

Brain Morphology in Nonsyndromic Unicoronal Craniosynostosis

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ABSTRACT

Studies of isolated craniosynostosis have shown biomechanical and biochemical influences on the craniofacial phenotype, resulting from both genetic and epigenetic factors. Much less attention has been directed toward the morphology of the brain, despite the interactive nature of the developing skull and developing brain. The aim of this study is to define the morphology of the brain in nonsyndromic unilateral coronal synostosis (UCS) in order to form more complete hypotheses about the cause of craniosynostosis. Landmark coordinate data were collected from 3D magnetic resonance image reconstructions of the brain in a sample of UCS patients and an age-matched morphologically normal cohort. These data were analyzed using Euclidean distance matrix analysis. The results of our study demonstrate that despite the basic similarity of overall shape of the brain and skull in UCS, the effects of craniosynostosis on the brain are not localized to structures immediately adjacent to the fused suture or to the endocranial surface of the skull. Rather, alterations are observed throughout the volume of the brain, with subcortical structures altered in conjunction with cortical changes. These results indicate that the morphological correlates are different for brain and skull and suggest that there is a large degree of independence in the developmental trajectories of the brain and skull. © 2005 Wiley-Liss, Inc.

Key words: craniosynostosis; brain; suture; development; morphometrics

Craniosynostosis is the premature fusion of one or more of the cranial sutures and occurs in roughly 1 in 2,000 live births (Cohen, 1986). Unilateral or bilateral fusion of the coronal suture is the second most common form of craniosynostosis and accounts for 20–30% of all craniosynostosis cases and has an estimated incidence of 0.8–1.0 in 10,000 live births (Hunter and Rudd, 1977; Lajeunie et al., 1995). Fusion of the coronal suture may be observed as one characteristic of numerous syndromes or as an isolated condition. The diagnostic phenotype of coronal craniosynostosis in infants is dysmorphology of the craniofacial complex (Fig. 1), confirmed by radiographic evidence of a closed suture. This study focuses on preoperative nonsyndromic isolated unilateral coronal synostosis (UCS).

Most of what is known about craniosynostosis comes from analyses of the skull and dura mater, the complex of

tissues assumed to be involved in both the cause and in the effect of premature closure of sutures (Cohen, 2000). The overtly dysmorphic skull of individuals with UCS has been described in several radiographic and 3D computed tomography (CT) studies (Fitz, 1981; Kreiborg et al., 1985;

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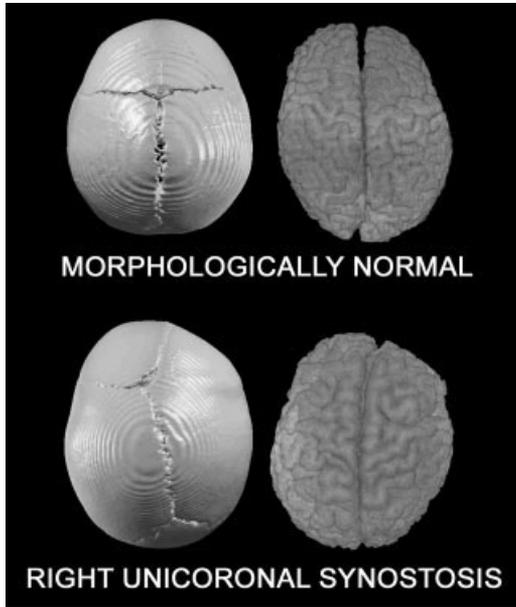


Fig. 1. Three-dimensional reconstructions of CT scans and MRIs of one child affected with right unicoronal synostosis, and one morphologically normal individual unaffected by craniosynostosis. These cases were chosen from our archive as examples and are not meant to represent average phenotypes.

Marsh and Vannier, 1986; Sakurai et al., 1998, 2001; Vannier, 2000; Captier et al., 2003). These descriptions of skull dysmorphology have led to numerous hypotheses regarding the developmental trajectory of the synostosed skull, including the role of the cranial vault and cranial base and treatment to be planned on the basis of these trajectories. As proposed originally by Virchow (1851), the shape of the skull in craniosynostosis is usually attributed to a lack of local growth perpendicular to the fused suture with compensatory growth occurring at adjacent patent sutures. This change in growth vectors is a simple variation on the sensitive and coordinated adjustment required of the normally developing head. It does not, however, explain why the suture fuses prematurely, but simply describes the ontogeny of head shape in craniosynostosis.

