Increased prenatal brain growth in a transgenic mouse model decreases cranial development: An expensive tissue hypothesis for the skull

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Our experimental study investigates the relationship between brain growth and cranial development in ß-catenin transgenic mice with remarkably increased levels of prenatal neurogenesis. To evaluate associated changes in skull form and control for variation in maternal resources among mouse litters, we directly compare data from transgenic and wild-type littermates. Ossification patterns in the limbs and skull were also analyzed to control for within-subject variation in skeletal formation. Transgenic mice, with relatively larger brains, are characterized by a corresponding decrease in the degree of cranial ossification for a given age, in contrast to the presence of similar rates of postcranial ossification between transgenic and wild-type mice. This disparity is most pronounced in the neurocranial vault, which is supplied by a greater number of vessels in common with the brain than the facial skull.

Mice with relatively larger brains had a decrease in cranial ossification. As modern humans are more encephalized than living apes and most extinct hominids, our findings provide unique insights into hominid evolution, particularly the “expensive tissue hypothesis” regarding energetic tradeoffs during neural and cranial development.

Increased Prenatal Brain Growth in a Transgenic Mouse Model Decreases Cranial Development: An Expensive Tissue Hypothesis for the Skull

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ABSTRACT

In vertebrates, it has been argued that the development and evolution of an enlarged brain requires an increased basal metabolic rate or a compensatory reduction in the resources devoted to the formation of other metabolically costly tissues, leading to a reduction in the size of such organs. While the latter scenario is indirectly supported by comparative data, especially in primates, this inherently ontogenetic phenomenon has not been addressed in a mechanistic framework.

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INTRODUCTION

The growth and maintenance of a metabolically costly organ, such as the brain, cannot be accomplished without a compensatory increase in resting metabolic rate, greater energy investment during prenatal growth by the mother (in placental mammals), or a decrease in the size and requirements of other "expensive" organ(s), including the digestive tract, musculature, or fat stores. Interspecific studies of adult vertebrates have supported evidence of this last trend in mammals, birds, and fish (Martin, 1981; Aiello and Wheeler, 1995; Martin, 1996; Wrangham et al., 1999; Aiello et al., 2001; Fish and Lockwood, 2003; Kaufman et al., 2003; Isler and van Schaik, 2006a,b, 2009; Barrickman and Lin, 2010; Navarrete et al., 2011; Kotrschal et al., 2013; Pontzer et al., 2014; Pontzer et al., 2016). For instance, the costs of increased encephalization in primates and fish are offset via reduced gut mass (Kaufman et al., 2003), while birds experience a trade-off between brain and locomotor muscle mass (Isler and van Schaik, 2006a). Notably, fish populations that were selected for increased relative brain size developed smaller guts (Kotrschal et al., 2013).

The energy demands of brain growth may also affect gestation length. Humans are born with brains that are relatively smaller and less developed than other primate newborns (DeSilva and Lesnik, 2006), and the length of the gestation period might be constrained by maternal metabolic rate and energy supply (Dunsworth et al., 2012). In addition to gestation, growth in childhood is also constrained by the energy demands of the brain. The brain's metabolic requirements are exceptionally high during childhood, and increases in the amount of energy used by the brain are linked to periods of slower growth in overall body size (Kuzawa et al. 2014).

Perhaps nowhere has such an argument gained considerable traction than in the study of human evolution, where the "expensive tissue hypothesis" has sought to explain the energetic trade offs during growth associated with increased relative brain size (Aiello and Wheeler, 1995; Aiello et al., 2001). Indeed, elevated levels of encephalization have long been posited to underlie a suite of features unique to the hominid skull, such as a flexed basicranium, a globular cranial vault, and a short orthognathic face (e.g., Ross and Ravosa, 1993; Ross and Henneberg, 1995; Enlow, 1996; Lieberman et al., 2004, 2008). Although these explanations are inherently mechanistic in nature, prior analyses regarding the morphological and especially metabolic correlates of greater encephalization have instead largely relied on comparative data, which can only at best indirectly address such important and longstanding questions. More recent studies, on the other hand, have directly measured the metabolic requirements of the brain and the total energy expenditure of the body (Kuzawa et al., 2014; Pontzer et al., 2014, 2016). They suggest that humans do, in fact, have a higher metabolic rate than expected for a primate of a similar size. Moreover, decreases in the size of the gut and locomotor efficiency have allowed for greater energy allocation to brain growth and maintenance, while minimizing increases in basal metabolic rate and total energy expenditure (Pontzer et al., 2016).

Here, we directly examine the influence of elevated prenatal neural development in a transgenic mouse model of encephalization (*Mus musculus*). Via targeted insertion of a transgene encoding a degradation-resistant form of ß-catenin into neuronal progenitor cells, ß-catenin transgenic mice develop enlarged brains, especially the surface area of the cerebral cortex, due to an increase in the number of proliferating neural precursor cells (Chenn and Walsh, 2002, 2003). Comparison of these transgenic mice to wild-type littermates for measures

of brain and body size as well as ossification levels in the skull and forelimb yields unique data regarding the prenatal correlates of increased encephalization.

