Published in final edited form as:

Orthod Craniofac Res. 2019 May ; 22(Suppl 1): 82–89. doi:10.1111/ocr.12276.

Mechanobiology of bone and suture – Results from a pig model

Katherine L. Rafferty¹, Michael C. Baldwin², Shean Han Soh¹, Susan W. Herring^{1,2}

¹Department of Orthodontics, University of Washington, Seattle, Washington

²Department of Oral Health Sciences, University of Washington, Seattle, Washington

Abstract

Objective: To compare the morphology and mechanical function of sutures in normal pigs and minipigs to those of Yucatan minipigs, a natural model for midfacial hypoplasia.

Setting and Sample Population: Research took place at the Department of Orthodontics at the University of Washington and used varying sample sizes of normal-snouted pigs and Yucatan minipigs.

Material and Methods: Skulls and heads were examined for morphology of the nasofrontal suture using computed tomography and histology. Strain gauge recordings were made of sutural strain during mastication and during cyclic tensile loading of the nasofrontal suture.

Results: Sutures in Yucatan pigs had narrower gaps than same-age normal pigs. The nasofrontal suture was simpler in construction and had more active osteoblasts on the bone fronts in Yucatan pigs. The sutural ligament was less well organized, and based on a small sample, masticatory strain appeared to be lower than in normal minipigs. However, sutures were not fused and showed similar strains in response to the cyclic loading procedure.

Conclusion: Midfacial hypoplasia in Yucatan pigs has the likely proximate cause of hyperossification. Yet prior to fusion, the sutures appear to be amenable to treatment that would promote their growth rate.

Keywords

biomechanics; mastication; midfacial hypoplasia; miniature swine; sutures

1 | INTRODUCTION

The fusion or patency of cranial sutures can be thought of as a competition between the inherently osteogenic surfaces of the articulating bones vs all the factors that tend to keep the bone fronts apart. Unlike the superficially similar periodontal ligament,¹ sutures do not seem to have innate molecular mechanisms that retard mineralization and inhibit fusion. Regional variation in TGF signalling from the dura mater governs the early post-natal fate of the dorsal midline sutures in rats,^{2,3} but a similar influence has not been found for other

Correspondence: Susan W. Herring, Department of Orthodontics, University of Washington, Seattle, WA 98195-7446, USA. herring@uw.edu.

Present Address: Shean Han Soh, Discipline of Orthodontics and Pediatric Dentistry, National University of Singapore, Singapore

sutures, and as there is no dura mater in the face, it is not clear that any such mechanism exists for facial sutures or even for other cranial sutures. In any case, rodents are poor mimics of human sutural biology in that no suture fuses except the posterior interfrontal, osseous remodelling is rare,⁴ and small size makes physiologic recording difficult.

Humans and other large mammals show progressive ossification of most sutures with earlier fusion associated with mechanically related phenomena such as size, muscularity and male gender.^{5,6} Genetic variants that contribute to bone density or thickness are involved as well.⁷ On the other side of the competition, tensile strain at the suture physically prevents fusion by separating the bone fronts. At the same time, the tension in the sutural ligament encourages osteogenesis,⁸ leading to the desirable result of elongated bones as well as patent sutures. This is the principle behind the success of distraction or spring treatments for craniosynostosis.

The pig, *Sus scrofa*, was the subject of classic work on sutural vs appositional growth of the braincase.^{9,10} The feeding apparatus of pigs is human-like,¹¹ and the mechanical strains produced at sutures by natural mastication are known.⁸ In contrast to wild pigs, many domestic pigs have edge-to-edge incisor occlusion or a slight under-bite, a trait which is exaggerated to the point of midfacial hypoplasia in the Yucatan minipig, a popular laboratory breed, and reaches extremes in the Yucatan micropig (Figure 1). The cause of midfacial hypoplasia in these pigs is unknown, but the sutures are affected at least secondarily. These commercially available animals provide a natural model to study the morphology and mechanical behaviour of poorly growing sutures. The present investigation compares these features in Yucatan minipigs and normal-snouted pigs, focusing on the nasofrontal suture.

