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Effects of Increased Muscle Mass on Mouse Sagittal Suture Morphology and Mechanics

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ABSTRACT

The purpose of this study is to test predicted form–function relationships between cranial suture complexity and masticatory muscle mass and biomechanics in a mouse model. Specifically, to test the hypothesis that increased masticatory muscle mass increases sagittal suture complexity, we measured the fractal dimension (FD), temporalis mass, and temporalis bite force in myostatin-deficient ($GDF8^{-/-}$) mice and wild-type CD-1 mice (all male, 6 months old). Myostatin is a negative regulator of muscle mass, and myostatin-deficient mice show a marked increase in muscle mass compared to normal mice. We predicted that increased sagittal suture complexity would decrease suture stiffness. The data presented here demonstrate that increased suture complexity (measured as FD) was observed in a hypermuscular mouse model ($GDF8^{-/-}$) with significantly increased temporalis muscle mass and bite forces. Hypermuscular mice were also found to possess suture connective tissue that was less stiff (i.e., underwent more displacement before failure occurred) when loaded in tension. By decreasing stiffness, suture complexity apparently helps to dissipate mechanical loads within the cranium that are related to chewing. These results suggest that cranial suture connective tissue locally adapts to functional demands of the biomechanical suture environment. As such, cranial sutures provide a novel model for studies in connective tissue mechanotransduction.

Key words: cranial sutures; fractal dimension; mechanotransduction; GDF8; myostatin; mastication; bite force

Sutures are synarthrotic joints that form between craniofacial skeletal elements. In a comparison of cranial bones with and without sutures, Jaslow (1990) discovered that while the presence of sutures decreases the strength of cranial bone in a three-point bending model, it also increases the energy per unit volume absorbed following impact loading. In addition, increased interdigitation of a suture increases its bending strength and toughness (Jaslow, 1990). Presumably, this relationship occurs because sutural interdigitation increases the surface area (with an increased amount of extracellular matrix between bony elements). The interlocking nature of cranial bone edges has also been credited with dissipating mechanical loads generated from impact (Jaslow and Biewener, 1995) and mastication (Herring, 1972; Herring and Mucci, 1991; Raf-

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ferty and Herring, 1999). Rafferty and Herring (1999) observed increased interdigitation in sutures that experienced compression, whereas sutures that underwent tension had a waveform pattern with less amplitude. While compression may result in increased interdigitation relative to tension, it remains to be demonstrated how increases in compression or tension relate to sutural waveform.

Early studies on cranial suture biology related suture morphology to its mechanical environment (Moss, 1954, 1957, 1961). These experiments showed that suture interdigitation and beveling were affected by mechanical unloading of the suture. Specifically, sutures transplanted into regions that did not experience mechanical loads altered suture morphology from beveled edges to butt edges. Also, subjecting parietals to compressive ligature (i.e., an abnormal mechanical environment) resulted in sagittal suture beveling in cross section (Moss, 1957). This suggests that beveling is a secondary response of bone, not an intrinsic characteristic of a particular suture. Additionally, sagittal sutures were mechanically isolated by functional temporalis muscle obliteration, with the result that interdigitation was lost (Moss, 1961). These experiments led to the conclusion that basic suture morphology is butt-ended, and that the functional matrix of the cranium helps to shape the suture's interdigitating and/or beveled morphology (Moss and Young, 1960). Given the contingency for biological growth in cranial tissues, suture interdigitation arguably adds strength to a necessary but compromising structure.

In the current study, the mouse sagittal suture was used to investigate suture biomechanics. The sagittal (or interparietal) suture is easily accessible and is believed to be loaded primarily in tension, since the temporalis is the only major masticatory muscle arising from the parietals, and its contraction generates tensile stresses along the sagittal suture (Behrents et al., 1978; Herring and Teng, 2000). Previous investigations described a linear relationship between the bite force of the temporalis and electromyographic (EMG) activity (Van Eijden, 1990; Van Eijden et al., 1990). In other words, bite force is proportional to the availability and recruitment of masticatory muscles, such as the temporalis. We predicted that mechanical loading from bite forces would relate to increases in sutural bone growth. We also tested the hypotheses that new bone formation would be increased in a hypermusclular mouse, and that these new regions of bone formation would be site-specific. Site specificity for bone formation allows for the development and growth of a complex line with interlocking lingulae.

We also predicted that suture complexity would increase as the tensile stresses acting on the sagittal suture increased. This would reflect the ability of the suture connective tissue to respond and adapt to strain distributions affected by increased tensional loads. Additionally, we hypothesized that the stiffness of the sagittal suture would decrease to compensate for increased loads. This may represent a mechanism whereby sutures are protected from fracture due to increased mechanical loading. These predictions were tested in a myostatin-deficient mouse model. The effects of increased muscle mass on skeletal architecture (Hamrick et al., 2000, 2003; Hamrick, 2003) were previously studied in this knockout mouse. In the present study, we used this model to study the effects of increased muscle mass on cranial sutures. Given that age is an important factor in cranial suture growth (Henderson et al., 2004; Sun et al., 2004), we used a combination of 4- and 6-month-old mice to assess growth-dependent aspects of suture biology. In the 4-month-old animals we evaluated growth processes in the sagittal suture, while in the 6-month-old animals we assessed the resulting morphological and functional manifestations of the observed developmental differences.