MATERIALS AND METHODS

Samples

The transgenic mice used have a constituently activated ß-catenin protein expressed in their central nervous system progenitor cells (Chenn and Walsh, 2002, 2003). An activated ß-catenin protein in the developing central nervous system results in an increase in the number of neural stem cells, ultimately resulting in a relatively larger brain among the transgenic mice. The use of these animals was approved by the Institutional Animal Care and Use Committee (IACUC) of Northwestern University.

This study examines wild-type and transgenic mice at E16.5, E17.5, and as neonates (P0). Sample sizes in each age cohort are as follows: E16.5, 14 wild-type and 13 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), then the head and one forelimb from each specimen were fixed in paraformaldehyde. The wild-type and transgenic mice are otherwise genetically similar to their littermates. A subset of specimens was imaged using microCT and MRI. See Table 1 for a list of all measurements obtained. The use of littermate comparisons allows us to control for in utero variation in the effects of maternal resources, whereas analyses of ossification data from the limbs and cranium for each specimen allows us to evaluate within-individual variation in resource allocation.

Measuring Ossification

The forelimbs of 23 mice and the heads of 29 mice were imaged using a Scanco Medical MicroCT 40 system. The amount of mineralized tissue in the skull and forelimb was recorded, as was the length of the humerus. The total bone volume of the forelimb was measured as the amount of mineralized tissue above the threshold value of 160 for E17.5 and P0 mice and 130 for E16.5 mice; the total bone volume of the skull was measured as the amount of mineralized tissue above the threshold value of 120. See López et al. (2008) for the determination of threshold levels. 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. There was not enough mineralized bone to accurately measure humeral length at E16.5.

Measuring Brain Size

Heads of mice were imaged on a Bruker 14.1 T MR microimager. 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 x 14 mm x 8 mm, and image matrix = 256 x 256 x150. This yielded a voxel size of 55 x 55 x 53 mm.

Brain volume was estimated by measuring the area of the brain in every sixth sagittal slice using the software program ImageJ, adding the areas, then multiplying by the distance between the slices (6 x 0.053 mm). Relative brain size was calculated relative to basicranial length.

RESULTS

Cranial Morphology

As would be expected from the action of the transgene, at all ages ß-catenin transgenic mice had significantly larger brains than did their wild-type counterparts, both absolutely and relative to basicranial length (Table 2). At each age, the average size of the brain in transgenic mice was more than double that for wild-type mice (Table 2). In addition to enlarged brains, transgenic mice also exhibited longer and wider cranial vaults, shorter and wider faces, and longer cribriform plates as compared to wild-type mice. Their basicrania were more ventrally flexed at the angle between the basisphenoid and basioccipital bones and where the cribriform plate meets the rest of the cranial base (López et al., 2008; López, 2011). These findings mirror those for interspecific comparisons of primates that vary in encephalization (e.g., Ross and Ravosa, 1993).

Somatic and Postcranial Development

In contrast to the skull, ß-catenin transgenic mice were not significantly different from wild-type littermates in body mass or crown-rump length at E16.5, E17.5, or P0 (Table 2, Figure 1). Wild-type and transgenic mice also had similar amounts of ossified bone in the forelimb and did not differ in humerus length (Table 2, Figure 1). These results suggest that the presence of the transgene had no effect on overall somatic development or postcranial ossification.

Cranial Ossification

Cranial ossification varied from litter to litter (Figure 2). At the three ages where wild-type and transgenic mice were compared controlling for litter, transgenic mice *always* developed reduced levels of ossification relative to their wild-type littermates. The wild-type and transgenic

mice showed similar patterns of variation by litter. The Spearman rank correlation for median cranial ossification of wild-type and transgenic mice within each litter is r = 1, indicating that ossification levels varied by litter identically in the two groups of mice. Furthermore, Levene's tests of equality of variances showed that the wild-type and transgenic mice did not differ in the variance of their cranial ossification at any age (E16.5: $F = 0.40$, $p = 0.548$; E17.5: not enough data to test; P0: $F = 0.13$, $p = 0.728$). Brain size varied in a similar way: the Spearman rank correlation of median brain size in wild-type and transgenic mice (paired by litter) was also r = 1, and the variances of wild-type and transgenic mice did not differ significantly (E16.5: $F = 2.78$, $p = 0.139$; E17.5: $F = 0.98$, $p = 0.427$; P0: $F = 0.94$, $p = 0.353$). Interestingly, variation across litters may be due to differences in maternal energy supply and investment in fetal growth, as suggested in a recent comparative study of human altriciality (Dunsworth et al., 2012).

The difference in cranial ossification in wild-type and transgenic mice was particularly marked in newborns (Table 2). In general, transgenic mice exhibited lower ossification in the bones of the cranial vault, a slighter decrease in ossification of facial elements, and similar amounts of ossification of the basicranium as their wild-type littermates (Figure 3).

Controlling for age, partial correlations were calculated between cranial ossification and brain size relative to basicranial length. The amount of cranial ossification was significantly negatively correlated with relative brain size ($r = -0.57$, $p = 0.008$). Ossification in the forelimb was not correlated with relative brain size ($r = 0.44$, $p = 0.171$). The negative correlation of cranial ossification and relative brain size contrasted with the positive (but non-significant) correlation of post-cranial ossification and relative brain size suggests that the growth of the brain might constrain cranial development, but that the effects of increased brain size are confined to the cranial region.

DISCUSSION

An Expensive Tissue Hypothesis for the Skull?

