A novel transgenic mouse model of fetal encephalization and craniofacial development

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Synopsis

There are surprisingly few experimental models of neural growth and cranial integration. This, and the dearth of information regarding fetal brain development, detracts from a mechanistic understanding of cranial integration and its relevance to the ontogenetic and interspecific patterning of the form of the skull. To address this shortcoming, our research uses transgenic mice expressing a stabilized form of β-catenin to isolate the effects of encephalization on the development of the basi- and neuro-cranium. These mice develop highly enlarged brains due to an increase in neural precursor cells, and differences between transgenic and wild-type mice are predicted to result solely from variation in relative brain size. By focusing on prenatal growth, this project adds to our understanding of a critically important period when major structural and functional interrelationships are established in the skull. Comparisons of wild-type and transgenic mice were performed using microcomputed tomography (microCT) and magnetic resonance imaging (MRI). These analyses show that the larger brains of the transgenic mice are associated with a larger neurocranium and an altered basicranial morphology. However, body size and postcranial ossification do not seem to be affected by the transgene. Comparisons of the rate of postcranial and cranial ossification also point to an unexpected effect of neural growth on skull development: increased fetal encephalization may result in a compensatory decrease in the level of cranial ossification. Therefore, if other life-history factors are held constant, the ontogeny of a metabolically costly structure, such as a brain, may occur at the expense of other cranial structures. These analyses indicate the benefits of a multifactorial approach to cranial integration using a mouse model.

Introduction

Neural expansion is often hypothesized to influence craniofacial form in humans and other mammals. For instance, the evolution of relatively greater brain size in early Homo is thought to have altered the cranial vault, basicranium, and facial skull of subsequent hominid lineages. Therefore, it is critical for the study of human evolution that we understand the morphological changes that are correlated with, and potentially caused by, encephalization. Changes in relative brain size are also seen across ontogeny. The degree of encephalization is remarkably greater during fetal and early postnatal growth, but neither the form of the skull nor the processes of skull growth at this stage of development are well studied. While enlargement of the brain is a long-recognized hallmark of human modernity, as well as prenatal development, explanations for correlated aspects of skull form are less clearly understood.

This study uses a transgenic mouse model for encephalization to investigate the interactions between relative brain size and cranial morphology, in particular, the morphology of the cranial base. The transgenic mice overexpress a truncated form of β-catenin in their neuroepithelial precursors, which increases the number of progenitor cells of the central nervous system (Chenn and Walsh 2002). These transgenic mice develop highly enlarged brains with increased cerebral cortical surface area and folds similar to the sulci and gyri in human and nonhuman primates (Chenn and Walsh 2002, 2003).

A major benefit of this mouse model is that neocortical proliferation of cells is thought to be a
primary mechanism of neural encephalization in primate and nonprimate mammals (Caviness et al. 1995; Rakic 1995). Moreover, as the altered β-catenin transgene is expressed only in neuroepithelial precursors, craniological differences between transgenic and wild-type mice are hypothesized to be a consequence of a relatively larger, or more encephalized, brain. Another unique aspect of this study is its focus on fetal encephalization. The prenatal period is hypothesized to have a singular influence on the skull and is a time when many important evolutionary changes and pathological conditions are thought to arise.

**Encephalization in human and primate evolution**

The extremely encephalized brain is one of the most distinctive traits of modern humans. Increases in both relative and absolute brain size have been documented throughout the evolution of the genus *Homo*, with modern humans being notable even among *Homo* for their high degree of encephalization (Ruff et al. 1997; Bruner et al. 2003; Rightmire 2004). Increases in relative brain size have likewise marked the origins of several taxa leading to modern humans. For instance, the early evolution of primates, anthropoids, and *Homo* all have been characterized by spectacular shifts in expansion of the brain, and such increases have been linked to morphological changes in the cranial vault, basicranium, circumorbital region, and facial skull (Radinsky 1974; McHenry 1976; Jerison 1979; Shea 1987; Martin 1990; Ruff et al. 1997; Barton 1998; Bruner et al. 2003; Rightmire 2004).

