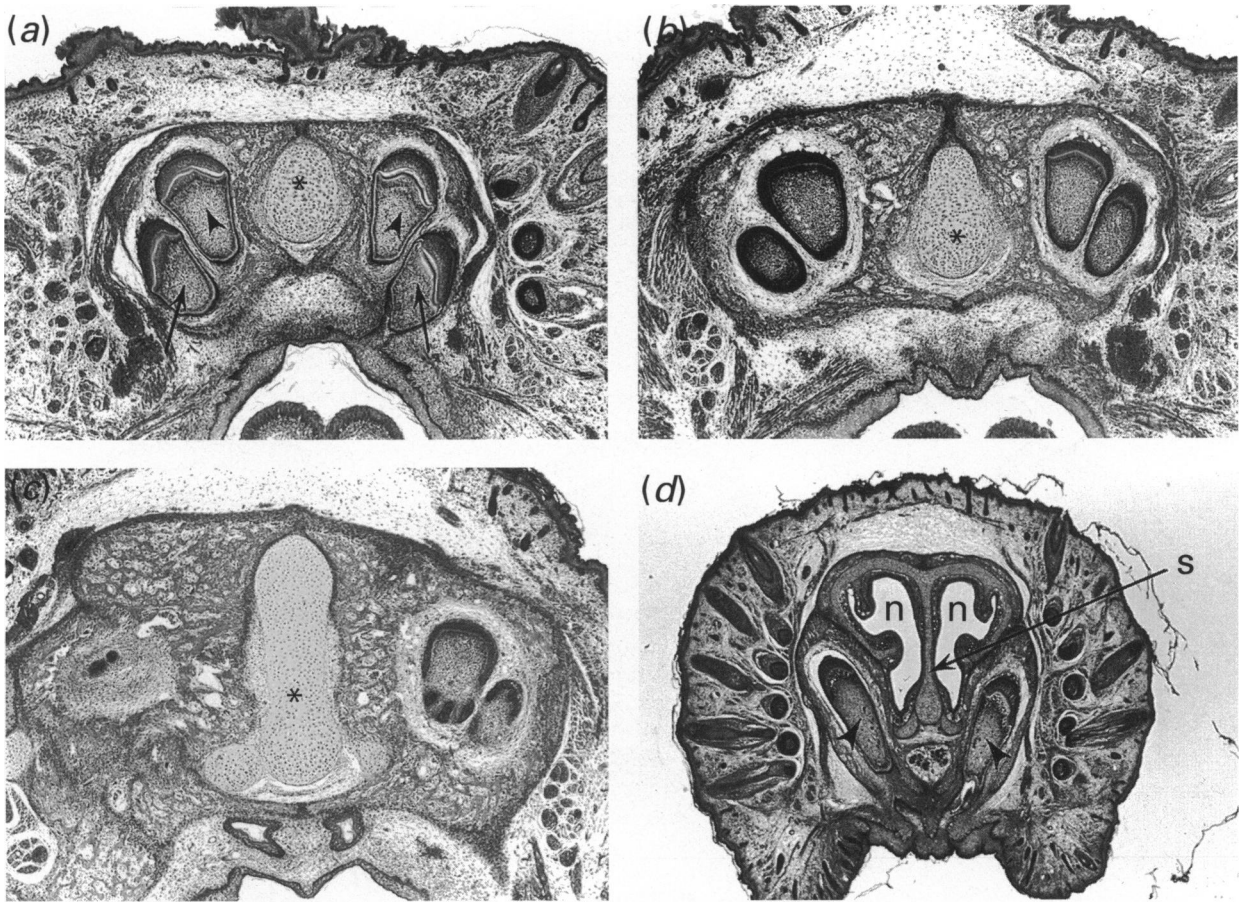

Fig. 4. *a–c*. Coronal sections through the premaxillary region of the same *Sey/Sey* mouse illustrated in Figure 3. This possessed 4 upper incisor teeth. The 2 normal upper incisors (arrowheads) are medially located, while the 2 'supernumerary' incisor teeth (large arrows) are laterally located. The midline cartilaginous rod-like structure (star) located between the most anterior part of the incisor teeth has a rounded profile in section (*a*). More posteriorly (*b*), it possesses a slight crest, and further posteriorly (*c*), it has a quite substantial crest superiorly and widens out inferiorly, where it is continuous with the rest of the cartilage primordium of the ethmoid bone. (*d*) Coronal section through a comparable region of a d 1 postnatal *Sey/+* mouse, displaying the normal appearance of this region. Two upper incisor teeth (arrow heads) are present, and the two nasal cavities (*n*) are separated by the cartilaginous nasal septum (*s*). (*a–c*, ×40; *d* × 25).