The "expensive-tissue hypothesis" (Aiello and Wheeler, 1995; Aiello et al., 2001) states that the evolution of a more encephalized brain, a metabolically expensive organ, is compensated by a reduction in size of the gut, and facilitated in humans via food processing and cooking. By lessening overall somatic energetic demands via a disproportionately smaller gastrointestinal tract, primates have evolved a relatively larger brain without a corresponding increase in basal metabolic rate. Analyses of ß-catenin transgenic mice support such a mechanistic assertion regarding ontogenetic increases in brain development occurring at the expense of other organs and tissues.

On one hand, the transgene affecting prenatal neurogenesis does not appear to alter the amount of nourishment available to prenatal mice, i.e., the mother does not over-express ß-catenin and the transgene does not affect placental growth. Thus, one can infer that wild-type and transgenic mice in the same litter receive similar maternal resources. This interpretation is consistent with the presence, in age-controlled comparisons, of similar body sizes *and* rates of forelimb ossification between transgenic and wild-type mice.

On the other hand, compared to age-matched wild-type forms, ß-catenin transgenic mice develop much greater encephalization *and* markedly lower rates of craniofacial ossification. The latter disparity characterizes bones of the cranial vault and, to a lesser extent, the facial skull (e.g., newborns in Figure 3). It is tempting to contrast the reduced osteogenesis of the vault and face, which occurs largely via intramembranous ossification, with the normal development of the basicranium, which ossifies endochondrally, to embryological factors. However, there is no

184 evidence that the ß-catenin transgene affects skeletal formation as its effects are confined to the
185 central nervous system (López, 2011). Moreover, although data on gut mass or postcranial
186 muscle mass are not available for these specimens, the lack of differences between transgenic
187 and wild-type mice in postcranial ossification and body size suggests that such tissues are
188 unaffected. In contrast, the fact that most nutrients to the brain, face and associated hard tissues
189 travel via branches of the common carotid artery suggests a phenomenon consistent with the
190 "expensive tissue hypothesis," whereby similar levels of finite cranial resources are differentially
191 allocated to neural, vs. skull, development in the transgenic mice.

192 There is evidence from the clinical literature to support this assertion regarding the nature
193 of cranial variation between wild-type and transgenic mice. In developing humans as well as
194 mammal experimental models, individuals with compromised vasculature, and correspondingly
195 reduced nutrient flow, experience underdeveloped craniofacial structures (Robinson et al., 1987;
196 Escobar and Liechty, 1998). In the case of the highly encephalized mice, as all observed skeletal
197 differences during ontogeny are restricted to the head, the presence of relatively smaller bony
198 elements can be used to infer ontogenetic reductions in resource allocation. Therefore, in order
199 to develop an enlarged brain in the presence of a finite energy supply, differentially greater
200 resources are diverted for the process of neurogenesis, with corresponding decreases in nutrient
201 levels supplied to developing hard tissues of the cranial vault and facial skull.

202 We found that both wild-type and transgenic mice showed identical patterns of variation
203 in brain size and ossification across litters, suggesting differences in maternal investment in
204 entire litters of mice. It is noteworthy that constraints on maternal investment can affect fetal
205 growth, birth size, and even health during adulthood (Barker, 1997; Gluckman and Hanson,
206 2004; Dunsworth et al., 2012).

Although much attention has been devoted to the link between maternal resources and brain size and relative gut size and encephalization, increased demands of a growing brain may likewise constrain the formation of cranial elements. This alternative mechanism of achieving an energetic trade off between increased prenatal brain growth and the rate of cranial ossification might result in heterochronic alterations in patterns of skull development among sister taxa. Indeed, subtle osteogenic changes during prenatal development, when the majority of brain growth occurs, can greatly affect adult cranial form. For instance, morphological differences, even between close relatives such as modern humans and Neanderthals, appear to arise very early in ontogeny (Ponce de Leon and Zollikofer, 2001; Ackermann and Krovitz, 2002; Bastir et al., 2007).

An alternative explanation for the decrease in cranial ossification may be changes in the tissue interactions that are normally found in the developing head. Cranial development in mammals is governed by a complex set of tissue interactions among the brain, dura mater, and surrounding bones (Greenwald et al., 2000; Opperman, 2000; Warren and Longaker, 2001; Jiang et al., 2002; Spector et al., 2002; Fong et al., 2003; Ravosa et al., 2016; Franks et al., 2017). If brain expansion in the transgenic mice similarly affects the growth of the dura mater, changes in dural mechanobiology could in turn alter skull ontogeny. However, as load-induced dura signalling appears to be largely *pro*-osteogenic (Spector et al., 2002; Fong et al., 2003), the 'expensive tissue hypothesis' remains the best mechanism to explain the lower rate of cranial ossification observed in our encephalized transgenic mice.