2 | METHODS

The investigation concentrated on 4- to 5-month-old animals, a rapidly growing age with a complete deciduous dentition. All procedures using live animals were reviewed and approved by the University of Washington Institutional Animal Use and Care Committee under protocols 2331–20 and 2331–21. Table 1 summarizes the animals used for each procedure.

2.1 | Morphology

Skulls of 4-month-old Yucatans of both sexes (n = 11) were compared with those of same-age normal Hanford minipigs (n = 11) for indications of sutural morphology and fusion. Medical CT scans were taken of 10-month-old skulls of one normal and one Yucatan micropig (no Yucatan minipig skulls of this age were available). Intact heads of 4- or 5-month-old normal domestic pigs (n = 3) and Yucatan minipigs (n = 5) were scanned at high resolution (NSI X5000).

Haematoxylin and eosin parasagittal sections of the nasofrontal suture were available for normal minipigs from previous studies (n = 4)¹² and for sham animals (1 normal domestic pig, 4 Yucatan minipigs) from a cyclic loading trial (see below). Undecalcified sections of the normal and 2–3 Yucatan shams were measured for the width of the nasofrontal and

coronal suture gaps.¹³ All available specimens were 4–5 months of age; both males and females were included.

2.2 | Masticatory strain

Suture strain during mastication is known for normal minipigs ($n = 11$).^{12,14} Masticatory strain was assessed in two 5-month female Yucatan minipigs which were concurrently used in a study of masseter botulinum toxin. This treatment produces only negligible effects in pigs.¹⁵ Under anaesthesia, single-element strain gauges were placed perpendicular to the nasofrontal and internasal sutures.¹² After awakening, masticatory strains were recorded for 10–15 min.

2.3 | Cyclic loading

The cyclic loading trial included a previous pilot study using normal domestic pigs¹³ and an ongoing project using Yucatan minipigs. The project will assess whether short-duration cyclic loading is effective in enhancing sutural growth.^{16,17} The pilot study developed a portable loading apparatus and established that the technique was successful in normal pigs.¹³ The ongoing project tests whether the poorly growing sutures of Yucatan minipigs can respond. Loading posts are surgically installed on the right frontal and nasal bones. After healing, daily tensile loading (3.0 Hz, 30 min) under anaesthesia is carried out for 5 days. Before loading on the final day, strain gauges are affixed over nasofrontal, coronal, interfrontal and internasal sutures. Sham animals receive the loading posts and daily anaesthesia with the apparatus engaged but not loaded, so there is no strain.

3 | RESULTS

3.1 | Morphology

Yucatan minipig skulls differed from those of same-age normal pigs in that the nasal, lacrimal and maxillary bones were much shorter. Premolars were crowded into a rotated position. Most sutures appeared narrow even when clearly patent. A few normal but most Yucatan skulls showed initial fusion of midline sutures (Table 2). The nasofrontal and coronal sutures appeared less interdigitated in Yucatan.

Parasagittal CT scans of 4- to 5-month-old heads showed that the nasofrontal suture of all pigs had interdigitations at least in some areas and was beveled with the nasal bone overriding the frontal. Oblique sections parallel to the plane of the snout dorsum (here-after ‘tangential’ sections) showed that Yucatan minipig nasofrontal sutures had fewer, shallower and less regular interdigitations than normal nasofrontal sutures. Especially in the Yucatan, the frontal sinuses were large and impinged on the suture. CT scans of the 10-month-old skulls showed an open, complex nasofrontal suture in a normal minipig but a simpler and medially fused nasofrontal suture in the Yucatan minipig (Figures 1 and 2).

