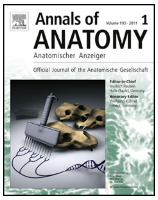
Annals of Anatomy 197 (2015) 59–66

Contents lists available at ScienceDirect

Annals of Anatomy

journal homepage: www.elsevier.de/aanat



Research article

Developmental covariation of human vault and base throughout postnatal ontogeny

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ARTICLE INFO

Article history:
Received 27 February 2014
Received in revised form
30 September 2014
Accepted 2 October 2014

Keywords:
Morphological integration
Neurocranial growth
Partial least squares
Modularity
Allometry
Geometric morphometrics

ABSTRACT

In the present study, we analyzed postnatal ontogenetic integration among morphological traits of the human neurocranium. Particularly, the covariation between the vault and the base during postnatal life was assessed. Since the association between these regions may depend on the generalized change produced by allometry, we tested its effect on their covariation. On a sample of adults and subadults ranging from 0 to 31 years, 3D coordinates of neurocranial landmarks and semilandmarks were digitized and geometric morphometric technics were applied. Main aspects of shape variation were examined using Principal Components analysis. Covariation between the vault and the base was examined by Partial Least Squares analysis. According to our results, the vault and the base covary strongly during postnatal ontogeny and their relation depends largely on allometry. Two size variables were studied: centroid size, which was obtained from the recorded morphometric points, and endocranial volume, taken as an estimation of brain size. Although growing brain was found to be a developmental process that contributes to covariation among neurocranial traits, there would be other factors that exert their influence during ontogeny. These results lead to reconsider cranial morphological evolution taking into account the developmental constraints given by ontogenetic patterns of integration and reinforcing the idea that in human evolution a suite of relevant characters may be fuelled by few developmental processes.

1. Introduction

Phenotypic variation is generated by several developmental processes where genetic and environmental factors intervene. As a result of these ontogenetic dynamics, a structure of association among morphological traits emerges (Mitteroecker and Bookstein, 2007; Jamniczky et al., 2010). This tendency to produce coordinated variation among different traits is known as morphological integration (Olson and Miller, 1958; Hallgrímsson et al., 2009). In the context of modern morphometrics, morphological integration is expressed by covariances of landmarks that describe anatomical structures (Klingenberg, 2009; Goswami and Polly, 2010).

One of the developmental processes that lead to coordinated variation of different traits is generalized increase of size. Those anatomical features simultaneously affected by size change would vary in a coordinated fashion to some extent. Allometry represents all size-related shape changes, which usually affect several parts of an anatomical region or even an entire organism (Chernoff and Magwene, 1999; Klingenberg, 2013). Ontogenetic allometry, in particular, deals with variation of traits associated with size change along ontogeny. Systemic and local processes could be related to allometric change during postnatal life. For example, growth hormone (GH) in mammals induces changes in most organs and structures, including the skull (Gonzalez et al., 2013). Locally, a growing organ, such as the brain, can produce size expansion and promote changes in different cranial regions, through mechanical interactions (Hallgrímsson and Hall, 2011).

The most accepted division of the mammalian skull distinguishes the face from the neurocranium (Cheverud, 1982, 2007; Enlow and Hans, 1998; Sardi and Ramírez Rozzi, 2005; Sardi et al., 2007). Furthermore, the neurocranium includes the vault

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http://dx.doi.org/10.1016/j.aanat.2014.10.002
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Table 1
Sample constitution.

Age group	Females	Males	Total
Infant-child	7	12	19
Juvenile	11	16	27
Adolescent	15	12	27
Adult	51	15	66
Total	82	53	139

and the base (Sperber, 2001; Lieberman, 2011). Various lines of evidence indicate that the brain and the meninges influence both neurocranial regions and constitute their main driving force of growth (Moss and Young, 1960; Bradley et al., 1996; Opperman, 2000; Richtsmeier et al., 2006). However, the vault and the base develop through different processes. The bones of the vault ossify intramembranously and grow by bone deposition in the margins of the sutures, whereas the base forms endochondrally and its anteroposterior elongation is produced by intrinsic factors acting in three synchondroses (Sperber, 2001; Morriss-Kay and Wilkie, 2005). Additionally, the base relates to facial structures and the vertebral column (Lieberman et al., 2000a).

While interactions between the neurocranium and the face have been assessed in several studies (Bastir and Rosas, 2006; Mitteroecker and Bookstein, 2008; Gkantidis and Halazonetis, 2011; Martínez-Abadías et al., 2011), covariation between the human vault and base has been less explored from a developmental perspective. Among the few antecedents, Bookstein et al. (2003) observed general coordinated shape change between sagittal traits of the vault and the base during ontogeny.

The main aim of the present study was to analyze postnatal ontogenetic integration among morphological traits of the human neurocranium. In particular, we evaluated the covariation between the vault and the base during postnatal life and assessed the effect of allometry in this pattern of association.