The cause of premature fusion of cranial sutures has been attributed either to physical constraint (Graham et al., 1980; Higginbottom et al., 1980) or to genetic mutation (Wilkie, 1997; Cohen, 2000; DeLeon et al., 2000; Wilkie and Morriss-Kay, 2001). Although some genetic mutations have been identified in individuals with craniosynostosis, the role of these mutations in pathways regulating suture patency and/or skull growth has not been characterized. To date, coronal craniosynostosis is the only isolated suture craniosynostosis that has been associated with specific genetic mutations. Screens of patients with apparently nonsyndromic coronal craniosynostosis have found varied proportions of these patients to carry a unique point mutation, P250R, in fibroblast growth factor receptor (FGFR) 3 (Bellus et al., 1996; Moloney et al., 1997; Muenke et al., 1997; Lajeunie et al., 1999). The current opinion is that the P250R mutation on FGFR3 causes a particular syndrome (Muenke syndrome) with variable expressivity (OMIM 602849) (Muenke et al., 1997) and

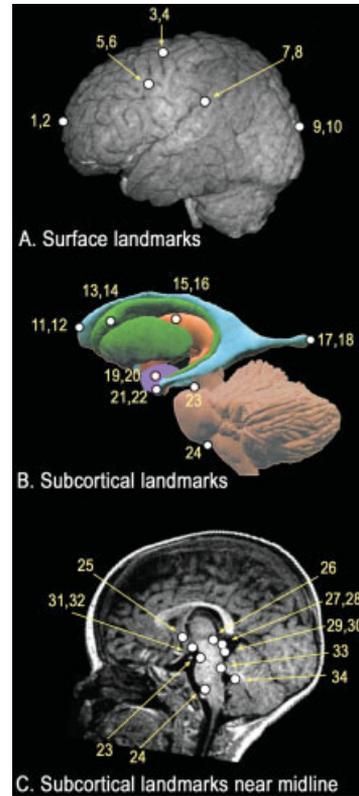


Fig. 2. Landmarks collected from the MRIs are illustrated on (A) 3D MRI reconstruction of the cortical surface, (B) a model of subcortical structures, and (C) a sagittal slice of an MRI of a morphologically normal child. Landmarks are as follows: 1,2, frontal pole; 3,4, intersection of superior frontal sulcus with precentral sulcus; 5,6, intersection of inferior frontal sulcus with precentral sulcus; 7,8, posterior termination of Sylvian fissure; 9,10, occipital pole; 11,12, anterior horn of lateral ventricle; 13,14, centroid of head of caudate nucleus; 15,16, centroid of thalamus; 17,18, posterior horn of lateral ventricle; 19,20, centroid of amygdala; 21,22, inferior horn of lateral ventricle; 23, superior-most point of pons in the midline; 24, inferior-most point of pons in the midline; 25, anterior commissure at midline; 26, posterior commissure at midline; 27,28, centroid of superior colliculus; 29,30, centroid of inferior colliculus; 31,32, centroid of mammillary body; 33, intersection of fourth ventricle with cerebral aqueduct; 34, posterior-most point of fourth ventricle.

incomplete penetrance (Lajeunie et al., 1999). Importantly, only 50% of isolated cases of coronal synostosis have been shown to carry the mutation (Bellus et al., 1996; Moloney et al., 1997; Muenke et al., 1997; Lajeunie et al., 1999). In short, although we have identified some of the players in the genetic basis of craniosynostosis, we have little idea of their function in the development of craniosynostosis phenotypes.

Similarly, physical constraint of the head does not always result in craniosynostosis. Despite studies suggesting that *in utero* constraint leads to craniosynostosis (Graham et al., 1979, 1980; Higginbottom et al., 1980), other studies have concluded that constraint leads only to deformation of the skull while sutures remain patent (Ferri et al., 1997; Bradley et al., 2000). Thus, we have little understanding of the relationship of physical constraint to suture fusion.

Genetic mutation and/or physical constraint may play a role in causing premature suture fusion, but neither can

affect the ontogenetic pathway of the skull and sutures without having an impact on the entire craniofacial system. The osseous elements of the skull do not develop in isolation, but rather within a complex interactive system with the developing brain and meninges. Studies of craniosynostosis have focused primarily on assessments of skull morphology, properties of dura directly underlying the sutures, or the identification of mutations in genes responsible for cranial bone formation and suture closure. Although we think of postnatal skull, brain, and dura mater as three separate units, they develop in intimate physical and biochemical contact with one another. Yet the nature of the interaction between these units of the system is unclear.

Many studies have demonstrated that the presence of dura mater is necessary to maintain suture patency, and further, that the signal mediating suture fusion involves soluble factors, rather than biomechanical factors or cell-cell interactions (Opperman et al., 1993, 1995, 1998). Additionally, studies have hypothesized that complex cell signaling from dura to osteogenic cell populations is responsible for patency of the suture (Opperman et al., 1993, 1995, 1998; Pensler et al., 1998; Mooney et al., 2001). However, the biochemical mechanisms necessary to the production of cranial vault phenotypes in craniosynostosis have not been identified.

