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Morphological integration between the cranial base and the face in children and adults

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Abstract

The primary aim of the present study was to assess morphological covariation between the face and the basicranium (midline and lateral), and to evaluate patterns of integration at two specific developmental stages. A group of 71 children (6–10 years) was compared with a group of 71 adults (20–35 years). Lateral cephalometric radiographs were digitized and a total of 28 landmarks were placed on three areas; the midline cranial base, the lateral cranial base and the face. Geometric morphometric methods were applied and partial least squares analysis was used to evaluate correlation between the three shape blocks. Morphological integration was tested both with and without removing the effect of allometry. In children, mainly the midline and, to a lesser extent, the lateral cranial base were moderately correlated to the face. In adults, the correlation between the face and the midline cranial base, which ceases development earlier than the lateral base, was reduced. However, the lateral cranial base retained and even strengthened its correlation to the face. This suggests that the duration of common developmental timing is an important factor that influences integration between craniofacial structures. However, despite the apparent switch of primary roles between the cranial bases during development, the patterns of integration remained stable, thereby supporting the role of genetics over function in the establishment and development of craniofacial shape.

Key words: covariation; development; geometric morphometrics; malocclusion.

Introduction

The craniofacial complex serves a multitude of functional demands in a tightly packed space and is, therefore, a challenging area where the concepts of modularity and integration can improve our understanding of developmental and evolutionary issues. At the coarsest scale, three main units can be identified: the cranial base, the cranial vault and the face. These units, each deriving from embryologically distinct regions and serving separate functional purposes, can be considered modules. The concept of modularity is difficult to define explicitly (Bolker, 2000). The term 'module', as used here, denotes a unit that is internally coherent due to strong interactions among its parts, but is relatively independent from other such units with which, if connected, it has weaker or fewer interactions (Klingenberg, 2009). Strong internal coherency leads to relatively independent morphological variation, as has been demonstrated for functional modules in general, and for the skull modules in particular (Cheverud, 1996; Lieberman et al. 2000b; Hallgrímsson et al. 2004; Sardi et al. 2007). In addition to serving functional demands, the independence of modules allows morphological evolution through separate, and thus more flexible, processes (Wagner et al. 2005; Smith, 2006; Hallgrímsson et al. 2007; Sardi et al. 2007).

However, morphological units cannot be completely isolated from each other as they exist within the coherent framework of the organism. Anatomical modules are considered integrated when there are mechanisms (embryological, developmental, functional or genetic) that create interactions between them and thus connect them in morphological and/or evolutionary respects (Cheverud, 1996; Rolian & Willmore, 2009). Such interactions can impose different levels of morphological integration (Moss & Young, 1960; Cheverud, 1982; Enlow, 1990; Hallgrímsson et al. 2007). The term 'integration', as used in the present study, refers to the morphological covariation between anatomical parts of individuals within a population. It is the interplay between modularity and integration that determines the final shape of the organism.

Considering the craniofacial complex, the cranial base module has been regarded as a major external determinant

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of the morphology of the facial module (Enlow, 1990; Lieberman et al. 2000a,b; Goodrich, 2005; Bastir & Rosas, 2006; Rosas et al. 2008). The cranial base is the center upon which the rest of the skull grows and attaches, and shows morphological and developmental conservatism in mammals compared with other regions of the skull (Lieberman et al. 2000a). During growth and development, the neurocranium interacts with the face and *vice versa* through the basicranium. Thus, the basicranium may have some influence on the growth and development of the face (Enlow, 1990).