Main evolutionary trends of morphological change are usually the result of modifications in the developmental processes that generate variation in correlated phenotypic characteristics (Raff, 1996; Lieberman et al., 2002). Since brain growth is thought to be a key developmental process leading to coordinated variation between different anatomical regions in the neurocranium, results of this study may provide insights into some important processes for human evolution. If traits of the vault and the base are integrated during ontogeny, single evolutionary changes in one region would result in global morphological modifications. In this context, changes in human neurocranial morphology may not be the consequence of isolated responses to diverse selective pressures but few developmental shifts would affect the direction or magnitude of variation upon related structures (Martínez-Abadías et al., 2012).

2. Materials and methods

2.1. Sample and data collection

In this study, we used head computed tomography (CT) images of 139 individuals from a dataset constructed at Fundación para la Lucha contra las Enfermedades Neurológicas de la Infancia (Buenos Aires, Argentina). The sample includes nonpathological humans from 0 to 31 years old of both sexes (Table 1). They were scanned with a General Electric Light Speed RT16 and, for each individual, 275 axial CT images with a resolution of 512 × 512 pixels and a voxel size equal to 0.449 × 0. 449 × 0.625 mm, were produced. A trial version of Avizo 6.0 software (Visualization Science Group) was used to handle CT images. From CT slices, a reconstruction in three dimensions (3D) was created using a chosen density threshold that corresponds to the Hounsfield unit scale (Spoor et al., 2000).

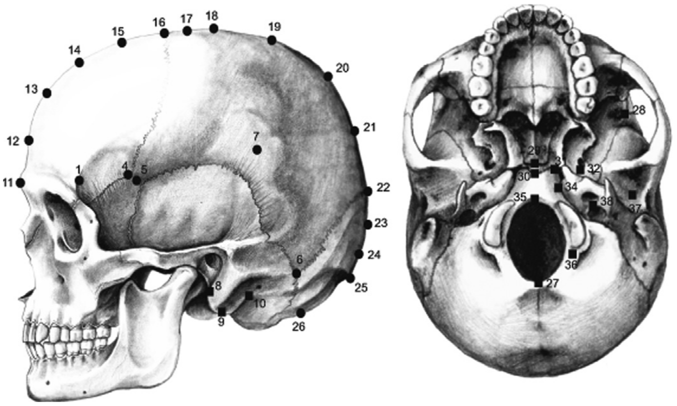

Fig. 1. Location of landmarks and semilandmarks. Circles represent vault points and squares represent base points. Detailed definitions of each point are provided in Table 2 as well as information about the neurocranial region to which they belong.

A threshold of 1150 Hounsfield units was empirically selected to show the maximum amount of bony tissue.

In order to provide a clear visualization of ontogenetic change in figures and descriptions, individuals were distinguished into the following age groups: infant-child (0–6 years), juvenile (7–12 years), adolescent (13–17 years) and adult (18–31 years), following Smith (1994).

On each individual, twenty-seven landmarks and 10 semilandmarks in 3D were digitized on the ectocranial surface (Table 2, Fig. 1). While landmarks are defined as discrete anatomical *loci* that can be recognized as the same *loci* in all the individuals of the sample (Zelditch et al., 2004), semilandmarks are frequently used to describe structures such as curves or surfaces, where landmarks are rare. In this study, a number of evenly-spaced semilandmarks were placed between specific landmarks to depict the sagittal contour of the neurocranium. As these contours have to be homologous among individuals, semilandmarks were resampled along the outline curve using a linear interpolation between the original curve points (Reddy et al., 2004).

Additionally, an estimation of the endocranial volume (EV) was obtained by semiautomatic segmentation of the endocranium. As an accepted indicator of brain size, EV provides a way to access to information about general size of this neural organ when soft tissues are not available (Conroy et al., 2000; Bienvenu et al., 2011).

To eliminate variation due to measurement error, an intra-observer error analysis was carried out by the author who collected the data (J.B.A.) prior to definite digitization. On a sample of 15 CTs, consisting of individuals of different ages, landmarks and semilandmarks were digitized three times and measurement error was estimated by means of different methodological approaches (Barbeito-Andrés et al., 2012). Following Corner et al. (1992), dispersion resulting of the placement of coordinates in repeated events of measure was assessed. Principal Component analysis (PCA) was carried out on the Procrustes coordinates obtained after Generalized Procrustes analysis (GPA) (see below for explanations about PCA and GPA). In a graphical representation, the position of each individual along the PCA axes that explain near 80% of variation were visualized taking into account that if repeated measures on the same individual are similar, they must occupy similar positions (O'Higgins and Jones, 1998). A complete description and discussion of the results of these analyses were presented elsewhere (Barbeito-Andrés et al., 2012). By means of this evaluation, problematic morphometric points were identified and their definitions were revised.

Intra-observer error analysis was repeated until the error was not significant ($p < 0.01$) according to an Analysis of the Variance for Repeated Measures and an Intraclass Correlation Coefficient.

Table 2
Collected landmarks and semilandmarks.