CONCLUSIONS

It has long been argued that the growth and maintenance of an enlarged brain requires some compensatory mechanism on the part of an organism such as an increased resting metabolic rate, increased maternal investment, or a decrease in the size of other metabolically expensive organs/tissues (Martin, 1981; Aiello and Wheeler, 1995; Martin, 1996; Wrangham et al., 1999; Aiello et al., 2001; Fish and Lockwood, 2003; Kaufman et al., 2003; Isler and van Schaik, 2006a,b, 2009; Barrickman and Lin, 2010; Navarrete et al., 2011; Kotrschal et al., 2013; Pontzer et al., 2014; Pontzer et al., 2016). Support for such arguments regarding somatic resource allocation have been substantiated by comparative and experimental data, but little evidence related to prenatal development. Using a transgenic mouse model of neurogenesis, we were able to address the issue of energetic trade offs more directly, demonstrating a causal link between decreased cranial ossification and greater encephalization. This disparity between wild-type and transgenic mice suggests that, if other life history parameters are held constant, development of a metabolically costly structure such as the brain may occur at the expense of the formation of other craniofacial structures. We also demonstrated the presence of maternal effects on variation in brain size and ossification across litters, which is of great significance for studies that emphasize such factors (Martin, 1996; Dunsworth 2012). In light of these results, it would be interesting to examine other animal models of altered brain size (e.g., the megencephaly mouse; Donahue et al., 1996) to determine if cranial ossification is similarly changed in those animals.

While prior comparative studies have suggested that the growth of the gastrointestinal tract has been offset so as to favor an enlarged brain, ontogenetic analyses herein provide

evidence about the existence of additional such mechanisms. Moreover, these findings highlight a novel alternative means of achieving such an energetic exchange during growth, with significant implications for understanding reduced prenatal rates of ossification in cranial elements. Although it may be the case that gut size has decreased to accommodate an encephalized brain in primates, our study indicates that there are multiple biological solutions to facilitate the development and evolution of a metabolically expensive tissue such as the brain.

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REFERENCES

Ackermann RR, Krovitz GE. 2002. Common patterns of facial ontogeny in the hominid lineage. *The Anatomical Record* 269:142-147.

Aiello LC, Wheeler P. 1995. The expensive-tissue hypothesis: the brain and the digestive system in human and primate evolution. *Current Anthropology* 36:199-221.

Aiello LC, Bates N, Joffe T. 2001. In defence of the expensive tissue hypothesis. In: Falk D, Gibson KR, eds. *Evolutionary Anatomy of the Primate Cerebral Cortex*. Cambridge: Cambridge University Press, 57-78.

Barker DJ. 1997. Maternal nutrition, fetal nutrition, and disease in later life. *Nutrition* 13:807-813.

Barrickman NL, Lin MJ. 2010. Encephalization, expensive tissues, and energetics: An examination of the relative costs of brain size in strepsirrhines. *American Journal of Physical Anthropology* 143:579-590.

Bastir M, O'Higgins P, Rosas A. 2007. Facial ontogeny in Neanderthals and modern humans. *The Proceedings of the Royal Society B - Biological Sciences* 274:1125-1132.

Chenn A, Walsh CA. 2002. Regulation of cerebral cortical size by control of cell cycle exit in neural precursors. *Science* 297:365-369.

Chenn A, Walsh CA. 2003. Increased neuronal production, enlarged forebrains and cytoarchitectural distortions in beta-catenin overexpressing transgenic mice. *Cerebral Cortex* 13:599-606.

DeSilva J, Lesnik J. 2006. Chimpanzee neonatal brain size: Implications for brain growth in *Homo erectus*. *Journal of Human Evolution* 51:207-212.

297 Donahue LR, Cook SA, Johnson KR, Bronson RT, Davisson MT. 1996. Megencephaly: a new
298 mouse mutation of chromosome 6 that causes hypertrophy of the brain. *Mammalian*
299 *Genome* 7:871-876.
300 Dunsworth HM, Warrener AG, Deacon T, Ellison PT, Pontzer H. 2012. Metabolic hypothesis for
301 human altriciality. *Proceedings of the National Academy of Sciences USA* 109:15212-
302 15216.
303 Enlow DH. 1996. *Handbook of Facial Growth.* 2nd Edition. Philadelphia: Saunders.
304 Escobar LF, Liechty EA. 1998. Late gestational vascular disruptions inducing craniofacial
305 anomalies: a fetal lamb model. *Journal of Craniofacial Genetics and Developmental*
306 *Biology* 18:159-163.
307 Fish JL, Lockwood CA. 2003. Dietary constraints on encephalization in primates. *American*
308 *Journal of Physical Anthropology* 120:171-181.
309 Fong KD, Warren SM, Loboa EG, Henderson JH, Fang TD, Cowan, CM, Carter DR, Longaker
310 MT. 2003. Mechanical strain affects dura mater biological processes: implications for
311 immature calvarial healing. *Plastic and Reconstructive Surgery* 112:1312-1327.
312 Franks EM, Scott JE, McAbee KR, Scollan JP, Eastman MM, Ravosa MJ. 2017. Intracranial and
313 hierarchical perspective on dietary plasticity in mammals. *Zoology* 124:30-41.
314 Gluckman PD, Hanson MA. 2004. Maternal constraint of fetal growth and its consequences.
315 *Seminars in Fetal and Neonatal Medicine* 9:419-425.
316 Greenwald JA, Mehrara BJ, Spector JA, Chin GS, Steinbrech DS, Saadeh PB, Luchs JS,
317 Paccione MF, Gittes GK, Longaker MT. 2000. Biomolecular mechanisms of calvarial
318 bone induction: immature versus mature dura mater. *Plastic and Reconstructive Surgery*
319 105:1382-1392.

Isler K, van Schaik C. 2006a. Costs of encephalization: the energy trade-off hypothesis tested on birds. *Journal of Human Evolution* 51:228-243.

