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Cleft Lip and Palate Associated with Other Malformations in a Neotropical Primate (*Saimiri ustus*)

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Cleft lip (with or without cleft palate) has been documented in several species of nonhuman primates, which in general are susceptible at similar doses and stages of gestation to the same teratogens as humans. Cleft lip can be unilateral or bilateral, isolated, syndromic, familial, or genetic. Here we report the first case of syndromic cleft lip and palate in a male bare-eared squirrel monkey (*Saimiri ustus*). Associated with the orofacial clefts, the monkey manifested absence of bones, malformation of vertebrae L3, only 4 fingers in each hand, and shortening of tendons leading to inflection of the hands and fingers. Previous reports describing cleft lip and palate in other squirrel monkeys (*Saimiri sciureus*) in other breeding units have suggested consanguineous mating as a possible cause. Although the etiology in the case we present is unknown, we discuss factors associated with orofacial clefts in humans and various nonhuman primates.

Development of the head and face comprises one of the most complex events during embryonic development, coordinated by a network of transcription factors and signaling molecules associated with proteins conferring cell polarity and cell-to-cell interactions. Disturbance of this tightly controlled cascade can result in a facial cleft, where the facial primordia ultimately fail to meet and fuse or form the appropriate structures. Collectively, craniofacial abnormalities are among the most common features of all birth defects in humans. The most frequent features are the orofacial clefts, cleft lip and cleft palate.[24] In humans, approximately 50% of patients with cleft lip also have cleft palate, which is thought to be a secondary effect resulting from the defect in facial prominence fusion that precedes palate formation. Cleft palate in isolation of other malformations therefore is considered to be etiologically distinct from cleft palate with cleft lip. Most (70%) cases of cleft lip and palate are regarded as nonsyndromic, where clefts occur without other anomalies. The remaining syndromic cases have additional features that can be subdivided into categories of chromosomal abnormalities (recognized as Mendelian single-gene syndromes), teratogenic effects, and several unknown syndromes. The high familial aggregation rates, recurrence risks, and increased concordance rates in monozygous versus dizygous twins provide evidence of a strong genetic component in cleft lip and palate.[17]

In general, both genes and environmental factors, acting either independently or in combination, are thought to be responsible for facial clefts. Orofacial clefts are among the most frequent congenital anomalies in nonhuman primates and have occurred as isolated birth defects[10,11,14,22,28,29] and as components of both syndromic[21] and nonsyndromic patterns of malformations.[3,13,25] Cleft lip and palate occurs more frequently in males, whereas the sex bias is reversed for isolated cleft palate, which is more common in females.[8] In nonsurviving *Saimiri* fetuses, the occurrence of cleft lip with or without cleft palate was more common in male than in female monkeys.[2]

Morphologic studies of nonhuman primates can provide valuable models to understanding human disorders, in addition to being important in their own right. Squirrel monkeys (Cebidae of the genus *Saimiri*) are the neotropical primates most commonly used in biomedical research. *Saimiri* monkeys comprise a complex of species and subspecies with broad morphologic plasticity.[15] Genetic studies[4,6,30] support Hershkovitz's[9] taxonomy, which proposes one Central American species (*S. oerstedii*) and 3 South American species (*S. sciureus*, *S. boliviensis*, and *S. ustus*). A new South American species, *S. vanzoline*, was described in 1985.[1] The various species of *Saimiri* share a chromosome number of 2N = 44. However, the number of chromosome arms (fundamental number) of their karyotypes differs among *Saimiri* spp. owing to pericentric inversions.[18,20] The phenotypes are similar, leading to problems in establishing pure, single-species colonies.

Here we describe for the first time spontaneous cleft lip and palate, associated with other embryonic malformations, in *Saimiri ustus*.

