

Central nervous system phenotypes in craniosynostosis

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Abstract

Though reduction in the number of cranial elements through loss of a suture is a recognized trend in vertebrate evolution, the premature closure of cranial sutures in humans, craniosynostosis, is considered a pathological condition. Previous research on craniosynostosis has focused primarily on the skeletal phenotype, but the intimate relationship between the developing central nervous system (CNS) and skull is well documented. We investigate the morphology of the CNS in patients with isolated craniosynostosis through an analysis of cortical and subcortical features using 3-D magnetic resonance images (MRI). Results show that a distinct CNS phenotype can be defined for specific diagnostic categories. Many differences in CNS morphology observed in the patient samples may be anticipated based on skeletal morphology, but others are not reflected in the skull. We propose a developmental approach to determining the cause of premature suture fusion, which includes investigation of the craniofacial complex as a system, rather than study of isolated tissues.

Key words brain; development; morphology; suture.

Introduction

Craniosynostosis is defined as the premature fusion of one or more of the cranial sutures and occurs in roughly 1 in 2000 live births (Cohen, 1986). Isolated sagittal synostosis, the most common form, accounts for 57% of isolated synostosis cases, with isolated coronal synostosis accounting for 18–24%, isolated metopic synostosis between 4 and 10%, and isolated lambdoid synostosis being the least common, making up only 1–4% of the cases (Cohen, 1986). The diagnostic phenotype in this disorder is dysmorphology of the craniofacial skeleton in infants, confirmed by radiographic evidence of a closed suture.

It has been noted that although the brain is 'normal' in craniosynostosis in the sense that all of its component structures are present, it is of abnormal shape (Marsh et al. 1997; Cooper et al. 1999; see Fig. 1).

Although craniosynostosis was first diagnosed on the basis of the skeletal phenotype (Virchow, 1851), abnormal growth of the brain was proposed by some as the primary factor leading to the overall phenotype observed in craniosynostosis (Moss, 1960). It is clear that craniofacial development is a complex and highly integrated process, involving multiple gene families, cell populations and tissue types. Many genes have been implicated in the formation of various processes and tissues that form the head (reviewed in Wilkie, 1997; DeLeon et al. 2000; Opperman, 2000; Wilkie & Morriss-Kay, 2001), and much is known about tissue interactions required to produce craniofacial tissues (e.g. Dunlop & Hall, 1995; Hall & Miyake, 1995, 2000), but few studies have investigated the interplay between established tissues over the course of development. There is some experimental evidence that the brain influences the form of the skull via their physical and developmental connection with the intermediate dura (Moss, 1960; Yu et al. 2001), leading to conclusions that growth of the brain places mechanical strain on sutural cells through their connections with the dura. Alternatively, experimental studies of dura and skeletal tissue indicate that dura alone is independently