Histological examination confirmed the simpler but less regular structure of Yucatan minipig nasofrontal sutures. The sutural ligament was generally less organized in interdigitated regions. Both normal and Yucatan sutures featured regions of suture widening filled with dense fibrous tissue, but these were more prominent in Yucatan because of the diminished

interdigitation. In addition, the bone fronts of Yucatans seemed more richly furnished with osteoblasts than those of normal pigs (Figure 3).

Measurements of the nasofrontal suture gap produced a width of 238 μm in the normal sham pig¹³ and an average of 180 μm (SD = 14) in Yucatan shams (n = 3, $P < 0.02$, one sample t test). The coronal suture gap in the normal sham was 146 μm ¹³ but averaged 72 μm (SD = 9, $P = 0.055$) in the Yucatan shams (n = 2).

3.2 | Masticatory strain

Although not completely out of the range of normal pigs, nasofrontal and internasal suture strains were very low in the two Yucatans tested (Table 2), and one of the animals showed tension at the internasal suture, in contrast to the compression typically observed.

3.3 | Cyclic loading

Cyclic tensile loading of the right nasofrontal suture produced a strain pattern very different from that of mastication regardless of breed. However, differences between normal and Yucatan pigs were minimal (Table 3). Tensile strains produced in the targeted (right) and opposite (left) nasofrontal suture were insignificantly larger in the Yucatans, and compression in the right coronal suture was insignificantly smaller. The absolute value of strain in the left coronal suture also tended to be smaller in Yucatans, but polarity of strain (compression vs tension) varied in both breeds. The internasal suture of Yucatans yielded relatively strong tension, in contrast to the inconsistent and smaller strains in normal pigs. The opposite pattern was seen in the interfrontal suture, where Yucatans showed smaller strains of inconsistent polarity and normal pigs had larger and consistently tensile strains.

4 | DISCUSSION

The midfacial hypoplasia of Yucatan minipigs is not obvious from the outside, because the anterior crossbite is obscured by the projecting nasal disc of pigs. Nevertheless, the typical Yucatan negative overjet of 10–12 mm has been noticed and exploited for studies of maxillary distraction.¹⁸ Our preliminary observations suggest that early ethmoidal ossification of the posterior cartilaginous nasal septum may occur in Yucatans, and this in itself could hamper snout elongation. However, early endochondral ossification might itself be a manifestation of a systemically heightened osteogenicity, which could directly affect all sutures.

Yucatan sutures are narrower than normal, as indicated by both their external appearance and by the available measurements on nasofrontal (30% narrower) and coronal (50% narrower) sutures. Interdigitations of the nasofrontal suture are reduced and coarser in Yucatans compared to normal pigs. Collagen fibres, although plentiful, seldom showed a clear orientation, whereas a compression-resisting orientation was often seen in normal nasofrontal sutures. The abutting nasal and frontal sutural bone fronts had strikingly large numbers of presumed osteoblasts compared to normal pigs of the same age. Although fusion was not seen in any suture, the combination of a narrow gap and heightened osteogenesis in Yucatan nasofrontal sutures would certainly enhance the likelihood of early synostosis.

These findings suggest that midfacial hypoplasia in Yucatan pigs involves both hyperostosis, accounting for the tight sutures and osteogenic bone fronts, and diminished growth of the nasal septum, accounting for failure to elongate the snout even in the absence of suture fusion.

The narrow sutural gap in Yucatans may also explain the low strains recorded at the nasofrontal and internasal sutures during mastication, as there is less space for bone movement. In addition, the short snout of these animals probably changes midfacial biomechanics by reducing the moment arm of the anterior bite force and hence reducing the tendency to shear the rostrum dorsally and to compress the internasal suture.¹⁹ Less consistent masticatory loading might also account for the lack of clear orientation of the sutural ligament in Yucatan nasofrontal sutures. Altered biomechanics due to skull proportions are also a likely explanation of the minor differences observed at non-targeted sutures during the cyclic loading trial. Yucatans tended to have higher facial suture (internasal) strain and lower cranial suture (coronal, interfrontal) strain than normal pigs, probably a consequence of their small face to braincase length ratio.