A functional approach to the study of skull form was introduced by van der Klaauw (1948–1952) and expanded on by Moss and colleagues (Moss and Young, 1960; Moss, 1962). In particular, Moss and Young (1960) presented a functional analysis to neurocranial growth, proposing that the size and shape of the cranial vault is determined by the form and orientation of the dura mater, which in turn is a direct reflection of the form of the brain. Citing Pupa (1936), Moss and Young (1960) point out that the brain is encapsulated by the dura mater, which is firmly attached to the chondrocranium from its initiation. Since the dura mater and skull base are so firmly integrated at specific sites, a system of forces is produced by growth of the brain, placing pressure against this capsule formed by the dura and skull tissues surrounding the brain. The dural folds produced by these attachment sites underlie the calvarial sutures and this relationship is proposed as playing a part in normal suture closure. Moss and Young (1960) suggested biomechanical forces produced by growth of the brain as the means of communication between the three adjacent tissues.

The role of biomechanical forces in signaling diffusion of growth factors in the communication among these tissues has been supported experimentally (Greenwald et al., 2000; Hunenko et al., 2001; Kirschner et al., 2002; Mao, 2002; Mao et al., 2003a, 2003b; Ogle et al., 2004), suggesting both biochemical and biomechanical influences on the craniofacial phenotype. Whatever the mechanism for communication, we know that a change in the growth trajectory of one of these tissue units influences changes in the trajectory of the others. For example, mechanical forces acting on the external neurocranium, such as binding of immature heads (Schendel et al., 1980; Cheverud et al., 1992) or a habitual sleeping position (e.g., Argenta et al., 1996; Najarian, 1999; Peitsch et al., 2002), changes the shape of the endocranium and the neural mass. Changes in arrangement of dural attachment sites by way of cranial base deformation (experimentally or naturally produced) alter the shape of the outer skull and the neural mass (Moss, 1959; Blechschmidt, 1976). So, too, changes

in brain volume such as hydrocephalus, anencephaly, and microcephaly result in adjustments in neurocranial shape (Siebert et al., 1987; Kjaer, 1995; Trenouth, 1996; Garg and Walsh, 2001).

Previous work has shown that although the human brain has been described as normal in craniosynostosis in the sense that all of its component structures are present, it is generally of abnormal shape (Marsh et al., 1997; Cooper et al., 1999). In an earlier study, we showed that in human cases of sagittal and metopic synostosis, the brain displays characteristic quantitative morphologies, with some aspects that reflect the shape of the skull and others that do not (Aldridge et al., 2002). The morphology of the brain in coronal synostosis in humans has not yet been systematically investigated.

One of the primary goals in the study of craniosynostosis is to determine the cause of premature suture fusion and its relationship to observed craniofacial dysmorphology. Beyond understanding the genetic mechanisms potentially underlying premature suture fusion, determination of the cause of craniosynostosis requires knowledge of the development of the entire craniofacial complex prior to, during, and following suture fusion. By the time children are diagnosed with craniosynostosis, the suture has already fused, and the associated dysmorphology is well established. Thus, the data required to test directly hypotheses related to the cause of suture fusion is not available in humans but must be sought in animal models. Studies of human data are constrained to the more modest goal of acquiring a quantitative depiction of the phenotypes associated with suture fusion. Within this context, morphology and growth can be evaluated in individuals with craniosynostosis and the findings compared to predictions based on competing hypotheses. We may then form clearer hypotheses to be tested in the appropriate animal models.

Recognizing that the trigger that initiates the condition we call craniosynostosis remains unknown, we propose that the role of the developing brain should be given the same attention already given to bone and dura mater in the study of potential developmental mechanisms in craniosynostosis. The aim of this study is to define the morphology of the brain quantitatively in UCS in order to evaluate two alternative hypotheses pertaining to the developmental mechanisms involved in the cause of brain dysmorphology in craniosynostosis.

Hypothesis 1

Brain dysmorphology in UCS is consequent to premature suture fusion. Under this hypothesis, we predict that suture fusion creates a constraint on the expanding brain, resulting in brain morphology that is increasingly different from normal with age, and that the most severe dysmorphology in the brain will be primarily observed in regions that are in physical contact with the skull near the fused suture, i.e., the cortical surface.

Hypothesis 2

Brain dysmorphology in UCS is a primary condition, rather than the result of suture fusion. Under this hypothesis, we predict that morphology of the brain is fundamentally different and remains so, but does not become increasingly different from normal with age, and that the most severe dysmorphology observed in the brain will not be restricted to the surface near the fused suture.