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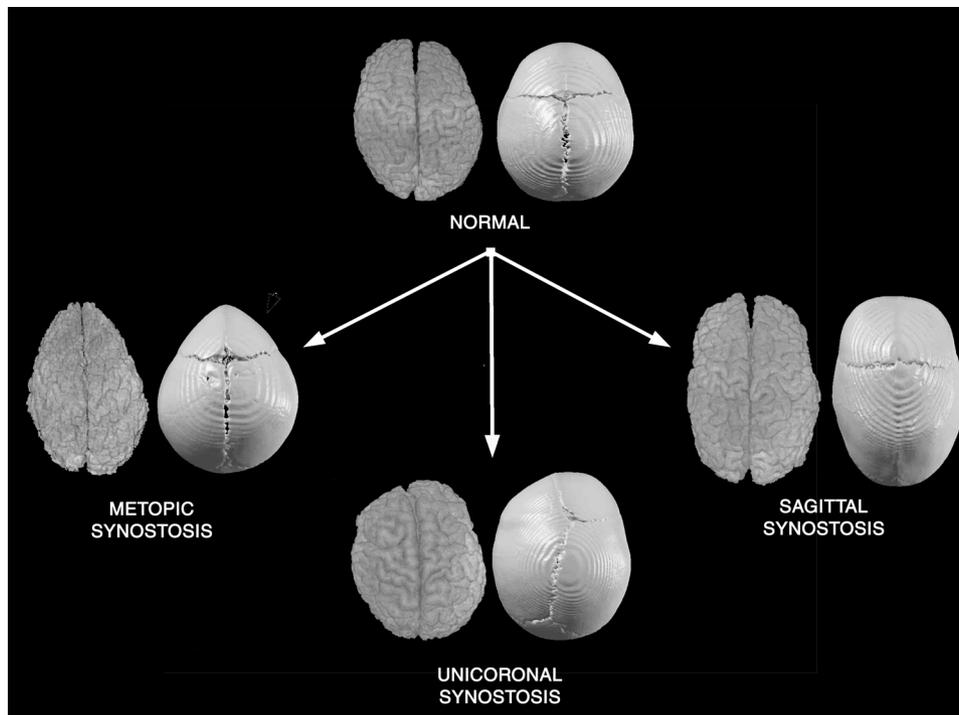


Fig. 1 Three-dimensional reconstructions of computed tomography (CT) scans and magnetic resonance images (MRI) of children affected with three forms of isolated craniosynostosis – sagittal, metopic and unicoronal, and one unaffected by craniosynostosis. These cases were chosen from our archive as examples of craniosynostosis phenotypes.

responsible for suture patency via signalling mechanisms (Opperman et al. 1993, 1995, 1998; Mooney et al. 2001). Though much knowledge can be gained by studying each tissue and the genes responsible for their development, the interplay of all developing tissues will eventually need to be considered.

Previous studies of the brain in craniosynostosis have reported primarily qualitative observations, or have measured relative volumes or sizes of the entire brain, with varying results. Differences in brain volume can indicate differences in absolute size, but these measures do not determine if the size difference is global or local. Put simply, volumetric studies cannot identify the locus of the difference (Fig. 2). Because generalized volume measures do not localize differences, they cannot suggest hypotheses about the processes responsible for size change, nor can they determine whether size changes are associated with changes in shape.

Neural organization refers to the spatial relationships between the individual component structures of the brain (Holloway, 1966). Although the brain is often viewed as a single organ, it is actually a heterogeneous structure in which different parts serve different functions (Harvey & Krebs, 1990; Aboitiz, 1996; Keverne

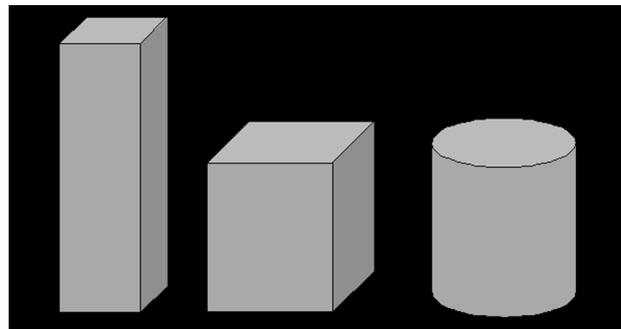


Fig. 2 Three forms of similar volumes, but of different shape, illustrate the inability of size measurements to provide information about differences in shape.

et al. 1996). These functionally distinct regions of the brain are considered to operate as a series of circuits, and any change in function or development of a specific region is associated with a gain or loss of circuitry in that area (Hofman, 1989; Aboitiz, 1996; Redies & Puelles, 2001). Localized changes in circuitry thus create differences in spatial relationships both within and between individual regions of the brain. The associated displacement of nearby structures results in localized form differences (Hofman, 1989; Harvey & Krebs, 1990; Aboitiz, 1996; Keverne et al. 1996; Barton & Harvey,

2000). Detailed studies of brain morphology summarize differences in spatial relationships between structures, and provide valid information pertaining to differences in neural organization. Changes in organization may reflect changes in developmental processes and in function, and therefore offer a window into the processes involved in the progression of craniosynostosis.