Isler K, van Schaik CP. 2006b. Metabolic costs of brain size evolution. *Biological Letters* 2:557-560.

Isler K, van Schaik CP. 2009. The expensive brain: a framework for explaining evolutionary changes in brain size. *Journal of Human Evolution* 57:392-400.

Jiang X, Iseki S, Maxson RE, Sucov HM, Morriss-Kay GM. 2002. Tissue origins and interactions in the mammalian skull vault. *Developmental Biology* 241:106-116.

Kaufman JA, Hladik CM, Pasquet P. 2003. On the expensive-tissue hypothesis: independent support from highly encephalized fish. *Current Anthropology* 44:705-707.

Kotrschal A, Rogell B, Bundsen A, Svensson B, Zajitschek S, Brännström I, Immler S, Maklakov AA, Kolm N. 2013. Artificial selection on relative brain size in the guppy reveals costs and benefits of evolving a larger brain. *Current Biology* 23:168-171.

Kuzawa CW, Chugani HT, Grossman LI, Lipovich L, Muzik O, Hof PR, Wildman DE, Sherwood CC, Leonard WR, Lange N. 2014. Metabolic costs and evolutionary implications of human brain development. *Proceedings of the National Academy of Sciences USA* 111:13010-13015.

Lieberman DE, McBratney BM, Krovitz G. 2002. The evolution and development of cranial form in *Homo sapiens*. *Proceedings of the National Academy of Sciences USA* 99:1134-1139.

Lieberman DE, Krovitz GE, McBratney-Owen B. 2004. Testing hypotheses about tinkering in the fossil record: the case of the human skull. *Journal of Experimental Zoology* 302:284-301.

López E. 2011. The influence of relative brain size on cranial morphology in a mouse model of encephalization. Ph.D Disseration. Evanston, IL: Northwestern University.

López EK, Stock SR, Chenn A, Taketo M, Ravosa MJ. 2008. A novel transgenic mouse model for fetal encephalization and craniofacial development. *Integrative and Comparative Biology* 48:360-372.

Martin RD. 1981. Relative brain size and basal metabolic rate in terrestrial vertebrates. *Nature* 293:57-60.

Martin RD. 1996. Scaling of the mammalian brain: the maternal energy hypothesis. *Physiology* 11:149-156.

Navarrete A, van Schaik CP, Isler K. 2011. Energetics and the evolution of human brain size. *Nature* 480:91-93.

Opperman LA. 2000. Cranial sutures as intramembranous bone growth sites. *Developmental Dynamics* 219:472-485.

Ponce de Leon MS, Zollikofer CP. 2001. Neanderthal cranial ontogeny and its implications for late hominid diversity. *Nature* 412:534-538.

Pontzer H, Brown MH, Raichlen DA, Dunsworth H, Hare B, Walker K, Luke A, Dugas LR, Durazo-Arvizu R, Schoeller D, Plange-Rhule J, Bovet P, Forrester TE, Lambert EV, Thompson ME, Shumaker RW, Ross SR. 2016. Metabolic acceleration and the evolution of human brain size and life history. *Nature* 533:390-392.

Pontzer H, Raichlen DA, Gordon AD, Schroepfer-Walker KK, Hare B, O'Neill MC, Muldoon KM, Dunsworth HM, Wood BM, Isler K, Burkart J, Irwin M, Shumaker RW, Lonsdorf EV, Ross SR. 2014. Primate energy expenditure and life history. *Proceedings of the National Academy of Sciences USA* 111:1433-1437.

366 Ravosa MJ, Menegaz RA, Scott JE, Daegling DJ, McAbee KR. 2016. Limitations of a
367 morphological criterion of adaptive inference in the fossil record. *Biological Reviews*
368 91:883-898.
369 Robinson LK, Hoyme HE, Edwards DK, Jones KL. 1987. Vascular pathogenesis of unilateral
370 craniofacial defects. *Journal of Pediatrics* 111:236-239.
371 Ross C, Henneberg, M. 1995. Basicranial flexion, relative brain size, and facial kyphosis in
372 *Homo sapiens* and some fossil hominids. *American Journal of Physical Anthropology*
373 98:575-593.
374 Ross CF, Ravosa MJ. 1993. Basicranial flexion, relative brain size, and facial kyphosis in
375 nonhuman primates. *American Journal of Physical Anthropology* 91:305-324.
376 Spector JA, Greenwald JA, Warren SM, Bouletreau PJ, Crisera FE, Mehrara BJ, Longaker MT.
377 2002. Co-culture of osteoblasts with immature dural cells causes an increased rate of
378 osteoblast differentiation. *Plastic and Reconstructive Surgery* 109:631-642.
379 Warren SM, Longaker MT. 2001. The pathogenesis of craniosynostosis in the fetus. *Yonsei*
380 *Medical Journal* 42:646-659.
381 Wrangham RW, Jones JH, Laden G, Pilbeam D, Conklin-Brittain N (1999) The raw and the
382 stolen: cooking and the ecology of human origins. *Current Anthropology* 40:567-594.

Figure 1(on next page)

Comparisons of measures of somatic growth and postcranial development.