TABLE 1. Sample sizes and age ranges

RUCS (n = 11)		Unaffected/(n = 10)	
Age (weeks)	Sex	Age (weeks)	Sex
6	M		
8	F	7	M
19	F	14	M
21	F	16	M
22	M	32	F
34	F	37	F
39	M	49	M
44	F	53	F
52	M	72	F
70	F	76	F
87	M	90	F
6 F, 5 M		6 F, 4 M	

Three-dimensional morphometric analysis of data collected from magnetic resonance images (MRIs) is performed in comparing the morphology of the brain in UCS to the normal condition to test the two hypotheses. Localization of dysmorphology of the brain to specific regions or structures elucidates the abnormal pattern of craniofacial development that occurs in craniosynostosis. We compare our observations with the predictions associated with each hypothesis and relate these findings to existing knowledge of tissue interaction and gene action in growth and development of craniofacial dysmorphologies.

MATERIALS AND METHODS

Study Sample

The study sample consists of whole brain 3D MRIs from a total of 21 infants of ages 6 to 90 weeks (Table 1), scanned at St. Louis Children's Hospital or Oklahoma University Medical Center. This number includes 11 infants diagnosed with isolated right unilateral coronal synostosis (RUCS) imaged prior to neurocranial surgery, and 10 age-matched infants unaffected by craniosynostosis (UN). Synostosis of the right coronal suture with patency of the remaining cranial sutures was documented. Infants unaffected by craniosynostosis were imaged due to suspected medical conditions (i.e., supposed concussion, unexplained seizures), but were subsequently determined to display no abnormalities. Four of the RUCS patients were screened for FGFR3 P250R mutations, with negative results. None of these infants presented with anomalies of the extremities or family history of craniosynostosis. Though none of these infants were determined on clinical presentation to have any defined craniosynostosis-related syndromes, the presence of a mild form of Muenke syndrome cannot definitively be excluded for six of the patients in the absence of FGFR3 P250R testing.

Image Acquisition, Data Collection, and Analysis

MRI scans were obtained in the sagittal plane using an MPRAGE sequence with the following parameters: matrix = 256×256 , field of view (FOV) between 19.9 and 25.8 cm, and slice thickness between 1.0 and 1.3 mm. The MRIs were analyzed using MEASURE software (Barta et al., 1997) written for a PC platform. This software allows visualization of MRI data in any three orthogonal planes and in a 3D

reconstruction. All nonneural tissue, including dura mater, was stripped from each image slice following a semiautomated procedure described by Aylward et al. (1997) and Buchanan et al. (1998). A 3D reconstruction of the remaining brain tissue is produced from the stripped slice data, which can be manipulated in virtual space and viewed from any direction.

Thirty-four anatomical landmarks were identified and defined for structures located on the cortical surface and on subcortical structures derived from the developmental forebrain, midbrain, and hindbrain (Fig. 2). Anatomical landmarks are biologically meaningful loci that can be repeatedly located with a high degree of accuracy and precision (Richtsmeier et al., 1995; Valeri et al., 1998). Three-dimensional landmark data provide a repeatable geometric representation of the relative location of these loci and allow investigation of spatial relationships between anatomical structures as well as localization of morphological differences to specific regions within structures when forms are compared (Richtsmeier and Lele, 1993).

Using MEASURE, the 3D coordinate locations of landmarks are determined using any of the three planar views or the 3D reconstruction. Measurement error for each of the 34 landmarks was evaluated following methods presented previously (Aldridge et al., 2000) and minimized statistically by digitizing each individual two times, evaluating these trials for gross error, and using the average of the two trials for analysis to reduce intraobserver error.

A landmark-based analysis of morphology used in conjunction with MRI technology allows simultaneous analysis of internal and external 3D neural morphology *in vivo*. The landmark coordinate data were analyzed using Euclidean distance matrix analysis (EDMA) (Lele, 1993; Lele and Richtsmeier, 2001) to compare the RUCS patient sample statistically to the sample of unaffected infants. EDMA is a linear distance-based morphometric method that does not rely on registration or smoothness criteria (Lele, 1993; Lele and Richtsmeier, 2001). Briefly, the matrices of the 3D landmark coordinates collected for each individual are converted into matrices of all possible unique interlandmark distances. A mean matrix of interlandmark distances is calculated for each sample. Each distance is compared across samples as a ratio of the mean values. A nonparametric bootstrapping algorithm estimates confidence intervals for each interlandmark distance to statistically evaluate the null hypothesis of similarity of individual linear distances ($\alpha = 0.10$). With relatively small sample sizes, confidence intervals are more useful than point estimates for statistical comparison. Confidence intervals have the additional advantage that they account for biological variability within samples. Linear distances determined to differ between samples by a magnitude of 10% or more and to be statistically significantly different are reported.

RESULTS

Results of our statistical analyses indicate that morphology of the brains of infants affected with RUCS differs markedly from those of morphologically normal infants. These differences are evident in both cortical and subcortical morphology. Details of the localized differences differing by 10% or more and statistically significantly different by confidence interval testing, are presented below and illustrated in Figure 3.