Number	Name	Description	Region
1	Fronto-temporal	The point where temporal line reaches the most anterior and medial position	Vault
2	Superior stephanion	The point where coronal suture and superior temporal line meet	Vault
3	Inferior stephanion	The point where coronal suture and inferior temporal line meet	Vault
4	Sphenion	Frontal–zygomatic–parietal intersection	Vault
5	Pterion	The middle point on the zygomatic–parietal suture	Vault
6	Asterion	The point where lamboidal, parietal and occipital suture meet	Vault
7	Euryon	Most lateral point of the braincase	Vault
8	Anterior mastoid	Most anterior point of mastoid process	Base
9	Mastoid	Most inferior point of mastoid process	Base
10	Posterior mastoid	Most posterior point of mastoid process	Base
11	Glabella	Most anterior midline point on the frontal bone, usually above the frontonasal juncture	Vault
12	Semilandmark 1	Between glabella and bregma	Vault
13	Semilandmark 2	Between glabella and bregma	Vault
14	Semilandmark 3	Between glabella and bregma	Vault
15	Semilandmark 4	Between glabella and bregma	Vault
16	Bregma	Midline point where the sagital and coronal sutures intersect	Vault
17	Semilandmark 5	Between bregma and vertex	Vault
18	Vertex	Most superior point of the skull in the midsagital line	Vault
19	Semilandmark 6	Between vertex and lambda	Vault
20	Semilandmark 7	Between vertex and lambda	Vault
21	Semilandmark 8	Between vertex and lambda	Vault
22	Lambda	Midline point where the sagital and lamboidal sutures intersect	Vault
23	Opisthocranion	Most posterior point of the skull in the midsagital plane	Vault
24	Semilandmark 9	Between opisthocranion and inion	Vault
25	Inion	The point where both occipital lines reach the sagital plane	Base
26	Semilandmark 10	Between inion and opisthion	Base
27	Opisthion	In the posterior border of the foramen magnum, the point where it intersects the sagital plane	Base
28	Sphenotemporal	Most external point in the sulcus placed anterior to the sphenotemporal crest	Base
29	Hormion	In intersection between the vomer bone and the sphenoid body, in the sagital plane, between both wings of the vomer	Base
30	Sphenobasion	The point where the inferior part of the spheno-occipital synchondrosis meet the sagital plane	Base
31	Lateral sphenobasion	Left lateral extreme of the spheno-occipital synchondrosis	Base
32	Ovale foramen	Most posterior point of the ovale foramen	Base
33	Carotid foramen	Most posterior point of the carotid foramen	Base
34	Middle petrous	Middle point in the internal border of the petrous bone	Base
35	Basion	In the anterior border of the foramen magnum, the point where it intersects the sagital plane	Base
36	Lateral foramen magnum	Most lateral point in the left border of the foramen magnum	Base
37	Glenoid fossa	Most posterior point in the glenoid fossa	Base

Both of them analyze the relation of variation between and within groups. Here, variation between groups (events of measure) is due to differences introduced by the observer. Therefore, these analyses provide an estimation of the repeatability of coordinate digitization.

2.2. Geometric morphometric and statistical analyses

A GPA on landmarks and semilandmarks was performed to eliminate the effect of scale, rotation and translation (Rohlf and Slice, 1990). As a result, shape variables (Procrustes coordinates) were obtained and used for successive tests. Centroid size (CS), which is defined as the square root of the summed distances between each landmark coordinate and the centroid of the landmark configuration, was used as a size variable (Rohlf and Slice, 1990; Bookstein, 1991).

To control for between-sexes variation, we performed statistical analyses using pooled within-sexes covariances matrices. Through this approach, we minimized the effect of sexual dimorphism, which is not of particular interest in this work.

As a first step, morphological changes in the neurocranium were described using PCA on Procrustes coordinates. From PCA, orthogonal axes (PCs) of maximal shape variation constructed from original variables were obtained. Each axis reflects an arrangement of traits that vary in a coordinated fashion and, consequently, is informative of the main patterns of covariation. For those PCs that were significantly correlated to age or centroid size, warped surfaces of a skull were created to depict the configurations corresponding to the negative and positive extremes of shape variation. In addition, the relations of age with CS transformed into its natural logarithm (CSnl) and EV were described through scatterplots, where the age variable was in the horizontal axis.

After exploring the main trends in ontogenetic morphological change, the association between the vault and the base was specifically evaluated with a 2-block Partial Least Squares (PLS) analysis performed with the whole ontogenetic series. This is a statistical technique used for exploring covariation between sets of variables previously defined. Since PLS is based on a singular value decomposition of the covariance matrix, it is also known as Singular Warps Analysis. This procedure finds uncorrelated pairs of axes (one axis *per* block) that represent new combinations of the original variables and account for decreasing amounts of covariation (singular values) between the blocks (Rohlf and Corti, 2000; Bookstein et al., 2003). Here, the blocks include shape variables of the vault and the base.