The purpose of this study is to quantify the differences in the shape of the brain in craniosynostosis and to relate these differences to what is known about skeletal morphology and craniofacial development. We do this through a quantitative investigation of patterns of neural organization in non-syndromic isolated sagittal (ISS) and isolated metopic (IMS) synostosis as compared to patterns in non-affected (NA) children using 3-D magnetic resonance images (MRI).

Materials and methods

Sample

The study sample consists of whole brain 3-D magnetic resonance images (MRIs) from 23 children of ages 7–39 weeks, scanned at St. Louis Children's Hospital. This number includes 10 children diagnosed with isolated sagittal synostosis (ISS), eight with isolated metopic synostosis (IMS) and five children unaffected by craniosynostosis (NA) of similar ages as those in the patient samples (Table 1). All scans were acquired in the sagittal plane using an MPRAGE sequence on a Siemens Magnetom Vision 3-D MRI scanner, with the following parameters: matrix = 256×256 , FOV ranging from 20.0 to 25.6 cm, slice thickness = 1.0 mm, and flip angle = 12° .

Data collection and analysis

The MR images were analysed using MEASURE software (Barta et al. 1997), written for a PC platform. This software allows visualization of MR image data in any three orthogonal planes and in a 3-D reconstruction. All non-neural tissue was stripped from each image slice

following a semi-automated procedure described by Aylward et al. (1997) and Buchanan et al. (1998). A 3-D reconstruction of the remaining brain tissue is then produced from the stripped slice data which can be manipulated in virtual space and viewed from any direction.

Anatomical landmarks represent biologically meaningful loci that can be repeatedly located with a high degree of accuracy and precision (Richtsmeier et al. 1995; Valeri et al. 1998). The relative locations of 3-D landmarks provide a repeatable geometric representation of form and allow investigation of spatial relationships between anatomical structures as well as localization of morphological differences to specific regions within structures (Richtsmeier & Lele, 1993). Therefore, the use of landmark data allows the analysis of multiple regions of the brain simultaneously as well as localization of differences to specific regions of the brain. A landmark-based analysis of form used in conjunction with MR imaging technology allows analysis of both internal and external 3-D morphology *in vivo*.

Using MEASURE, landmarks can be placed on any of the three planar views or directly on the reconstruction of the external surface, or on surfaces internal to the brain. Thirty-four landmarks were identified and defined for structures located on the cortical surface as well as subcortical structures derived from the developmental forebrain, midbrain and hindbrain (see Fig. 3 for definition and illustration). Measurement error was evaluated following methods presented previously (Aldridge et al. 2000) and minimized statistically by digitizing each specimen multiple times, and using the average of the various trials for analysis to reduce intra-observer error.

The landmark coordinate data recorded in MEASURE were analysed using Euclidean Distance Matrix Analysis, or EDMA (Lele & Richtsmeier, 2001), to compare each patient sample to the sample of unaffected children. Statistical tests of the null hypothesis of similarity of shapes for biologically relevant subsets of landmarks (reported as *P*-values in Table 2) and confidence interval testing for statistical evaluation of similarity of individual linear distances (using an α level of 0.10) are reported. These landmark subsets were designed to reflect specific anatomical regions or developmental origins of the brain.