Box plots show distributions of somatic growth measures for wild-type and transgenic mice at various ages. Outliers (values more than one and a half times the interquartile range above the third quartile or below the first quartile) are indicated by open circles. (A) Body mass, (B) Crown-rump length, (C) Forelimb ossification, (D) Humerus length.

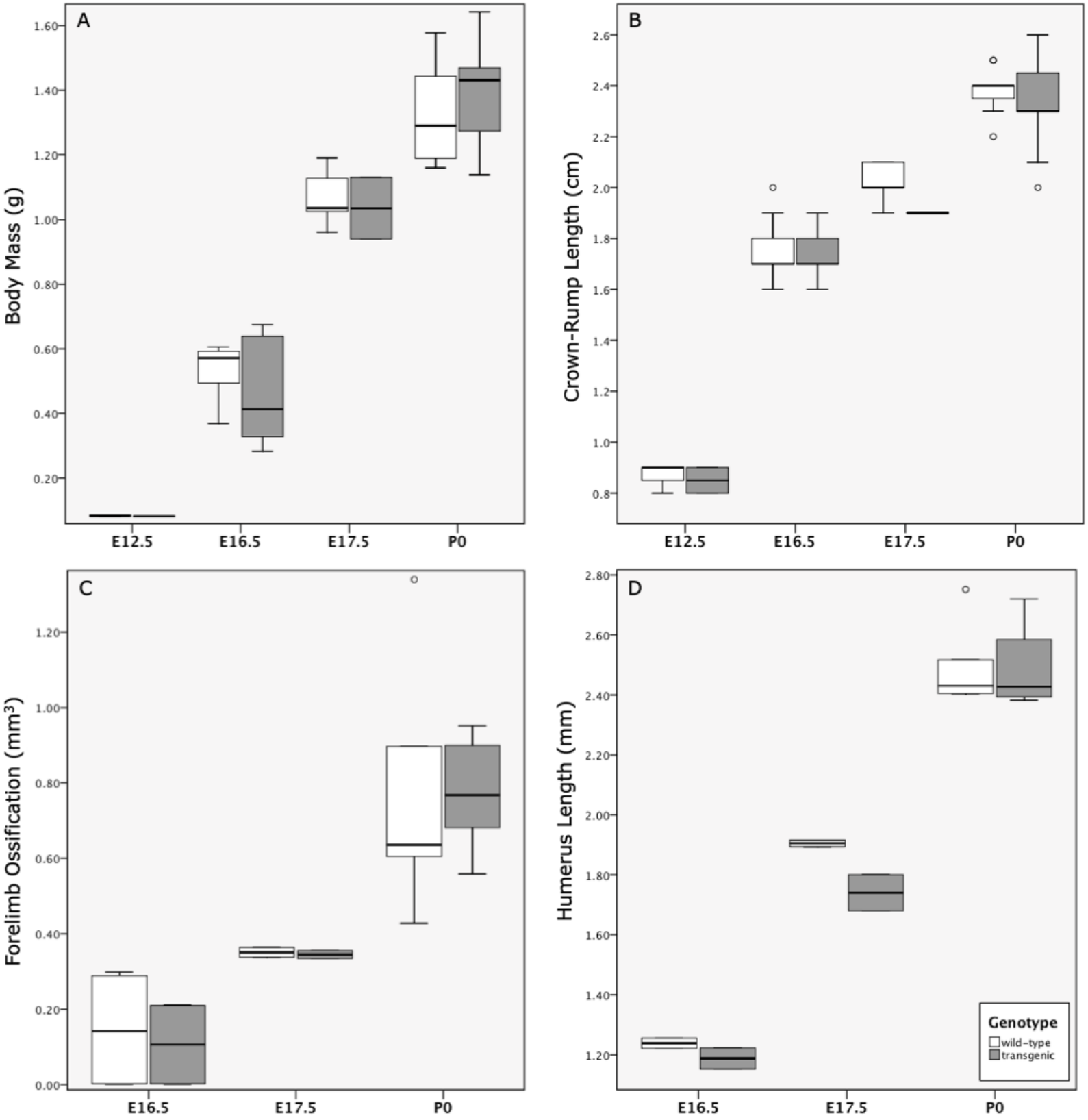

Figure 2(on next page)

Comparisons of cranial ossification by litter.

Box plots show distributions of somatic growth measures for wild-type and transgenic mice at various ages, separated by litter. Each litter had at least one mouse from each group, except the second E17.5 litter, which only provided data for a wild-type mouse. Within each litter, left = wild-type, right = transgenic.