Case Report

The squirrel monkey (*Saimiri ustus*) reported here belongs to the Center for Laboratory Animal Breeding (Cecal, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil), where it was kept in a social group. This colony started in 1989 with 57 wild-caught animals from Rondonia, Northern Brazil. The animals are housed in groups of 8 to 12 individuals per cage, comprising a single male, 6 females, and their respective offspring. The monkeys are maintained in cages made from concrete and metal bars that received anticorrosive treatment. All cages are covered with domed fiberglass roofs so that the animals can benefit from natural light. The cages are arranged side by side and in 2 rows and are furnished with climbing structures and perches, stainless steel feeders, and waterers. The cage floor is made of rough concrete

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and covered with autoclaved wood shavings. The monkeys are fed a commercial primate food (Monkey Chow 6030, Nuvital, Colombo, Brazil) supplemented with fruits and vegetables: bananas, oranges, pineapples, pumpkins, carrots, eggplants, apples, papayas, corn, pimientos, beets, French beans, tinamou quail eggs, and insect larvae. They also receive a solution of 10% sugar-cane syrup offered ad libitum. All cages are washed with water with no disinfectant solution. This breeding colony is maintained in compliance with Brazilian law, registered in the Brazilian Institute of the Environment and Renewable Natural Resources and cleared by the Ethics Commission for the Use of Animals of the Oswaldo Cruz Foundation. The facility also follows published regulations for the use of laboratory animals.[12]

The male squirrel monkey presented here was born with several malformations including bilateral cleft lip and palate. The gestation was unremarkable and full-term; no drugs were administered during gestation. However delivery was excessively long due to dystocia; the dam tried to remove the fetus and then cannibalized most of its lower body. The dam was preanesthetized with ketamine (10 mg/kg), midazolam (0.1 mg/kg), and atropine (0.02 mg/kg) for cesarean section to remove the remainder of the fetus. To allow intubation, lidocaine was sprayed directly in the larynx, and then propofol (2.5 mg/kg IV) was administered. Isoflurane gas was used to provide surgical anesthesia. For analgesia, tramadol chlorhydrate (3 mg/kg) was used for 3 d after surgery.

According to colony records, the parents of the affected fetus were not related. The sire was 7 y old; the dam 10 y of age, and both parents had normal karyotypes. The dam had had a single stillborn fetus prior to the offspring presented here. Because this progeny had been dead for several hours, chromosome analysis was not performed. Due to the condition of the fetus, its sex was identified by PCR of *SRY* (a Y-chromosomal gene) sequences by using the SW2 forward primer (5′ CTT GAG AAT GAA TAC ATT GTC AGG G 3′)[31] and a reverse primer (5′ CGG TAA AAA GGA GAG TCT GCG TAG 3′) to amplify an approximately 900 bp fragment.[19] Genomic DNA was isolated from liver by using ammonium acetate precipitation;[7] DNA extracted from the blood of male and female *Saimiri ustus* adults were used as positive controls. Each PCR reaction contained 1.0 or 1.25 U *Taq* DNA polymerase, 0.25 µM of each dNTP, 50 pmol of each primer, 1.5 mM $MgCl_2$, and 1× reaction buffer in a final volume of 50 µL. After initial denaturation for 5 min at 94 °C, amplifications proceeded with 30 cycles of 94 °C for 1 min, 50 to 55 °C for1 min, and 72 °C for 1 min. PCR products were separated in a 0.8% agarose gel containing ethidium bromide (10 mg/mL) and visualized on a UV transilluminator. This molecular sexing analysis indicated that the fetus was male.

Gross necropsy of the presented animal revealed no abnormalities of the heart, lung, kidneys, liver, trachea, esophagus, brain, stomach, or spleen with no abnormalities; due to partial cannibalization of the fetus, the intestines, bladder, and genitalia were not available for evaluation. Bilateral cleft lip and palate were discovered at gross necropsy (Figure 1). In addition, bilateral radial aplasia, malformation of vertebra L3, and only 4 fingers on each hand with bilateral absence of thumbs (Figure 2) were present radiographically. In the absence of the radii, the hands were contracted toward the radial faces of the forearms.[23] The ears of the fetus were low in position (Figure 3). No histologic evaluation was performed.