Because the brain closely overlies the basicranium, changes in neural proportions are predicted to affect the growth and orientation of the base of the skull. Thus, basicranial flexion—the extent to which the anterior cranial base is deflected posteroventrally toward the posterior cranial base—should covary with encephalization of the brain in primates and other mammals (Ross and Ravosa 1993; Strait 1999; Strait and Ross 1999; Lieberman et al. 2000). Across major primate clades (primates, haplorhines, platyrrhines, and catarrhines), the angle of basicranial flexion is negatively correlated with relative brain size (Ross and Ravosa 1993; Spoor 1997). However, this relationship does not hold across lower-level taxa such as hominoids, hominids, and modern *Homo sapiens* (Ross and Ravosa 1993; Ross and Henneberg 1995). Moreover, depending on the analysis, humans possess either a flatter cranial base than expected for a hominid of its brain size (Ross and Henneberg 1995) or an appropriate amount of basicranial flexion based on data for nonhominid primates or anthropoids (Spoor 1997; McCarthy 2001). These seemingly contradictory findings may be due to differing methods of calculating relative brain size and basicranial flexion in *H. sapiens* (Lieberman and McCarthy 1999; Lieberman et al. 2000; McCarthy 2001) as well as small sample sizes for hominoids and hominids (Ross and Ravosa 1993).

**Encephalization in ontogeny**

The fetal brain and neurocranium are relatively larger than the corresponding adult structures (Dobbing and Sands 1973; Epstein 1979; Deacon 1990; Beals and Joganic 2004). Therefore, the prenatal period is an especially important stage for investigating the role of brain size in patterning craniofacial development. Changes in relative brain size can be expected to affect the skull base across ontogeny. Across primate species and human evolution, a relatively larger brain is associated with a more flexed cranial base (Ross and Ravosa 1993; Lieberman et al. 2000). However, this is not the case during all stages of prenatal development (Jeffery and Spoor 2002, 2004; Jeffery et al. 2007). Furthermore, while most increases in brain size occur prenatally or in infancy (Epstein 1979), and brain growth ceases in humans by age 7 (Jolicoeur et al. 1988; Cabana et al. 1993), some studies have noted postnatal changes in basicranial flexion after this time (Bjork 1955; May and Sheffer 1999). Others have found no significant increase in flexion after eruption of the first molar, instead showing that human basicrania flex rapidly, with the basicranial angle decreasing during the first two postnatal years (George 1978; Lieberman and McCarthy 1999), the period marked by rapid growth of the brain (Epstein 1979; Lieberman and McCarthy 1999). In contrast to the human pattern, nonhuman primate basicrania extend postnatally after cessation of brain growth, suggesting that postnatal cranial base extension in these taxa might be influenced by processes of facial growth (Michejda 1975; Lieberman and McCarthy 1999; May and Sheffer 1999; Lieberman et al. 2000). Given the apparent lack of correspondence between interspecific and ontogenetic patterns, as well as between prenatal and postnatal data, it is unclear which human cranial traits, if any, directly result from neural expansion during development.

As a large proportion of ontogenetic increases in brain size occur prenatally, it is particularly beneficial to study fetal growth to investigate the influence of relative brain size on the morphology of the skull. If there is little variation in relative brain size during the postnatal period, then the role of encephalization...
in affecting postnatal variation in cranial morphology will be less important than that of other influences that occur largely postnatally. Indeed, among factors posited to affect basicranial flexion, prenatal phases of ontogeny are singularly characterized by significant increases in expansion of the brain. This is in contrast to postnatal growth, in which increases in the size of the brain are diminished and other influences on basicranial flexion are much more pronounced (i.e., masticatory forces and feeding behavior, respiratory activity, and posture and locomotion). Thus, if basicranial flexion is unrelated to prenatal neural expansion, then observed interspecific associations with encephalization must be spurious and due instead to the presence of other factors characteristic of postnatal ontogeny.