MATERIALS AND METHODS

Localization of Bone Formation in the Sagittal Suture

We performed a bone-labeling experiment to localize bone-forming regions on the convex and concave parietal bone fronts, using an antibiotic that is incorporated into newly forming bone (Nakamura et al., 2000; Singh et al., 2002; Starck and Chinsamy, 2002). It was expected that convex, advancing bone fronts would incorporate the antibiotic label, demonstrating that new bone formation occurs at these regions. It was also expected that concave, retreating fronts would not show label uptake, due to bone resorption at those locations. We gave 4-month-old mice intraperitoneal injections of tetracycline (Sigma-Aldrich Co.) (20 mg/kg; n = 9 per group). Five days later, the mice were given a CO_2 overdose and thoracotomy according to AVMA-recommended procedures approved by the Institutional Review Board of the Medical College of Georgia. We chose 4-month-old animals for this experiment in order to appreciate differential growth within the sagittal suture between experimental groups. Undecalcified crania were embedded in methyl methacrylate and ectocranially viewed with a Zeiss LSM 510 confocal laser scanning microscope (Carl Zeiss, Thornwood, NY) with a Meta System equipped with a Coherent Mira 900 tunable Ti:Sapphire laser (Coherent, Santa Clara, CA) for multiphoton excitation. Multiphoton microscopy allowed laser excitation of tetracycline at its appropriate wavelength (390 nm). Digital images of emission were acquired at 560 nm by means of a computer interface using LSM 5 Image Examiner software (Carl Zeiss, Thornwood, NY). Since this antibiotic is incorporated into newly formed bone, and fluoresces under the proper light, fluorescent regions in the images represent new bone formed over a 5-day interval.

Temporalis Mass

We employed a hypermuscular mouse model (mice lacking myostatin (GDF8)) to explore the effects of increased muscle mass on sagittal suture complexity. Myostatin is a negative regulator of muscle growth, and mice lacking myostatin have significantly increased muscle mass (McPherron and Lee, 1997; Lee and McPherron, 1999). Myostatin is expressed only in muscle, and not in normal bone or other connective tissue (McPherron and Lee, 1997). We compared 6-month-old male $GDF8^{-/-}$ mice on the CD-1 background with age- and sex-matched, wild-type CD-1 mice to observe the effects of increased muscle mass and masticatory forces on sagittal suture morphology and mechanics. We chose 6-month-old animals for these analyses because mice at this age represent the end-point of skeletal growth and thus reflect differences in mechanical loading during development. We predicted that increases in temporalis mass would be accompanied by increases in sagittal suture complexity. All of the mice

were fed hard chow ad libitum. First, 6-month-old male GDF8$^{-/-}$ and normal mice were given a CO_2 overdose, followed by a thoracotomy (n = 8 per group). The temporalis muscles were dissected free and weighed to the nearest 0.001 g. The normal distribution of the temporalis mass was verified with the use of probability plots, and analyzed for statistical significance by Student's *t*-test for comparing two independent sample means.

Temporalis Bite Force

According to previous investigations, there is a positive relationship between the temporalis bite force and electromyographic (EMG) activity (Van Eijden, 1990; Van Eijden et al., 1990). In GDF8$^{-/-}$ animals, we tested the hypothesis that maximum bite forces would increase with increased temporalis muscle mass. We anesthetized 4-month-old mice from each genotype (n = 9 per group) with 3 mg ketamine and 0.4 mg xylaxine/25 g by intraperitoneal injection, and subjected them to electrical field stimulation of the right temporalis muscle. We chose this age group in order to establish that differences in muscle mass and contractility are observable during skeletal development. A Grass SD9 stimulator from Grass Instruments (Astro-Med) with platinum electrodes was used to deliver a 12-Hz, 1-msec pulse with 10-sec duration stimulus at 0, 2, 4, 6, 8, 10, 12, 14, 16, and 18 volts. A 10-sec recovery period was allowed between each successive stimulation voltage to allow for muscle recovery. The 12-Hz frequency was chosen for its ability to elicit a maximal force response. Maximal force was measured with a subminiature load cell (catalog #AL322; Sensotec Honeywell, Columbus, OH) that was placed between the upper and lower incisors. This load cell interfaced with an amplifying unit and computer data acquisition system (Polyview; Astro-Med, Grass Instruments). We analyzed differences in bite forces between genotypes for statistical significance using Student's *t*-test for comparing two independent sample means.

Suture Fractal Dimension (FD)

Articulated parietal bones with intact sagittal sutures were dissected free from 6-month-old mice (n = 9 per group). We decided to use this skeletally mature age group to compare differences in suture morphology manifested as a result of functional differences in developing crania. To image the sagittal suture's waveform, we performed a clearing and staining protocol. This technique uses potassium hydroxide (3% KOH) to digest soft tissues, such as the periosteum, that overlie the bone. This step effectively clears the tissue, and the addition of alizarin red stains the bone (Wassersug, 1976). Next, the sagittal sutures of these specimens were captured by means of a Leica S6D stereomicroscope with Pixera digital camera (Vashaw Scientific, Inc., Norcross, GA) and Dell computer interface. Digital images of mouse sagittal sutures were traced in Adobe Photoshop 6.0 with a single-pixel line (Fig. 3A) and measured by the ruler dimension method with Benoit 1.3 FD analysis software (Trusoft International®). The FD is a measure of a shape's self-similarity upon successive magnification. It has proven to be a successful alternative to traditional Euclidian geometry for analyzing forms in nature (Mandlebrot, 1967, 1977; Hartwig, 1991; Long and Long, 1992; Cross, 1997; Monteiro and Lessa, 2000; Lynnerup and Jacobsen, 2003).