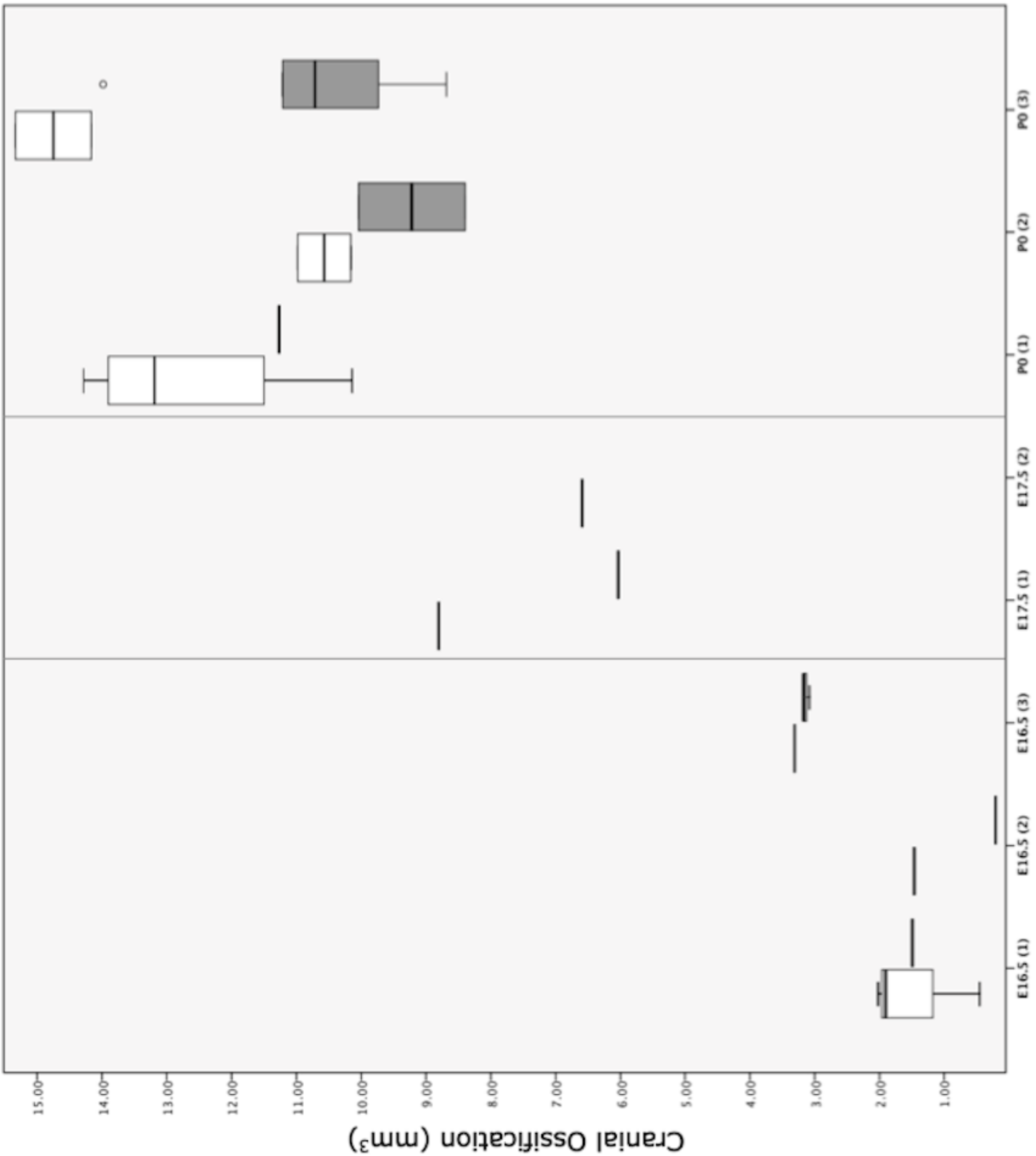
Cranial Ossification (mm³)
15.00
14.00
13.00
12.00
11.00
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
E16.5 (1)
E16.5 (2)
E16.5 (3)
E17.5 (1)
E17.5 (2)
P0 (1)
P0 (2)
P0 (3)

Figure 3

Comparisons of cranial ossification in newborn mice.

These are typical examples of microCT images of wild-type and transgenic newborn mice. Images display mineralized tissue above the threshold value of 120. Left = wild-type, right = transgenic.