cartilaginous rod-like structure which extended forwards to reach the subcutaneous tissues at the tip of the snout. The rod appeared to be continuous with, and to be an anteriorly-directed extension of, the cartilaginous primordia of the ethmoid and more posteriorly the basisphenoid.

In the premaxillary region, the median cartilaginous rod-like structure had a dorsal crest which was principally present in the posterior part of the region between the incisor teeth. The rod extended anteriorly and gradually reassumed its previous circular profile just in front of the midpoint of the incisor teeth. Evidence of the cartilaginous rod-like structure is seen as early as 14.5 d p.c., while its precartilaginous primordium is seen from about 13.5 d p.c. A representative series of intermittent coronal sections through the frontonasal region in a *Sey/Sey* newborn mouse which possessed 4 upper incisor teeth is presented in Figure 3.

In none of the *Sey/Sey* mice was there any evidence of a readily recognisable vertically directed nasal septum. The 'cavity' above the fused palatal shelves in these mice was accordingly bounded dorsally by the roof of the oropharynx since no derivatives of the nasopharynx had formed. In addition to the absence of a nasal septum, no indication either of vomeronasal organs or olfactory nerves was present.

(*b*) *The number of upper incisor teeth present*. In this strain, the earliest developmental age at which the total number of upper incisor teeth was present was found to be 14.5 d p.c.

In order to confirm that only the normal complement of 2 upper incisor teeth was present in *Sey/+* and +/+ mice, 6 *Sey/+* and 3 +/+ embryos of 14.5 d p.c., or older (for details of ages, see section (*a*) above) and 4 newborn *Sey/+* mice were serially sectioned in the coronal plate. Analysis of the upper incisor dentition revealed that, as expected, all the