Relative size of the brain and dysmorphology

Interspecific comparisons and studies of cranial dysmorphology in humans suggest that patterns of cranial growth and form—both normal and abnormal patterns—are determined prenatally (May and Sheffer 1999a, 1999b; Nuckolls et al. 1999; Wong et al. 2001; Ackermann and Krovit 2002; Zumpano and Richtsmeier 2003; Zollikofer and Ponce de Leon 2004). For example, microcephalic and anencephalic skulls are often reported as having flatter cranial bases as a result of relatively diminished growth of the brain in utero (Weidenreich 1941; Babineau and Kronman 1969; Melsen and Melsen 1980). However, some report a more flexed basicranial angle in microcephalics or anencephalics (Fields et al. 1978; Trenouth 1989). As dysmorphologies, like interspecific differences in morphology, are associated with an early alteration of fetal growth patterns in the skull (Siegel et al. 1991; Mooney et al. 1992; Hallgrimsson et al. 2004; Cox et al. 2006), a characterization of prenatal growth is essential for our understanding of the basis of normal human cranial organization and evolution as well as the onset and progression of dysmorphologies (Plavcan and German 1995).

Studies of ontogeny, and perturbations therein, can shed light on the developmental mechanisms underlying evolutionary transformations. Thus, an understanding of prenatal development can elucidate patterns of variation during phylogenetic events (Gilbert et al. 1996; Gerhart and Kirschner 1997; Minelli 2003; Cobb and O’Higgins 2004). For example, it is proposed that during the evolution of the human brain, genes that regulate neural size during development (such as ASPM and MCPH1, two genes involved in microcephaly) were differentially targeted by natural selection (Gilbert et al. 2005). As the β-catenin transgene likewise regulates the proliferation of neural precursor cells, this mouse model has significant potential for increasing our understanding of encephalization and its role in patterning early craniofacial development.

The main goal of this article is to test the hypothesis that prenatal increases in relative brain size are correlated with variation in cranial form. The β-catenin transgenic mouse model is especially suited to address this hypothesis. Because the expression of the transgene and the corresponding increase in brain size begins early in development, this mouse model can be used to study the interactions between brain size and major regions of the skull during prenatal development. The prenatal period is critically important for understanding craniofacial organization—craniofacial organization occurs prenatally, and many significant craniofacial anomalies and evolutionary shifts in neural expansion and cranial morphology arise at this time (Siegel et al. 1991; Mooney et al. 1992; Plavcan and German 1995; May and Sheffer 1999a, 1999b; Nuckolls et al. 1999; Wong et al. 2001; Ackermann and Krovit 2002; Hallgrimsson et al. 2004; Zollikofer and Ponce de Leon 2004; Cox et al. 2006).

Mice have long been used as model organisms in scientific research due to the fact that they have a relatively low cost, a short breeding cycle, a well-known genome and developmental sequence, and genetic and physiological similarities to humans. With the development of various types of transgenic mice, the mouse genome can be precisely manipulated, and we can more accurately establish causal relationships between genotypes and phenotypes. This specific mouse model has several advantages for the study of the relationships between neural growth and cranial morphology. Because the β-catenin transgene is expressed only in neural precursors, the effects of relative brain size on the skull can be isolated. Furthermore, the wild-type and transgenic mice are genetically similar, and are often littermates, which controls for variation in genetic and in utero effects on cranial morphology. Finally, the use of a prenatal model controls for postnatal influences on cranial form (such as mastication, respiration, and posture; see DuBrul 1950; Riesenfeld 1966; Radinsky 1968; Beecher et al. 1983; Kiliaridis et al. 1985; Jaanuson 1987; Scarano et al. 1998; Ravosa et al. 2008) and also focuses on a period of development when relative brain size is predicted to have the most singular, and potentially significant, effect on cranial morphology.
Materials and methods