In fractal analysis, one can quantify complex, planar shapes by assigning them a unit-free measure that lies between one and two dimensions (1.0–2.0). With the ruler dimension method (also known as the structured walk), one calculates the FD by using a ruler whose length varies according to a constant value (the coefficient of decrease) to measure the length of a jagged line. For complex objects, overall length becomes longer as the ruler used to measure length becomes shorter. The ruler dimension (i.e., D_r or FD) is taken as the slope of a line where the log ruler length is on the x-axis and the log *N* of rulers (overall line length) is on the y-axis. D_r is a scaling value that relates changes in ruler length to changes in overall shape length. The steeper this slope is, the more complexity the shape demonstrates. We compared differences in FDs between genotypes, and analyzed them for statistical significance using Student's *t*-test for comparing two independent sample means.

Mechanical Testing

In vitro mechanical testing of sagittal suture samples was carried out in 6-month-old GDF8$^{-/-}$ and wild-type mice (n = 8 per group). We chose this age group in order to test predictions of differential mechanical properties in cranial sutures of mature animals. The animals were killed according to the above-described protocol, and rectangular coupons (2 mm × 10 mm) were removed from the anterior and posterior segments of each specimen's sagittal suture with the use of a high-speed dental handpiece turning a fine diamond bur. Each coupon retained a section of suture connective tissue with parietal bone on each side. The mechanical properties of the mouse sagittal sutures were tested by means of a Vitrodyne V1000 Universal Tester (John Chatillon and Sons, Greensboro, NC). First, the parietals were affixed to a split test jig by cyanoacrylate glue. Then the coupons were tensionally pulled at a rate of 10 μm/sec until failure occurred. The resulting load-displacement curves for the two genotypes were analyzed with the use of Mann-Whitney's test for two independent groups. Distribution-free statistical techniques were utilized because there was a lack of normality with the load-displacement parameters.

RESULTS

Localization of Bone Formation in the Sagittal Suture

The cranial sutures enable intramembranous bone to grow peripherally. To ensure continued bone growth, the cranial sutures must remain patent with resident mesenchymal cells in an undifferentiated and proliferative state (Opperman et al., 1995; Opperman, 2000; Ignelzi et al., 2003; Warren et al., 2003). As cranial bone grows at the suture, pluripotent mesenchymal cells differentiate into a more committed cell type as they are recruited into the growing bone front. If opposing fronts were to actively form bone, the suture would become obliterated, losing the stem cell population from which new bone is formed. On the basis of these contingencies, we evaluated the hypothesis that suture growth proceeds by one growth front that forms new bone while the opposite front remains less active (Fig. 1). Figure 2 shows an ectocranial view of a mouse sagittal suture at 4 months of age. Newly formed (fluorescing) bone appears disproportionately along the convex lingulae (red perimeter), which is the active

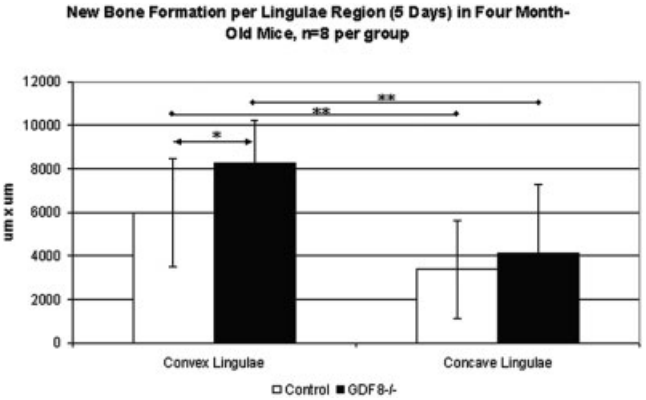

Fig. 1. Comparison of new bone formation in 4-month-old GDF8$^{-/-}$ and CD-1 controls. Comparisons between convex and concave lingulae within each genotype were carried out with the use of Student's paired *t*-test. Double asterisks denote statistical significance at $P < 0.01$. Comparisons of bone formation between genotypes were carried out with Student's *t*-test for two independent groups. Single asterisks denote statistical significance at $P < 0.05$, which was achieved in the comparison of bone formation at convex fronts.

growth front. The opposite front incorporated little fluorochrome label (blue perimeter). A comparison of total new bone formation between genotypes and between lingulae regions suggests that new bone formation is more prevalent along convex suture fronts. Furthermore, a comparison between genotypes suggests that GDF8$^{-/-}$ mice with increased masticatory contractile forces shows increased bone formation along convex fronts over a 5-day interval when compared to wild-type controls (Fig. 1).

Temporalis Mass, Bite Force, and Suture FD

Using the myostatin-deficient mouse (GDF8$^{-/-}$) model, we tested the hypothesis that increased muscle mass would be associated with increased bite forces. Because the knockout mouse shows significant systemic increases in skeletal muscle mass (Lee and McPherron, 1999), we expected that the temporalis and masseter should also show increases in the knockout mouse. Indeed, masseter muscle mass showed a 56% increase in the knockout mouse (Student's *t*-test, $P < 0.01$), while temporalis mass increased by 61% (Student's *t*-test, $P < 0.01$; Fig. 3B) in the 6-month-old mice. Data from bite force analysis following electrical stimulation of temporalis demonstrated that myostatin-deficient animals had increased maximal forces (Fig. 4A). The knockout mice bit with forces that were on average 0.15 Newtons, or 33% greater than the wild-type mice, at 12 volts ($P < 0.05$), 14 volts ($P < 0.01$), 16 volts ($P < 0.05$), and 18 volts ($P < 0.01$) (Fig. 4A,B) at 4 months of age.

