A Comparison of Craniofacial Growth in Normal and Cleft Palate Rhesus Monkeys

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In an attempt to better understand the etiology of middle ear disease in humans with cleft palate, a rhesus monkey model was developed. In this study, the model was extended to deal with the specific problem of otitis media in infants with cleft palate. Fifteen rhesus monkeys, *Macaca mulatta*, were utilized in the present investigation. Soft palate clefts were surgically produced in ten of the subjects, and five served as unoperated controls. Longitudinal data on middle ear status and craniofacial growth were collected until an approximate age of 2 years. Comparisons of linear regression coefficients representing size and rates of growth of craniofacial dimensions were made between operated and control group animals. No significant ($p < .05$) differences were found between the groups. It is concluded that surgically produced clefts of the soft palate alter middle ear function and produce ear disease but do not change the rates of growth of the measured components of the craniofacial complex.

Recently, in an attempt to better understand the etiology of middle ear disease (otitis media) in humans with cleft palate, a rhesus monkey model of cleft palate was developed (Doyle et al, 1980). Since otitis media is a nearly universal finding in infants with cleft palate (Paradise et al, 1969; Stool and Randall, 1967), our goal was to produce an animal model of this condition.

Previously, hypotheses offered concerning the mechanism by which palatal defects are related to middle ear disease centered around alterations of craniofacial growth (Subtelny, 1959) and subsequent abnormal musculoskeletal relations and function (Bluestone, 1971). However, to date no evidence has been offered which can support a hypothesis in which growth alteration is a necessary prerequisite for the production of middle ear disease in individuals with cleft. Such a hypothesis is tested with the data presented in the present study.

Although numerous studies have been conducted concerning the normal growth of the craniofacial complex in monkeys of the genus *Macaca* (Sirianni and Newell-Morris, 1980; Sirianni and VanNess, 1978; Tarrant, 1975, Elogyhen et al, 1972; Enlow, 1966; Pihl, 1959; Gans and Sarnat, 1951; Moore, 1949), there is much variability in technique, species, time period studied, and landmarks utilized. For these reasons, in the present study of craniofacial growth of the rhesus monkey (*Macaca*

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mulatta) with cleft palate, direct comparisons are made only to a series of normal (control) animals studied simultaneously with the same techniques and maintained under the same laboratory conditions in our facility.

Similarly, some other studies have investigated the growth consequences of surgically produced clefts of the hard palate in rhesus monkeys (Chierici et al, 1970). However, since our concern was with modeling the human cleft condition with respect to otitis media, we were interested in the minimally traumatic conditions sufficient to produce measurable changes in middle ear function or obvious middle ear pathology or both. The present study was, thus, limited to a consideration of clefts of the soft palate and was designed to test the hypothesis that the association of middle ear disease and cleft is the result of altered craniofacial growth.

MATERIALS AND METHODS

Fifteen (11 male and 4 female) rhesus monkeys *Macaca mulatta*, less than 6 months old were studied. They were observed from 7 to 12 weeks preoperatively, and data collection continued until an approximate age of 2 years. Baseline eustachian tube (ET) function and middle ear status were assessed, and then surgery was performed on the animals in the cleft group (N=10) at approximately[1] 6 months of age. The animals were sedated with ketamine hydrochloride 10 mg/kg IM, and an incision was made along the palatal midline from the uvula to the posterior border of the palatine bone. The midline *septum membranosus* was excised. Each month, lateral and frontal radiographs were taken of all animals, with the animals secured in a specially designed cephalostat. The animals were tranquilized with ketamine hydrochloride, 10 mg/kg, IM. A Norelco Oralix dental x-ray unit was used, at an exposure of 7 ma, 50 kv for 3 seconds. The tube-to-cassette distance was held constant at 152 cm (60 in). The radiographs were viewed on a light box, and the landmarks under study were assigned Cartesian coordinates through the use of a SAC GP6 microprocessor/digitizer and Olivetti P6060 minicomputer. Data storage and analysis were carried out by on-line transmission to a DEC PDP10 computer. The cranial landmarks: sella, nasion, vertex, maximum occipital point, gonion, menton, lower alveolar point, prosthion, posterior nasal spine, and basion were recorded for the lateral films (Fig. 1), and left and right zygomatic arch and left and right orbital margins were recorded on the frontal films. Point identification and data entry were carried out by a "blinded" orthodontist experienced in cephalometic tracing, with the films being randomly selected from the two study groups. Sixteen facial dimensions were calculated which defined cranial, facial, and mandibular dimensions of lengths and breadths, all measured to the nearest 0.01 cm. These included maximum bizygomatic breadth (MBYZIG), maximum biorbital breadth (MBIORB) (Fig. 2), basion-nasion (BASNAS), sella-nasion (SELNAS), maximum occipital point-nasion (MAXNAS), menton-nasion, (MENNAS), prosthion-nasion (PRONAS), posterior nasal spine-nasion (PNSNAS), posterior nasal spine-prosthion (PNSPRO), sella-prosthion (SELPRO), maximum occipital point-prosthion (MAXPRO), vertex-prosthion (VERPRO), basion-prosthion (BASPRO), vertex-basion (VERBAS), lower alveolar point-gonion (LAPGON), menton-gonion (MENGON). Means and standard deviations for these dimensions were calculated and compared between groups at two postoperative ages using Student's t test. As well, linear regression coefficients of length against the independent variable of age were calculated. The y intercept here, represents the size of a given dimension at birth, as calculated by regression analysis based on measures at known ages (i.e., from monthly x-ray films), and the slope represents the rate of growth of the dimension during the study period. Since it was determined that during the study period males and females exhibited no significant ($p < .05$) size or growth rate differences