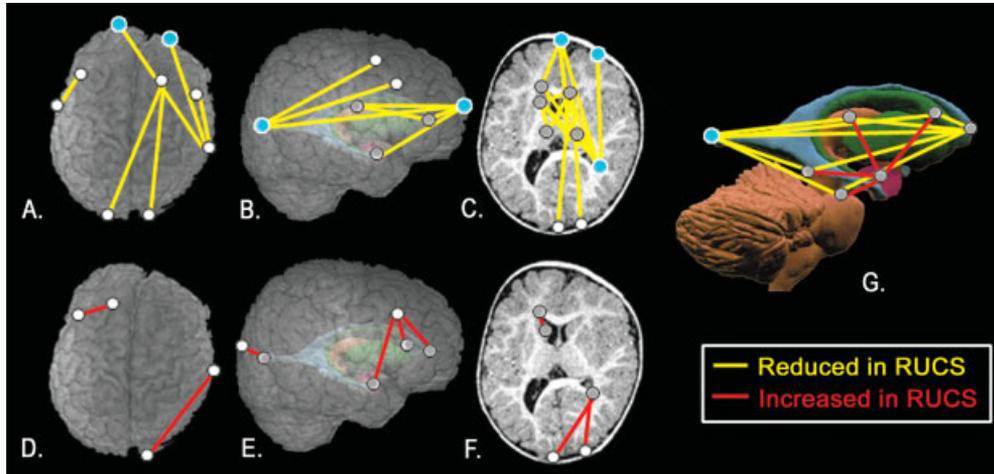


Fig. 3. Results of tests of the null hypothesis of similarity in form between RUCS and unaffected samples. Cortical landmarks are illustrated in white; subcortical landmarks are gray. **A** and **D**: Superior cortical view used to illustrate linear distances between cortical landmarks. **B** and **E**: Lateral cortical view with subcortical model ghosted underneath used to illustrate linear distances between a cortical landmark and a subcortical landmark. **C** and **F**: Axial slice used to illustrate linear distances between subcortical landmarks. **G**: Subcortical model

used to illustrate linear distances between subcortical landmarks. Yellow lines illustrated in A–C and G indicate significant differences that are smaller in the RUCS sample. Red lines illustrated in D–F and G indicate significant differences that are greater in the RUCS sample. These results are illustrated on an individual chosen as an example from the RUCS sample and is not meant to represent an average RUCS phenotype.

Linear distances measured between CNS structures that are significantly reduced in RUCS (Fig. 3, yellow lines) can be primarily attributed to three landmarks: the right and left frontal poles and the posterior horn of the right lateral ventricle (these landmarks are marked in blue in Figure 3A, B, C, and G). Linear distances connecting the posterior horn of the right lateral ventricle with many subcortical landmarks (including the posterior commissure, superior pons, the right thalamus, and both caudate nuclei and anterior horns of the lateral ventricles; Fig. 3C and G), the left frontal pole (Fig. 3B and C), and the right superior and inferior frontal sulci (Fig. 3B) are reduced in RUCS as compared to normal. Distances joining the frontal poles with subcortical structures in the anterior and middle portions of the right hemisphere (i.e., caudate nuclei, thalami, and amygdalae; Fig. 3B and C) and with the right Sylvian fissure (Fig. 3A) are reduced in RUCS. Additionally, the distance between each of the occipital poles and the superior frontal sulcus and the anterior horn of the lateral ventricle on the right side are reduced in RUCS (Fig. 3A). Finally, the distance between the inferior frontal sulcus and Sylvian fissure is reduced bilaterally in the RUCS group (Fig. 3A).

Linear distances connecting the right inferior frontal sulcus with the anterior ventricles, the genu of the corpus callosum, and the right caudate nucleus and amygdala are significantly increased in RUCS as compared to normal (Fig. 3E), as is the linear distance between the left inferior frontal and superior frontal sulci (Fig. 3D). Linear distances connecting each amygdala with similarly sided caudate nuclei and thalami are also significantly greater in RUCS (Fig. 3G, red lines). Further, the distances joining the amygdala on the side of suture fusion with the pons and contralateral thalamus are significantly increased in RUCS. Finally, the linear distances joining the right Sylvian fissure with the right occipital pole (Fig. 3D) and joining the posterior horn of

the right lateral ventricle with both occipital poles (Fig. 3F) are significantly greater in RUCS.

Taken together, these results indicate that the brain in RUCS is characterized by increased height of frontal cortex relative to the subcortical structures on the side ipsilateral to the synostosed suture and an inferior displacement of the right temporal pole (as described by the position of the amygdala). Further, the hemisphere ipsilateral to the synostosed suture displays decreased anteroposterior length, but this shortening is accomplished through a retraction of the frontal region relative to the rest of the brain, rather than an overall anteroposterior decrease in the hemisphere as a whole.