The hypermuscular mouse suture also showed a significant increase in the FD of the sagittal suture (1.36 ± 0.099) compared to the wild-type mice (1.24 ± 0.089; Student's *t*-test, $P < 0.05$; Fig. 3C). In other words, the FD analysis demonstrated a significantly increased sagittal suture complexity in the mice with significantly increased temporalis muscle mass and bite forces. To further evaluate suture complexity, we compared the relative lengths of the sagittal suture as calculated by Jaslow (1990). This technique measures the path-length of the suture and divides this value by the linear distance between points bregma and lambda (the beginning and end points of the sagittal suture). The resulting unit-free value describes the sagittal suture path-length in relation to total length, and supports the FD data (Fig. 3C). This lends support to the hypothesis that increased muscle mass increases sutural waveform complexity.

Suture Mechanics

Load-displacement curves of sagittal sutures taken from 6-month-old wild-type and myostatin-deficient mice were compared (n = 8 per group). We used 6-month old mice in this experiment in order to assess differences in biomechanical properties according to differential suture morphology in skeletally mature animals. We used the myostatin-deficient mouse model to test the hypothesis that increased sagittal suture complexity would yield sutures with decreased stiffness. This hypothesis assumes that increased suture complexity relates to increased masticatory muscle forces. Therefore, observed mechanical differences should relate connective tissue adaptation to increased stresses in the mechanical loading environment.

The GDF8$^{-/-}$ mice possess sutures that allow significantly more elongation before failure occurs (386 ± 138 μm) compared to normal mice (260 ± 55 μm;, Mann-Whitney, $P < 0.01$; Fig. 5A). Additionally, the knockout mice also showed decreased stiffness in sagittal sutures (1.17 ± 0.71 g/μm) compared to wild-type individuals (1.55 ± 0.62 g/μm;, Mann-Whitney, $P < 0.05$; Fig. 5B). Figure 6 represents mean load-displacement profiles fit with power curves for knockout mice vs. wild-type mice. When considering these data, it is important to recognize that GDF8$^{-/-}$ mouse sagittal sutures do not demonstrate significantly increased breaking strength. Rather, the observed difference in stiffness is the result of an increase in suture ligament elongation at failure. This verifies the predicted connective tissue adaptation to increased mechanical loading.

DISCUSSION

Experimental and theoretical evidence suggests that the mechanical loading environment plays a significant role in determining the complexity, or FD, of cranial sutures. Our data from myostatin knockout mice support the prediction that increasing masticatory muscle mass and bite force would increase sagittal suture waveform complexity. This phenomenon can be explained as tissue adaptation to a particular mechanical loading regime that is achieved by differential bone growth at the suture. This idea is supported by the fact that caiman species (genus *Caiman,* family Alligatoridae) that exploit hard foods have more complex cranial sutures than closely related caiman species that do not process such foods (Monteiro and Lessa, 2000). We have also observed a similar pattern among neotropical primates: *Cebus* species that exploit tough fruits have more complex sagittal sutures than closely related congenerics that do not feed on these foods (Byron et al., 2004). In the case of caimans and *Cebus* primates, members with higher FDs process tougher foods that consistently require more masticatory force. These comparative observations, in addition to the experimental data from mice provided in the present work, support the notion that there is a functional relationship between masticatory muscle strength and cranial suture complexity.