However, recent research, which has mainly focused on the midline cranial base, has failed to establish a definite relationship between it (its shape, size and/or flexion) and the morphology of the face, including malocclusion patterns (Lieberman et al. 2000a; Bastir & Rosas, 2006; Polat & Kaya, 2007; Proff et al. 2008). In an attempt to resolve this issue, morphometric studies have focused on the role of the lateral cranial base structures instead (Bastir et al. 2004; Bastir & Rosas, 2005). These studies have analyzed basicranial and mandibular covariation and suggested that, because of spatial and temporal relations, the middle cranial fossa (encompassing lateral structures), rather than the midline cranial base, may be more relevant to the morphological development of the mandible. Also, findings of high morphological integration between lateral base and facial structures, compared to almost no integration between midline base and face in adults (Bastir & Rosas, 2006), and studies of ontogenetic maturation (Chang et al. 2005) all indicate that the effective interface between the neurocranium and the face might be the lateral basicranium. A more recent study of endocranial base variation in modern humans strengthened the evidence for the dissociation between midsagittal and lateral components of the basicranium (Bruner & Ripani, 2008).

Developmental and ontogenetic factors that may account for low correlations between facial patterns and basicranial angulation (Lieberman et al. 2000a), or low integration between facial and midline base shape in adults (Bastir & Rosas, 2006) have not been adequately investigated so far. However, it is important to explore variations in patterns of integration during growth and development (Arthur, 2002) and to know the processes that underlie integration in the mature organism (Boughner & Hallgrímsson, 2008). This helps to understand mechanisms that are responsible for the final shape configuration of the craniofacial complex.

Bastir et al. (2006) investigated the ontogeny of the human skull in a longitudinal sample using 2D geometric morphometric methods and concluded that the midline cranial base achieves adult shape at 7–8 years, while the lateral cranial floor attains adult shape at 11–12 years. The face achieves adult shape at 15–16 years (Bastir et al. 2006), thus sharing more common developmental timing with the lateral cranial floor compared to that of the midline basicranium. These findings are generally in line with those of traditional studies that used linear or angular measurements (Buschang et al. 1983; Lieberman & McCarthy, 1999). In the present study, the term common developmental time is used to express common ontogenetic periods when shape changes occur within structures. These biological procedures occur through coordinated developmental processes, which may finally result in increased morphological integration.

To test these interpretations, we studied two different aged human groups using geometric morphometric methods and partial least squares analysis. According to longitudinal ontogenetic data of morphological maturation of the human skull (Bastir et al. 2006), the younger group (prepubertal children) contained subjects with all three modules in active growth and development (exhibiting common developmental timing), whereas in the older group (adults), the shape of all structures had been completed long ago (first the middle cranial base, then the lateral base and finally the face), presumably giving sufficient time for loss of any transitory morphological integration due to development to occur. Nevertheless, this second group incorporated a longer period of common developmental timing for the lateral base and the face. According to the authors' knowledge there is no other study evaluating and comparing patterns of morphological covariation between the face and the lateral basicranium (anterior, middle and posterior cranial fossa) with covariation patterns between the face and the midline cranial base from an ontogenetic and developmental point of view. The study of Bastir & Rosas (2006), which first showed the different covariation patterns between midline base shape and face compared to lateral basicranium and face, included only adult subjects with acceptable occlusion that derived from geographically distinct regions. Another unique characteristic of the present study is that the two groups included subjects of the same origin, who presented a wide range of dentofacial deformities. The inclusion of subjects with different facial patterns in the study groups aimed to test for possible interrelationships between cranial base shape and certain malocclusion patterns (Class I, I, and III), and to assess whether and how these covariation patterns change through ontogeny. When we refer to malocclusion we focus on skeletal jaw discrepancies and not on dental relationships.

The primary objective of the present study was to test the null hypothesis of no difference in strength and patterns of morphological covariation between the lateral basicranium and the face compared to that of the midline basicranium and face, in subjects with various skeletal malocclusions at two specific developmental stages. By this, we aimed to investigate whether common developmental timing is a factor that significantly affects morphological integration patterns between these structures (increased morphological integration associated with increase duration of common developmental timing), and to evaluate the way these patterns change during the development of the organism.