In order to determine whether age is a factor in the dysmorphology of the brain in RUCS, we performed regression analyses for each linear distance against age for the two samples. Comparison of the slopes of the morphologically normal and RUCS samples indicates that the slopes do not differ significantly for any of the linear distances compared (four examples are shown in Fig. 4). This indicates that the degree of dysmorphology does not increase or decrease with age, suggesting that the brain dysmorphology in RUCS we describe here is present prenatally and does not worsen with growth.

To test the effect of age, we also performed a principal coordinates analysis application of EDMA (Richtsmeier et al., 1998) on the entire data set (Fig. 5). The first principal axis shows a high correlation with age ($R^2 = 0.87$); however, the individuals in the two samples do not cluster separately along this axis. The second principal axis shows a distinction between RUCS and normal, but shows a very low correlation with age ($R^2 = 0.23$). The linear distances highly correlated with the second axis score characterize the S-I height of the frontal cortex, M-L breadth of the subcortex in the right temporal lobe, and A-P length of the subcortex in the right frontoparietal region. These results again suggest that the

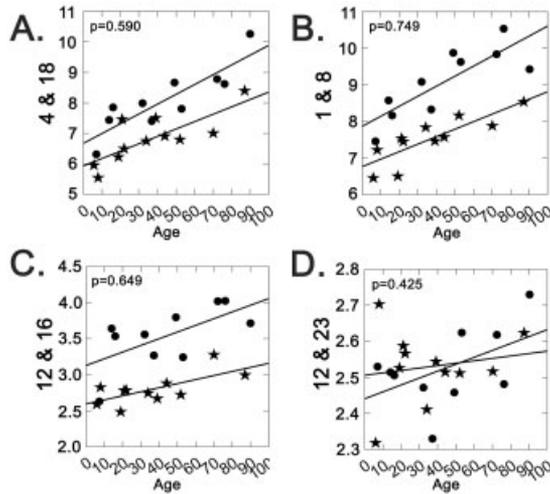


Fig. 4. Examples of linear distances plotted against age in weeks. A–C: Three examples of linear distances that differ significantly between RUCS and unaffected samples. Landmark numbers refer to those given in Figure 2. **A:** A linear distance between a cortical landmark and a subcortical landmark (4 and 18). **B:** A linear distance between two cortical landmarks (1 and 8). **C:** A linear distance between two subcortical landmarks (12 and 16). **D:** An example of a linear distance that does not differ significantly between the RUCS and unaffected samples (between landmarks 12 and 23). The *P* values in each panel represent results of statistical comparison of the slopes of the regression lines for each sample. Nonsignificant values indicate that the differences between RUCS and unaffected samples do not increase or decrease with age. Circles represents individuals in the unaffected sample. Stars represents individuals in the RUCS sample.

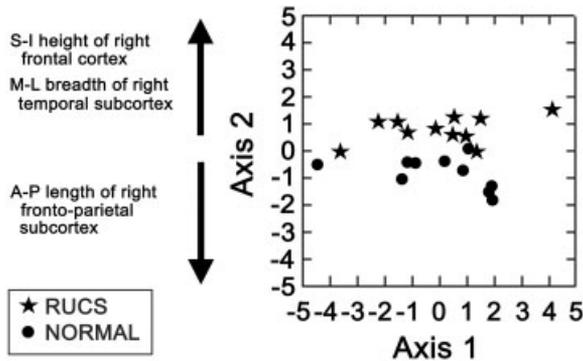


Fig. 5. Principal coordinates analysis of RUCS and unaffected individuals. The first principal axis accounts for 52% of the sample variability; the second principal axis accounts for 13% of the sample variability. Circles represents individuals in the unaffected sample. Stars represents individuals in the RUCS sample.

dysmorphology distinguishing RUCS from normal is present early in infancy and does not increase with age.

DISCUSSION

Previous studies of brain morphology in craniosynostosis have reported primarily qualitative observations or have measured relative volume or size of the entire brain or cranial cavity with varying results. It has been stated that changes in the form of the skull are mirrored by similar changes in the underlying brain in craniosynostosis (Gault et al., 1992), and results of this study indicate

that many aspects of brain morphology in RUCS reflect skull dysmorphology. For example, the flattened frontal bone observed in the skull in RUCS (Marsh and Vannier, 1986; Vannier, 2000; Sakurai et al., 2001) is mirrored in the retraction of the frontal region of the brain on the right side (ipsilateral to the premature suture fusion). Likewise, the bulging of the squamous temporal and anterior displacement of the petrous temporal of the cranium on the side contralateral to suture fusion (Fitz, 1981; Marsh and Vannier, 1986) are reflected in the inferiorly displaced temporal poles and anterior displacement of the Sylvian fissure in the brain. However, and importantly, other aspects of brain morphology do not reflect the overlying skeleton. For example, the calvarial rotation and deviation of the midline cranial base toward the side of suture fusion described for the skull in RUCS (Kreiborg et al., 1985; Marsh and Vannier, 1986; Sakurai et al., 1998, 2001; Captier et al., 2003) are not observed in any features of the brain. Instead, we observe superoinferior changes in the position of the temporal poles and the frontal cortex ipsilateral to the fused suture.