Even though abnormal in structure and function, the mechanical response to cyclic loading in the Yucatan nasofrontal sutures was at least as great as that of normal sutures. The explanation for this similarity in sutural deformation probably lies in the fact that sutural strain occurs almost entirely at the sutural gap, which has a much lower Young's modulus than the neighbouring bone.²⁰ Thus strain would not be affected by greater osteogenesis or an accumulation of osteoblasts in Yucatan sutures. The narrower gap in Yucatans might have reduced strain somewhat, but because the collagenous tissue is less organized in Yucatans, Young's modulus is probably lower as well, causing relatively greater deformation under a given load. Furthermore, the reduced interdigitation of Yucatan sutures would facilitate relatively higher strains because of the lesser total amount of collagenous tissue and smaller surface area of the bone fronts.

Whatever the cause, the strain response of Yucatan sutures to cyclic loading implies that even abnormal sutures, provided they have not begun to fuse, could respond favourably to this treatment. However, the current sample size is still very small, and we have not yet evaluated whether the cyclic loading regime actually enhances sutural growth in Yucatans as it does in normal pigs.¹³ Thus clinical efficacy is not yet demonstrated. Although our approach was invasive because of the surgical installation of implants, less traumatic clinical approaches to treatment of slow-growing midfacial sutures can easily be envisioned, such as motorized reverse-pull headgear.

5 | CONCLUSIONS

Yucatan minipigs are characterized by midfacial hypoplasia accompanied by maxillary dental crowding and anterior cross bite. The nasofrontal sutures of Yucatans differ from normal pigs in being narrower and simpler, with less organized orientation of the sutural ligament and more active osteoblasts at the bone fronts. During mastication they show relatively low strain. Nevertheless, the sutures are not synostosed and can respond mechanically to cyclic loading as well as normal sutures.

ACKNOWLEDGMENTS

We thank Drs Xian-Qin Bai for histological processing and Murat Maga for CT scanning and processing. We thank Dr David Shin, Ms Kelsey Smith, Mr Ben LaCourse and Ms Jay Park for measurement of undecalcified sections. This research was supported by the University of Washington Orthodontic Alumni Fund, the Dr Douglass L. Morell Research Fund and NIH PHS award R21 DE024814.