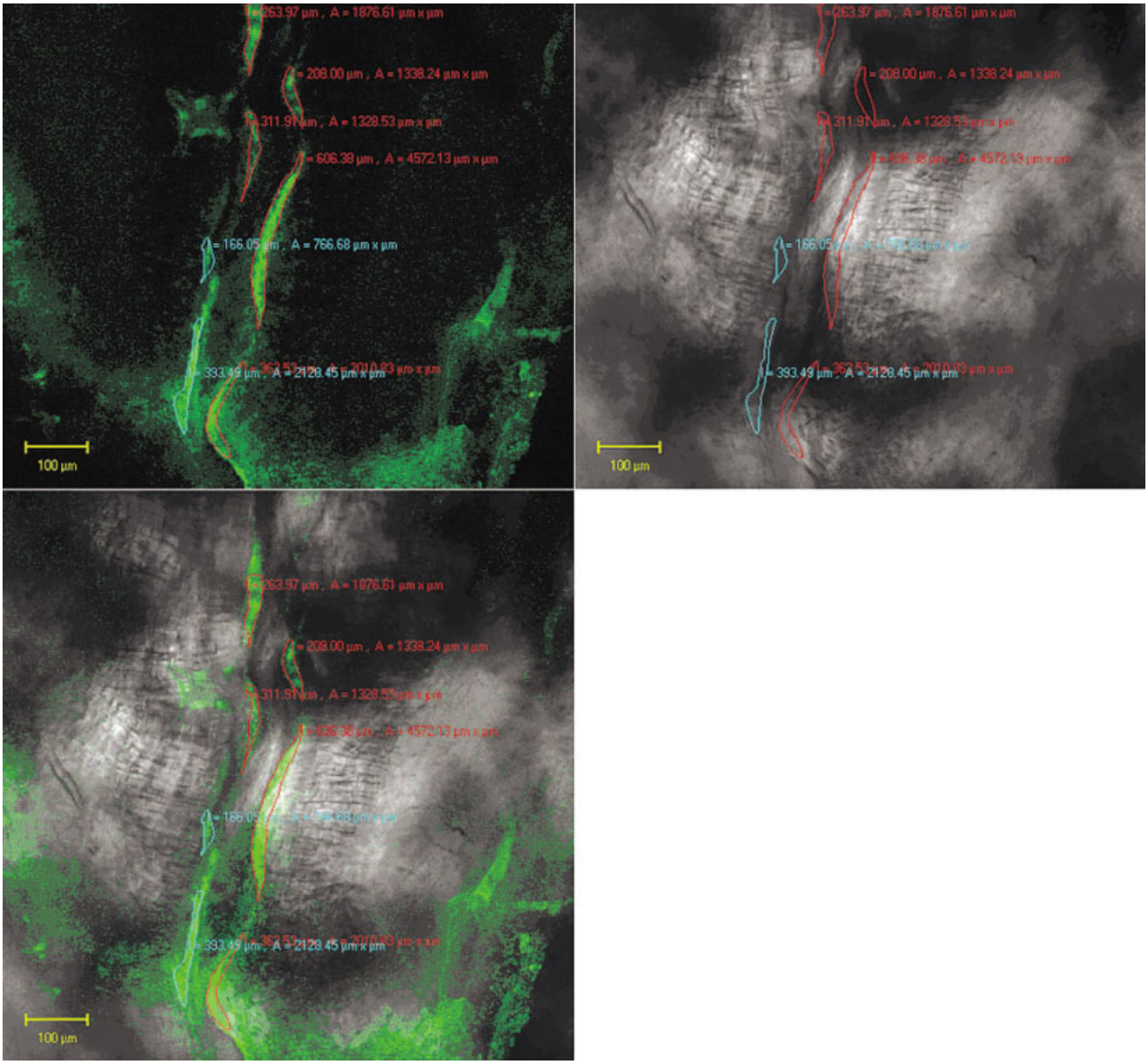

Fig. 2. An exemplary ectocranial view of a mouse sagittal suture labeled with tetracycline. The regions where new bone formed are labeled with green fluorescence. Notice the difference in total label between the convex and concave surfaces.

Mechanistically, this phenomenon can be explained at the cellular level in relation to the strain distribution applied to a line with a waveform shape. Two-dimensional finite element analysis predicts that sinusoidal lines experience differences in stress between opposing bone fronts (Yu et al., 2003). The retreating fronts experience higher shear stresses, while the advancing bone front experiences higher tensile stress. It is hypothesized that this establishes a differential tissue response on either side of the suture. Predictably, higher shear stresses along concave suture lingulae induce cell death, the recruitment of osteoclasts, and a further retreat of this front. On the other hand, we propose that growing fronts experience cell wounding, transient Ca^{2+} entry sufficient for osteogenic gene expression, and new bone formation. The iteration of these events would reinforce the specific pattern of complexity formed early in life, except that the amplitude of the suture would be expected to increase.

The hypotheses relating increased suture complexity to increased bone formation at convex lingulae, increased muscle mass, and decreased stiffness are supported by the data presented here. The fact that sagittal suture complexity, temporalis mass, and temporalis contractile force are associated leads us to suggest that suture complexity in part reflects the degree of masticatory loading experienced by the suture environment. Therefore, differences

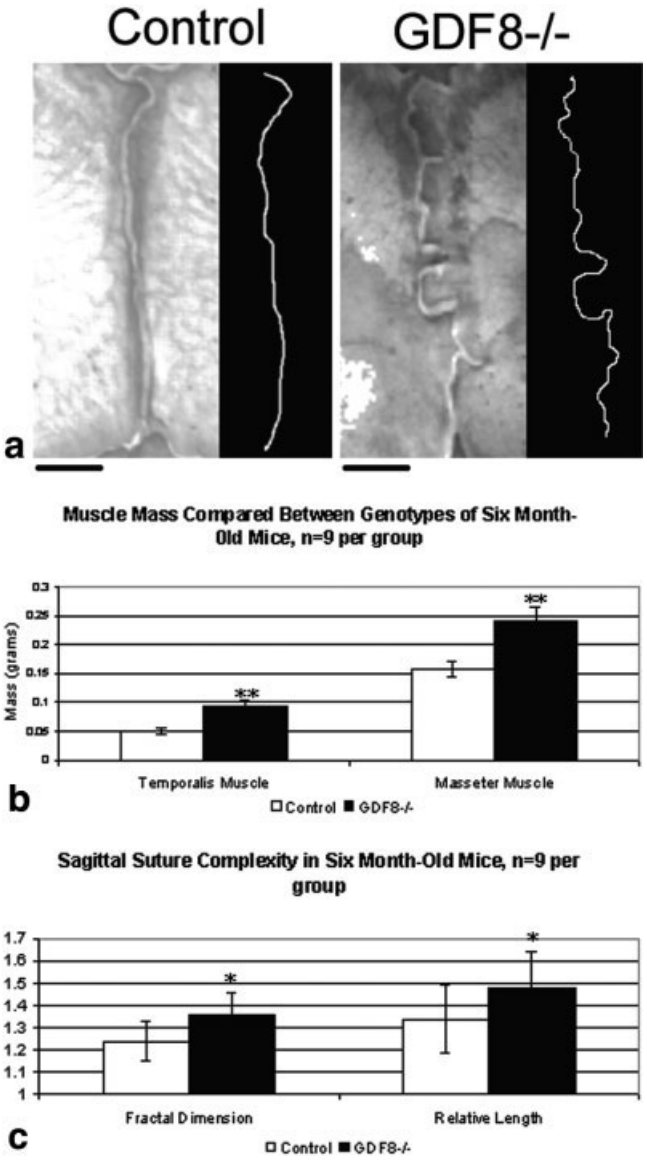

Fig. 3. **A:** Images depicting control and GDF8$^{-/-}$ sagittal suture phenotypes, and their corresponding tracings. This figure illustrates the method used to measure the FD, and shows both the original image of the sagittal suture and the tracing from which the FD was calculated. Scale bar = 1 mm. **B:** Histograms depicting the mean masseter and temporalis mass for each genotype (6-month-old mice). Double asterisks denote statistical significance at $P < 0.01$. **C:** Histograms depicting sagittal suture FD and relative length (as calculated following Jaslow (1990)). Single asterisk denotes statistical significance at $P < 0.05$.