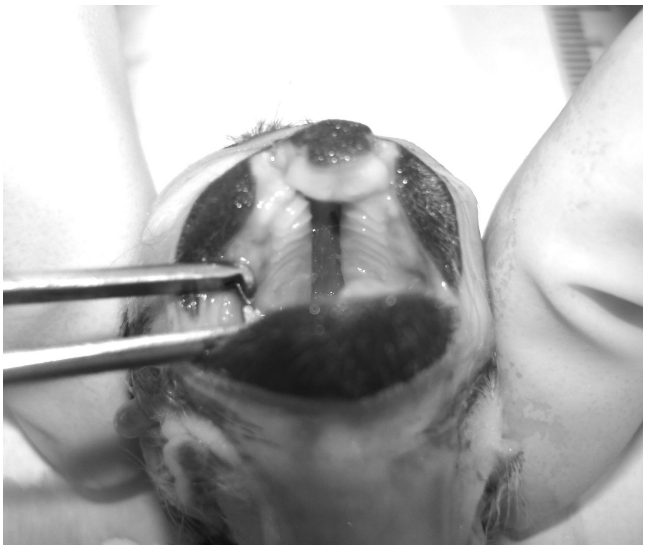

Figure 1. Bilateral cleft lip and cleft palate in a squirrel monkey.

Discussion

As with all clinically recognizable congenital syndromes, cases of syndromic cleft lip and palate can be subdivided into 4 categories: 1) part of a characterized Mendelian disorder (single gene defect), 2) due to structural abnormalities of chromosomes, 3) parts of syndromes associated with teratogens, and 4) those whose etiology remains obscure. Among 3110 human cases of orofacial clefts, 653 were associated with multiple congenital abnormalities[22,27] Of these, 60 (9.2%) had a known etiology (monogenic, 25 [3.8%]; chromosomal, 31 [4.7%]; teratogenic, 4 [0.6%]). Furthermore 70.8% of cases of cleft lip and palate occurred as isolated anomalies whereas 29.2% were associated with other defects, such as multiple congenital anomalies.[5]

The monkey we describe presented with cleft lip and palate in association with bone anomalies. In humans, associated malformations were more frequent in infants who had both cleft lip and palate (28%) than in those with isolated cleft palate (22%) or cleft lip (8%). Malformations of the upper or lower limbs or vertebral column were the most common additional anomalies and accounted for 33% of all associated defects.[16] In the case of cleft lip and palate we present here, the affected squirrel monkey was male; cleft lip and palate has previously been reported to show male predominance in nonhuman primates[8] and humans.[2]

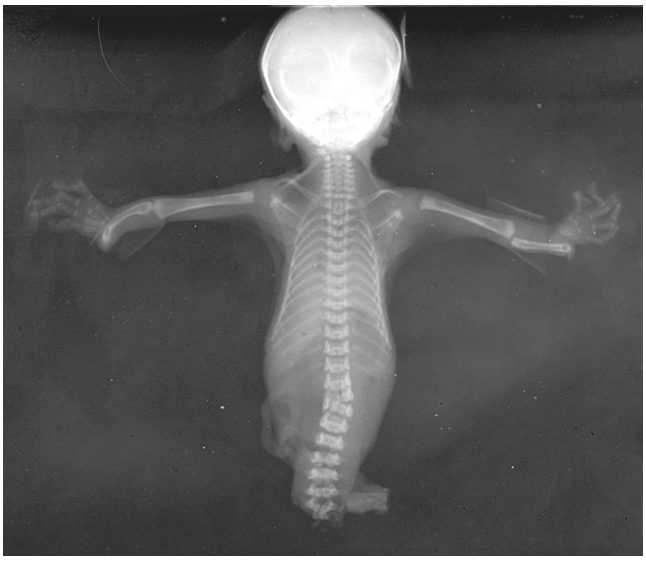

Figure 2. Absence of the 2 radii, malformation of vertebra L3, and only 4 fingers on each hand, with absence of the thumbs.

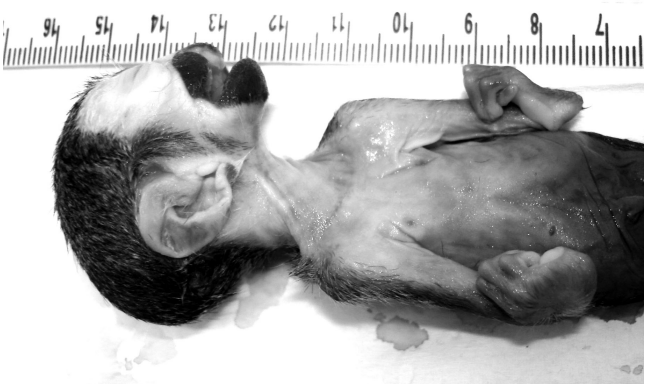

Figure 3. In the absence of radii, the hands and fingers contracted toward the radial face of the forearm. The ears were low in position.