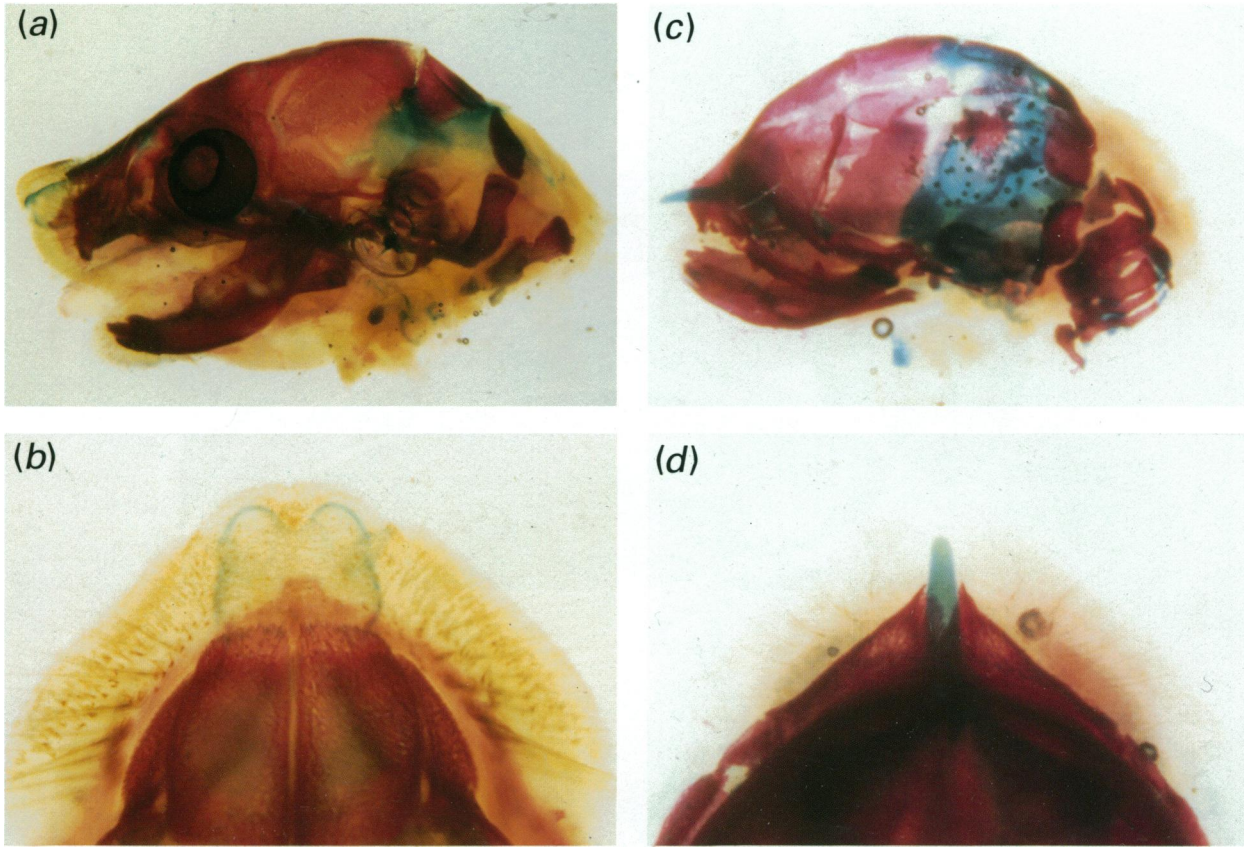

Fig. 5. 'Cleared' heads of d 1 postnatal normal (*a*, *b*) and *Sey/Sey* (*c*, *d*) mice, double-stained with Alizarin red S and Alcian blue, to display bone and cartilage, respectively. Note the presence of the eye and nasal cartilages in the normal embryo, and the absence of eyes in the *Sey/Sey* embryo. Note also the presence in the latter of the cartilaginous rod-like structure that is directed forwards towards the tip of the snout region, giving the embryo a unicorn-like appearance. The increased amount of cartilaginous material displayed in the skull illustrated in (*c*) compared with that seen in the normal skull (*a*) is probably a reflection of the fact that the homozygous mutant mouse is, in developmental terms, somewhat less advanced than its morphologically normal sibling at the time of birth.

embryos and newborn mice in this series possessed a total of 2 incisor tooth germs with enamel, dentine and pulp tissues present, as would be consistent with their developmental ages.

In all, the upper incisor dentition of 8 *Sey/Sey* embryos isolated on 14.5 (3), 15.5 (1), 16.5 (1) and 17.5 (3) d p.c., and 12 newborn *Sey/Sey* mice was analysed. Only 4 mice (20%) in this series possessed the normal complement of 2 upper incisor teeth; 5 mice (25%) possessed a single supernumerary (i.e. supplemental) incisor tooth on the lateral side of the normally located left upper incisor tooth, while 2 mice (10%) possessed a single supernumerary incisor tooth on the lateral side of the normally located right upper incisor tooth. Nine mice (45%), however, possessed 2 supernumerary teeth, 1 on each side of the upper jaw lateral to each normally positioned incisor tooth (see Fig. 4).

In only 1 out of 15 (6.7%) of the supernumerary teeth examined (a supernumerary left upper incisor tooth) in the newborn mice was the appearance different from the expected; this tooth was found to be smaller and less well differentiated than the 2 normally located upper incisor teeth present in this mouse.

The lower incisor teeth were in all regards normal in all groups.

(*c*) *Analysis of the facial skeleton in Alizarin red S and Alcian blue stained 'cleared' specimens.* Analysis of the Alizarin red S and Alcian blue stained 'cleared' crania of 6 normal newborn mice revealed that both the maxilla and premaxilla were ossified and distinguishable as discrete units. Similarly, the locations of the upper incisor teeth, within their sockets, were also quite clearly defined. From the nasal region, the nasal cartilages which surrounded the vestibule of the nose protruded for some considerable distance. Although the incisal edges of the upper and lower incisor teeth almost met, the upper jaw nevertheless protruded for some considerable distance in front of the lower jaw, since the lower incisors were directed forwards and upwards while the upper incisors were directed backwards and downwards.