Finally, allometric effect on developmental covariation between the vault and the base was assessed. In order to capture size-related shape changes along ontogeny, the complete sample from infantile to adult individuals was included in the same analyses. Residuals of multivariate regression of Procrustes coordinates on CS and EV were used to repeat PLS analysis (Monteiro, 1999; Klingenberg, 2009; Martínez-Abadías et al., 2011). Information

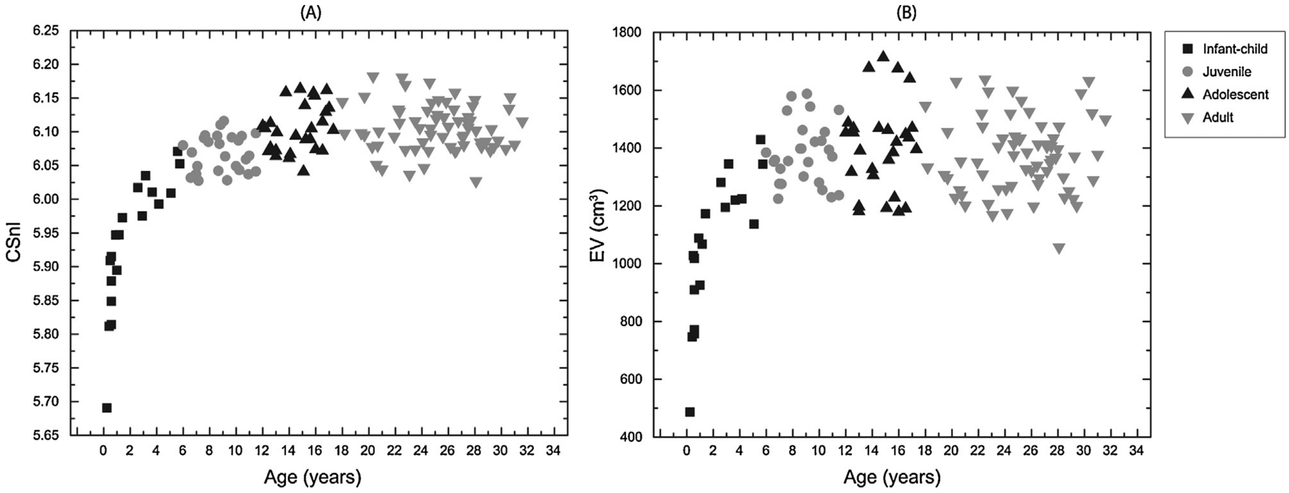

Fig. 2. Distributions of size variables in relation to age. (A) Age *vs* CSnl, (B) Age *vs* EV.

about shape variation that is not predicted by the regression model is retained in these residuals and, therefore, these new PLS analyses estimated vault/base covariation once size effect on shape was adjusted. If the size-adjusted PLS analyses indicate that the vault and the base are still morphologically associated, it would suggest that other processes, apart from general growth, are involved. While EV-adjusted PLS characterizes the effect of the endocranial content (*i.e.* basically the brain) on this pattern of covariation, CS-adjusted PLS expresses the results once the influence of additional factors that produce size variation has been corrected. Both analyses provide a complementary view and offer a more precise idea about the effect of the growing brain as an integrating process.

Visualization of shape change along the axes is shown by means of surfaces generated in Avizo. To this end, PCA and PLS vectors were visualized by warping a surface that represented the average shape. The *p*-values for PLS analyses were established by means of permutation tests. Morphometric and statistical analyses were carried out using MorphoJ (Klingenberg, 2011) and R 2.13.0 (R Development Core Team, 2011).

3. Results

3.1. Neurocranial variation and covariation during postnatal ontogeny

Neurocranial size (CSnl) changed dramatically during the first 4 years of postnatal life and then varied until age 6 (Fig. 2A). Similarly, EV increased markedly during the first 3–4 years of postnatal life and it is stabilized near the age of 6 years (Fig. 2B).

The first 16 PCs explained the 81.62% of shape variation and when association of age and size with PCs scores was examined with the Pearson's correlation coefficient, PC1 (23.89% of variation) was the only one that was significantly correlated to CSln ($r = -0.723$, $p < 0.0001$), EV ($r = -0.455$, $p < 0.0001$) and age transformed into its natural logarithm ($r = -0.796$, $p < 0.0001$). The trajectory of PC1 expresses a strong change until the age of 12–15 years old (Fig. 3). According to PC1, infant-children were characterized by relatively wide vaults, whereas adults showed a slight anteroposterior elongation of the vault, an outstanding development of the mastoids, a well-developed glabella and the frontal bone loses its vertical configuration (Fig. 3). It is noticeable that the vault was relatively wider in young individuals than in adults, especially in the frontal region. Adult bases were relatively wider in the posterior region as well. The foramen magnum displayed a central position in adults whereas it was more posteriorly placed in the youngest individuals and had larger relative size (Fig. 3).