Results

Results indicate that neural organization of the brains of children affected with both forms of craniosynostosis

Table 1 Sample sizes and age distributions

Diagnosis	<i>N</i>	Age (weeks)
Isolated sagittal synostosis (ISS)	10	14–39
Isolated metopic synostosis (IMS)	8	11–27
Non-affected (NA)	5	9–37
Total	23	9–39

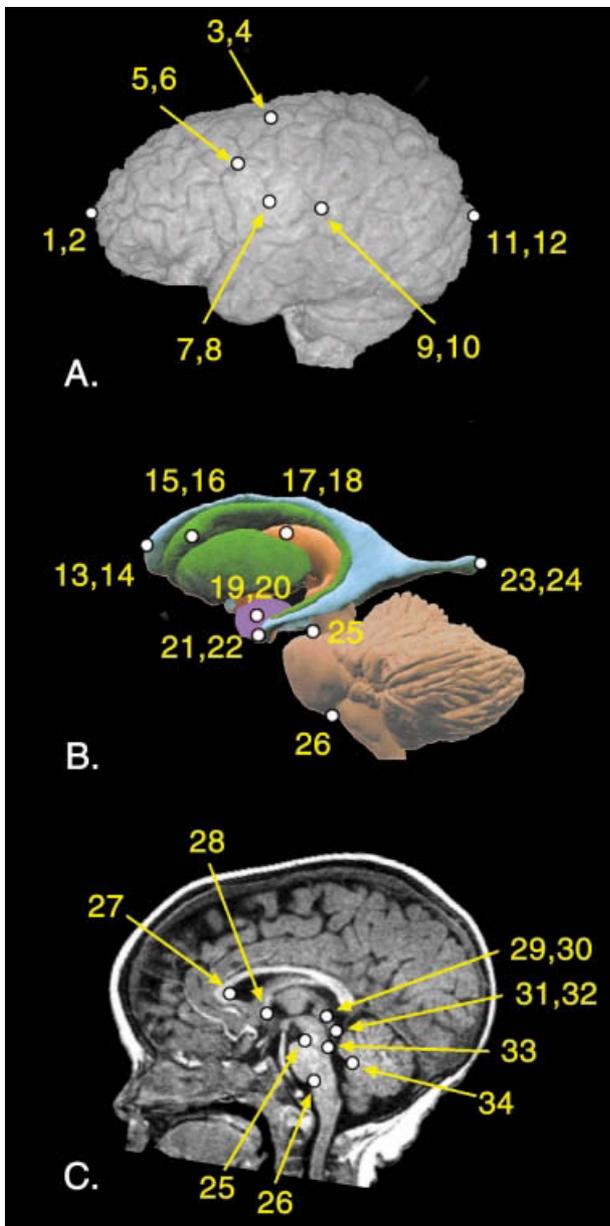


Fig. 3 Landmarks collected from the MRIs are illustrated on (A) 3-D MRI reconstruction of the cortical surface, (B) a model of subcortical structures and (C) a sagittal slice of an MRI of a child with ISS. Landmarks are as follows: 1,2 Frontal pole; 3,4 Posterior termination of the superior frontal sulcus; 5,6 Posterior termination of the inferior frontal sulcus; 7,8 Inferolateral termination of the central sulcus; 9,10 Posterior termination of the Sylvian fissure; 11,12 Occipital pole; 13,14 Anterior horn of the lateral ventricle; 15,16 Centroid of the head of the caudate nucleus; 17,18 Centroid of the thalamus; 19,20 Centroid of the amygdala; 21,22 Inferior horn of the lateral ventricle; 23,24 Posterior horn of the lateral ventricle; 25 Midline of the most superior aspect of the pons; 26 Midline of the most inferior aspect of the pons; 27 Midline of the genu of the corpus callosum; 28 Midline of the anterior commissure; 29,30 Centroid of the superior colliculus; 31,32 Centroid of the inferior colliculus; 33 Junction of the cerebral aqueduct and the 4th ventricle; 34 Posterior-most aspect of the 4th ventricle.

differs substantially from those of children without synostosis. These differences are evident not only in cortical morphology, but in subcortical morphology as well.

Brain morphology associated with ISS

Statistical testing was conducted using biologically relevant landmark subsets (where sample size exceeded the number of landmarks). All interlandmark distances that were statistically significantly different are illustrated in Fig. 4 and *P*-values for landmark subsets are reported in Table 2.