Samples, measures, and collection of data

The transgenic mice used are the offspring of two lineages of mice, one possessing the floxed β-catenin allele (β-catenin<sup>loxEx3</sup>) and the other expressing cre-recombinase under the control of the enhancer element in the second intron of the nestin gene. The cre-induced deletion of exon 3 of the β-catenin allele in the transgenic mice from this crossing result in an activated β-catenin protein (β-catenin<sup>+/C1Ex3</sup>), lacking N-terminal sequences necessary for β-catenin degradation (Harada et al. 1999; Chenn and Walsh 2002; Zechner et al. 2003). As the cre-recombinase is under the control of the enhancer contained in the nestin gene, the activated β-catenin protein is expressed only in progenitor cells of the central nervous system (Yaworsky and Kappen 1999) (note that the naturally occurring gene for β-catenin is not affected, and β-catenin is expressed normally in all other cells). β-catenin is an intracellular protein that regulates the expression of signaling molecules that regulate cell division and cell differentiation (Parr et al. 1993; Peifer and Polakis 2000). An activated β-catenin protein in the progenitor cells of the central nervous system results in a twofold increase in the number of progenitor cells that divide, rather than differentiate, leading to an increase in the size of the neural precursor pool, and ultimately a larger brain (Chenn and Walsh 2002).

A previous investigation has shown that β-catenin transgenic mice develop larger and more convoluted brains than do their wild-type counterparts by embryonic day 15.5 (E15.5), midway through cortical neurogenesis (Chenn and Walsh 2002). This study examines wild-type and transgenic mice at E16.5, E17.5, and as neonates (p0). The sample sizes in each age cohort are as follows: E16.5, 9 wild-type and 10 transgenic; E17.5, 9 wild-type and 2 transgenic; p0, 11 wild-type and 13 transgenic. Body mass and crown-rump length were measured (to the nearest gram and millimeter, respectively). After measurements of body size were recorded, the head and one forelimb from each specimen were fixed in paraformaldehyde. A subset of specimens was imaged using microCT and MRI. See Table 1 for a list of all measurements obtained.

The forelimbs of 17 mice and the heads of 24 mice were imaged using a Scanco Medical MicroCT 40 system (Fig. 1). The microfocus X-ray tube was operated at 45 kV and 177 μA, and the beam passed through a 0.13 mm thick Be window on the X-ray tube and through a 0.50 mm thick Al filter before

### Table 1 List of measurements taken from specimens

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass (g)</td>
<td>Measured to the nearest (g)</td>
<td>E16.5: 6 wt, 10 tg; E17.5: 9 wt, 2 tg; p0: 11 wt, 13 tg</td>
</tr>
<tr>
<td>Crown-rump length (cm)</td>
<td>Measured to the nearest (mm)</td>
<td>E16.5: 9 wt, 10 tg; E17.5: 9 wt, 2tg; p0: 11 wt, 11 tg</td>
</tr>
<tr>
<td>Forelimb ossification (mm&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>The amount of mineralized tissue in the forelimb above the threshold level of 160 (E17.5, p0) or 130 (E16.5)</td>
<td>E16.5: 2 wt, 2 tg; E17.5: 2 wt, 2tg; p0: 4 wt, 5tg</td>
</tr>
<tr>
<td>Humeral length (mm)</td>
<td>The length of the humerus measured in the center of the shaft of the humerus</td>
<td>E17.5: 2 wt, 2tg; p0: 4 wt, 4tg</td>
</tr>
<tr>
<td>Cranial ossification (mm&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>The amount of mineralized tissue in the skull above 120</td>
<td>E16.5: 3 wt, 2tg; E17.5: 2 wt, 1tg; p0: 8 wt, 8tg</td>
</tr>
<tr>
<td>Brain size (mm&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>The sum of the visible brain area in every 6th slice, multiplied by 6 x the interslice distance</td>
<td>E16.5: 1 wt, 2tg; p0: 7 wt, 5tg</td>
</tr>
<tr>
<td>Cranial-vault length (mm)</td>
<td>The distance between landmarks found on MR images (opisthion to nasion)</td>
<td>E16.5: 2 wt, 2tg; p0: 9 wt, 8tg</td>
</tr>
<tr>
<td>Cranial-vault width (mm)</td>
<td>The distance between landmarks found on MR images (euryon to euryon)</td>
<td>E16.5: 1 wt, 2tg; p0: 7 wt, 6tg</td>
</tr>
<tr>
<td>Cranial base length (mm)</td>
<td>The sum of the individual lengths of the basicranial bones and the intervening synchondroses</td>
<td>E16.5: 2 wt, 2tg; p0: 8 wt, 6tg</td>
</tr>
<tr>
<td>Cranial base angles (°)</td>
<td>The angles between the presphenoid and basipectenoid, basisphenoid and basiocipital, cranial base and cribriform plate, and cranial base and foramen magnum</td>
<td>E16.5: 2 wt, 2tg; p0: 9 wt, 8tg</td>
</tr>
</tbody>
</table>