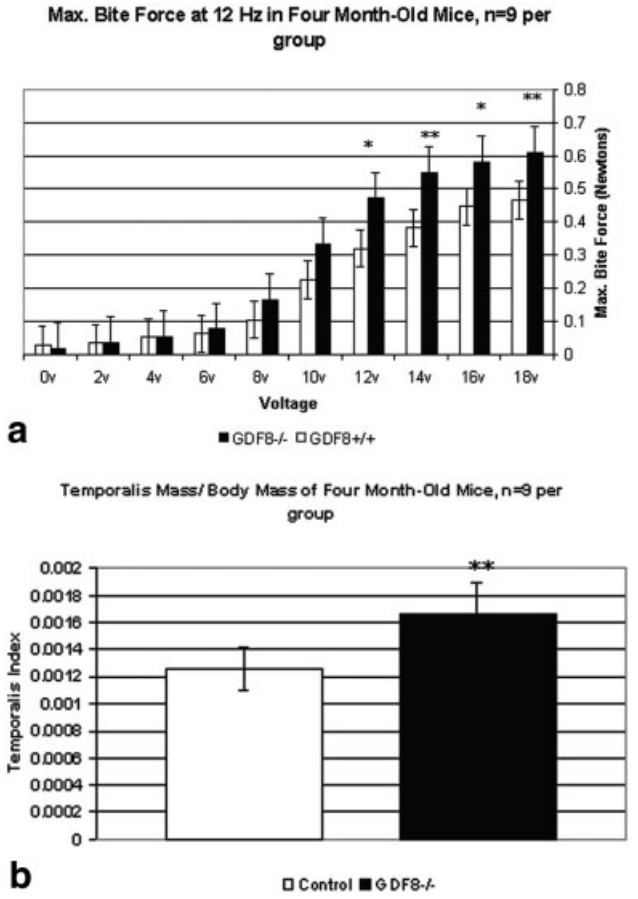

Fig. 4. **A:** Comparison of maximum bite force values between genotypes (4-month-old mice, n = 9 per group). Significance values were computed with Student's *t*-test for two independent groups. Single asterisks denote significance at $P < 0.05$. Double asterisks denote significance at $P < 0.01$. Error bars represent standard error. **B:** Comprison of temporalis muscle indices between genotypes (4-month-old mice). Double asterisks denote significance at $P < 0.01$. Body mass within the 4 month-old mouse comparison was significantly different between genotypes. Therefore, body mass was used as the denominator in a temporalis mass to body mass ratio. Values represent normalized temporalis mass per unit body mass.

in the extensibility and stiffness observed between samples with varying sagittal suture complexities are interpreted to be a consequence of increased tensional force resulting from temporalis contraction, and not simply because the suture is more interlocked.

One possible criticism of this interpretation is that there are differences within the connective tissue environment between sutures of varying complexity that might relate to extensibility and stiffness rather than just muscle force. Figure 7 depicts a scanning electron micrograph taken of the ectocranial surface of a mouse sagittal suture. The collagen fibers shown bridging the gap between each parietal bone presumably affect mechanics. However, one hypothesis currently being tested is that the density of these fibers is ultimately a response to increases in muscle contractile force. Therefore, mechanical differences observed between varying suture complexity, which appear to be simply a result of suture waveform, may actually reveal a more proximate relationship to muscle contractile force.

It is possible that increasing tensile stress in the sagittal suture due to the increased contractile forces of the temporalis causes local connective tissue to adapt so that the suture environment becomes more compliant, to resist higher loads. This may represent a secondary tissue response to dissipate tensile forces, such that:

$$\text{Load Dissipation} = f(\text{Tensile Stress}) + g(\text{Stress Relaxation})$$