[1]The exact age of these captive-bred animals is known and used in all computations of regression analysis.

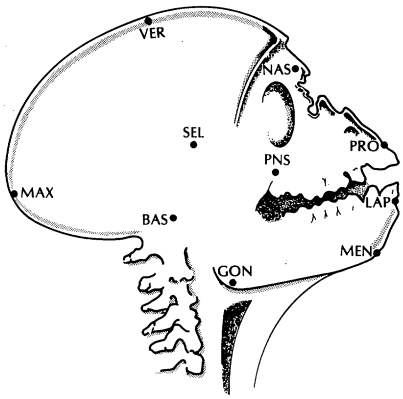

FIGURE 1. Cranial landmarks utilized from lateral x-ray films. VER = vertex, NAS = nasion, SEL = Sella, MAX = maximum occipital point, PNS = posterior nasal spine, PRO = prosthion, BAS = basion, LAP = lower alveolar point, MEN = menton, GON = gonion

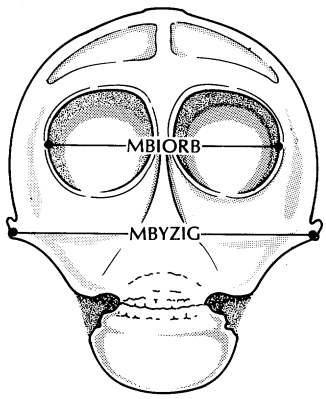

FIGURE 2. Dimensions calculated from frontal x-ray films. MBIORB = maximum biorbital breadth, MBYZIG = maximum bizygomatic breadth.

with regard to the dimensions under study[2], males and females were combined without regard to sex in each of the two study groups (cleft and control). This is consistent with growth data reported for *Macaca* during the first $2^1/_2$ years of life by Swindler and Sirianni (1973) and with data on hormones and tooth formation (Swindler et al, 1982).

Middle ear status in all animals was monitored throughout the 2-year period of data collection. A detailed description of the testing procedures as well as a description of the pathophysiology is available (Doyle et al, 1984). In the infant monkeys, the cleft induced a disease condition which was chronic in nature and of significant duration. This is congruent with the chronic otitis media with effusion which purportedly affects children with unrepaired clefts of the palate (Paradise et al, 1969). All of our sample continued to exhibit open clefts of the soft palate and associated impaired middle ear function during the entire study period.[3]

Results

Student's T test indicated that the mean initial dimensions were not significantly different ($p < .05$) between the groups based on the intercepts (Table 1), nor were they significantly different ($p < .05$) at 6 or 14 months postsurgery (Table 2). For the sixteen computed dimensions, slopes of the regression lines (growth rates) were not significantly different ($p < .05$) between the operated and unoperated groups (Table 1). The regression coefficients presented are the averages computed for all animals in a group for actual data collection ages over the entire study period. Since x-ray films were taken once a month for all animals, and all were not born on the same day of the month, means calculated at 6 and 14 months postsurgery represent a "box design", where the age of the animals in a given cell can vary by as much as thirty days.

Discussion and Conclusions

From the results presented, it is apparent that the animals were of approximately equal size with regard to the sixteen defined craniofacial dimensions at the onset of the study. For a period of 2 years

[2]Additionally, it was found that body weight did not differ significantly ($p < .05$) between sexes or between groups.

[3]Three animals underwent repeated (3 times) attempts at cleft repair, with all exhibiting a breakdown of the repair, thus assuring an open cleft. If anything, these procedures should have had an additive affect on growth retardation, but none was evidenced by our data.