Interestingly, the statistically significant and substantial differences in the RUCS brain are observed primarily on the right side, while fewer differences of this magnitude are observed on the left. In particular, the obvious anterior protrusion of the left frontal bone in cases of RUCS (Marsh and Vannier, 1986) is not mirrored in our measures of the underlying left frontal lobe. This does not suggest that the entire left side of the brain is unaffected in RUCS. Rather, many linear distances on the contralateral side do differ significantly, but the magnitude of difference is relatively less than those reported for the right side (< 10%). Thus, the side ipsilateral to the fused suture is affected disproportionately as compared to the contralateral side, but is not affected in isolation.

These findings have important implications for the role of the brain in craniosynostosis. Previous studies of the craniofacial phenotype in craniosynostosis have directed their focus toward the skull, suture, and dural tissues due to the implicit belief that the fused suture is the primary cause of the overall dysmorphology, which impacts the brain secondarily. This theory is based on the fact that one role of cranial sutures is to serve as growth sites for the calvarial bones (Opperman, 2000). However, the results of this study show that many of the changes in the brain are not reflected in skull morphology.

We posed two alternative hypotheses pertaining to the cause of brain dysmorphology in unicoronal craniosynostosis, with predictions associated with each: hypothesis 1, brain dysmorphology in UCS is consequent to premature suture fusion; hypothesis 2, brain dysmorphology in UCS is a primary condition, rather than the result of suture fusion. In order to determine whether our data support either hypothesis, we compare our results with the predictions resulting from each hypothesis by answering two key questions.

One, which areas of the brain show the most severe dysmorphology? Many of the significant differences in brain morphology are observed in close proximity to areas of the skull that are also markedly altered in RUCS. For example, the frontal bone and the frontal lobe on the side ipsilateral to suture fusion are affected similarly. Likewise, many more differences are observed on the side of the brain ipsilateral to suture fusion as compared to the contralateral side of the brain. These results support the hypothesis that brain dysmorphology in UCS is conse-

quent to suture fusion. In contrast, there are features of the brain dysmorphology that are not observed in skeletal changes. For example, the differences in height measures of the temporal lobes have not been reported for corresponding regions of the skull. Further, many of the changes occur in structures deep beneath the cortex, not in proximity to the site of suture fusion. These results then suggest brain dysmorphology in UCS to be a primary condition, not the result of suture fusion.

Two, does brain morphology become increasingly different from normal with age? Results of both the principal coordinates analysis and the regression analyses indicate that brain morphology differs from normal at a very early age and does not become increasingly different with postnatal growth. This suggests that the brain is fundamentally different from normal at birth, and that the developmental mechanisms responsible for brain dysmorphology are active prior to birth. These results provide provisional support for the idea that brain dysmorphology is a primary condition, rather than secondary compensation following suture fusion.

Taken together, our findings suggest a degree of independence in the dysmorphology of the brain on the one hand and the skull on the other in cases of unicoronal craniosynostosis. In addition, though the brain has been implicitly thought to be a passive player in craniosynostosis, our study suggests a more reciprocal relationship between brain and other craniofacial tissues, with no single tissue playing a primarily directive role postnatally. However, because we do not know the precise timing of suture fusion in these infants, as they are diagnosed after the suture has fused and dysmorphology is obvious, we cannot rule out the possibility that at least some aspects of the differences in brain morphology are secondary compensatory changes in brain morphology following suture fusion. In a study of a rabbit model of coronal craniosynostosis, Cooper et al. (1999) found that brain morphology appeared normal shortly after birth and acquired dysmorphic features later. They then concluded that brain dysmorphology in craniosynostosis is due to secondary compensatory growth changes following suture fusion. The results of our study conflict with the findings in the rabbit model, as we see a great degree of dysmorphology in the brain shortly after birth, with no increase in the degree of dysmorphology with growth.