Materials and methods

Sample

The records of the Department of Orthodontics of the Dental School, University of Athens, were searched to identify orthodontic patients for inclusion in the study. At first, subjects aged 6–10 and 20–35 years before orthodontic treatment were selected, irrespective of sex and type of malocclusion. Cases with congenital malformations, systemic diseases or syndromic conditions were excluded. None of the selected patients had previously undergone any kind of orthodontic intervention or had any kind of pathological disorder. The skeletal maturation stage of each child was evaluated using the CVM method (Baccetti et al. 2005) to retain only children before the peak of pubertal growth (stage CS1 or CS2). The pretreatment lateral cephalometric radiographs of the selected patients that were of good quality and depicted a reference ruler on the cephalostat for exact measurement of the magnification factor were used for the study.

In total, 153 pretreatment radiographs of 82 children and 71 adults fulfilled the inclusion criteria. These subjects presented a wide range of dental and craniofacial patterns as expected for an orthodontic population (Proffit et al. 2007). This option was adopted because, considering the large percentage of malocclusions compared to what is considered ideal occlusion in humans (Proffit et al. 1998), some scientists consider aspects of malocclusion not to be a true pathological entity, but in many cases a part of physiologic variation (Mew, 2004). Furthermore, disagreement among epidemiological studies regarding malocclusion reveals the difficulty of establishing a definite limit that separates normal from abnormal dental or skeletal traits (Proffit et al. 2007). In the present study, all subjects were considered healthy, in terms of pathology, according to their medical and dental history, diagnostic radiographs and photographs. Thus, any malocclusion was regarded as normal skeletal variation and not as an abnormal condition or pathological entity.

Reduction of the sample was deemed necessary because, in 2-block partial least squares analysis (PLS), the correlation between PLS scores increases with the number of variables and decreases with the number of cases (Mitteroecker & Bookstein, 2007). Thus, to obtain valid comparisons, it was necessary to exclude 11 children to achieve an equal number of subjects in each group. We opted to retain the younger children, to ensure that all three modules (midline base, lateral base and face) were still in active growth and development, or, in the case of the midline cranial base, when it had just completed its adult shape configuration (Bastir et al. 2006). Consequently, the younger group comprised subjects with all modules having common developmental timing. In contrast, the older group included subjects with a longer common developmental period for the lateral base and the face compared to that of the midline base and the face. Furthermore, the older group was characterized by the establishment of adult facial morphology and the developmental and functional maturity of all structures of the craniofacial complex. In this group, a considerable amount of time had passed since all structures had attained their adult shape (Bastir et al. 2006), presumably giving sufficient time for any transitory covariation attributed to common developmental time to fade.

The final material consisted of 142 pretreatment lateral cephalometric radiographs of white patients of Greek ethnic origin, divided into two age groups: 71 pre-pubertal children (32 males and 39 females) aged 6–10 years (mean age 8.5, SD 1.0, range 6.4–9.8), and 71 adults (23 males and 48 females) aged 20–35 years (mean age 25.4, SD 4.0, range 20.0–34.5).

The cephalometric radiographs were scanned at 150 dpi, a resolution considered sufficient for accurate landmark identification (Held et al. 2001), and a set of 30 landmarks was digitized on screen using the VIEWBOX 4 software (dHAL Software, 2009) (Fig. 1). Paired bilateral landmarks were digitized by averaging the left and right sides (Enlow & Hans, 1996). The landmarks represented three craniofacial units, reflecting the three-dimensional form of the head; the lateral cranial floor (Latbase: six landmarks), the midline cranial base (Midbase: five landmarks) and the face (Face: 17 landmarks). The midline cranial base and the lateral cranial base were represented by similar number of landmarks because, when studying integration among several anatomical regions, comparable results can be obtained only when those regions are captured by the same number of landmarks (Mitteroecker & Bookstein, 2007). These cephalometric points (Allpoints: 28 landmarks) were adopted from Bastir & Rosas (2006) to obtain comparable results. The two landmarks (Porion and Orbitale) which define the Frankfurt