In the *Sey/Sey* mice, the dorsal/anterior surface of

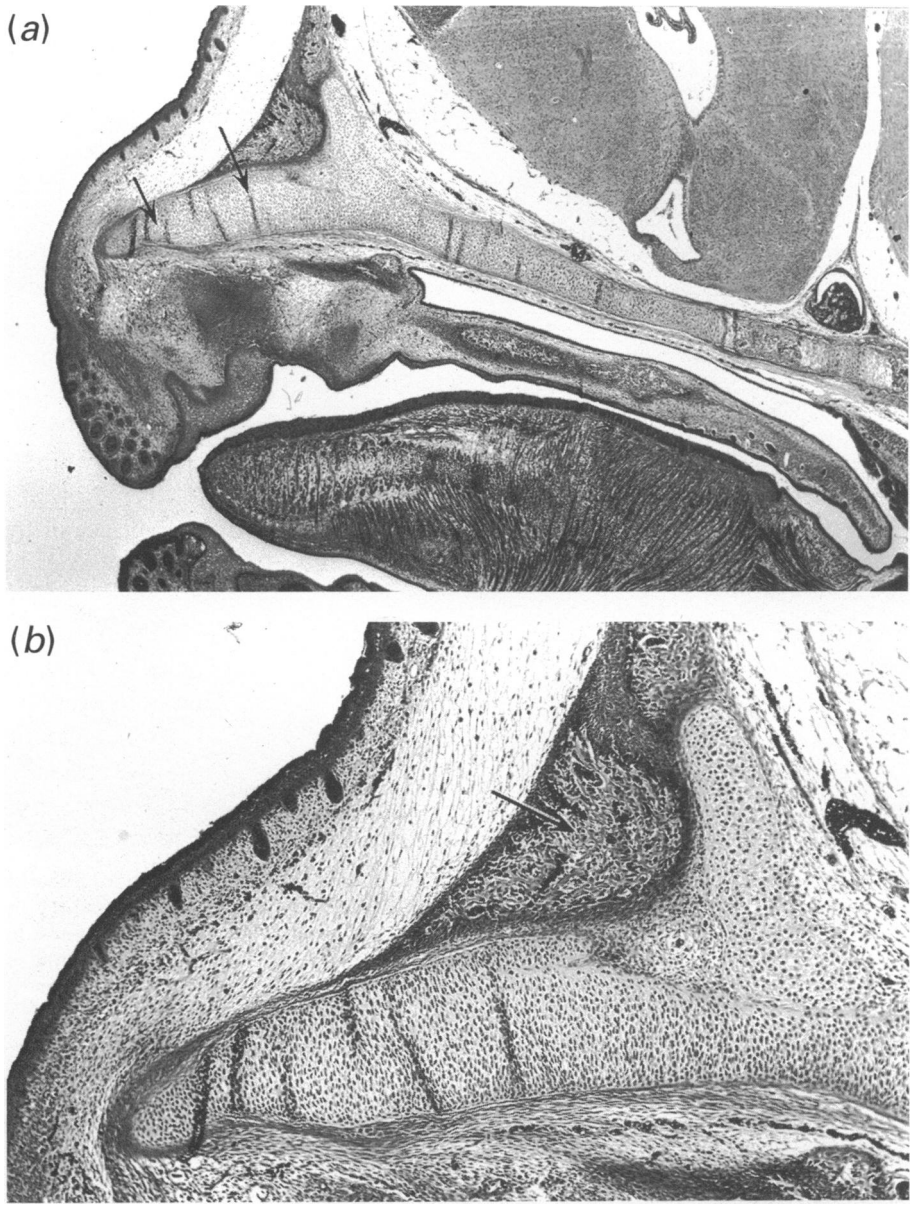

Fig. 6. (*a*) Median sagittal section through the cephalic region of a 17.5 d p.c. *Sey/Sey* mouse embryo. Note that the cartilaginous rod-like structure (arrows) extends forwards almost to the tip of the snout, and appears to be a precursor of the ethmoid bone and is additionally continuous with the basisphenoid bone. The horizontal 'lines' seen in association with the cartilaginous 'horn' are artefactual, and are corrugations in the section. Haematoxylin and eosin, ×25. (*b*) Close up view of the most anterior part of the cartilaginous rod-like structure. Note the presence of a mass of cancellous bone which is continuous across the midline (arrow). The latter is of maxillary origin, and is in close association with the proximal part of the 'horn'. ×63.

the maxillary bones appeared to be continuous with the anterior/upper surface of the premaxilla, and the face was foreshortened and broader than normal (see Fig. 1). The face also had a characteristic prognathous appearance, and the incisal edges of the upper and lower incisor teeth failed to meet, because the upper incisor teeth were invariably located some distance behind the lower incisor teeth.