The results of the PLS analysis showed that the first pair of axes (PLS1) was the only one that explained a significant part of covariation (75.85%) between the vault and the base, indicating that there is coordinated variation between these blocks (Table 3). Individuals were distributed along the PLS1, being the youngest ones at the lowest scores, juveniles at an intermediate position and adolescents overlapped with adults at the opposite extreme (Fig. 4). As score values (and age) increase, the vault becomes longer in an anteroposterior direction, covarying with the relative reduction of the foramen magnum and its forward displacement (Fig. 4). From the superior view, there was an ontogenetic shift from infantile globular vaults with predominance of width dimensions to adult narrow and long anteroposteriorly configurations (Fig. 4). However, some features such as mastoid processes did not show changes when covariation between the vault and the base was examined. This suggests that the ontogenetic change in these traits is independent from the integration between the vault and the base.

3.2. Allometric effect on neurocranial integration

Multivariate regressions were carried out in order to statistically adjust for the effects of allometry. According to these results, CSnl predicted 10.61% ($p < 0.0001$) of shape variation in the vault and 10.65% in the base, whereas EV predicted 6.75% of shape variation in the vault and 7.04% in the base.

When the allometric effect was adjusted with the residuals of CSnl regression, covariation between the vault and the base was over the level of $p < 0.0001$, thus became not highly significant and individuals of different ages overlap (Fig. 5). When PLS was carried out using the residuals of EV regression, association between the vault and the base remained significant and the PLS1 explained more than 60% of covariation (Table 3). Infant-children and juveniles overlapped and were separated from adolescents and adults along EV-adjusted PLS1 (Fig. 5).

Since a large amount of size variation has been found in the sample during ontogeny (Fig. 2), additional analyses were carried out. Instead of pooling all individuals to a common mean, regressions were computed adjusting size within groups. As previously described, it can be noticed that CSnl and EV grow markedly until approximately the age of 6 years (Fig. 2). To be consistent with the dynamics of the observed size change, we computed the regression of shape on CSln and EV within the Infant-child group and within a set that includes the rest of

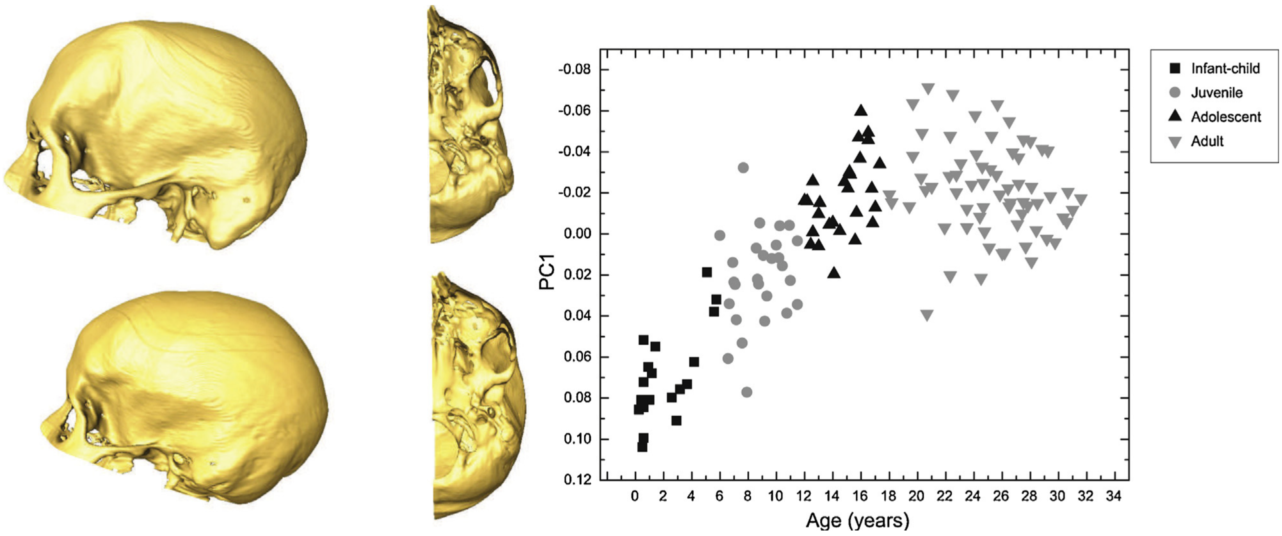

Fig. 3. Ontogenetic change along PC1. Warping of Infant-children (positive scores) and Adults (negative scores). Only shape changes in the vault and the base have to be taken into account, variation in regions where landmarks were not recorded (*i.e.* face, zygomatic arch) are artifacts of the warping procedure.

the individuals (juvenile + adolescent + adult). These “CSnl-within-groups adjusted PLS” and “EV-within-groups adjusted PLS” were based on these within-groups mean-centred regression residuals and covariation explained by the PLS1 was 38.10% ($p = 0.006$) and 47.588% ($p > 0.0001$), respectively (Table 3). According to the “CSnl-within-groups adjusted PLS”, the trend towards a reduction of the covariation when allometry is adjusted was conserved even when ontogenetic size variation within the sample was considered. Similarly, the results for the “EV-adjusted PLS” did not changed noticeably when the regressions were computed within ontogenetic groups.