Although we could not perform chromosomal evaluation of our monkey, the case bears similarities to those of human trisomy 18, with features of radial aplasia, vertebral anomalies, thumb hypoplasia or aplasia, and low-set ears, although radial aplasia and vertebral anomalies have been documented to occur in only a few cases.[28]

Studies in *Saimiri sciureus* have indicated a high incidence of cleft lip or palate (or both) related to consanguineous matings.[2,11,26] Although our animal demonstrated vertebral malformation as have other squirrel monkeys,[26] inbreeding likely is not a key factor in our animal's malformations, because we use rotation for unrelated breeding, and at our institution, the offspring generally are not available for breeding because they are used experimentally. Factors pointing toward a possible genetic component to the etiology of the present case include the dam's history of a previous underweight stillbirth and the absence of other factors such as inadequate housing conditions and advanced age of the dam. An understanding of the etiology of malformations in nonhuman primates will require detailed cytogenetic analyses, information on the extent of genetic variability in the colony, and efforts to identify environmental teratogens. One important factor in nonhuman primate teratology studies is the background incidence of congenital anomalies in the colony. Accurate interpretation of experimental data is fundamental because safety assessment studies generally use a minimal number of nonhuman primates. In addition, long-term monitoring of spontaneous occurrences of orofacial anomalies is also important for recognizing trends and identifying potential patterns of defects. This information is useful not only in managing a healthy nonhuman primate colony but also in evaluating the incidences of malformations in experimental studies.

In summary, here we describe the first incidence of cleft lip and palate in *S. ustus*. Further information is needed to compare the etiology, incidence, and hallmarks of cleft lip and palate between *S. sciureus* and humans, although features of our case suggest similarities between nonhuman primates and humans. Additional studies are necessary to obtain a more accurate assessment of cleft lip and palate in *Saimiri ustus*.