tg, β-catenin transgenic mouse; wt, wild-type mouse.
encountering the sample. Reconstruction was with 17 $\mu$m isotropic voxels (volume elements) using 250 projections of 512 samples and 0.2 s integration time per projection. The total bone volume of the forelimb was measured as the amount of mineralized tissue above the threshold value of 160 [The value of the threshold is on a linear scale from 0 to 1000 which corresponds to linear attenuation coefficients $\mu$ of 0–8 cm$^{-1}$, respectively]. When evaluating the microCT data to measure ossification, care was taken not to include any of the scapula (when evaluating the forelimb) or the vertebrae (when evaluating the skull). The length of the humerus was measured from the microCT images in E17.5 and p0 mice by measuring the length in the midline of the visible bone on the slice midway through the humerus (Table 1). There was not enough mineralized bone to accurately measure humeral length at E16.5.

Heads of E16.5 and newborn mice were imaged on a Bruker 14.1 T MR microimager operating at a proton frequency of 600 MHz (Fig. 1). T2-weighted MR images were obtained using a 3D rapid acquisition with relaxation-enhancement (RARE) pulse sequence using the following parameters: TR/effective TE = 3000 ms/60 ms, 2 averages, RARE factor (echo train length) = 8, field of view = 14 mm $\times$ 14 mm $\times$ 8 mm, and image matrix = 256 $\times$ 256 $\times$ 150. This yielded a voxel size of $\sim$55 $\times$ 55 $\times$ 53 $\mu$m$^3$.

Linear and angular measurements were obtained from MR images in the software program VolView. The volume of the brain was estimated by measuring the area of the brain in every sixth slice using the software program ImageJ, adding the areas, then multiplying by $6 \times 0.053$ mm (the distance between the slices). As some specimens were only imaged in a midsagittal section, brain volume could only be measured for 15 mice (Table 1).

MR images were used to measure linear and angular dimensions. The linear measurements were cranial-vault length (opisthion to nasion), maximum cranial-vault width (euryon to euryon), and basicranial length (the sum of the individual lengths of the presphenoid, basisphenoid, and basioccipital bones, together with the synchondroses between them, measured in the midsagittal plane). Using MR images in the midsagittal plane, four angles describing cranial base morphology were measured: (1) the angle between the presphenoid and the basisphenoid, (2) the angle between the basisphenoid and the basioccipital, (3) the angle between the cranial base (defined as the line drawn from the front of the presphenoid to the back of the basisphenoid) and the cribiform plate, and (4) the angle between the cranial base and the foramen magnum (Table 1).