As would be expected from the scaphocephalic skeletal morphology in ISS, the underlying brain is significantly longer anteroposteriorly as indicated by analysis of the anatomical poles and the cortical surface subsets. This lengthening is particularly evident posteriorly. Though the subcortical forebrain and parieto-occipital regions do not differ significantly overall between ISS and NA, many individual interlandmark distances do differ significantly, again indicating a lengthening of the posterior structures, especially between the posterior horns of the lateral ventricles and the occipital poles. Results also indicate a significant mediolateral expansion of structures in the anterior frontal region. Conversely, analysis of the parieto-temporal region indicates a significant mediolateral constriction, notably on the left side. Finally, the hindbrain and base regions differ significantly between groups. Examination of confidence intervals for individual interlandmark distances indicates a postero-inferior expansion of these regions. In summary, the ISS brain is expanded along the A-P axis posteriorly and inferiorly, mediolaterally expanded in the anterior frontal region, and mediolaterally constricted temporally, as compared to the non-affected brain.

Brain morphology associated with IMS

Statistical testing was conducted using biologically relevant landmark subsets (where sample size exceeded the number of landmarks). *P*-values are listed in Table 2, and all interlandmark distances determined to be significantly different by confidence interval testing are illustrated in Fig. 5. Significant differences between the IMS sample and the NA sample are localized to a few specific anatomical regions. First, regions of the cortical surface differ significantly between the two