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References

1. **Ayres JM.** 1985. On a new species of squirrel monkey, genus *Saimiri*, from Brazilian Amazonia (Primates, Cebidae). Papéis Avulsos Zoologia **36:**147–164.
2. **Baker CA, Hendrickx AG, Cooper RW.** 1977. Spontaneous malformations in squirrel monkey (*Saimiri sciureus*) fetuses with emphasis on cleft lip and palate. J Med Primatol **6:**13–22.
3. **Benirschke K, Kumamoto AT, Bogart MH.** 1981. Congenital anomalies in *Lemur variegatus.* J Med Primatol **10:**38–45.
4. **Boinski S, Cropp SJ.** 1999. Disparate data sets resolve squirrel monkey (*Saimiri*) taxonomy: implications for behavioral ecology and biomedical usage. Int J Primatol **20:**237–256.
5. **Calzolari E, Pierini A, Astolfi G, Bianchi F, Neville AJ, Riviera F.** 2007. Associated anomalies in multimalformed infants with cleft lip and palate: an epidemiologic study of nearly 6 million births in 23 EUROCRAT registeries. Am J Med Genet **143:**528–537.
6. **Cropp S, Boinski S.** 2000. The Central American squirrel monkey (*Saimiri oerstedii*): introduced hybrid or endemic species? Mol Phylogenet Evol **16:**350–365.
7. **FitzSimmons NN, Moritz C, Moore SS.** 1995. Conservation and dynamics of microsattelite loci over 300 million years of marine turtle evolution. Mol Biol Evol **12:**432–440.
8. **Fraser FC.** 1970. The genetics of cleft lip and cleft palate. Am J Hum Genet **22:**336–352.
9. **Hershkovitz P.** 1984. Taxonomy of squirrel monkey genus *Saimiri* (Cebidae, Platyrrhini): a preliminary report with description of a hitherto unnamed form. Am J Primatol **7:**155–210.
10. **Hill WCO, Sabater PJ.** 1970. Notes on 2 anomalies in mandrills (*Mandrillus sphinx* Linn). Folia Primatol (Basel) **12:**290–295.
11. **Hoopes CW, Jerome CP.** 1987. Oral–facial clefts and associated malformations in the squirrel monkey (*Saimri sciureus*). J Med Primatol **16:**203–209.
12. **Institute for Laboratory Animal Research**. 1996. Guide for care and use of laboratory animals. Washington (DC): National Academies Press.
13. **Koford CB, Farber PA, Windle WF.** 1966. Twins and teratisms in rhesus monkeys. Folia Primatol (Basel) **4:**221–226.
14. **Kraus BS, Garrett WS.** 1968. Cleft palate in a marmoset: report of a case. Cleft Palate J **5:**340–345.
15. **Lavergne A, Catzeflis F, Lacote S, Barnaud A, Bordier M, Mercereau-Puijalon O, Contamin H.** 2003. Genetic analysis of the *Saimiri* breeding colony of the Pasteur Institute (French Guiana). J Med Primatol **32:**330–340.
16. **Milerad J, Larson O, Hagberg C, Ideberg M.** 1997. Associated malformations in infants with cleft lip and palate: a prospective, population-based study. Pediatrics **100:**180–186.
17. **Mitchell LE, Risch N.** 1992. Mode of inheritance of nonsyndromic cleft lip with or without cleft palate: a reanalysis. Am J Hum Genet **51:**323–332.
18. **Moore CM, Harris CP, Abel CR.** 1990. Distribution of chromosomal polymorphism in 3 subspecies of squirrel monkey (genus *Saimiri*). Cytogenet Cell Genet **53:**118–122.
19. **Moreira MAM.** 2002. *SRY* evolution in Cebidae (Platyrrhini: Primates). J Mol Evol **55:**92–103.
20. **Mudry MD, Ponsa M, Borrel A, Gorostiaga M, Garcia M.** 1995. La citogenética en la Taxonomia de los Platirrinos con especial enfasis en cébidos de Argentina y países limítrofes. Marmosiana **1:**79–86.
21. **Pugsley SL.** 1985. Congenital malformations in a common marmoset (*Callithrix jacchus*) similar to human 13-trisomy syndrome. Lab Anim **19:**123–124.
22. **Sarkozi A, Wyszynski DF, Czeizel AE.** 2005. Oral clefts with associated anomalies: findings in Hungarian congenital abnormality registry. BMC Oral Health **5:**4.
23. **Schuler AM, Gibson SV, Brady AG, Abee CR, Scammell JG.** 2007. Congenital radial and thumb aplasia in a neonatal owl monkey (*Aotus nancymaae*). J Am Assoc Lab Anim Sci **46:**72–74.
24. **Schultz AH.** 1956. The occurrence and frequency of pathological and teratological conditions and of twinning among nonhuman primates. Primatologia **1:**965–1014.
25. **Stanier P, Moore GE.** 2004. Genetics of cleft lip and palate: syndromic genes contribute to the incidence of nonsyndromic clefts. Hum Mol Genet **13:**R73–R81.

26. **Stills HF Jr, Bullock BC.** 1981. Congenital defects of squirrel monkey (*Saimiri sciureus*). Vet Pathol **18:**29–36.
27. **Stoll C, Rosanob A, Botto LD, Erickson D, Khoury MJ, Olney RS, Castilla EE, Cocchi G, Cornel MC, Goujard J, Bermejo E, Merlob P, Mutchinick O, Ritvanen A, Zampino G, Mastroiacovo P.** 2001. On the symmetry of limb deficiencies among children with multiple congenital anomalies. Ann Genet **44:**19–24.
28. **Sugayama SMM, Kim CA, Utagawa CY, Albano LMJ, Bertola DM, Koiffmann CP, Gonzalez CH.** 1999. Estudo genéticoclínico de 24 pacientes com trissomia 18 (síndrome de Edwards). Pediatria **21:**133–143.
29. **Swindler DR, Merrill OM.** 1971. Spontaneous cleft lip and palate in a living nonhuman primate, *Macaca mulatta.* Am J Phys Anthropol **34:**435–439.
30. **Vandeberg JL, Williams-Blangero S, Moore C, Cheng M, Abee C.** 1990. Genetic relationships among 3 squirrel monkey types: implications for taxonomy, biomedical research, and captive breeding. Am J Primatol **22:**101–111.
31. **Whitfield LS, Lovell-Badge R, Goodfellow PN.** 1993. Rapid sequence evolution of the sex-determining gene *SRY*. Nature **364:**713–715.