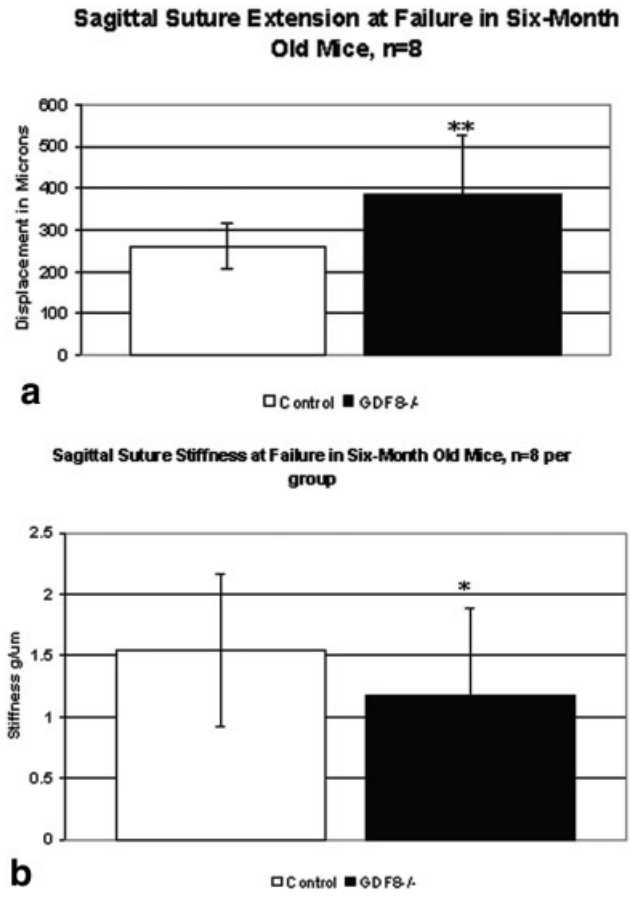

Fig. 5. **A:** Histogram comparing displacement differences between each genotype. Note that the sutures of the hypermuscular mice allow significantly more stretch before failure occurs. Double asterisks denote statistical significance at $P < 0.01$. **B:** Displacement differences lead to the observed differences in stiffness, since stiffness = load/displacement. Single asterisk denotes statistical significance at $P < 0.05$. For both A and B, standard deviations preclude the use of parametric statistical techniques.

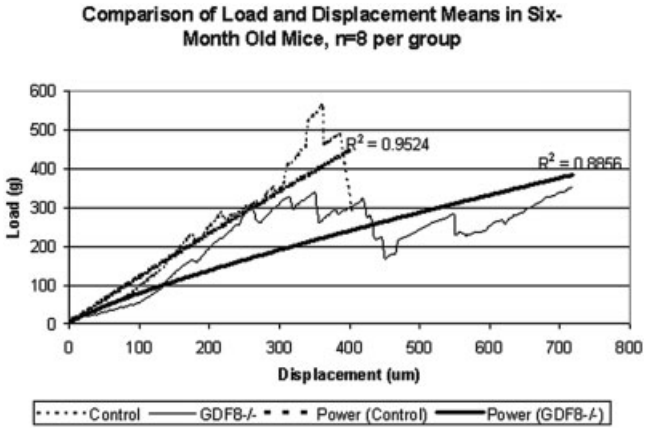

Fig. 6. Mean load-displacement profiles for each genotype fit with power curves. Note that the slopes of the least-square lines differ, and the power curve of the GDF8$^{-/-}$ mice extends further along the x-axis (displacement).

This relationship predicts that mechanical loads transmitted through the parietal bones will dissipate so that tissue and cellular homeostasis can be maintained. As tensional stress increases, there is a concomitant increase in the ability of the suture to relax (i.e., displacement) so that the suture's ability for load dissipation can increase in proportion to the functional demands of such tissue. It is acknowledged that the sutural loading environment is complex, and that compressive and shear stresses may also occur within regions of the suture in vivo (Herring and Teng, 2000; Rafferty et al., 2003). However, given that tension is known to occur in sagittal sutures with contraction of the temporalis (Behrents et al., 1978; Herring and Teng, 2000; Sun et al., 2004), we believe that this represents the primary loading regime in the rodent model described in this work.

Previous studies of suture complexity reported that compressive forces are associated with increased sutural interdigitation relative to tensile forces (Rafferty and Herring, 1999). We do not attempt to refute those findings. In this study we considered only tensional forces that act on suture interdigitation, and did not perform a comparison with sutures that experience compression. However, a novel finding of the present study is that increased mechanical loading in tension induces a growth response in suture tissue that ultimately increases interdigitation. Given these results, it would be interesting to investigate whether an increase in the compressive forces in other cranial sutures would induce increased interdigitation, and whether the amplitude would also increase relative to the increases observed in the sagittal suture.

Overall, it appears that sutures play an important role in dissipating external and internal mechanical loads placed on the skull. Internally, the enlargement of the braincase places tensional loads along sutures that may modulate growth early in life, when cranial vault bones are thin. Henderson et al. (2004) calculate that porcine and murine sutural strain decreases with age, and that this relates to decreases in sutural bone deposition rates. This raises the question of whether strains experienced by mature sutures in vivo influence the biology of osteoblasts. While internal strains delivered to the suture as a result of brain growth may be insufficient to induce suture growth in mature individuals, external strains are potential mediators of suture mechanobiology. External mechanical loading of the skull occurs when muscles of the craniofacial complex contract, such as during mastication or when the cranium receives a physical impact. In a pig study, Sun et al. (2004) concluded that masticatory strain is a likely candidate for new bone formation along cranial sutures. Jaslow and Biewener (1995) suggested that in goats, sutures function as shock absorbers during impact loading (i.e., head-butting).