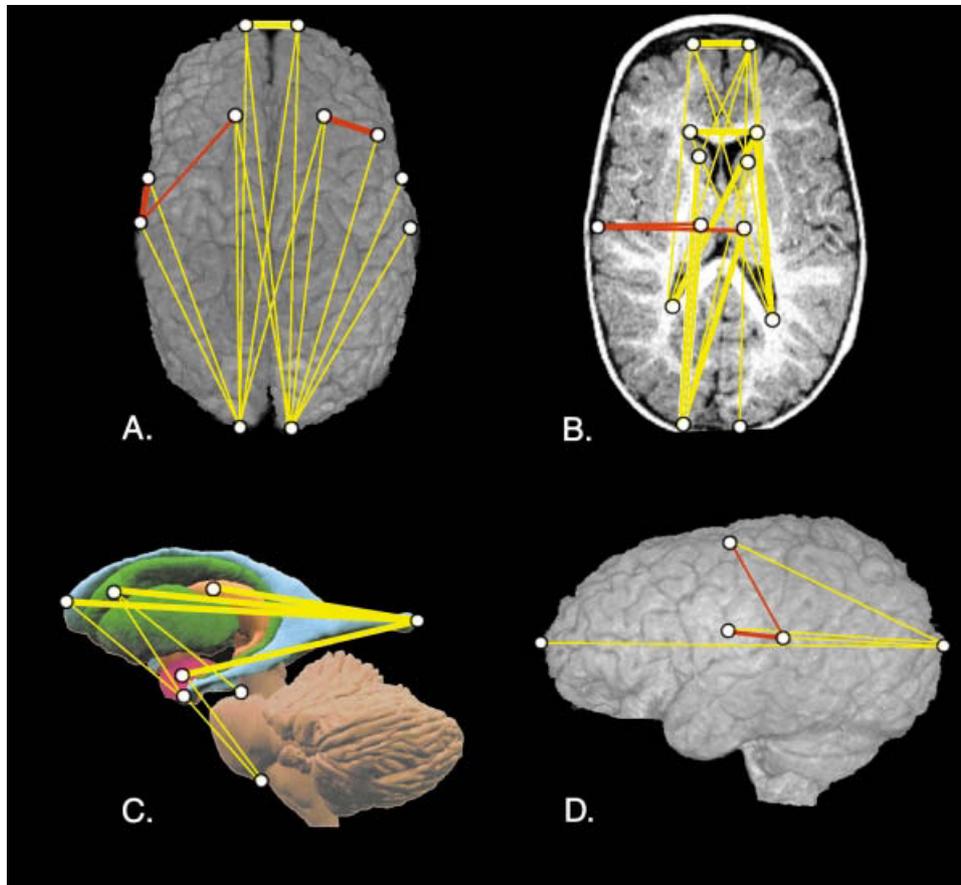


Fig. 4 Results of tests of the null hypothesis of similarity in form between ISS and NA samples. Yellow lines indicate significant differences which are greater in the ISS sample. Red lines indicate significant differences which are smaller in the ISS sample. Heavy lines indicate significant differences of 15% or greater; thin lines indicate significant differences of 5–14%. These results are illustrated on an individual chosen as an example from the study sample and is not meant to represent an average ISS phenotype.

Table 2 Results of statistical tests of the null hypothesis of similarity in form for the comparisons of ISS (column 3) and IMS (column 4) to the NA sample. Landmark subsets representing neuroanatomical components are used to ensure that the number of landmarks in each test is smaller than the number of individuals in the samples. Landmarks are illustrated in Fig. 3

Landmark subset	Landmarks included in subset	<i>P</i> -values, ISS	<i>P</i> -values, IMS
Cortical surface	1 2 3 4 9 10 11 12	0.030	0.005
Subcortical forebrain	15 16 17 18 19 20 23 24	0.214	0.697
Hindbrain	25 26 33 34	0.005	0.925
Midbrain	29 30 31 32	0.239	0.070
Ventricles	13 14 21 22 23 24	0.119	0.473
Poles	1 2 9 10 11 12	0.010	0.299
Frontal region	1 2 3 4 5 6 15 16	0.030	0.060
Parieto-occipital region	9 10 11 12 17 18 23 24	0.168	0.881
Parieto-temporal region	7 8 9 10 17 18 19 20 21 22	0.030	0.010
Basal region	19 20 21 22 25 26 33 34	0.020	0.198

samples. This difference is particularly evident in the frontal region, as would be expected, given the premature closure of the metopic suture and the trigonocephalic shape of the cranium. One interpretation of

these changes is that the frontal cortical surface has been displaced in a posterolateral direction. Secondly, the parieto-temporal region shows a significant mediolateral expansion in the anterior portion of this

