The Origin and Development of the Upper Lateral Incisor and Premaxilla in Normal and Cleft Lip/Palate Monkeys Induced With Cyclophosphamide

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Objective: **Cleft lip/palate (CLP) is a common human congenital defect in which the maxillary lateral incisors are often absent, malformed, and malpositioned. The present study was designed to examine the origin of the upper primary lateral incisor relative to the medial nasal process (MNP) and maxillary process (MP) fusion area and to the premaxillary/maxillary (incisive) suture in monkeys.**

Method: **Scanning electron microscopy, histology, skeletal staining, and drying techniques were used to study facial development in embryo and fetal monkey specimens. A teratogenic dose of cyclophosphamide was administered to pregnant monkeys prior to fusion of the MNP and MP and fetuses were examined for CLP.**

Results: **Formation of the anterior maxilla involved fusion of the MNP and MP at stages 14–18. At stages 18–20, the palatal portion of the MNP had formed the medial and lateral incisive mounds. By stage 22, the upper primary lateral incisor has formed within the MP, lateral to the MNP/MP fusion area and to the ossifying premaxilla. Ossification of the premaxilla begins in the MNP and subsequently spreads laterally across the MNP/MP fusion area into the MP. Accordingly, the lateral incisor undergoes a complex positional shift (mainly medial) relative to the incisive suture both prenatally and postnatally and is finally located medial to the suture. Examination of the cyclophosphamide-induced CLP fetuses showed that the lateral incisor is located lateral to the alveolar cleft and does not shift medial to the incisive suture.**

Conclusion: **Understanding the origin of the lateral incisor (the tooth closest to the cleft) and the shift after its formation provides clues to high incidence of malformations and ectopia of this incisor in cleft patients.**

KEY WORDS: *cleft lip/alveolus/palate, cyclophosphamide, incisive mounds, lateral incisor, monkey (macaque), nasopalatine duct, premaxilla*

The upper lateral incisor is unique in terms of variations and abnormalities in humans. In cleft lip/palate patients, the upper lateral incisor is the most vulnerable to injury in the region of the cleft in both deciduous and permanent dentitions (Ranta, 1986). These injuries are manifested later as delayed or ectopic eruption, hypoplasia, or aplasia (Suzuki et al., 1992; Solis et al., 1998). In noncleft patients, the permanent upper lateral incisor is the third most common congenital missing tooth, second to the third molars and the mandibular second premolar. This permanent incisor is the most commonly found microdontia (small tooth, peg lateral) with a prevalence of 0.8% to 8.4% of the population (Neville et al., 1995). Additionally, structural abnormalities such as dens invaginatus

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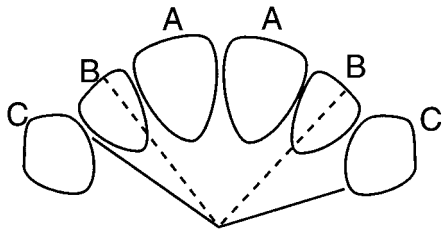

(Ferenczy, 1958) (Lisson and Kjaer, 1997)

FIGURE 1 Controversy over the origin of the maxillary lateral incisor in humans (modified from Ferenczy (1958) and Lisson and Kjær, 1997). Palatal view. Dashed and solid lines represent the medial nasal/maxillary process fusion area and premaxillary/maxillary suture, respectively. A, B, and C represent the primary upper central and lateral incisors and canine, respectively.

(dens in dente) most frequently involve the upper lateral incisor among the permanent teeth (0.04% to 10% of all patients). It appears that the tooth buds of both deciduous and permanent maxillary lateral incisors are located in an area that is susceptible to insult during development.

During the fourth and sixth weeks of human embryonic development, the upper jaw, from which the lateral incisors originate, forms by fusion of the paired medial nasal processes (MNP) and maxillary processes (MP). As the medial nasal processes fuse with each other, they form the premaxilla, including the medial portion of the upper lip (philtrum) and the primary palate. There is controversy over the exact origin of the maxillary lateral incisor relative to the MNP/MP fusion area and the location of the premaxillary/maxillary suture in the human. The MNP/MP fusion area may be medial to the lateral incisor or at the medial or middle one-third of the lateral incisor (Ferenczy, 1958; Ooé, 1958; Lisson and Kjær, 1997). The premaxillary/maxillary suture may be between the lateral incisor and canine or at the middle third of the canine (Ferenczy, 1958; Lisson and Kjær, 1997) (Fig. 1). These differing views are based on clinical or human embryological studies. It is our intention that a combination of teratological and embryological studies in the monkey, an animal model that closely mimics human development, may provide a resolution to this controversy.

TABLE 2 Teratological Analysis

	MMU* (Macaca mulatta)	MCY** (Macaca fascicularis)
CP-treated fetuses	10	4
Fetuses with clefting	4	1
Examination	GD 75	GD 160 (at term)

*McClure et al., 1979; **Hendrickx et al., 1991.

Human teeth in general have two origins: ectoderm and mesoderm. The ectoderm gives rise to the enamel, whereas the mesoderm produces the dentin, pulp, cementum, and periodontal ligament. Invagination of the oral epithelium in the presumptive upper and lower jaws leads to the formation of the vestibular and dental laminae. The vestibular lamina differentiates to form the vestibule and gingiva. The superficial layer of the stratified squamous gingival epithelium is abundant in glycoprotein stained with periodic acid-Schiff reagents (Itoiz and Carranza, 1996). Continued and localized proliferation in the dental lamina results in the formation of a series of epithelial growths into the underlying mesoderm at sites corresponding to the positions of the future deciduous teeth. Subsequent growth of both ectodermal and mesodermal components proceeds in three stages: the bud, cap, and bell. Typically, the permanent (succedaneous) tooth bud is derived from the dental lamina lingual to the deciduous counterpart. Tooth matrix formation is followed by calcification (Ten Cate, 1998).

In the present study, we used two macaque species, the rhesus (*Macaca mulatta*) and long-tailed (*M. fascicularis*) macaque, which have many similarities in craniofacial anatomy and prenatal development. Both species have moderately long snouts, specialized molars, narrow nasal openings with outward facing nostrils, and a narrow interorbital region. Many of these features are common to the genus *Macaca* (Fleagle, 1988). The embryological development of these two species is also remarkably similar. For example, the first and second pharyngeal arches first appear on gestation day (GD) 23, and

TABLE 1 Number Of Monkeys Used For Normal Development Analysis At Different Developmental Stages

	MCY (Macaca fascicularis)				MMU (Macaca mulatta)			
	Scanning Electron Microscopy	*Histology*	*Skeletal Staining*	*Dried Skeleton*	*Scanning Electron Microscopy*	*Histology*	*Skeletal Staining*	*Dried Skeleton*
Stage 12	3							
Stage 13	1							
Stage 14	2							
Stage 16	5				1			
Stage 17	4				1			
Stage 18	2							
Stage 20	2				1			
Stage 21	3				1			
Stage 22		1						
Stage 23	1	1						
Fetal	2		15			1	2	7
Postnatal			2					12
Total	25	2	17		4	1	2	19