In the absence of nostrils, no nasal cartilages were present, and furthermore, there was no recognisable nasal septum. However, a single midline cartilaginous rod-like structure was clearly seen which protruded between the maxillary/premaxillary complex, giving the 'cleared' specimens a quite characteristic 'unicorn-like' appearance (see Fig. 5). The medial borders of the maxillae appeared to splay out slightly in the region where the cartilaginous rod emerged between them, giving the impression that it had forced its way through their apposed medial borders. This was clearly not the case, however, as evidenced by the fact that the precartilaginous precursor of the rod-like structure was already in its definitive location well before evidence of ossification was seen in the maxilla/premaxilla primordium complex (see section

(*a*) above). When the skull was disarticulated, and this cartilaginous structure was viewed in isolation, its relationship to the various components of the chondrocranium was more clearly seen, and seemed to confirm its continuity with the ethmoid and basisphenoid.

Analysis of median sagittal sections through the cephalic region of 2 17.5 d p.c. embryos confirmed that continuity existed between the cartilaginous rod-like structure and the ethmoid as well as the basisphenoid and possibly also the basioccipital bones (see Fig. 6).

DISCUSSION

It is of interest to note that the incidence of the homozygous *Small eye* embryos in our prenatal series was very close to the predicted ratio of 25% and similar to that reported previously (Hogan et al. 1986). The fact that this is a dominant mutation that is lethal in the homozygous state had earlier been reported in the original paper in which this mutation had been described (Roberts, 1967), where the heterozygote:normal ratio was close to 2:1. This finding was consistent with the fact that no homozygotes were observed, presumably because they had all been eaten by the mothers at the time that the litters were inspected.

Ocular findings

Previous workers (Hogan et al. 1986, 1988) have reported that at 10.5 d p.c., a reasonably normal optic vesicle is present in *Sey/Sey* embryos, but by 11–11.5 d p.c., because the lens placode fails to differentiate, the normal inductive interaction between these two tissues which is needed to facilitate more advanced ocular differentiation fails to occur. Consequently, by 11.5 d p.c., the optic vesicle becomes very distorted, and no remnant of the ocular apparatus is seen by 12.5 d p.c. beyond a blind-ending optic stalk which extends only minimally beyond the optic chiasma. Curiously, primitive eyelids are formed, and fuse in the region overlying the presumptive orbit. Within the orbit itself, however, disorganised muscle masses develop, and presumably represent the extrinsic ocular musculature.

Palate development

Analysis of *Sey/Sey* embryos at 12.5 and 13.5 d p.c. consistently revealed the presence of the palatal shelves of the maxillae, and demonstrated that their degree of development was consistent with that of the differentiation of the other craniofacial structures. At 14.5 d p.c., however, the time at which palatal shelf elevation, apposition and some degree of fusion across the midline might have been expected to have occurred, this finding was only seen in 1 out of 3 embryos studied at this stage of development. In 2 of these embryos, no evidence of palatal shelf elevation was seen. In the single 15.5 d p.c. embryo studied, the palatal shelves were still seen to be located on either side of the tongue mass, whereas in all embryos that were more advanced than 16.5 d p.c., the normal sequence of events associated with fusion of the palatal shelves appeared to have occurred.

At about 15.5 d p.c., in genetically normal and *Sey/+* mice, the inferior surface of the anterior part of the nasal septum initially makes contact with the upper surface of the secondary palate. Over the period of the next few days, the inferior border of the nasal septum progressively fuses with the secondary palate until, with the exception of its most posterior part, in the region overlying the soft palate, the 2 definitive nasal cavities are formed (Kaufman, 1992).

If nasal derivatives had been present in *Sey/Sey* mice the region above the palatal shelves would have formed the nasopharynx. However, in these mice the space that is delineated by the closure of the palatal shelves is in fact a dorsal subdivision of the oropharynx. Clearly no evidence of olfactory epithelium would be expected to be present in the anterior part of the roof of this region in these embryos, because of the absence of all nasal derivatives, and none was recognised.