**Statistical analyses**

Controlling for age, two-tailed Mann–Whitney U-tests were used to compare wild-type and transgenic mice in the following measurements: body mass, crown-rump length, forelimb ossification, humeral length, brain volume, skull ossification, cranial-vault length, cranial-vault width, cranial base length, angle between the presphenoid and basisphenoid, angle between the basisphenoid and basioccipital, angle between the cranial base and cribiform plate, and angle between the cranial base and foramen magnum (Table 1). Because of small sample sizes, a nonparametric test was used. There were no significant differences in body mass, crown-rump length, forelimb ossification, or humeral length (see below) and hence cranial measurements were compared without first adjusting for size or another measure of overall development.

**Results**

**Somatic and postcranial development**

The transgenic mice did not differ from their wild-type counterparts in overall body size: there were no significant differences between wild-type and transgenic mice in either body mass or crown-rump length at E16.5, E17.5, or p0 (Table 2, Fig. 2).
Wild-type and transgenic mice also had similar amounts of ossified bone in the forelimb and did not differ in the length of the humerus (Table 2, Fig. 2).

**Cranial morphology**

Newborn transgenic mice were found to have significantly larger brains as compared to wild-type mice; a significant difference was not found between wild-type and transgenic mice at E16.5 (Table 3, Fig. 3). Wild-type and transgenic mice did not differ in the amount of ossified bone in the skull at E16.5; however, newborn wild-type and transgenic mice did differ significantly in cranial ossification (Table 3, Fig. 3).
The MR images of E16.5 and neonate mice were used to measure length and width of the cranial vault. Transgenic mice had significantly larger values as compared to wild-type mice for each of these distances at p0, but not at E16.5 (Table 4, Fig. 4). There was no significant difference in length of the cranial base between wild-type and transgenic mice at either E16.5 or p0 (Table 4, Fig. 5). Although wild-type and transgenic mice did not differ in cranial-base length, they did differ in morphology of the base of the cranium. Four angles in the cranial base were measured (Fig. 6). Two of the four angles measured differed significantly between wild-type and transgenic specimens in newborn mice (Table 5).

**Table 3** Brain size and cranial ossification

<table>
<thead>
<tr>
<th></th>
<th>E16.5</th>
<th>E17.5</th>
<th>p0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>wt</td>
<td>tg</td>
<td>P-value</td>
</tr>
<tr>
<td>Brain size (mm³)</td>
<td>5.09 (4.13)</td>
<td>10.69 (4.13)</td>
<td>0.221</td>
</tr>
<tr>
<td>Volume of ossified bone in skull (mm³)</td>
<td>1.46 (0.88)</td>
<td>0.85 (0.91)</td>
<td>0.248</td>
</tr>
</tbody>
</table>

Cells contain mean and standard deviation (in parentheses) or P-value of Mann–Whitney U-test comparing wild-type and transgenic means. Statistically significant P-values (P<0.05) are italicized.
The angles between the cranial base and the cribiform plate and between the cranial base and the foramen magnum are smaller (i.e., more flexed or less retroflexed) in transgenic mice than in wild-type mice.

**Discussion**

An understanding of prenatal skull development and the complex interactions among various cranial components (e.g., cranial base and brain) is fundamental for testing hypotheses about the link between cranial development and evolution as well as the etiology of cranial dysmorphologies. The highly encephalized brains of the β-catenin transgenic mice facilitate their use as an experimental model in which to study ontogenetic (and potentially evolutionary) transformations in the skull due to neural expansion. The prenatal period in all mammals is similar in being a time of considerable neural development, and thus encephalization likely has a larger influence on skull form than during postnatal ontogeny. An examination of this phase in mice is more relevant to the human condition than it would be in a postnatal study, in which interspecific differences in skull form may be influenced by marked taxic variation in masticatory plasticity, respiration, and posture. Furthermore, the β-catenin transgenic mice have enlarged brains from early in prenatal development, which makes this mouse model ideal for studying interactions between major regions of the skull during fetal development. Indeed, a study of fetal development is necessary because cranial organization occurs prenatally, and many significant craniofacial anomalies and evolutionary shifts in neural expansion arise before birth.