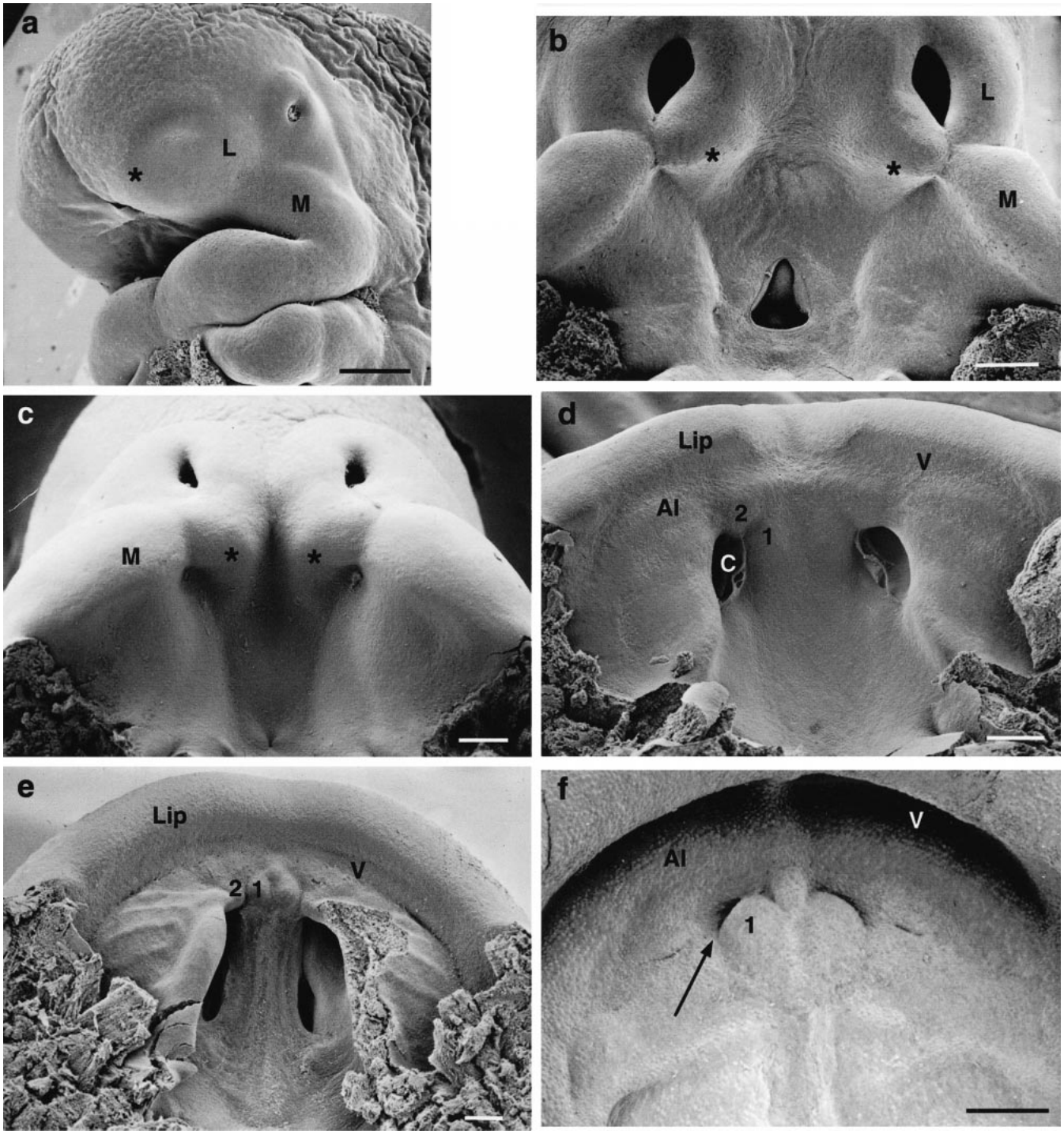

FIGURE 2 Normal development of the facial processes in monkeys (MCY). At stage 14 (a), the maxillary process (M) adjoins smoothly with the olfactory placode bordered medially by the medial nasal process (*) and laterally by the lateral nasal process (L). At stage 16 (b), there is a distinct groove between M and *. Note the approximation of the medial nasal processes between stages 16 and 17 (c). At stage 18 (d), the terminal end of the medial nasal process ventral to the primary choana (C) is divided into the incisive mounds 1 and 2. The upper lip is separated from the upper alveolus (A1, 2, and probably 1) by the vestibule (V). The groove between 1 and 2 is more distinct at stage 20 (e) and forms an opening (arrowhead) at early fetal stage (GD 52; f). Bar = 200 μm.

the secondary palatine shelves fuse on GD 45 in both species. Both direct measurements of surgically recovered embryos and *in utero* monitoring by ultrasonography indicate that the greatest length and major morphological features are identical throughout the embryonic period (Tarantal and Hendrickx, 1988; Makori et al., 1996).

Differences in the rhesus and long-tailed macaque are more readily seen later in pregnancy and at birth. The gestation pe-