REFERENCES

1. Bartold PM. Bone and tooth interface: periodontal ligament In: McCauley LK, Somerman MJ, eds. Mineralized Tissues in Oral and Craniofacial Science. Ames, IA: Wiley-Blackwell; 2012:219–230.
2. Rawlins JT, Opperman LA. Tgf-b regulation of suture morphogenesis and growth. *Front Oral Biol.* 2008;12:178–196. [PubMed: 18391501]
3. Shibazaki-Yorozuya R, Wang Q, Dechow PC, Maki K, Opperman LA. Changes in biomechanical strain and morphology of rat calvarial sutures and bone after Tgf-b3 inhibition of posterior interfrontal suture fusion. *Anat Rec.* 2012;295:928–938.
4. Meta IF, Fernandez SA, Gulati P, Huja SS. Alveolar process anabolic activity in C3H/HeJ and C57BL/6J inbred mice. *J Periodontol.* 2008;79:1255–1262. [PubMed: 18597609]
5. Todd TW, Lyon DWJ. Endocranial suture closure. *Am J Phys Anthropol.* 1924;7:325–384.
6. Kokich VG. The biology of sutures In: Cohen MMJ, ed. *Craniosynostosis: Diagnosis, Evaluation and Management.* New York, NY: Raven Press; 1986:81–103.
7. De Pollack C, Renier D, Hott M, Marie PJ. Increased bone formation and osteoblastic cell phenotype in premature cranial suture ossification (craniosynostosis). *J Bone Miner Res.* 1996;11:401–407. [PubMed: 8852951]
8. Herring SW. Mechanical influences on suture development and patency. *Front Oral Biol.* 2008;12:41–56. [PubMed: 18391494]
9. Brash JC. Some problems in the growth and developmental mechanics of bone. *Edinburgh Med J.* 1934;41:305–387.
10. Mednick LW, Washburn SL. The role of the sutures in the growth of the brain case of the infant pig. *Am J Phys Anthropol.* 1956;14:175–191. [PubMed: 13362486]
11. Herring SW. TMJ anatomy and animal models. *J Musculoskel Neuronal Interact.* 2003;3:391–394.
12. Rafferty KL, Herring SW. Craniofacial sutures: morphology, growth and *in vivo* masticatory strains. *J Morphol.* 1999;242:167–179. [PubMed: 10521876]
13. Soh SH, Rafferty K, Herring S. Cyclic loading effects on craniofacial strain and sutural growth in pigs. *Am J Orthod Dentofacial Orthop.* 2018;154:270–282. [PubMed: 30075929]
14. Al Dayeh AA, Rafferty KL, Egbert M, Herring SW. Deformation of nasal septal cartilage during mastication. *J Morphol.* 2009;270:1209–1218. [PubMed: 19434723]
15. Liu ZJ, Rafferty KL, Ye W, Herring SW. Differential response of pig masseter to botulinum neurotoxin serotypes A and B. *Muscle Nerve.* 2015;52:88–93. [PubMed: 26039454]
16. Peptan AI, Lopez A, Kopher RA, Mao JJ. Responses of intramembranous bone and sutures upon *in vivo* cyclic tensile and compressive loading. *Bone.* 2008;42:432–438. [PubMed: 18032124]
17. Kopher RA, Mao JJ. Suture growth modulated by the oscillatory component of micromechanical strain. *J Bone Miner Res.* 2003;18:521–528. [PubMed: 12619937]
18. Papadaki ME, Troulis MJ, Glowacki J, Kaban LB. A minipig model of maxillary distraction osteogenesis. *J Oral Maxillofac Surg.* 2010;68:2783–2791. [PubMed: 20971370]
19. Rafferty KL, Herring SW, Marshall CD. The biomechanics of the rostrum and the role of facial sutures. *J Morphol.* 2003;257:33–44. [PubMed: 12740894]
20. Popowics TE, Herring SW. Load transmission in the nasofrontal suture of the pig, *Sus scrofa*. *J Biomech.* 2007;40:837–844. [PubMed: 16690062]
21. Herring SW, Teng S. Strain in the braincase and its sutures during function. *Am J Phys Anthropol.* 2000;112:575–593. [PubMed: 10918130]

FIGURE 1.

A and B. Dorsal views of surface reconstructions from CT scans of skulls of a 3-mo-old normal domestic pig (A) and Yucatan minipig #511 (B) from the sham loaded group (implant engagement but no loading). Note the less complex outline of the nasofrontal suture (white arrows) and the relatively tight appearance of the coronal suture in the Yucatan. Note also that the lower incisors jut in front of the premaxilla in the Yucatan because of its midfacial hypoplasia but normal mandible. The asterisks mark the *os rostrale*, an ossification in the anterior terminus of the pig nasal septum. (C) and (D) are tangential CT cuts through the nasofrontal sutures (white arrows) of a 10-mo-old normal Hanford minipig and a

Yucatan micropig, respectively, showing the simpler morphology and partial fusion of the suture in the latter

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

FIGURE 2.

CT cuts through the nasofrontal sutures of the normal (A, A' and C, C') and Yucatan (B, B' and D, D') pigs (same individuals as Figure 1A,B). A and B are tangential sections through the suture, and A' and B' are enlargements of the boxed areas. C and D and their enlargements C' and D' are parasagittal sections through the suture. Sutural interdigitations near the dorsal surface of the Yucatan are fewer, shorter and less regular than those of the normal pig. The frontal sinus appears to be encroaching on the suture, especially in the Yucatan. Brightness and contrast were adjusted to facilitate comparison

FIGURE 3.