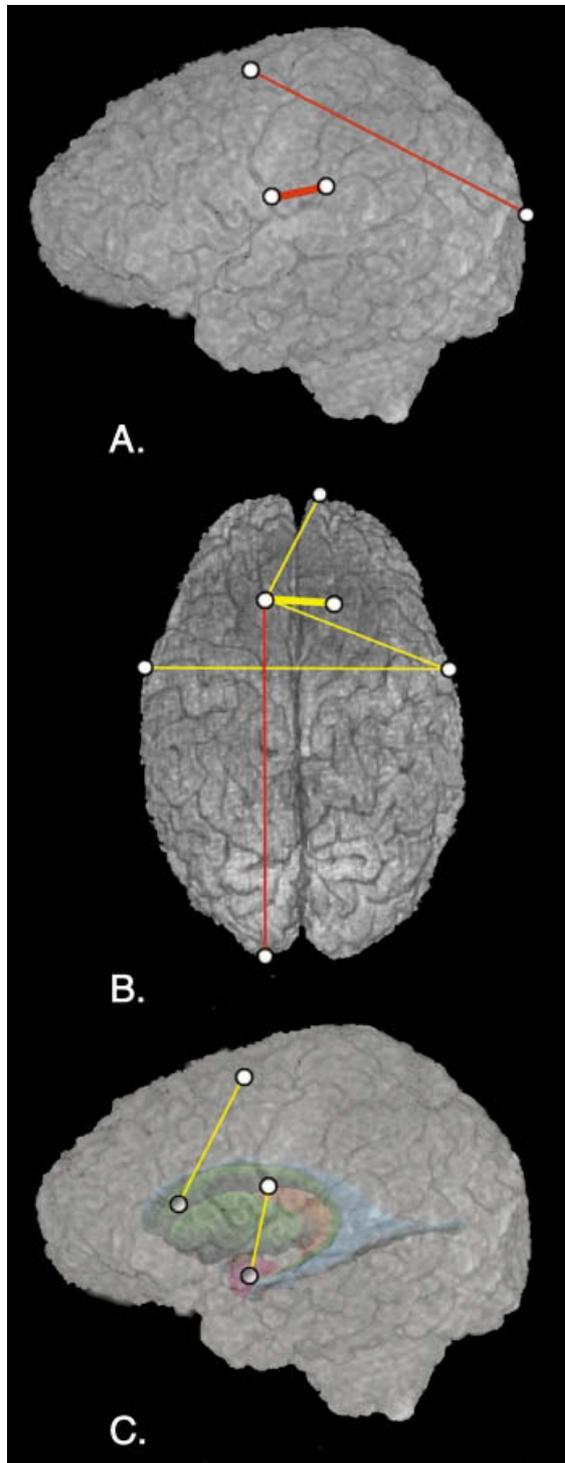


Fig. 5 Results of tests of the null hypothesis of similarity in form between IMS and NA samples. Yellow lines indicate significant differences which are greater in the IMS sample. Red lines indicate significant differences which are smaller in the IMS sample. Heavy lines indicate significant differences of 15% or greater; thin lines indicate significant differences of 5–14%. These results are illustrated on an individual chosen as an example from the study sample and is not meant to represent an average IMS phenotype.

region. Finally, the midbrain shows a substantial medio-lateral constriction in the IMS sample.

Our analysis indicates that the IMS brain displays a posterior and lateral rotation of the frontal cortex, a mediolateral constriction of the anterior parieto-temporal cortex, and a mediolateral constriction of the midbrain as compared to NA.

Discussion

The ultimate goal of scientific investigation of craniosynostosis is to understand its aetiology in order to determine how better to repair and ultimately prevent the condition. The identification of genetic mutations associated with various forms of craniosynostosis (e.g. Jabs et al. 1993; Steinberger et al. 1996; Johnson et al. 2000) has provided valuable tools for diagnosis, but little in the way of explanation of the process of premature suture closure. Thorough investigations of the phenotypic variation associated with this condition are necessary to meet this goal. The skull and cranial sutures are the most readily visible dysmorphic tissues in craniosynostosis, and surgical tools have been developed to change their configuration. The advent of MRI has allowed researchers a window to the underlying brain, leading to gross observations that the overall shape of the brain is also dysmorphic in craniosynostosis (Proudman et al. 1995; Tokumaru et al. 1996; Marsh et al. 1997). The results of this study show that the cortical and the subcortical organization of the brain is dysmorphic in craniosynostosis, and though many of the organizational changes mirror the skeletal dysmorphism, many do not. Preliminary analyses of the brain in isolated unicoronal synostosis and lamboid synostosis indicate significant and unique differences in the shape of the brain in these conditions as well (Aldridge et al. 2001).

ISS has been associated with an anteroposteriorly expanded neurocranium, increased head circumference, bony ridging over the sagittal suture, biparietal and bitemporal narrowing of the skull, inferior displacement of parietal bony tubers, and frontal and occipital prominences (Marsh & Vannier, 1986; Kaiser, 1988; Richtsmeier et al. 1991, 1998). In ISS, the overall scaphocephalic skull shape is mirrored in the observed anteroposterior expansion of the brain. However, this expansion is primarily observed in a posteriorly directed expansion of the brain, rather than an overall A-P increase. Neither the posterior nor inferior expansion

of the hindbrain and inferior forebrain structures determined from this study are evident from skull studies. Furthermore, the observed mediolateral expansion of the frontal region may be reflected in frontal bossing of the neurocranium, but quantification of this region has not shown significant differences from the normal condition (Richtsmeier et al. 1998).