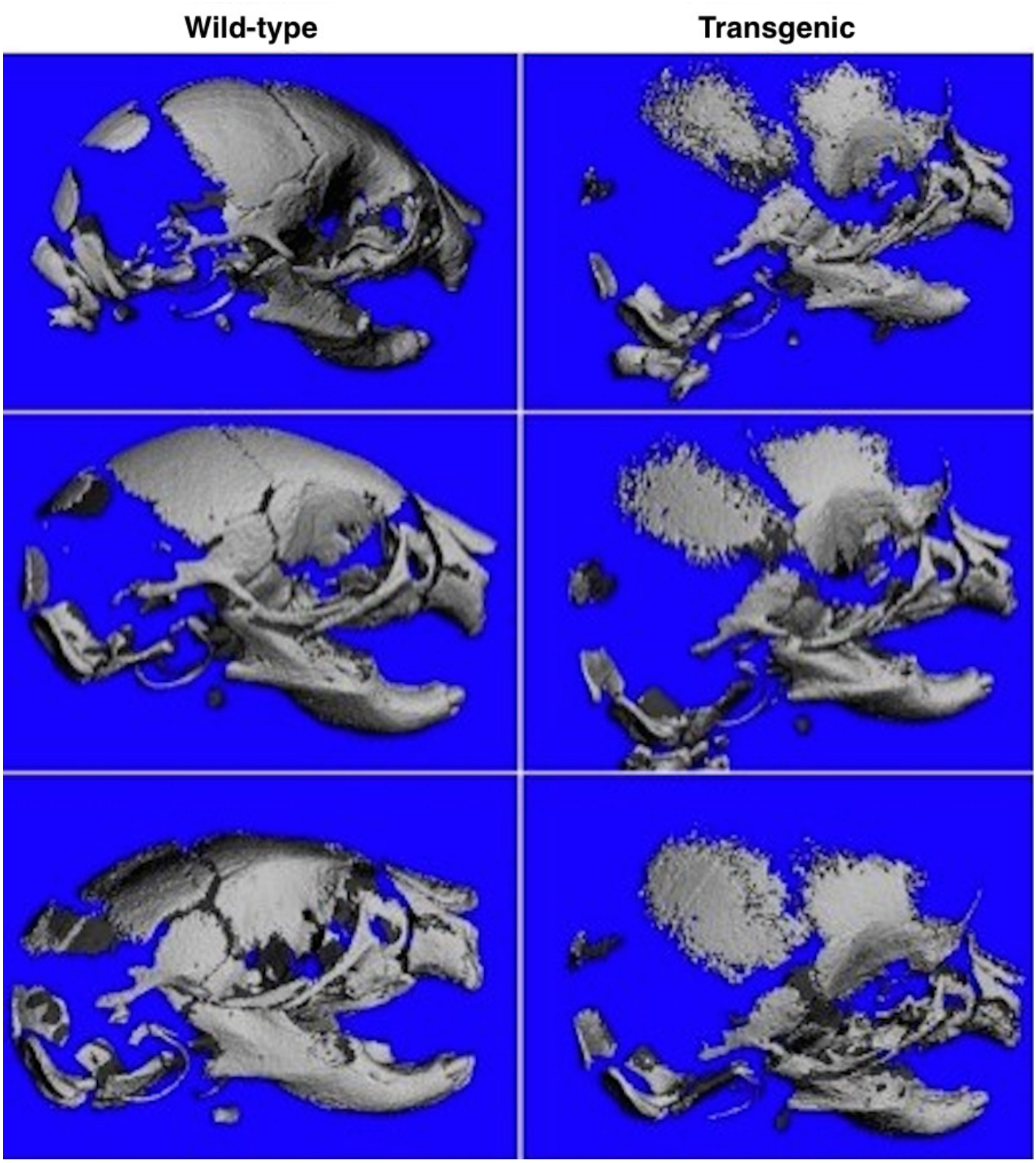

Table 1(on next page)

Measures of skeletal size and development.

wt = wild-type mice; tg = transgenic mice

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Variable	Description	Sample Size
body mass (g)	measured to nearest g	E16.5: 11 wt, 13 tg; E17.5: 9 wt, 2 tg; P0: 11 wt, 13 tg
crown-rump length (cm)	measured to nearest mm	E16.5: 14 wt, 13 tg; E17.5: 9 wt, 2 tg; P0: 11 wt, 11 tg
forelimb ossification (mm^3)	amount of mineralized tissue in forelimb above threshold level of 160 (E17.5, P0) or 130 (E16.5); see Lopez et al. 2008 for the determination of threshold levels	E16.5: 4 wt, 4 tg; E17.5: 2 wt, 2 tg; P0: 6 wt, 5 tg
humeral length (mm)	length of humerus along center of bony shaft	E16.5: 2 wt, 2 tg; E17.5: 2 wt, 2 tg; P0: 6 wt, 4 tg
cranial ossification (mm^3)	amount of mineralized tissue in skull above 120	E16.5: 5 wt, 5 tg; E17.5: 2 wt, 1 tg; P0: 8 wt, 8 tg

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Table 2(on next page)

Comparisons of cranial and postcranial size and development.

wt = wild-type mice; tg = transgenic mice

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	E16.5 [mean, (SD), N]			E17.5 [mean, (SD), N]			P0 [mean, (SD), N]		
	wt	**tg**	**p-value**	**wt**	**tg**	**p-value**	**wt**	**tg**	**p-value**
body mass (g)	0.53 (0.08) 11	0.47 (0.16) 13	0.543	1.07 (0.07) 9	1.04 (0.13) 2	0.637	1.33 (0.16) 11	1.39 (0.15) 13	0.297
crown-rump length (cm)	1.74 (0.12) 14	1.73 (0.09) 13	0.795	2.02 (0.08) 9	1.90 (0.00) 2	0.080	2.38 (0.09) 11	2.35 (0.19) 11	0.563
forelimb ossification (mm^3)	0.15 (0.17) 4	0.11 (0.12) 4	0.386	0.35 (0.02) 2	0.34 (0.01) 2	0.439	0.76 (0.32) 6	0.77 (0.16) 5	0.465
humeral length (mm)	1.24 (0.02) 2	1.19 (0.05) 2	0.439	1.90 (0.02) 2	1.74 (0.08) 2	0.121	2.49 (0.14) 6	2.49 (0.16) 4	0.748
brain volume (mm^3)	46.27 (8.83) 6	123.50 (40.98) 3	*0.020*	63.05 (28.78) 3	174.59 1	0.180	97.57 (15.26) 7	219.84 (30.65) 7	*0.002*
brain volume$^{1/3}$/ cranial base length	1.06 (0.07) 5	1.30 (0.08) 3	*0.025*	0.93 (0.12) 3	1.39 1	0.180	0.97 (0.03) 7	1.23 (0.08) 7	*0.002*
cranial ossification (mm^3)	1.83 (1.03) 5	2.23 (1.34) 5	0.754	7.70 (1.56) 2	6.04 1	0.221	12.68 (2.01) 8	10.50 (1.76) 8	*0.046*

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