Alternatively, GDF8 deficiency may directly affect suture connective tissue biochemistry. Analyses of mice deficient in GDF5 have revealed that the tendons are structurally weakened, more compliant, and show a decrease in large-diameter collagen fibers (Clark et al., 2001; Mikic et al., 2001, 2002; Chhabra et al., 2003). It is known that the GDF8 (myostatin) receptor is the Activin type IIB (ActR-IIB) receptor (Rebbapragada et al., 2003); however, this receptor has not yet been identified in ligaments or tendons. The GDF5 receptor is the BMP receptor type IB

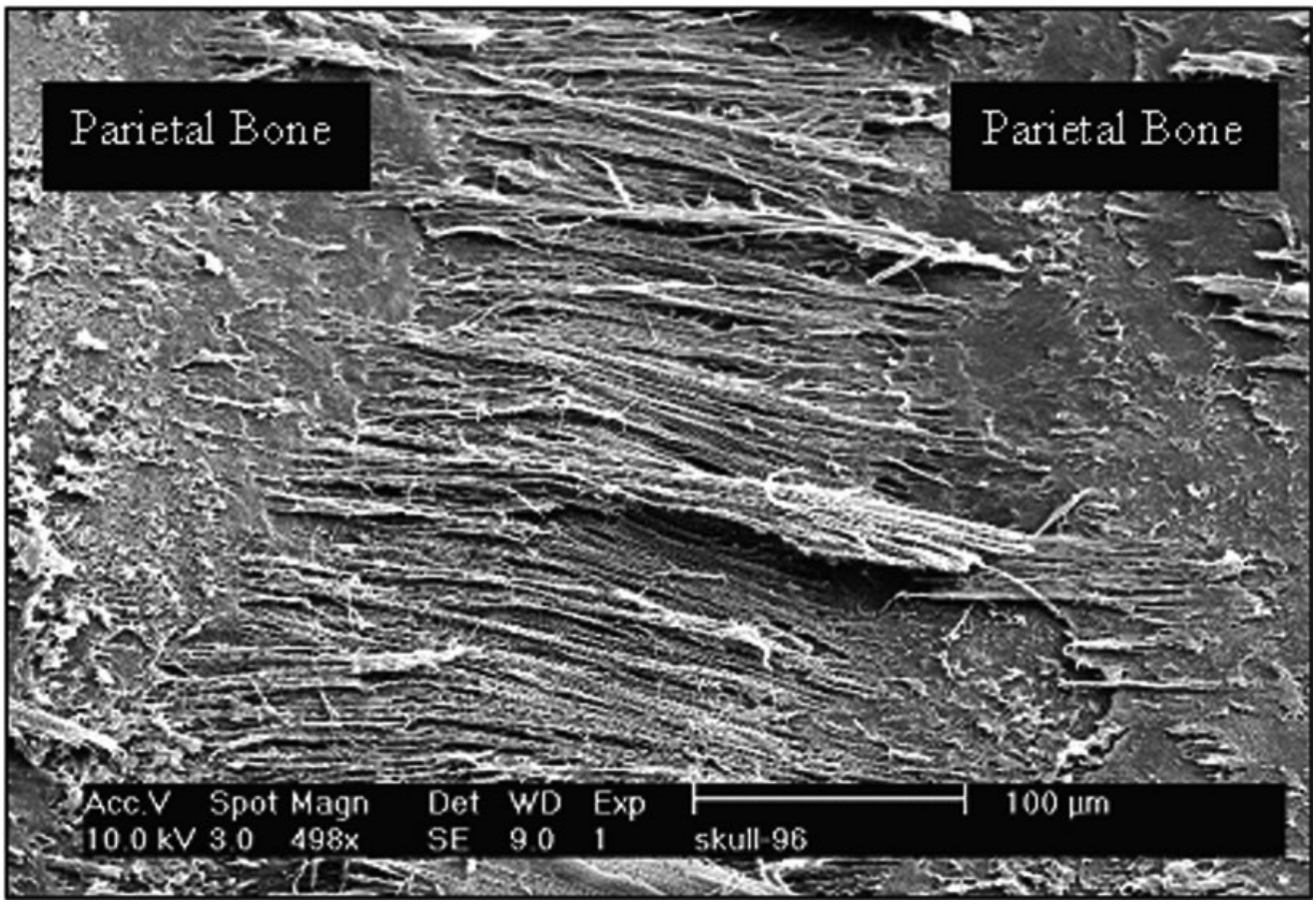

Fig. 7. Scanning electron micrograph depicting the sagittal suture of a mouse from the ectocranial perspective. Bundles of collagen fibers are clearly visible bridging the gap between parietal bones. The average sagittal suture width between bone fronts for each genenotype was approximately 75 μm. The image indicates that collagen fibers extend beyond the suture gap as they are incorporated into the parietal bone matrix.

(BMPR-IB) (Nishitoh et al., 1996; Yi et al., 2000). GDF5 is known to convey signals by inducing the formation of a heteromeric complex consisting of three BMP receptors, including the myostatin and the GDF5 receptors (Nishitoh et al., 1996). It is possible that in myostatin-deficient mice, the ActR-IIB receptor misfunctions by overinteracting with the GDF5 signalling pathway. Additionally, it is possible that the ActR-IIB receptor is present within connective tissue, such as ligaments and tendons, but has not yet been identified.

Data from 4-month-old mice indicate that as the musculoskeleton approaches maturity, muscle mass, contractile forces, and new sutural bone formation are positively related. Growth labeling data support a model for suture osteogenesis whereby local growth occurs within convex osteogenic fronts at the sagittal suture, and is increased in individuals with larger muscle contractile forces. We conclude that this functional relationship is a primary contributor to the resulting cranial suture morphology and mechanics in mature 6-month-old mice. An FD analysis of the suture waveform in a 6-month-old mouse model suggests that increased loads due to increased masticatory muscle mass relate to increases in sagittal suture complexity, or interdigitation. Our results also suggest that increasing sutural complexity is associated with decreases in suture stiffness in 6-month-old mice. These results corroborate the hypotheses that cranial suture connective tissue adapts to specific mechanical loading environments, and the suture waveform functions to dissipate mechanical loads. Finally, by combining these results with those of future investigations, we hope to elucidate the basic mechanisms underlying intra- and interspecific variation in cranial suture morphology.

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