In sum, results indicated that shape covariation between the vault and the base was structured by size but CSnl and EV changes explained different ontogenetic changes.

4. Discussion

In this paper, we analyzed ontogenetic variation and covariation among morphological traits of the vault and the base to recognize postnatal patterns of association between the major regions of the neurocranium and to infer which processes would be involved in their integration. According to some of our results, the vault and the base covary strongly during postnatal ontogeny and their relation depends to some extent on allometry.

In agreement with our findings, many authors have supported that the skull is an integrated structure, where different regions interact dynamically (Lieberman et al., 2000b, 2002; Bookstein et al., 2003; Mitteroecker and Bookstein, 2008; Hallgrímsson et al., 2009; Martínez-Abadías et al., 2009, 2012; Bastir et al., 2010). The processes responsible for this pattern may be multiple and may act at different spatiotemporal scales, overlapping their effects and producing a very complex structure of covariation (Hallgrímsson et al., 2009).

We found that the main neurocranial modifications, which affected both the vault and the base, were related to anteroposterior elongation (Figs. 3 and 4). Some studies described similar trends of morphological changes considering the sagittal profile (Bookstein et al., 2003; Anzelmo et al., 2013) while in the present work, lateral variation in the vault and the base was also evaluated. Here, it was shown that infant globular vaults are characterized not only by shorter and higher configurations but also by marked laterally expanded frontoparietal regions (Figs. 3 and 4). Some authors have stated that cranial globularity in anatomically modern humans (AMH) is the result of a “globularization phase” that takes place in the early ontogeny, between birth and the emergence of deciduous dentition (Gunz et al., 2010; Neubauer et al., 2010). Based on our complete postnatal ontogenetic series, there is a reduction of this globular configuration in advanced ontogenetic stages as also observed by Neubauer et al. (2010) after this “globularization phase”. This pattern of anteroposterior elongation and vertical shortening in the neurocranium was also observed by Sardi et al. (2007), who analyzed individuals between 32 and 47 weeks of gestational age, and by Bookstein et al. (2003), using a sample of individuals from 3 months of postnatal life. Also for postnatal brain development, variation from high and short profiles to anteroposteriorly elongated configurations was reported (Moss and Young, 1960; Bruner et al., 2010; Ventrice, 2011).

Our results do not challenge the concept of “globularization phase” (Gunz et al., 2010) since they are derived from a wide range of postnatal ages, from birth to adulthood. A more detailed study on the first year of life with a better representation of neonate individuals and the digitization of landmarks that captured specific globular features previously described by other authors is required to discuss this idea in particular.

The covariation between the vault and the base involved an important morphological change related to the anteroposterior

Table 3
PLS analyses.

	Singular value PLS1	*p*-Value	% Cov PLS1	Corr PLS1	*p*-Value
Procrustes coordinates PLS	0.00121	<0.0001	75.851	0.764	<0.0001
CSnl-adjusted PLS	0.00051	0.0016	42.406	0.553	0.003
CSnl-within-groups adjusted PLS	0.00049	0.0060	38.098	0.598	<0.0001
EV-adjusted PLS	0.00079	<0.0001	60.451	0.691	<0.0001
EV-within-groups adjusted PLS	0.00058	<0.0001	47.588	0.621	<0.0001

p-Values were obtained after permutation 10,000 random iterations.