Though primarily thought of as growth sites, sutures serve functions in addition to osteogenesis. Evolutionarily, sutures serve as fibrous joints between cranial elements, reacting to and dissipating stress experienced by the skull both intrinsically and extrinsically (Herring, 2000; Herring and Teng, 2000). Numerous experimental studies have demonstrated effects of biomechanical stresses on sutures *in vivo*, including differences in trabecular bone patterns (Ozaki et al., 1998), increased bone growth at suture sites (Mao, 2002; Mao et al., 2003a, 2003b), increased proliferation of immature dural cells (Fong et al., 2003), and increased release of TGF- β 1 (Fong et al., 2003) and of FGF-2 (Yu et al., 2001; Fong et al., 2003), which have previously been identified in both normally fusing sutures (Most et al., 1998; Greenwald et al., 2000; Gosain et al., 2004) and in those induced to close (Iseki et al., 1997; Mathijssen et al., 1999; Moursi et al., 2002). These studies show that increased biomechanical stresses affect suture biology, suggesting a role of biomechanical influences on suture patency.

Alternatively, some experimental studies suggest that biochemical signaling mechanisms are independently re-

sponsible for suture patency and the associated craniofacial morphology, not biomechanical factors (Opperman et al., 1993, 1995, 1998; Mooney et al., 2001). A series of *in vitro* and *in vivo* studies of suture biology concluded that the presence of dura mater is necessary to maintain suture patency, and further, that the signal mediating suture fusion involves soluble factors alone (Opperman et al., 1993, 1995, 1998; Warren and Longaker, 2001). These studies suggest that the development of skeletal and central nervous system tissues is indirectly affected by the biochemical factors determining suture patency.

The scenario depicting suture fusion resulting from biochemical signaling contrasts with that holding biomechanical forces responsible for fusion of the suture. These perspectives, however, are not mutually exclusive. Recent experimental studies have shown that mechanical constraint alters the expression of TGF- β (Hunenko et al., 2001; Kirschner et al., 2002; Fong et al., 2003), FGF2 (Yu et al., 2001; Fong et al., 2003), FGFR1 (Ogle et al., 2004), and FGFR2 (Hunenko et al., 2001; Kirschner et al., 2002; Ogle et al., 2004) in the dura and osteogenic fronts. This suggests multiple possibilities for the etiology of craniosynostosis. For example, dural tissue under mechanical strain may reversibly or irreversibly alter the normal pattern of cell signaling. Epigenetic factors or disproportionate growth in skeletal and neural tissues may produce tension, increasing expression of FGF-2, for example, as described by Yu et al. (2001), or FGF receptors (Hunenko et al., 2001; Kirschner et al., 2002; Ogle et al., 2004), leading to craniosynostosis. Alternately, genetic mutation may lead to increased production of FGF-2 by CNS cells, which leads to increases in growth of the CNS (Gremo and Presta, 2000), producing disproportionate tension at the suture and ultimately premature fusion.

Though numerous studies of children with various forms of isolated craniosynostosis have concluded that their mental development is within normal parameters (Kapp-Simon et al., 1993; Arnaud et al., 1995; Speltz et al., 1997), others have concluded that the rate of learning disorders and mental retardation is greater in these children (Kapp-Simon, 1998; Panchal et al., 2001; Magge et al., 2002; Shipster et al., 2003). In a study of patients with simple bilateral or unilateral coronal synostosis, Hunter and Rudd (1977) found no evidence for increased mental retardation. The contradictory results of these studies may arise from the pooling of patient populations with various sutures synostosed, or possibly due to very small sample sizes. Thus, it is unclear whether isolated coronal craniosynostosis is associated with altered cognitive function. If changes in cognition are present, it seems that their presentation is very mild. Therefore, the substantial brain dysmorphology described for RUCS in this study does not appear to have marked neurologic or cognitive consequences. This suggests that the brain is remarkably plastic with respect to the development of its structure-function relationship at the anatomical level, while retaining highly precise relationships at the level of neural connection or topology of neural networks.

The results of our study demonstrate that the overall shape of the brain reflects general skull shape in UCS, suggesting that we cannot think of skull, dura, and brain as independent units. On the other hand, despite the basic similarity of overall shape in brain and skull, details of these morphologies differ in the two tissues. Further, the effects of craniosynostosis on the brain are not localized to structures immediately adjacent to the fused suture, or to

the endocranial surface of the skull, or to the cortical surface of the brain. Rather, alterations are observed throughout the volume of the brain, with subcortical structures altered in conjunction with cortical changes. These results indicate that there is a lack of direct correspondence in the physical effects of UCS in the skull and in the brain and suggest there is a large degree of independence in the developmental trajectories of the brain and skull despite their physical and chemical connections between the units of the craniofacial complex.

Our results prompt us to reject both hypotheses posed at the start, which are based on previous ideas of skull and dura as directing brain growth or vice versa. Instead, we suggest the entire skull, the entire brain, and the meninges are parts of an integrated developmental network, continually interacting in the production of their respective phenotypes. Premature closure of a suture is simply an event in development and does not signal the initiation of novel morphogenetic events.

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