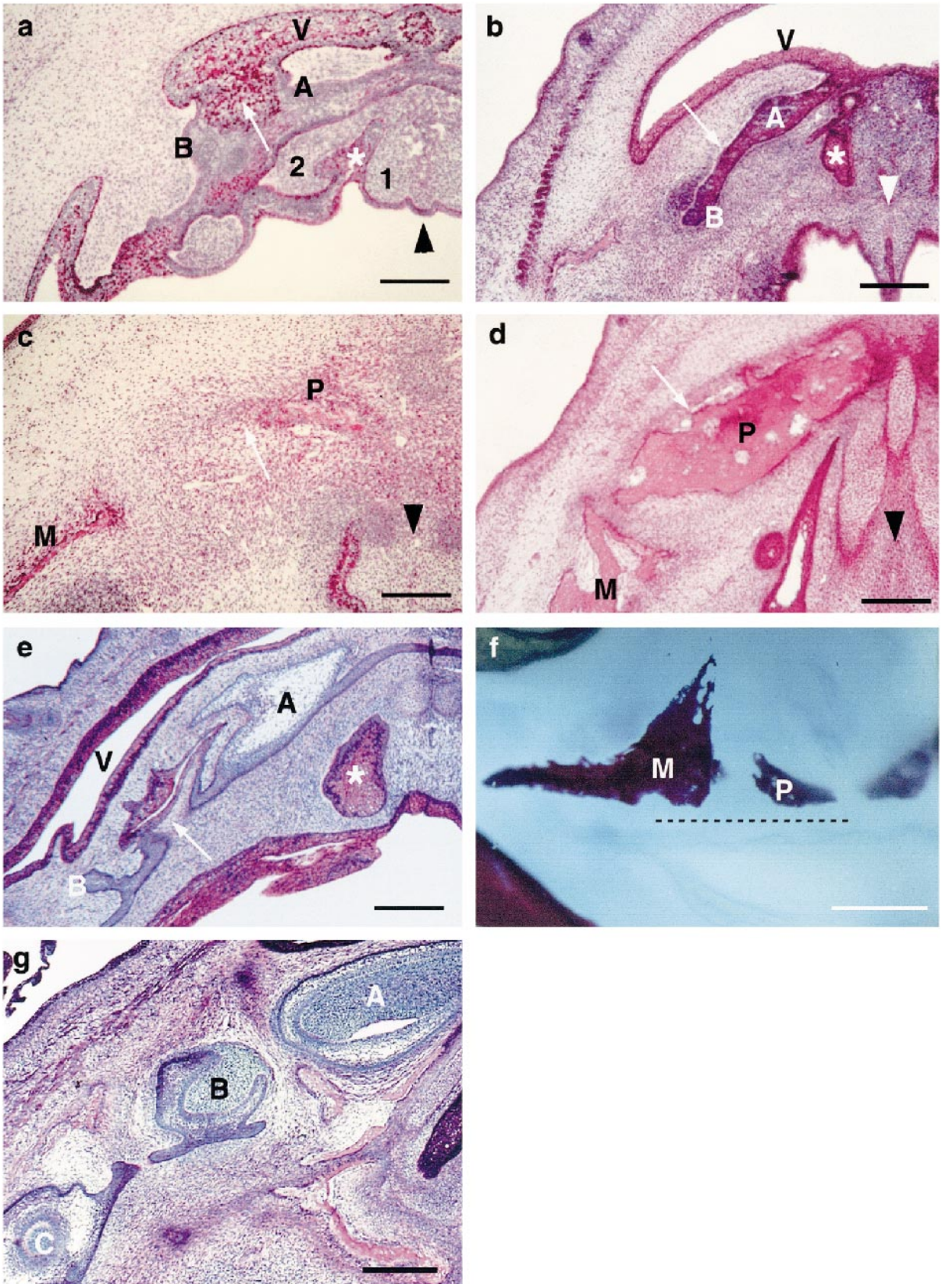

FIGURE 3 Normal development of the primary upper lateral incisor and nasopalatine duct in macaques. a–e, g: periodic acid-Schiff and hematoxylin staining. A, b, and e: transverse sections of the alveolar ridge (gingival portion) of the developing upper jaw of different staged embryos/fetuses, section plane being indicated by a dashed line in f, ventral to the top. c, d: transverse sections (through the premaxilla and maxilla) rostral to a,b, respectively. Note the midline (arrowheads) of different sections of the same embryo (a versus c, b versus d) is aligned at the approximately same level.

riod is slightly longer in the rhesus (165–175 days) than in the long-tailed (155–165 days) macaque. The mean birth weight of rhesus and long-tailed macaques is 475 and 345 g, respectively (Hendrickx and Dukelow, 1995). The biparietal diameter (BPD) of the fetal skull is also indicative of the smaller size of the long-tailed macaque fetus; the decrease in this parameter is first recognized by GD 50–60. At birth, the BPD is approximately 3 mm shorter in the long-tailed macaque than in the rhesus (Tarantal and Hendrickx, 1988).

The medial nasal process and maxillary process fuse at approximately GD 33. In the rhesus monkey, primary teeth begin to emerge at 1½ months of age and finish eruption at 8½ months (Kenney, 1975). The mixed dentition characterized by emergence of the first permanent molars begins approximately 1 year later. Completion of the permanent dentition with the eruption of the third molars has occurred in almost all rhesus monkeys by 7 years of age.

An obvious difference in maxillary development between the human and monkey is that there is a distinct premaxilla in the macaque, whereas a separate premaxillary center appears to be absent in the human (Crelin, 1969; Wood et al., 1969; Collins, 1995). The distinct premaxillary/maxillary boundary in monkeys allows us to determine the positional changes of the upper anterior teeth after their formation. In addition, spontaneous cleft lip malformations with or without cleft palate have not been reported in macaques (Hendrickx and Prahalada, 1986; Peterson et al., 1997), in contrast to a 1:500–1,000 incidence in humans (Murray et al., 1997). Despite these differences, the monkey models the human in morphogenesis and general structures of the jaw and teeth (Kenney, 1975).

Experimental induction of cleft lip with or without cleft palate in rhesus monkeys has been reported using cyclophosphamide (CP; McClure et al., 1979). CP, a commonly used chemotherapeutic agent and immunosuppressant, is a potent teratogen in several animal models (Mirkes, 1985). Malformations are often found in the axial skeleton, central nervous system, and craniofacial region (Chernoff et al., 1989; Francis et al., 1990).

The objectives of the present study were threefold: (1) to determine the origin of the upper deciduous lateral incisor and its positional change relative to the medial nasal/maxillary process fusion area (early stage) and to the premaxilla/maxilla suture pre- and postnatally; (2) to determine the sensitive window for experimental induction of cleft lip/palate in long-tailed monkeys; and (3) to analyze CP-induced cleft lip/palate in monkey fetuses relative to the position of the lateral incisor.

Materials and Methods

Animals

Two species of monkeys, rhesus (*M. mulatta*, MMU) and long-tailed (*M. fascicularis*, MCY), were housed and bred under standard housing conditions (Hendrickx and Hummler, 1992). Untreated control embryos/fetuses of varying gestational ages were collected by hysterotomy and fixed as specified by the experimental protocol described below. The experiments were carried out in accordance with the ''Principles of Laboratory Animal Care'' (NIH publication No. 86-23, revised in 1985) in addition to the standards established by the United States Federal Animal Welfare Act and Institute for Laboratory Animal Resources. The criteria for staging of nonhuman primate embryos are very similar to that for the human (Hendrickx, 1971).

Scanning Electron Microscopy

Twenty-five MCY and four MMU control embryos and fetuses were examined by scanning electron microscopy (SEM) for early facial development (Table 1). Specimens were fixed in 2.0% glutaraldehyde in 0.085 M cacodylic buffer for 24 hours, postfixed in 1% OsO_4 for 1–2 hours, dehydrated in a graded ethanol series, and critical point dried with CO_2. The embryos/fetuses were then mounted on aluminum stubs with double-sided tape, sputter coated with gold/palladium in an argon-vacuum container, and examined at 10–11 kV on a Philips SEM.

Histology

Two MCY and one MMU control embryos/fetuses on GD 45–61 were examined to determine the origin of the primary upper lateral incisor (Table 1). The heads of fixed embryos/fetuses were embedded in paraffin, sectioned at 5 μm, stained with periodic acid-Schiff, and counterstained with hematoxylin.

←

a: stage 22 (GD 45) the vestibular lamina (V) is connected dorsally (posteriorly) to the dental lamina (represented by A,B, precursors to the primary upper central and lateral incisors) by an ''epithelial bridge'' (arrow). *: an epithelial invagination between the incisive mounds (1, 2) as mentioned in Figure 1. b: stage 23 (GD 48) the dental lamina (A, B) is still connected to V at the area indicated by an arrow (demonstrated in a section 20 μm caudal to b). *: nasopalatine duct. c: stage 22 (GD 45) 150 μm rostral to a. Initial stage of formation (matrix and/or ossification) of the premaxilla (P) and maxilla (M), with a large space between them. Arrow: the lateral aspect of the premaxilla, corresponding to the epithelial bridge in a. d: stage 23 (GD 48) 210 μm rostral to b. The premaxilla is more developed, the lateral portion of the premaxilla (lateral to the arrow) being just formed. e: early fetal stage (GD 61) the epithelial bridge (arrow) is between cap-staged A and B. *: nasopalatine duct. f: a GD 56 fetus the ventrolateral view of skeletal staining. Dashed line: section plane at the gingiva of the upper jaw. g: early fetal stage (GD 61) 210 μm rostral to e. C: canine. Bar = 160 μm (a–d), 190 μm (e), 1 mm (f), 200 μm (g).