Studies of the skeletal phenotype associated with IMS have described a midline ridge or keel overlying the metopic suture, ethmoidal hypoplasia, orbital hypotelorism and slanted orbital roofs, a shortened anterior cranial fossa, decreased superoinferior cranial height, bitemporal and bicoronal narrowing, and biparietal widening of the skull (Fernbach & Naidich, 1986; Friede et al. 1990; Posnick et al. 1994; Kolar & Salter, 1997; Zumpano et al. 1999). In the IMS brain, the mediolateral expansion of the anterior parieto-temporal region mirrors the trigonocephalic skull shape, but the decreased cranial vault height and anterior frontal constriction described in the IMS anterior cranial base and vault are not observed in neural structures. Instead, we observe a posterolateral rotation of the cortex in this region as might fit with the observation by Fernbach & Naidich (1986) of slanted orbital roofs in IMS. Furthermore, observed midbrain constriction cannot be reflected in skeletal morphology due to its location deep within the CNS.

Expression studies have shown that many of the genes implicated in craniosynostosis are expressed in the cranial skeleton, in the suture, and/or the dura underlying the suture. However, many of these genes are also expressed in other tissues, including neural tissues (Liu et al. 1995; Bourgeois et al. 1996; Thompson et al. 1991). Gene expression does not necessarily indicate a causative relationship; any single gene may be involved in a developmental cascade, or may have multiple targets. Genes are often expressed jointly with genes up- or downstream within a particular pathway, and effects of mutation may be mediated by modifier genes as well as environmental influences.

Conclusion

The results of this study show that the organization of the brain is dysmorphic in craniosynostosis, and that this dysmorphology includes both cortical and sub-cortical features. Our observations of CNS dysmorphology that are not reflected in skull shape indicate that the developing CNS may play a role in the production of

the craniosynostosis phenotype. The craniofacial complex is a system that includes the musculoskeleton, CNS and dura, and should be studied as a system. The mechanism triggering premature suture fusion may involve altered environmental conditions, anomalous genetic cascades, cell signalling mechanisms, biomechanical forces or some combination of these factors. Furthermore, the trigger may not necessarily be the same for each affected individual, depending upon the specifics of genetic background, variation in developmental timing of specific events, environment and biomechanical influences. Given that the central nervous system is intimately involved in skull development (Hanken & Thorogood, 1993), it should not be ignored in considering the aetiology of craniosynostosis. The fusion of the suture has been viewed as the point of origin in the production of the craniosynostosis phenotype, leading to the observed dysmorphogenesis associated with each craniosynostosis condition. Since the development of craniosynostosis is a process, not an isolated event, we may do better to consider fusion of the suture as one point in a pathway of processes and interactions rather than the defining feature and cause of the craniosynostosis phenotype.

Developmental processes are not clearly illustrated by anatomy at any given age. We cannot definitively determine the processes responsible for the production of the craniosynostosis phenotype through the assessment of craniofacial morphology after suture fusion has occurred. Instead of considering morphology at a fixed point in time, i.e. fusion of the suture, we must consider the course of development preceding and following fusion of the suture. This is not possible in humans, but already developed animal models (e.g. Mooney et al. 1994) and those currently being developed in other laboratories may be ideal for a developmental approach to craniosynostosis.

Over the course of vertebrate evolution, the trend has been toward a reduction in the number of elements in the cranial skeleton, termed 'Williston's Law' (Gregory et al. 1935; Sidor, 2001), which is viewed as a normal process. This rearrangement in the skull may correspond with alteration in the shape, size, or orientation of the brain or sense organs (Hanken, 1983). Given the intimate and integrated nature of skull and brain development, the most likely basis for the appearance of new skeletal phenotypes and disappearance of cranial elements over the course of evolution lies in the interactive processes of CNS and musculoskeletal

development. The aetiology of craniosynostosis, though a pathological condition in humans, may follow mechanisms similar to those responsible for the evolution of skull phenotypes.

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