Wild-type and β-catenin transgenic mice were compared at ages E16.5, E17.5, and p0. At none of these ages did wild-type and transgenic mice differ significantly in overall body size (as measured by body mass and crown-rump length) or postcranial development (as measured by forelimb ossification and humeral length). These measures of development indicate that the β-catenin transgenic mice grow at a normal rate as compared to their wild-type littermates. Therefore, cranial dimensions were compared

### Table 4 Length and width of the cranial vault and the length of the cranial base

<table>
<thead>
<tr>
<th></th>
<th>E16.5</th>
<th>p0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>wt</td>
<td>tg</td>
</tr>
<tr>
<td>Cranial-vault length (mm)</td>
<td>6.29 (0.28)</td>
<td>6.83 (0.66)</td>
</tr>
<tr>
<td>Cranial-vault width (mm)</td>
<td>4.90</td>
<td>5.14 (0.28)</td>
</tr>
<tr>
<td>Cranial base length (mm)</td>
<td>3.61 (0.23)</td>
<td>3.97 (0.35)</td>
</tr>
</tbody>
</table>

Cells contain mean and standard deviation (in parentheses) or P-value of Mann–Whitney U-test comparing wild-type and transgenic means. Statistically significant P-values (P < 0.05) are italicized.

### Table 5 Cranial base angles

<table>
<thead>
<tr>
<th></th>
<th>E16.5</th>
<th>p0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>wt</td>
<td>tg</td>
</tr>
<tr>
<td>Presphenoid–basisphenoid angle (°)</td>
<td>183.45 (3.87)</td>
<td>185.60 (1.21)</td>
</tr>
<tr>
<td>Basisphenoid–basioccipital angle (°)</td>
<td>176.46 (0.87)</td>
<td>173.90 (0.34)</td>
</tr>
<tr>
<td>Cribriform plate angle (°)</td>
<td>192.55 (2.52)</td>
<td>184.89 (4.05)</td>
</tr>
<tr>
<td>Foramen magnum angle (°)</td>
<td>252.05 (5.75)</td>
<td>246.17 (1.78)</td>
</tr>
</tbody>
</table>

Cells contain mean and standard deviation (in parentheses) or P-value of Mann–Whitney U-test comparing wild-type and transgenic means. Statistically significant P-values (P < 0.05) are italicized.

![Box-plots for length of the cranial base.](https://academic.oup.com/doi/fig/10.1093/icb/icz083)
between wild-type and transgenic mice of the same age group without adjusting for body size.

As would be expected from the action of the transgene, newborn transgenic mice had significantly larger brains than did their wild-type counterparts. The average size of the brain in transgenic mice at both E16.5 and p0 was more than double that for wild-type mice, although the difference in brain size was not statistically significant in E16.5 mice, probably due to the small sample sizes. Along with their larger brain size, transgenic mice showed an increase in the length and width of the neurocranium.

At each of the three ages examined, transgenic mice had lower levels of ossified bone in the skull as compared to their wild-type counterparts. The different levels of cranial ossification was only statistically significant in neonates. This difference in ossification levels between wild-type and transgenic mice could indicate that, if other life history parameters are held constant, the ontogeny of the brain (an extremely metabolically costly structure) may occur at the expense of other structures of the skull.

