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Effects of induced premaxillary suture fusion on the craniofacial morphology in growing rats

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ABSTRACT

Objective: Due to premaxillary rapid development and fusion with the maxilla at the fetus stage, the functions of the premaxillary suture still remain unclear. This study was designed to explore the effect of artificial induced premaxillary suture fusion on craniofacial morphology.

Methods: Thirty Sprague Dawley rats were divided into control and experimental groups, with 3 week, 5 week and 8 week subgroups of five animals each. An incision was made in each rat along the premaxillary suture and cyanoacrylate was administered to immobilize the exposed premaxillary suture for experimental rats. No glue was applied to controls. Weights, dental impressions and radiographs were taken before and after surgery until sacrifice and used to determine the differences between groups using the one-way ANOVA test.

Results: After immobilizing the premaxillary suture, significant changes in the craniofacial morphology were measured at the different time points. In the experimental groups, local changes occurred at the 3rd week. A global alteration in craniofacial morphology was apparent at the 8th week in the experimental group compared to the control. At each successive time point, craniofacial morphological alterations increased in rats with fused premaxillary sutures.

Conclusions: Induced premaxillary suture fusion can inhibit the growth of the premaxilla and cause extensive craniofacial morphological changes. These findings suggest that premaxillary suture fusion may be related to craniofacial malformation or malocclusion and to the formation of the flattened craniofacial profile in humans.

1. Introduction

In humans, the premaxilla is an important midfacial component derived from the premaxillary anlage and composed of an alveolar process, four upper incisors, the palatine plate of premaxilla and the *Processus Stenonianus*. The premaxillary bone development is related to the movement of the nasal septum during the fusion of the secondary palate.[1] In humans, the premaxillary suture (PMS) fuses around birth. Only the labial side of PMS fuses while the other sides remain patent much later, such as fusing with the vomer at the age of 15.[1] Thus, some authors even suggest class III malocclusion can be treated by pushing this bone block forward.[2]

Due to its rapid development and fusion with the maxilla at the fetus stage, the functions of the PMS still remain unclear. PMS is an essential growth site for the adjacent bones[3] and it

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has been speculated that PMS function is to affect deciduous incisor size and position.[4,5] Rudimentary premaxillary bones are generally found to be combined with other defects. In fetuses with holoprosencephaly, the premaxilla is often absent or reduced.[6,7] In bilateral cleft lip and palate patients, the premaxilla floats and moves forward due to premaxillo-vomerine suture growth without restraint from the premaxillomaxillary suture.[8]

From published documents, the fusion period of the PMS may be related to craniofacial morphology. PMS from individuals of European ancestry fuse earlier than those of African-American ancestry and produced a more prominent anterior nasal spine.[9] In addition, the fused PMS differs between modern and ancient peoples. Maureille and Bar found Neandertal children had a persistence of the PMS at a young age in comparison to extant chidren. Furthermore, they found synostosis of the PMS was later in Neandertal children than in modern ones. Persistence of an open PMS represents the potential for an extended period of growth in the midface of Neandertals.[10]

Many studies have shown that premature synostosis of cranial sutures affects craniofacial and mandibular morphology.[11–17] One study found that unilateral artificial synostosis of the frontonasal and frontopremaxillary sutures in rats could make the snout bend towards the treated side and rotate around the sagittal plane.[18] But so far, no evidence related to PMS fusion and its effect on the craniofacial morphology has been published. Therefore, the hypothesis is proposed that the fusion of PMS can affect craniofacial morphology and may be related to the unique facial profile in humans.

2. Materials and methods

Thirty female Sprague Dawley rats aged 3 weeks (Harlan, Indianapolis, IN) were used in this study. The animals were divided into two groups, an experimental and a control, of 15 rats each. The groups were further separated into three subgroups ($n = 5$) according to different time periods, 3 weeks, 5 weeks and 8 weeks. An animal protocol was reviewed and approved by the Committee for Care and Use of Laboratory Animals at the Medical College of Georgia.

Before surgery, all animals received intramuscular injections of an anesthetic cocktail with ketamine hydrochloride (100 mg/mL), xylazine (20 mg/mL) and acepromazine (10 mg/mL) at a dosage of 0.4–0.6 mL/kg.

All rats received a 1–2 cm incision along the left PMS. A scalpel was used to abrade the periosteum adjacent to PMS. For experimental groups, methyl cyanoacrylate (Barristo, Chicago, IL) was applied to the exposed suture surface. Zap It accelerator (Dental Ventures of America, Corona, CA) was used to shorten polymerization time. For both groups, incision closure was obtained with a simple interrupted suture of 5-0 nylon and removed 1 week later.

Weight, dental impressions and radiographs were taken pre-operatively and post-operatively weekly for both groups under general anesthesia. Superfast Regisil Rigid polyvinylsiloxane (Dentsply, Milford, DE) was used to take dental impressions of each rat maxilla. A Faxitron Series 43807N soft X-ray system (Hewlett-Packard, Palo Alto, CA) and Kodak Insight dental film were used to take lateral and dorsoventral cephalograms. Soft X-rays were taken for 5 s at 2.0 mA and Kvp of 45. The head of each animal was fixed with a custom-made frame oriented vertically to the median sagittal plane.

2.1. *Preparation of sections and histology*

Under deep anesthesia, rats were perfused transcardially with 100 mL of 10% formalin solution until death. The soft tissue was removed from the skull and each sample resulted in a sagittal hemi-maxillae. Specimens were sectioned using a low-speed saw (Isomet, Buehler Ltd., Lake Buff, IL) under constant water irrigation and were demineralized for 6 weeks in 0.1 M EDTA and 0.1 M NaOH. The samples were processed through gradients of ethanol and xylene before paraffin embedding.

Serial sections of 5 μm thickness were cut with a Microm rotary microtome (Walldorf, Germany) in the sagittal plane. Sections were mounted on slides and heat-fixed overnight at 50 °C and processed for routine hematoxylin–eosin staining.

2.2. *Morphometric analysis*

Radiographs and impressions were scanned into a computer for measurement. Each impression was scanned with its occlusal plane oriented parallel to the horizon. Adobe Photoshop CS software was used to denote landmarks on radiographs and the UTHSCSA Image Tool software (Dental Diagnostic Science, San Antonio, TX) was used to measure 23 linear (Fig. 1) and 5 angular (Fig. 2) parameters. Radiographs were calibrated with a custom-made 10 mm standard marker, while linear impression measurements were calibrated with a ruler. The means and standard deviations of each measurement were calculated.

2.3. *Statistical analyses*

The measurements were recorded and the one-way ANOVA test was used to determine the differences between the control and experimental groups (for the comparison of the treated and untreated dental arch length in the experimental groups, paired t test was used). Statistical significance was defined as 5%.

3. Results

The body weight continuously increased without any significant difference between control and experimental groups, which indicated no substantial influences of surgical and experimental procedure on the overall growth and development of the animals.

The gross morphological changes are shown in Fig. 3. The snout deviated toward the treated side in the experimental groups from the 3rd week until to the end of the experiment while the snout of the controls displayed no deviation.

3.1. *Craniofacial length*

Fifteen craniofacial length measurements from the lateral radiographs are identified in Table 1. At the 3rd week, the