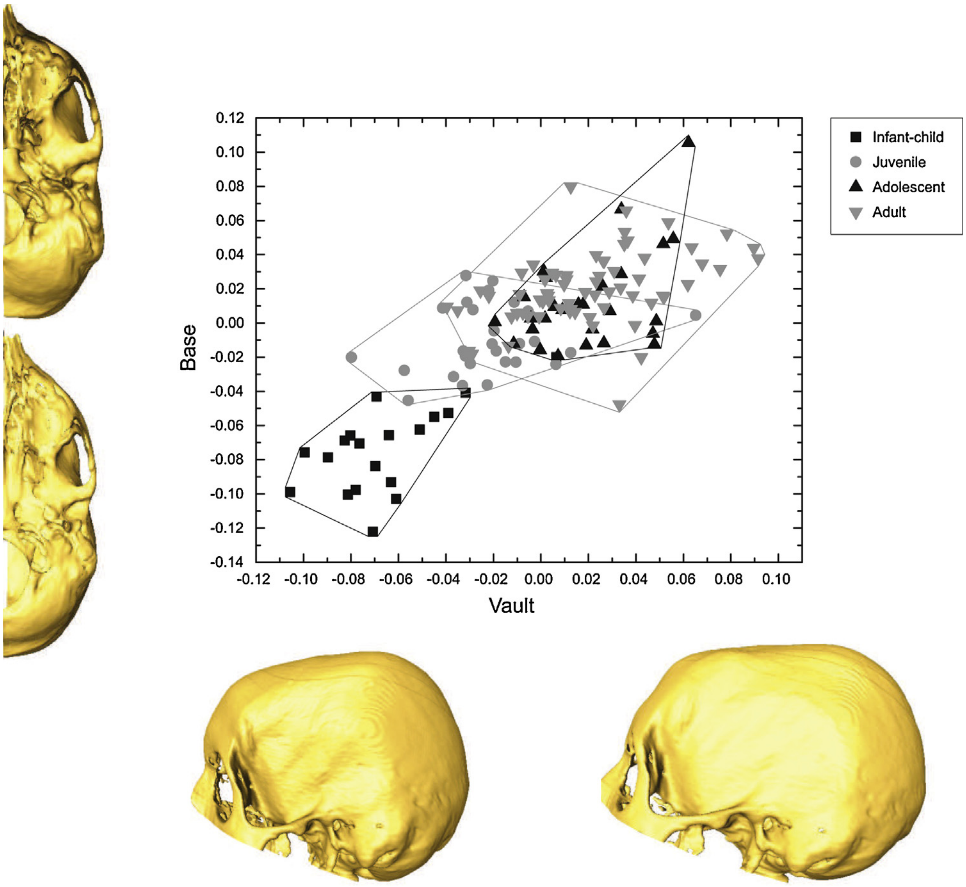

Fig. 4. Covariation of the vault and the base along PLS1. Warping of Infant-children (negative scores) and Adults (positive scores). Only shape changes in the vault and the base have to be taken into account, variation in regions where landmarks were not recorded (*i.e.* face, zygomatic arch) are artifacts of the warping procedure.

elongation, which is the relatively forward position adopted by the foramen magnum (Fig. 4). This description agrees with the patterns of covariation found for adult great apes and other *Homo* specimens, where rounded and expanded cranial vaults were associated with an anteriorly placed foramen magnum (Singh et al., 2012). In other vertebrate species, a relation between cranial size and the position of the foramen magnum has also been described (Kulemeyer et al., 2009; Marugán-Lobón and Buscalioni, 2006). Martínez-Abadías et al. (2012) showed that the expansion of the braincase and the forward shift of the foramen magnum are two genetically integrated changes that distinguish AMH. They suggested that these features are part of a whole suite of transformations that may have occurred as a result of localized processes that caused changes in different structures because of their integration. Here, we showed that some cranial features of AMH, as vault expansion and position of the foramen magnum in the base, are ontogenetically integrated.

At least part of the neurocranial ontogenetic variation seemed to be unrelated to interactions between the vault and the base. We found that enlargements in mastoid processes, glabella and some other morphological traits usually named “robust traits” (Lahr and Wright, 1996; Gonzalez et al., 2010) were displayed by PC1, which is strongly correlated with age and size. These anatomical

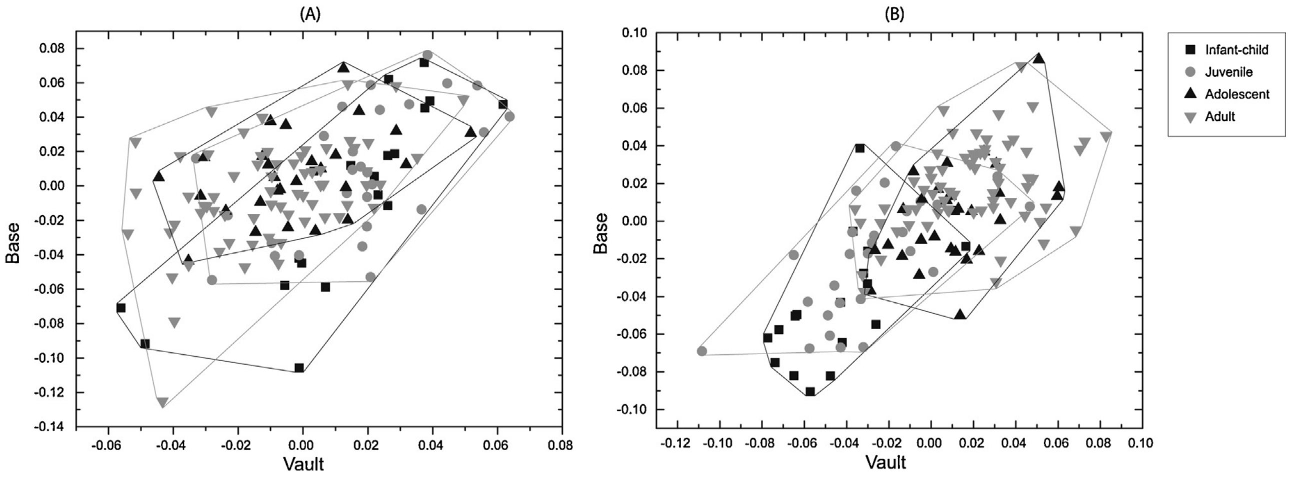

Fig. 5. Size-adjusted PLS analyses. CSnl-adjusted PLS1 (A) and EV-adjusted PLS1 (B).

structures developed slightly in infants and were clearly evident in adults (Fig. 3) and, because they do not relate to the covariation between the vault and the base (Fig. 4), they must be explained by other processes. This result corroborates the findings of Lieberman et al. (2000b), who stated that while some changes in the cranial base affect vault morphology, others, such as the thickening of the outer table that results in occipital bun, do not rely on vault/base interactions.