Skeletal Stain

Seventeen MCY and two MMU control fetal, infant, and adult heads (Table 1) were fixed in 95% ethanol or 10% formalin. The specimens were processed for alizarin red and alcian blue stain (Inouye, 1976).

Dried Skull Preparation

Nineteen skulls of MMU control monkeys from GD 100 to postnatal 5.5 years old (Table 1) were used for this procedure. The maceration of the soft tissues (without fixation) involved incubation in graded solutions of potassium hydroxide at elevated temperature, manual removal of the softened soft tissues, and water rinse. Bleaching of the skulls was conducted in 10% hydrogen peroxide with subsequent air drying of the skull (McKinney, 1978).

Teratogenic Studies

In the initial study, MMU dams received CP on two different treatment regimens: 7 mg/kg on GD 27–29 or 10 mg/kg per day on GD 27 and 29 (McClure et al., 1979). The fetuses were delivered by cesarean section on GD 75 (Table 2). The heads of 10 MMU fetuses were reexamined grossly and then stained with alizarin red and alcian blue for further examination of the craniofacial skeleton.

In addition, four MCY pregnant females were treated by CP (7 mg/kg per day) on GD 27–29. Fetuses were collected at term (~GD 150) for teratological analysis (Table 2; Hendrickx et al., 1991). CP (Cytoxan, 47721, Mead Johnson Laboratories, Evansville, IN) was dissolved in sterile distilled water and injected intramuscularly (20 mg/mL = injection concentration; 0.35 mL/kg = injection volume).

Results

Development of the Facial Processes

At stage 12 (~GD 27), the stomadeum is bordered rostrally by the prominent frontonasal processes, caudally by mandibular processes and laterally by the relatively primitive maxillary processes. The maxillary process enlarges appreciably at stage 14 (~GD 30; Fig. 2a). The ventral portion of the maxillary process adjoins with the lateral (dorsal) aspect of the frontonasal process (i.e., the olfactory placode) with a smooth and shallow depression in between. The medial and lateral nasal processes border the olfactory placode. At stage 16 (GD 31–35), the medial and lateral nasal processes are well developed with formation of the olfactory pit (Fig. 2b). At this stage, there is a distinct groove at the junction between the maxillary and medial nasal processes. The distinct junction between the olfactory placode and maxillary process is absent at stage 14.

Between stages 16 and 17 the bilateral medial nasal processes are approximating each other (Fig. 2b and 2c). The approximation continues at subsequent stages. At stage 18 (GD 35–38), the upper lip is separated by the shallow vestibule from the upper alveolus (Fig. 2d). Obliteration of the groove between the medial nasal process and maxillary process lags behind in the alveolus, compared with that in the lip. Ventral to the primary choana the terminal portion of the medial nasal process (alveolar portion) appears to be divided into two distinct areas mediolaterally: incisive mounds 1 and 2. Note that mound 2 is ventrolateral to mound 1. The incisive mounds are more distinct at stage 20 (~GD 40; Fig. 2e). Also, the incisive mounds rotate between stages 18–20 so that at stage 20 the incisive mounds are almost at the same ventrodorsal level. The groove between mounds 1 and 2 deepens and is more defined at an early fetal stage (GD 52; Fig. 2f). The oral opening is ventro- (instead of dorso-) lateral to mound 1. The right and left sides of the mound 1 and the median mound between them appear to form the incisive papilla prenatally and postnatally. Mound 2 is less distinct and apparently forms the most anterior (previously ventral) portion of the alveolus at the early fetal stage.

In brief, the medial nasal process and maxillary process undergo fusion to form the upper lip/alveolus between stage 14 and stage 18 (GD 30–38). The subdivision of the medial nasal process at stages 18–20 is the initial step to form the nasopalatine duct, confirmed by histology below.

Origin of the Primary Upper Lateral Incisor

Embryos and fetuses were analyzed histologically to determine the origin of the deciduous maxillary lateral incisor relative to the fusion area of the medial nasal and maxillary processes. The transverse section of the future gingiva/alveolar ridge of the developing upper jaw (plane indicated by the dashed line in Fig. 3f) showed that at stage 22 (GD 45) two epithelial structures form ventral to the primary palate, the glycoprotein-rich vestibular lamina, and the relatively glycoprotein-scanty dental lamina (Fig. 3a). Lateral to the midline, the dorsal portion of the vestibular lamina is connected by an ''epithelial bridge'' to the ventral portion of the dental epithelium. The epithelial bridge is also glycoprotein abundant, suggesting a role of a lining epithelium, similar to the vestibular lamina. Therefore, the epithelial bridge most likely represents the fusion area of the medial nasal and maxillary processes. Immediately lateral and medial to the epithelial bridge, there are two thickened areas on the ventral portion of the dental lamina, which likely represent the precursors to the primary upper central and lateral incisor tooth buds. The section, approximately 150 μm rostrally, showed the initially ossified premaxilla and maxilla with a wide space between them (Fig. 3c). Note that the lateral incisor is lateral to the ossifying premaxilla at this early tooth developmental (prebud) stage.

At stage 23 (GD 48), the upper central and lateral incisor primordia associated with the anterior dental lamina were at the bud stage (Fig. 3b). The epithelial bridge (presumably the fusion area between the medial nasal/maxillary processes) is also found between these two tooth buds in more caudal sec-

tions (~20 μm). The premaxilla is enlarged with a narrower space between the premaxilla and maxilla (Fig. 3d), as compared with that in the embryo mentioned above (Fig. 3c). The premaxilla is now lateral to the fusion area of the medial nasal/maxillary processes and appears to accommodate both the central and lateral incisors as compared with Figure 3b. The relative position of the premaxilla, maxilla and the alveolar ridge can be visualized in an early-staged fetus (GD 56; Fig. 3f).

At an early fetal stage (GD 61), the anterior teeth are at the cap stage (Fig. 3e and 3g). Note the expansion of these tooth buds, compared with those at the earlier stage (Fig. 3b). The glycoprotein-rich epithelium trapped between the tooth buds of the central and lateral incisors represents the earlier medial nasal/maxillary process fusion area (Fig. 3e).

In brief, sequential histological analyses during GD 45–61 demonstrate that the primary maxillary central and lateral incisors are derived from the medial nasal and maxillary processes, respectively, immediately bordering the fusion area of these two facial processes. The lateral portion of the premaxilla is derived from the most medial aspect of the maxillary process.

Development of the Nasopalatine Duct

As demonstrated by SEM at stage 18–20, the terminal part of the medial nasal process is divided into the incisive mounds 1 and 2, immediately ventral to the primary choana (Fig. 2e and 2f). The division is confirmed by transverse sections at stage 22 (Fig. 3a). Between the incisive mounds is an epithelial invagination, which communicates dorsally with the primary choana. At stage 23, the dorsal communication is closed (Fig. 3b); the epithelial invagination forms a complete ''duct,'' which persists at the early fetal stage (Fig. 3e). Serial sections do not indicate that the primary choana appears to be directly involved in forming this nasopalatine duct.