Development of the upper incisor teeth

Another unexpected finding reported here was the influence of the homozygous *Small eye* phenotype on the total number of upper incisor teeth present. While there are normally 2 upper incisor teeth present in the mouse, in 80% of the homozygous mutant embryos and neonates studied, in addition to a pair of normally located upper incisors, either 1 or, more commonly (in 45% of cases), 2 supernumerary incisor tooth germs were present, each being invariably located lateral to its ipsilateral normal tooth. In 35% of the embryos or neonates, the 2 normal upper incisors were accompanied by a single supernumerary incisor tooth germ which was just over twice as commonly located on the left compared to the right side of the upper jaw. Colyer (1936), in a comprehensive study of dental variations in animals, noted that positional variations and impaction of teeth was in general rare in rodents,

and in only a few species (not *Mus*) did he find supernumerary molars.

In the human, while the absence of one or more teeth is relatively commonly encountered, the presence of supernumerary teeth is only very rarely described; they probably occur in only 1–2% of the population, and the commonest sites are in the maxillary third molar region and between the maxillary incisors (Fuller & Denehy, 1984). The presence of supernumerary teeth is said to be an occasional feature in Gardner's syndrome, a condition principally associated with the presence of intestinal adenomatous polyps, epidermal fibromatous lesions and osteomata of the calvarium, mandible, face and elsewhere (Gardner, 1962), though there appears to be no consistent pattern of supernumerary dentition in affected individuals.

A similar situation has been reported in relation to the Oral-Facial-Digital syndrome (Gorlin & Psaume, 1962; Doege et al. 1964) where the presence of supernumerary teeth is also an occasional feature. In two-thirds of cases in some series, the upper lateral incisors were missing, while in some of these cases the lower lateral incisors were also missing. The presence of extra incisors and doubling of the upper canines have also occasionally been reported.

It is of interest that despite the absence of eyes and nasal derivatives, no dental or palatal abnormalities were reported in a stillborn human infant that was almost certainly homozygous for the aniridia gene (Hodgson & Saunders, 1980; Hodgson, personal communication).

The initiation of normal tooth development and the factors that influence their subsequent shape is controlled by genes such as *msx1* and *msx2* (previously named *Hox-7* and *Hox-8*) (MacKenzie et al. 1991*a*, *b*, 1992), though little is known of the interactions of inductive and organising mechanisms during dental morphogenesis and differentiation (see, however, recent study by Jowett et al. 1993). However, because the homozygous *Small eye* mice analysed in the present study had a mutation in the *Pax-6* gene, it would seem that the latter may also have an effect on the pattern of tooth development. This may be a direct effect, or an interactive effect with the *msx* genes indicated above. Equally, the roles of neural crest cells (Lumsden, 1988) and innervation (Lumsden & Buchanan, 1986) in the initiation of tooth germ development have yet to be elucidated. Duplication of a tooth may occur spontaneously in otherwise normal animals either by the production of an additional tooth bud from the dental lamina or by division of an existing tooth germ while it is developing. The occasional production of supernumerary teeth may be caused by local factors within the jaw. While Sperber (1967) speculated on the presence of supernumerary tooth genes, their formation is more likely to be due to the aberrant expression of *msx1* and *msx2* during early craniofacial development.

The consistent finding of supernumerary teeth in the present study suggests that incisor tooth number and position were genetically controlled in the mutant strain of mice examined, and the strain may be a suitable animal model for the study of supernumerary tooth production and development.

Anomalous features of the skull in Sey/Sey *mice*

In the absence of nasal derivatives, the premaxilla and maxilla appeared to fuse in the *Sey/Sey* mice to form a single complex skeletal unit. In addition, and possibly the most unexpected finding reported here, was the presence of the midline cartilaginous rod-like structure which protruded between the 2 maxillary bones to give the 'cleared' skull its characteristic unicorn-like appearance. This rod-like structure appeared to be a rostral extension of the ethmoid/basisphenoid, and was the nearest equivalent in these mice to a cartilaginous nasal septum. There seems no evidence to suggest, however, that it represents a median structure derived from the amalgamation of the 2 nasal cartilages, and we would wish to suggest that it might develop as a consequence of their absence.

It is clearly essential to follow up these studies with appropriate molecular analyses which, we believe, may shed light on the genetic factors that control the establishment of the dentition in the region of the primary palate, and the various abnormal components of the craniofacial region described in this paper.

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