The cranial base was examined via MRI in E16.5 and p0 mice. Brain size relative to basicranial length is often thought to influence basicranial flexion (Ross and Ravosa 1993; Lieberman et al. 2000); transgenic mice exhibited an increase in brain size, but not in basicranial length, leading to an increase in brain size relative to the cranial base (and relative to body size), in addition to an absolutely larger brain. In fact, transgenic mice did have an altered pattern of cranial-base angulation. Newborn transgenic mice were found to have a more horizontally inclined cribriform plate as compared to wild-type mice, indicating that the encephalized brain may affect the

Fig. 6 Boxplots for cranial base angles. (A) Angles between presphenoid and basisphenoid, (B) basisphenoid and basioccipital, (C) cranial base and cribriform plate, and (D) cranial base and foramen magnum.
positioning of the face via a change in angulation of the cribriform plate. Transgenic mice also had a more horizontally inclined foramen magnum; a more horizontal and anterior foramen magnum is a trait often associated with an upright posture (Dart 1925; Schultz 1942, 1955; DuBrul 1950; Le Gros Clark 1950; Ashton and Zuckerman 1951). During the postnatal period, the increase in flexion at these two sites might constrict the size of the pharynx and affect swallowing and respiration. Although the need to maintain a patent airway could limit the amount of flexion during the evolution of basicranial shape, this constraint is not present in this particular model system.

The wild-type and transgenic mice already seem to differ in brain size and in the angles between the basicranium and the cribriform plate and foramen magnum by E16.5 (Figs 3 and 6). Therefore, the results of this study cannot determine whether the increased flexion is due to the enlarged brain flexing the basicranium or to it inhibiting retroflexion (Lieberman et al. 2000; Gould 2002; Ross et al. 2004; Jeffery et al. 2007). A future analysis including a younger cohort may shed light on this issue.

The morphological changes seen in the cranial base of the β-catenin transgenic mice show that an increase in relative brain size during prenatal development can affect the shape of the basicranium in the absence of other factors affecting the growth of the skull. If future studies of this mouse model consistently show that traits such as basicranial flexion are associated with relative brain size, that finding would be important for the systematic analysis of primate cranial morphology. For instance, if various craniofacial characters are found to be structurally, and perhaps functionally, correlated with encephalization, then it would be unwise to consider such features as independent characters when scored for phylogenetic analysis (Lieberman et al. 2000; Strait 2001). Along with basicranial flexion, orbital frontation is also positively correlated with increased relative brain size, both ontogenetically and phylogenetically, although the extent to which variation in orbital position is independent of variation in relative brain size and basicranial flexion is unclear (Ravosa et al. 2000, 2006; Ravosa and Savakova 2004; Heesy 2005). Again, an understanding of the correlations of characters would not only provide information about the patterning of cranial evolution during phylogenetic shifts in encephalization (e.g., origins of primates, anthropoids, and Homo), it also would be useful in identifying independent characters for systematic analyses.

A need for understanding the cranial correlates of encephalization is underscored by the recent discovery of Homo floresiensis (Brown et al. 2004; Morwood et al. 2005). Although this diminutive Late-Pleistocene hominid has an endocranial capacity of only about 400 cc, considerable debate exists as to whether it is a remarkably primitive recent species or simply a representative of a microcephalic family (Falk et al. 2005, 2007; Weber et al. 2005; Martin et al. 2006a, 2006b). Studying the interactions of encephalization and cranial form in a mouse model can shed light on developmental patterns of integration directly relevant to interpretation of the evolutionary morphology of H. floresiensis and other extinct hominids.

**Conclusions**

This study compared β-catenin transgenic mice to genetically similar wild-type mice at two prenatal ages and as neonates. Transgenic and wild-type mice did not differ in their overall body size or in terms of postcranial development. The two groups also had similar basicranial lengths, although transgenic mice had statistically significantly larger brains than did wild-type mice, with correspondingly longer and wider neurocrania. The encephalized brain of the transgenic mice was accompanied by reduced cranial ossification in the neonate age group. Finally, the morphology of the cranial base was affected by the enlarged brain of the transgenic mice, as evidenced by a more horizontal cribriform plate and foramen magnum in the transgenic mice as compared to the wild-type mice.

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Transgenic mouse model for encephalization