In brief, the nasopalatine duct appears to be derived from an epithelium entrapped between the dividing portions of the medial nasal process, ventral to the primary choana.

Development of the Premaxilla Relative to the Upper Lateral Incisor

To determine the development of the premaxilla relative to the upper lateral incisor, a total of 38 monkey specimens were analyzed using either skeletal staining or dried skulls (Table 1). Development of the premaxilla was divided arbitrarily into three stages: prenatal, deciduous dentition, and permanent dentition.

1. Premaxillary development at prenatal stages (between GD 49 and term, n = 24)

The premaxilla and maxilla were shown to be ossified on GD 49 in monkeys. Although the deciduous upper maxillary tooth primordia are not calcified until approximately GD 75, the developing upper teeth and their position relative to the premaxilla/maxilla can be identified by the outline of developing teeth and the corresponding tooth sockets. Between GD 49 and GD 60, there is a relatively wide space (suture) between the premaxillary and maxillary ossification centers (Fig. 3f). Around GD 75, the suture is narrow (Fig. 4a). From the labial view, the central incisor and canine are located beneath the developing premaxilla and maxilla, respectively, and the lateral incisor is at the suture between these two ossification centers. However, from the palatal view, the suture appears to pass posterior to the lateral incisor. On GD 100, most (10% to 80%) of the calcified lateral incisor is located medial (anterior) to the premaxillary/maxillary suture (Fig. 4b). At term the lateral incisor is nearly completely medial to the narrowing but persistent premaxillary/maxillary suture (Fig. 5d and 5f).

2. Premaxillary development at the deciduous dentition or early mixed dentition stage (n = 9)

Macaque infants/adolescents examined at this stage (10 months to 1 year 10 months) are characterized by the presence of all of the deciduous teeth with or without the first permanent molars. There is a wide space between the upper lateral incisor and canine, 3–4.5 mm, known as the primate space of the upper jaw (Fig. 4c). There is no space between any two other neighboring upper teeth. Labially, the premaxillary/maxillary suture crosses the primary space at the junction of the middle and posterior (dorsal, distal) one-thirds. Palatally, the suture terminates medially at the posterior aspect of the nasopalatine canal (Fig. 4d). Note the septum (two thin bony plates) between the nasopalatine canals.

3. Premaxillary development at the permanent dentition stage (n = 5)

Macaque adults analyzed at this stage (~5½ years) have complete permanent dentition with the third molars erupted or being erupted. The upper primate space is 3–4.5 mm, similar to that in the previous age group, without presence of any other interdental spaces in the maxilla. The premaxillary/maxillary suture is located in the anterior portion of the alveolar socket for the prominent permanent canine, both labially and palatally (Fig. 4e and 4f). The anterior one-third of the palatal wall of the canine socket may be formed entirely by the premaxillary bone (four out of five monkeys; Fig. 4f) or by a thin septum from the maxillary bone (one out of five; Fig. 4e). Interestingly, one monkey has a unilateral supernumerary erupted permanent lateral incisor, which is also anterior to the premaxillary/maxillary suture. In summary, there is a continuous posterior shift of the premaxillary/maxillary suture relative to the upper deciduous or permanent lateral incisor in the prenatal and postnatal macaque life.

Teratological Analysis

Five out of 14 CP-treated monkey fetuses (36%) exhibit unilateral or bilateral cleft lip, alveolus, or palate (Table 2), which is characterized by failure of fusion of the medial, lateral nasal, and maxillary process derivatives (Fig. 5b and 5c as compared with control in 5a). Although the lateral portion of the lip (pre-