Neurocranial size was assessed using two variables obtained from the ectocranial (CSnl) and the endocranial (EV) surfaces, and we observed that allometry influences ontogenetic covariation between the vault and the base (Table 3). The main organ that affects neurocranial size and shape, particularly at early stages of life, is the growing brain (Richtsmeier et al., 2006; Sardi et al., 2007; Hallgrímsson et al., 2009). When the allometric effect was adjusted by the CSnl, this covariation decreased. However, the association remained significant when allometry was adjusted by the EV, which is a better proxy of brain morphology. This point suggests that other factors beyond the brain may exert their influence upon neurocranial morphological integration during ontogeny, probably in an additive way. The putative factors responsible for the integration between the vault and the base range from gene networks and molecules with pleiotropic effects over different parts of the skull, such as FGF/FGFR, SHH, Retinoic acid, GH/IGF, to the extended growth at the sutures and synchondrosis (Helms et al., 2005; Tapiada et al., 2005; Mitteroecker and Bookstein, 2008; Hallgrímsson et al., 2009; Martínez-Abadías et al., 2011). Despite it is out of the scope of this study to assess the particular role of gene networks, hormones or growth factors, their influence on craniofacial development has been widely demonstrated and their effects on skull phenotype have been documented (Helms et al., 2005; Tapiada et al., 2005; Martínez-Abadías et al., 2011; Gonzalez et al., 2013). Traits with a common genetic basis are expected to be inherited together and, therefore, to vary coordinately (Cheverud, 1996; Mitteroecker and Bookstein, 2008). Similarly, factors such as FGF/FGFR are responsible for pleiotropic effects since they affect the development of various craniofacial structures as shown in studies of dysmorphologies (Anderson et al., 1998; Martínez-Abadías et al., 2011).

In brief, our results provide evidence to support a more relevant influence of size change on shape and on the interaction among cranial structures. Ontogenetic allometry is not just a factor that obscures actual patterns of morphological integration or inflates covariation but it has to be considered to understand the observed patterns of covariation. This result is informative since the influence of allometry was extensively demonstrated for the face (Aiello and Dean, 2002; Rosas and Bastir, 2002; Anzelmo et al., 2012) but its relevance regarding neurocranial shape patterning has not been extensively queried. Other studies have reported a more moderate influence of allometry on craniofacial integration. Gkantidis and Halazonetis (2011) found that allometry does not affect patterns but only slightly the magnitude of integration in some particular regions of the skull. However, they analyzed lateral and midline basicranial as well as facial traits and they worked with two different age stages separately (6–10 and 20–35 years) where the percentage of shape variation explained by size was small. It is worth noting that, in our results, this trend of reduction of covariation was also found even when allometry was adjusted within groups according to the stages of ontogenetic change in size. Recently, Sardi et al. (2014) analyzed ontogenetic covariation among endocranial traits in *Pan troglodytes* and also suggested that allometry plays a key role in integrating these features. Although these authors studied neurocranial shape features in chimpanzees that are very different to human traits, there are similarities in the observed patterns of covariation and the influence of allometry with the present work.

5. Conclusion

This study provided developmental insights into neurocranial morphological integration and the ontogenetic processes that affected the vault and the base simultaneously, producing coordinated variation between them. Molecular and genetic bases of craniofacial development have been extensively studied (Tapiada et al., 2005; Chai and Maxson, 2006). Nevertheless, the production of phenotypic variation in complex structures (*e.g.* human neurocranium) requires other complementary levels of analysis to be understood. In this study, we assessed the potential role of some developmental factors and processes that are essential to explain how complex shape craniofacial traits develop. Information regarding the role of brain growth, developmental interactions between neurocranial traits and relative independence of other features is valuable to encourage future research that aims to assess particularly hypotheses derived from comparative studies such as this work.

Our results contribute to reconsider cranial morphological evolution taking into account the constraints given by ontogenetic patterns of integration and reinforce the idea that in human evolution a suite of relevant characters were fuelled by few developmental processes (Martínez-Abadías et al., 2012). Growing organs, such as brain, may be strong integrating processes for those bones that are related to them. However, there would be other factors that contribute to the structure of integration in the neurocranium. This shows how cranial morphology is the complex result of multiple interactions at different hierarchical levels overlapping along pre and postnatal ontogeny (Hallgrímsson et al., 2007).

Acknowledgements

We thank the Fundación para la Lucha contra las Enfermedades Neurológicas de la Infancia (FLENI) for providing the tools to build the database used in this work. This study was carried out with the financial support of the Agencia Nacional de Promoción Científica y Tecnológica, Argentina (PICT 1822) and the Universidad Nacional de La Plata, Argentina (PI N531). We are indebted to M. Cristina Muñe for her valuable help editing this manuscript.

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