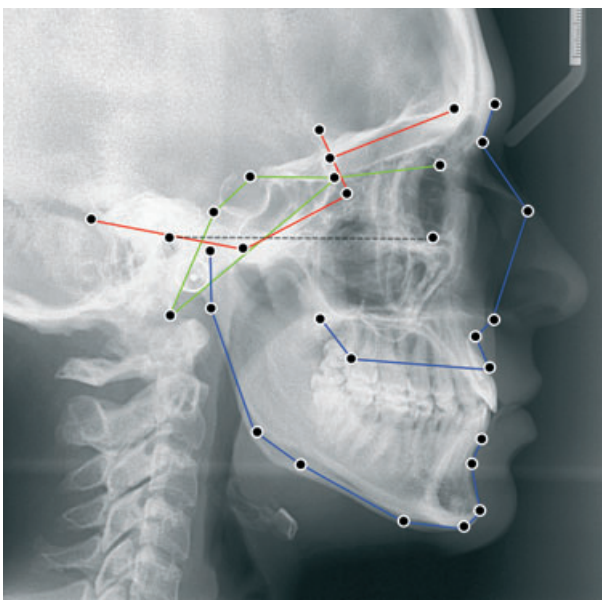

Fig. 1 Lateral cephalometric radiograph showing the craniofacial regions and landmarks analyzed in the study. The blue line illustrates facial structures represented by 17 landmarks: Glabella, Nasion, Rhinion, ANS, A Point, Supradentale, Posterior maxillary alveolar (most posterior cementoenamel junction not including 3rd molars), PNS, Infradentale, B Point, Pogonion, Menton, Inferior mandibular border, Antegonial notch, Gonion, Ramus flexion, Mandibular Condyle (most superior point). The green line illustrates midline cranial base structures represented by five landmarks: Anterior Cribriform, Posterior Cribriform, Posterior Spenoid plane, Base of Dorsum Sellae, Basion. The red line illustrates lateral cranial base structures represented by six landmarks: Anterior orbital roof, Posterior orbital roof, Spheno-parietal junction (center), Anterior greater sphenoid, Inferior on MCF, Petroso-parietal junction (center). The black dotted line illustrates Frankfurt horizontal plane defined by two landmarks: Porion, Orbitale.

Horizontal plane were not included in the analyses, but were essential for digitization of Type III landmarks (Bookstein, 1991), such as Pogonion.

Method error

To test the error of point identification, 20 radiographs were re-digitized 10 days after the first digitization by the same investigator (N.G.). Random error was evaluated by assessing: (i) differences between repeated measures of *x* and *y* landmark coordinates using Dahlberg's formula (Houston, 1983), and (ii) Euclidean distances between the first and second location of each landmark. The average random error of the *x* and *y* point coordinates was 0.70 mm (range 0.12–3.74 mm, SD 0.69 mm). The average value of the landmark distances between repeated measurements was 1.03 mm (range 0.28–4.32 mm, SD 0.94 mm).

Systematic error was evaluated by paired *t*-tests of the *x* and *y* coordinates of each landmark (Houston, 1983). Because of the large number of *t*-tests, we performed a Bonferroni correction of the traditional level of statistical significance ($P = 0.05$) to avoid Type I errors. The *P*-value was adjusted by dividing the initial *P*-value by the number of *t*-tests (60) (Zelditch et al. 2004). No systematic error was detected in any measurement.

Geometric morphometrics and statistical analysis

The four landmark sets (Allpoints, Latbase, Midbase, Face) were subjected to generalized least squares (GLS) Procrustes superimposition (Rohlf, 1990; Bookstein, 1991; Dryden & Mardia, 1998) to obtain a set of shape variables. Another set of variables was obtained from thin-plate splines (TPS) interpolation, which provided the partial warps and uniform component scores for the sample. Size was determined by using the natural logarithm of centroid size (lnCS) (Bookstein, 1991; Dryden & Mardia, 1998).