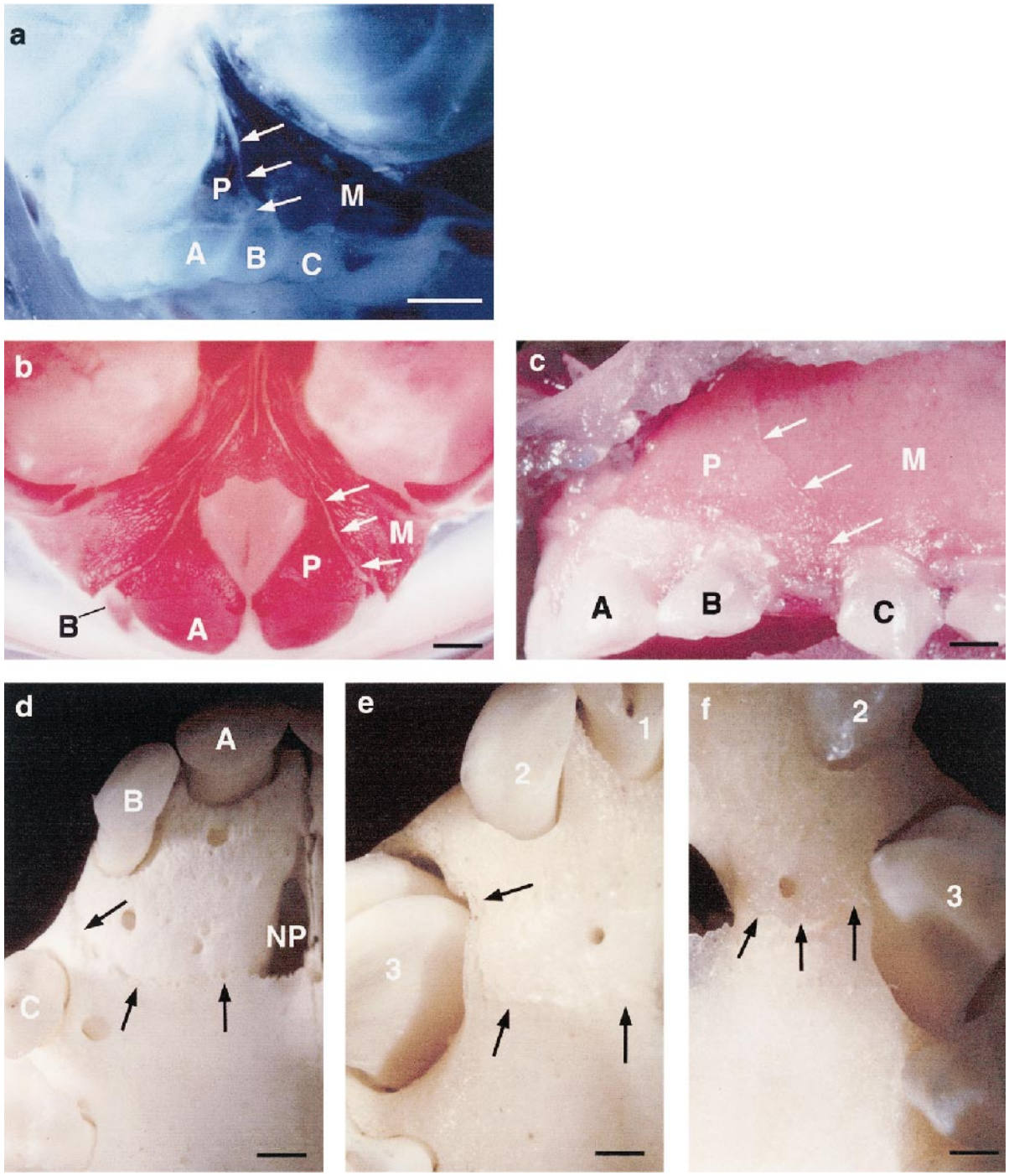

FIGURE 4 Normal development of the premaxilla. a–c: ventral or ventrolateral view of the upper jaw, d–f: palatal view. a: the primary upper lateral incisor (B, uncalcified) is below the premaxillary (P)/maxillary (M) suture (arrows) of a GD 75 monkey fetus. A: central incisor; C: canine. b: the majority of the calcified lateral incisor (B) is medial to the P/M suture in a GD 100 monkey fetus. c, d: the P/M suture (arrows) crosses the middle and distal (posterior) one-third of the upper primate space between primary lateral incisor (B) and canine (C) during the mixed dentition stage in monkeys. NP: nasopalatine canal. During the permanent dentition stage in monkeys, the P/M suture (arrows) may cross the medial (anterior) and middle one-third of the palatal wall of the socket of the upper canine (3) in a straightforward (f) or more complex (e) manner. 1, 2: permanent central and lateral incisors. Bar = 2 mm (a, c–g), 1 mm (b).

sumably derived from the maxillary process) is deficient in tissue volume and does not extend medially, the median portion of the lip and associated premaxilla fail to descend and rotate to varying degrees (observed in three out of five fetuses with clefting, 3/5). The typical (lateral) cleft lip/alveolus is sometimes associated with median cleft lip/alveolus. The two affected, unfused portions of the premaxilla consisted of two relatively prominent upper primary central incisors, and the hypoplastic and hypocalcified ossification centers (Fig. 5e and 5g, compared with the control in 5d and 5f). The long axis of the central

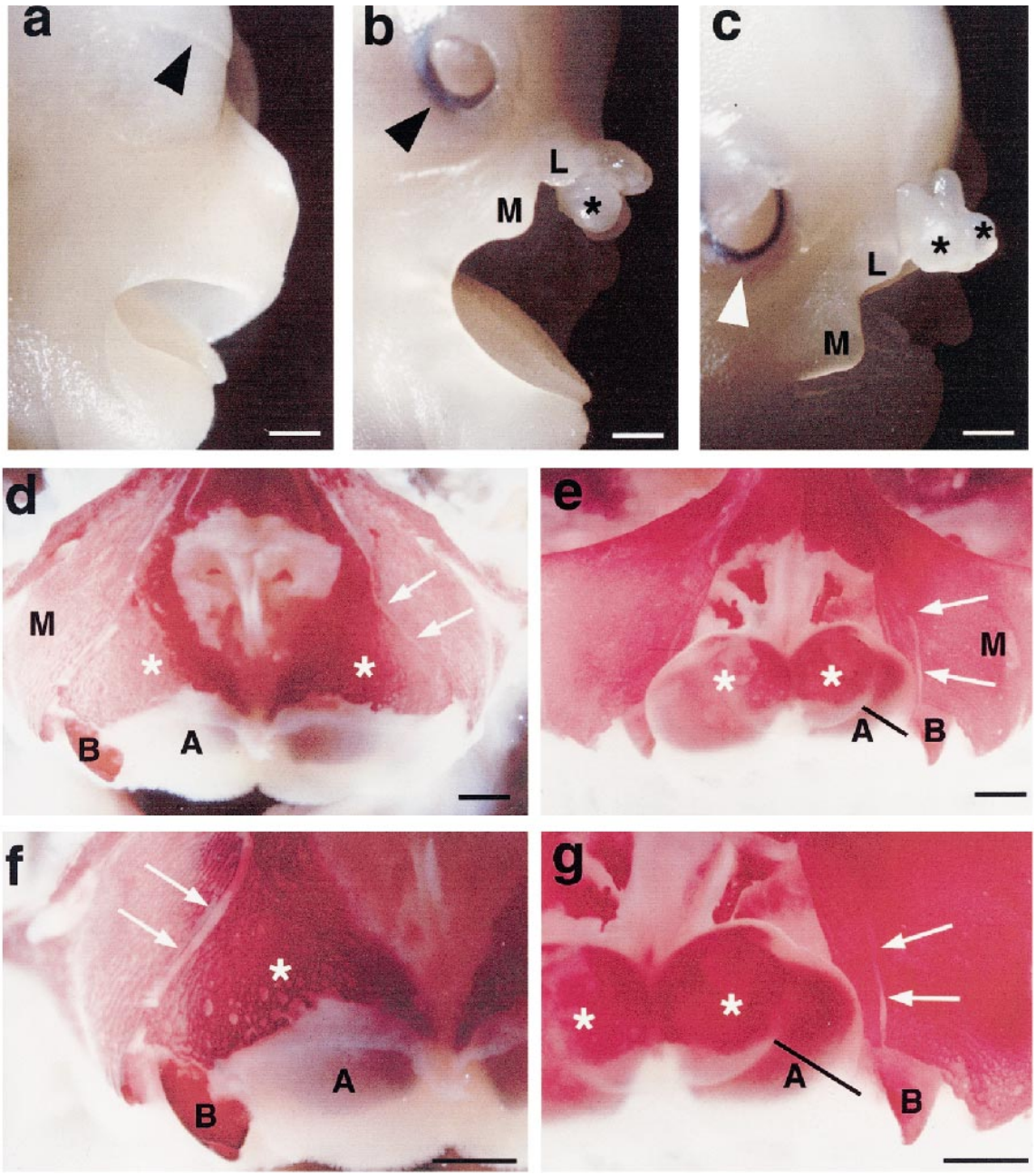

FIGURE 5 CP-induced facial clefts and associated malformations in monkeys. Lateral (a–c) and anterior (d–g) view of the face of control (a, d, and f) and affected (b, c, e, and g) fetuses on GD 75 (a–c) and at term (d–g, skeletal staining). Arrowhead: linear closed palpebral fissure in the control fetus (a) and irregular, open palpebral fissure in the affected fetuses (b, c). *: premaxilla. L and M: superior labial structures presumably derived from the lateral nasal process and maxillary process, respectively. d, f: primary upper central (A) and lateral (B) incisors are both medial to the premaxillary (*)/maxillary (M) suture (arrows) in the control. In the treated fetus (e, g), A and B are medial and lateral to the cleft, respectively. The line beside A represents the abnormal rotation of the long axis of A. The majority portion of B is lateral to the premaxillary/maxillary suture. Bar = 2 mm (a–g).

incisors in affected fetuses is abnormally rotated. By contrast, the lateral incisor in affected fetuses is lateral to the alveolar clefting (5/5). The premaxillary/maxillary suture line is lateral to the alveolar clefting bilaterally. It is interesting that approximately 70% of the lateral incisor is distal (posterior) to the suture in affected fetuses (Fig. 5e and 5g), whereas in similarly staged noncleft controls, the lateral incisor is anterior to the suture (Fig. 5d and 5f). In addition, the lower palpebral lid is defective in affected fetuses (Fig. 5b and 5c).