TABLE 1. Mean Intercepts (initial size in cm) and Mean Slopes of Regression Lines (cm/day) (Growth Rates) of Craniofacial Dimensions

	Intercepts		*Slopes*	
	Control	*Operated*	*Control*	*Operated*
MBYZIG	5.4	5.4	.0032	.0032
MBIORB	4.3	4.3	.0015	.0016
BASNAS	5.0	5.0	.0012	.0014
SELNAS	3.5	3.3	.0009	.0013
MAXNAS	8.1	7.9	.0009	.0011
MENNAS	4.5	4.8	.0022	.0018
PRONAS	2.5	2.6	.0019	.0019
PNSNAS	3.0	2.8	.0014	.0018
PNSPRO	2.5	2.6	.0020	.0020
SELPRO	3.9	3.9	.0024	.0024
MAXPRO	8.5	8.6	.0028	.0024
VERPRO	6.3	6.5	.0024	.0021
BASPRO	4.7	4.8	.0025	.0023
VERBAS	5.2	5.2	.0005	.0008
LAPGON	3.4	3.5	.0031	.0030
MENGON	2.6	2.6	.0023	.0022

the rates of growth of these dimensions did not differ significantly between the cleft and control group animals. Although the surgical creation of a soft palate cleft resulted in middle ear disease and ET function abnormalities, it did not contribute to the production of any alteration of facial growth as measured. Looking at the sixteen chosen dimensions, two categories may be defined: those which would not be expected to change due to surgical clefting and those which might. The former group includes dimensions of facial width (MBYZIG and MBIORB), cranial height (VERBAS), and mandibular length (LAPGON and MENGON), while the latter group contains anterior vectors of the cranial base, face, or both. It is important to note that those expected to change exhibited no greater differences between groups than the dimensions expected to remain stable. One can conclude that in this animal model altered ET function is not a consequence of altered growth, and consequently, such altered growth is not a necessary prerequisite for altered ET function.

Functional matrix theory (Moss, 1968, 1971) predicts that alteration of nasopharyngeal function in growing subjects would be translated into changes in growth of the craniofacial complex. Radical changes in the palate (e.g., hard palate clefts) have been shown to alter growth, and septal resection in some animals has resulted in altered growth rates (Siegel, 1972; Siegel and Sadler, 1981). In the present study, the minimal surgery necessary to produce compromised ET function was carried out with no detectable effect on growth rates of the structures studied. The impaired middle ear function may have resulted from the changed pressure-volume rela-

TABLE 2. Means (and Standard Deviations) of Craniofacial Dimensions Reported at Two Postoperative Ages During the Study Period

	6 Months Post Surgery		*14 Months Post Surgery*	
	Control	*Operated*	*Control*	*Operated*
MBYZIG	6.7 (.11)	6.7 (.27)	7.3 (.18)	7.1 (.42)
MBIORB	4.9 (.11)	4.9 (.13)	5.2 (.06)	5.2 (.17)
BASNAS	5.6 (.24)	5.5 (.15)	5.8 (.42)	5.8 (.23)
SELNAS	3.9 (.13)	3.8 (.28)	4.2 (.31)	4.0 (.36)
MAXNAS	8.5 (.29)	8.4 (.16)	8.8 (.37)	8.5 (.21)
MENNAS	5.6 (.13)	5.5 (.10)	6.0 (.24)	5.9 (.22)
PRONAS	3.4 (.24)	3.4 (.10)	3.7 (.39)	3.7 (.21)
PNSNAS	3.7 (.12)	3.6 (.20)	3.9 (.17)	3.9 (.24)
PNSPRO	3.3 (.11)	3.4 (.23)	3.8 (.22)	3.7 (.34)
SELPRO	4.8 (.14)	4.8 (.20)	5.5 (.15)	5.2 (.37)
MAXPRO	9.6 (.19)	9.6 (.31)	10.3 (.33)	10.0 (.23)
VERPRO	7.2 (.26)	7.4 (.14)	7.7 (.31)	7.4 (.27)
BASPRO	5.6 (.17)	5.6 (.20)	6.3 (.21)	6.1 (.23)
VERBAS	5.4 (.18)	5.7 (.11)	5.5 (.20)	5.7 (.20)
LAPGON	4.7 (.16)	4.7 (.17)	5.3 (.17)	5.2 (.38)
MENGON	3.5 (.08)	3.4 (.18)	3.9 (.22)	3.8 (.29)

tions produced by the palatal defect. If any muscle function impairment resulted, it was not sufficient to produce significant changes in bone growth.

Further refinement of the present model to investigate changing ET function associated with soft-palate cleft repair will contribute to a better understanding of the conditions existing in a large proportion of the humans with cleft palate. Studies of individuals exhibiting abnormal growth must await a different type of experimental investigation.

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