Histological parasagittal sections (haematoxylin and eosin) through the nasofrontal sutures of normal (top pair) and three Yucatan (bottom three pairs) pigs, all sham group animals from the cyclic loading experiment. Low power views in the left column show that interdigitations were fewer (absent in #507), shorter and less regular in the Yucatans than in the normal pig, and higher power views in the right column suggest a greater number of osteoblasts (large purple cells) clustered on the bone front surfaces. In the normal sham, the sutural ligament shows regions of fibres oriented to resist compressive strain (double-headed arrows in upper pair) which are not seen in the Yucatans. The frontal sinus (asterisks) can be seen impinging on the suture in Yucatans #511 and #507. Images were sharpened digitally,

and some were flipped for consistency of view. F – frontal bone; N – nasal bone; S – nasofrontal suture

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

TABLE 1

Breeds, ages and sample sizes for procedures

	Domestic pigs	Hanford or Sinclair minipigs	Yucatan minipigs	Yucatan micropigs
External morphology	-	4 mo, n = 11	4 mo, n = 11	-
CT scans	4–5 mo, n = 3	10 mo, n = 1	4–5 mo, n = 5	10 mo, n = 1
Decalcified histology (morphology)	4–5 mo, n = 1 ^a	4–5 mo, n = 4 ¹²	4–5 mo, n = 4 ^a	-
Undecalcified histology (suture gap width)	4 mo, n = 1 ^a	-	4–5 mo, n = 3 ^a	-
Masticatory strain	-	4–5 mo, n = 11 ^{12,14}	5 mo, n = 2	-
Cyclic loading strain	4 mo, n = 3 ¹³	-	4–5 mo, n = 4	-

^aThese animals were sham controls from the cyclic loading trial.

TABLE 2
External morphology and masticatory strain of sutures of 4- to 5-month-old minipigs

Breed	Hanford or Sinclair	Yucatan
Suture		
Ant. Interfrontal		
Morphology	Straight	Straight
Possible fusion	1/11 skulls	9/11 skulls
Masticatory strain	+1036 μe (SD 400, n = 6) ¹²	No data
Post. Internasal		
Morphology	Straight	Straight
Possible fusion	3/11 skulls	9/11 skulls
Masticatory strain	-440 μe (SD 238, n = 6, range -109 to -761) ¹²	-110 μe (n = 1), +118 μe (n = 1)
Nasofrontal		
Morphology	Complexly interdigitated, wide except in one skull	Less complex, narrow
Possible fusion	0/11 skulls	0/11 skulls
Masticatory strain	-2012 (SD 770, n = 11, range -682 to -2892) ^{12,14}	-909 (SD 340, n = 4 gauges on two pigs, range -353 to -1265)
Coronal		
Morphology	Complexly interdigitated	Less complex, narrow
Possible fusion	0/11 skulls	0/11 skulls
Masticatory strain	+278 (SD 150, n = 7) ²¹	No data

TABLE 3

Suture strains resulting from cyclic tension applied to the right nasofrontal suture^b
^ag

Average strain μm	Nasofrontal suture			Coronal suture			Internasal suture	Interfrontal suture
	Loaded side	Opposite side	Loaded side	Loaded side	Opposite side	Loaded side		
Normal (domestic) pig (n = 3) ¹³	+1689 $\pm$ 489	+1005 $\pm$ 368	-836 $\pm$ 464	-283 to +113 ^c	-68 to +245 ^c	+270 $\pm$ 236		
Yucatan minipig (n = 4)	+1992 $\pm$ 432	+1056 $\pm$ 130	-328 $\pm$ 91	-47 to +86 ^c	+453 $\pm$ 127	-217 to +173 ^c		

^aTensile strains are positive (+), and compressive strains are negative (-). Differences between breeds were not significant.

^bValues were measured on the fifth and final day of loading during the first minute of the 30-min session.

^cStrain was tensile in some animals and compressive in others, so range rather than average is presented.