DISCUSSION

Window of Induction of Cleft Lip, Alveolus, and Palate by Cyclophosphamide in Monkeys

The results of our SEM analysis of embryos show that the medial nasal and maxillary processes begin approximation and fuse at stages 14–18 (GD 29–38). The results of the present MCY teratology study confirm the results of the earlier MMU

teratological study (McClure et al., 1979) indicating that the sensitive period for induction of cleft lip/palate by CP is between GD 27 and GD 29, prior to the normal fusion process. Understanding stages of normal medial nasal/maxillary process fusion and the window of cleft lip/palate induction in MCY lays a foundation for our future pathogenetic studies to elucidate how CP interferes with the fusion process, leading to cleft formation in the monkey.

The teratogenic mechanism of action for CP involves induction of cell death in rapidly proliferating cells by inhibiting DNA synthesis. CP binds to DNA during the S phase of the mitotic cycle and prevents cells from completing mitosis (Mirkes, 1985; Chernoff et al., 1989). In the present study, embryonic exposure to high doses of CP occurred during the time that neural crest cells are proliferating within the pharyngeal arch region. These proliferating cells would be likely targets for this antimitotic drug. Insufficient mesenchymal cells in the developing facial region may decrease the size of specific anlagen (premaxilla/maxilla), prevent the anlagen from making contact at the critical time, and lead to facial clefting. Conversely, studies of cleft lip/palate in human fetuses suggest that even though normal growth mechanisms may be operating, these mechanisms may be operating in an abnormal milieu or within altered anatomic relationships (Mooney et al., 1991).

The Origin of the Upper Lateral Incisor in Monkeys

In cleft lip, alveolus, and palate patients, the primary upper lateral incisor is often found lateral to the clefting (Suzuki et al., 1992). This phenomenon is also present in CP-induced facial cleft monkeys, an animal model most closely related to humans. Serial histological sectioning and skeletal staining at different developmental stages demonstrate the complex relationship of the lateral incisor to the medial nasal/maxillary process fusion area and to the premaxillary/maxillary suture in monkeys. The results of the present sequential embryological studies indicate that the primary upper lateral incisor is derived from the dental lamina immediately lateral to the fusion area between the medial nasal and maxillary processes. By contrast, the origin of the lateral incisor in humans is controversial. The medial nasal/maxillary process fusion area may be anterior (medial) to the lateral incisor (Ooé, 1958; consistent with findings in monkeys) or crosses the medial one-third or the middle of the lateral incisor (Ferenczy, 1958; Lisson and Kjær, 1997; Fig. 1). The controversies may be due to different study methods and examination at different stages. In any case, the maxillary lateral incisor tooth bud in both humans and monkeys is the closest to the postulated cleft site, which may account for the fact that it is the most frequently affected tooth in the cleft patient.

Unlike the human, the premaxilla in the monkey has a separate ossification center. The suture between the premaxilla and maxilla, also called the incisive suture, is a distinct structure, which persists until at least 5.5 years of age in the macaque. This characteristic allows us to precisely determine the relative position of the lateral incisor to the premaxilla during development. At stage 22 (prior to bud stage), the lateral incisor is lateral to the premaxilla. At stage 23 or older, the premaxilla appears to grow rapidly laterally so that the lateral incisor is medial to the incisive suture (Fig. 6a). At tooth cap and bell stages (early fetal stages), the expansion of the anterior tooth buds exceeds the lateral growth of the premaxilla so that the lateral incisor appears to be pushed relatively more laterally than the premaxilla and is found at the incisive suture (Fig. 6b). From that stage on, the lateral growth of the premaxilla is predominant. The lateral incisor is progressively situated medial relative to the incisive suture (Fig. 6c and 6d). In summary, the lateral incisor and the incisive suture undergo complex relative shifting after their formation. This may explain the different observations in humans of the position of the incisive suture relative to the primary lateral incisor and canine (Ferenczy, 1958; Lisson and Kjær, 1997, Fig. 1). Teratogenic insults causing cleft formation likely interfere with the complex relative shifting of the lateral incisor and the incisive suture. This may explain why the major part of the lateral incisor of cleft monkeys at term (~GD 160) is typically lateral to the incisive suture (Fig. 6e and 6f).

The Role of the Premaxillary/Maxillary Suture in Nonhuman Primates

In young human skulls, there is a distinct suture between the premaxilla and maxilla observed *palatally*, which obliterates later in life (Moore and Persaud, 1993). However, there is no corresponding suture between these two bones observed labially even in young humans (Crelin, 1969; Wood et al., 1969; Collins, 1995). In the chimpanzee, a sexually dimorphic association in premaxillary/maxillary suture patency has been reported (Mooney and Seigel, 1991). In the ''typical'' male specimens, the facial component of the premaxillary/maxillary suture was patent while the ''typical'' female exhibited a greater than 50% fusion of this suture. In contrast, the premaxillary/maxillary suture in both rhesus and long-tailed monkeys is persistent throughout their dentition development and undergoes continuous relative positional changes, from the middle of the deciduous lateral incisor, lateral to the lateral incisor, and finally to the medial aspect of the permanent canine. This suggests that the premaxillary/maxillary suture should be an important growth center for maxillary mesiodistal length development in monkeys.

There is a distinct difference of the upper primate space between the human and the monkey. In the human, the upper primate space between the primary lateral incisor and the canine is associated with other interdental spaces between the anterior teeth (primary spacing) in approximately 70% of children with primary dentition (Dale, 1994). The presence of the primary space/spacing appears to indicate the increase in maxillary width to accommodate larger permanent anterior teeth. At the deciduous/early mixed dentition stage in macaques, the upper primate space is the sole space in the maxilla. During permanent canine eruption, this space disappears and then reappears (Kenney, 1975). This suggests that the growth of the

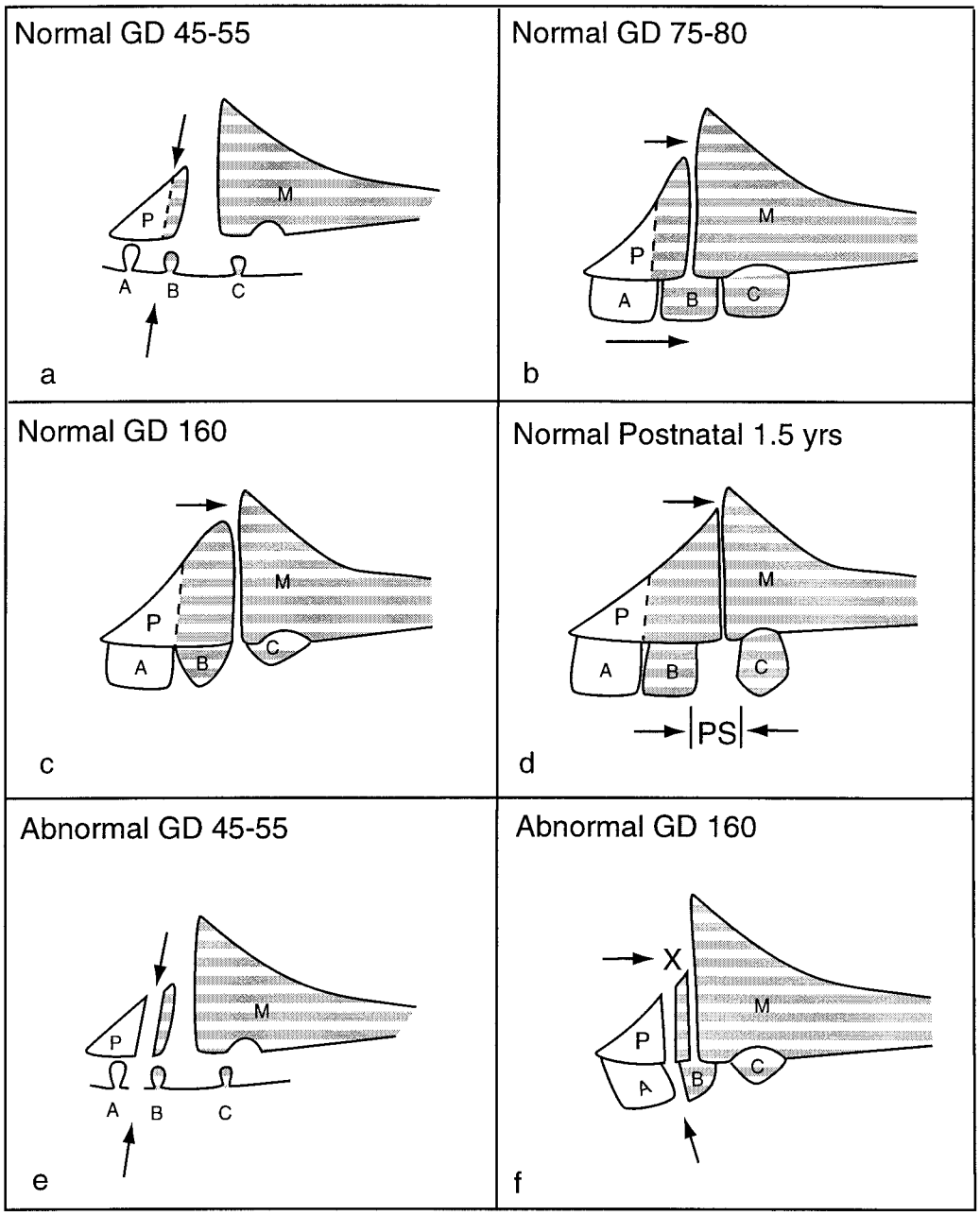

FIGURE 6 Illustration of normal (a–d) and abnormal (e, f) development of the primary upper anterior teeth, premaxilla, and maxilla in monkeys (front [labial] view, medial [anterior] to the left in each panel). a: in normal early dental lamina development (GD 45–55), the medial portion of the premaxilla (P) and the central incisor (A) are derived from the medial nasal process (medial to arrows and dashed line), whereas the lateral portion of the premaxilla, maxilla (M), and lateral incisor (B) and canine (C) teeth are derived from the maxillary process (striped area, lateral to arrows and dashed line). b: at later stages of monkey development (GD 75–80), the differential growth, indicated by different lengths of arrows, of the premaxilla, and of the upper incisors (bell stage) positions the lateral incisor at the premaxillary/maxillary suture. Continuous lateral growth of the premaxilla (transverse arrow) will eventually cause the lateral incisor to be located medial to the premaxillary/maxillary suture at term (GD 160, c) and result in the formation of the upper primate space (PS) postnatally (1.5 years, d). It is speculated that on GD 45–55 following cyclophosphamide exposure on GD 27–29, failure of fusion of the medial nasal and maxillary processes (arrows) leads to the separation of the medial and lateral portions of the premaxilla as well as the central incisor and lateral incisor-canine primordia associated with the dental lamina (e, as compared with a). It appears that lateral growth of the premaxilla may also be retarded/disrupted (–>(??)X) so that the relatively medial shift of the lateral incisor does not occur. The lateral incisor is located between the lateral portion of the premaxilla and medial portion of the maxilla on GD 160 (f, as compared with c).

upper primate space in macaques may be a major mechanism to accommodate the dimensional (mesiodistal) discrepancy of the anterior teeth between deciduous and permanent dentitions. The close relationship of the upper primate space and the premaxillary/maxillary suture suggests that the growth of the latter may play an important role in maintenance of the former in nonhuman primates (Fig. 6d).

The Premaxillary/Maxillary Suture Versus the Medial Nasal/Maxillary Process Fusion Area in Monkeys

The results of the analysis of CP-induced cleft lip, alveolus, and palate in monkey fetuses show that the alveolar clefting (presumably occurring at the medial nasal/maxillary process fusion area) is medial to the premaxillary/maxillary suture. The noncoincidence of these two structural boundaries is confirmed by histological investigation in late macaque embryos and early fetuses prior to the medial nasal/maxillary process fusion. The histological analysis suggests that the lateral portion of the premaxilla forms from the maxillary process (Fig. 6a). The noncoincidence between the medial nasal/maxillary process fusion area and the premaxillary/maxillary suture has been supported by human embryological studies (Ferenczy, 1958; Lisson and Kjær, 1997; Fig. 1).

The results of analyses of human/monkey cleft lip/alveolus patterns and early dental lamina histology of these two species point out that the medial nasal/maxillary process fusion area is located between the central and lateral incisors, instead of between the lateral incisor and canine. Understanding of this issue may help solve the controversy over the origin of globulomaxillary cyst. Typically this cyst is often found between the roots of the upper permanent lateral incisor and canine and traditionally regarded as a fissural cyst because it is presumably derived from the epithelium trapped between the globular process of His (medial nasal process) and the maxillary process. More recently pathological analysis of the lining epithelium of globulomaxillary cyst suggests that this cyst is more likely of odontogenic origin (Neville et al., 1995). The results of the present embryological studies support the nonfissural origin theory of globulomaxillary cyst, since most of the cyst is located between the lateral incisor and canine, lateral to the medial nasal/maxillary process fusion area.

Formation of the Incisive Mounds

The results of the present studies show that at stages 18–20 the palatal portion of the medial nasal process forms the medial and lateral incisive mounds, which have not been described in the literature before. Furthermore, the incisive mounds are found to be more closely related to formation of the nasopalatine duct than the primary choana. Traditionally, the nasopalatine duct is considered to be derived from the primary choana (Collins, 1995). Therefore, the results of the present studies provide an alternative hypothesis for the origin of the nasopalatine duct.

Conclusion

The medial nasal and maxillary processes in monkeys undergo fusion at stage 14–16. Embryonic exposure to a teratogenic dose of CP prior to the fusion is essential to induce cleft formation. The primary maxillary lateral incisor and the lateral portion of the premaxilla are derived from the most medial aspect of the maxillary process in monkeys. In addition, the lateral incisor undergoes a complex position shift relative to the premaxillary/maxillary (incisive) suture. The analysis of monkey fetuses with cleft lip/palate induced by cyclophosphamide exposure shows that the primary upper lateral incisor is lateral to alveolar clefting, consistent with the observation of human cleft cases and with early embryological studies on the origin of the lateral incisor. The teratogenic insult also interferes with the complex relative shift of the lateral incisor. The origin of the tooth bud of the upper lateral incisor close to the cleft formation region and complex movement of the tooth after its formation may provide evidence of the vulnerability of this tooth to different teratogenic insults. The results of the present study also provide the evidence that the nasopalatine duct in monkeys is likely not derived from the primary choana but from the division of the medial nasal process.

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