Sexual dimorphism and size differences

Because of the unbalanced male/female ratio (approximately 1 : 2) in the adult sample, we tested the presence of sexual dimorphism within groups. This was performed by permutation tests using the Procrustes distances between group means as the test criterion (VIEWBOX 4 software, 10 000 permutations) (Good, 2000).

Furthermore, because allometry is a factor that may influence morphological integration between structures (Klingenberg, 2009), size differences between groups (children vs. adults) and within groups (males vs. females) were evaluated by unpaired *t*-tests on lnCS.

Principal components analysis (PCA)

PCA was used to assess the overall variation in the sample and the distribution of individuals in shape space (Rohlf, 1996) using VIEWBOX 4 software. Partial Procrustes superimposition was applied to all 142 subjects, including all 28 landmarks. Principal components (PC) were supplied as both deformations (coefficients of how the shape coordinates jointly shift) and scores. PC scores were visualized with plots, and shape differences with TPS transformation grids.

Allometry – regression analysis

Patterns of morphological integration can be influenced by the presence of allometry (Klingenberg, 2009). Ontogenetic growth allometry is expected for the child group because it encloses a long period of active growth (6–10 years), and static allometry is expected for the adult group because of the male/female ratio (1 : 2), males being on average larger than females (Rosas & Bastir, 2002).

Thus, to test for ontogenetic growth allometry in children and static allometry in adults, we performed multivariate regression of shape variables on size (Monteiro, 1999), independently for the two groups, using tpsRegr (Rohlf, 2009). The landmark coordinates were imported into tpsRegr and subjected to GLS Procrustes superimposition and TPS interpolation, which provided the partial warp and uniform component scores. These capture the shape variation of the sample and constitute the dependent variables of multivariate regression, with size (lnCS) as the independent variable. The multivariate tests of significance for the general linear model are provided by Wilks' Lambda.

Because size differences were found both within and between groups, and allometry was evident in both developmental groups (see Results), we decided to remove the effect of size on shape and obtain a new set of shape variables that were not influenced by allometry. These new shape variables were obtained as the residuals of the aforementioned multivariate regression of shape variables on lnCS and represent shape variation after subtracting allometry. This procedure was performed six times, separately for each block of shape variables (Face, Midbase, Latbase; one each for children and adults). Thus, we were able to explore morphological integration with and without the effect of allometry.

Partial least squares and singular warp analysis

PLS and singular warp analysis were performed to assess patterns of covariation/morphological integration between the lateral, the midline cranial base and the face, in the two age groups. Separate GLS Procrustes superimpositions were performed in each case to examine the individual shape variation of each structure irrespective of its position within the craniofacial system, and thus other structures. The PLS analysis was performed twice, first including the effect of size on shape and secondly after removing the effect of allometry on shape variables as described above. In this analysis, the blocks of landmarks are defined *a priori*. In the present study, 12 blocks of shape variables (Face, Midbase and Latbase, for children and adults, with and without allometry) were constructed to make eight assessments: (i) Face/Midbase 6–10 years, (ii) Face/Latbase 6–10 years, (iii) Face/Midbase 20–35 years, and (iv) Face/Latbase 20–35 years, with and without the effect of allometry.

To further test the possibility that the mixed sex effects in our sample (unbalanced male/female ratio in adults) might have influenced the results, we also repeated the PLS and singular warp analysis including only female subjects (39 children and 39 adults). We selected this option instead of applying any statistical correction to our original data because we preferred to retain them in their actual biologic form.

Shape variables were imported into tpsPLS (Rohlf, 2006) for PLS analysis, which provided pairs of covariance-maximizing linear combinations (singular values) between two blocks of variables. PLS treats the variables of both blocks symmetrically, and therefore we obtained variables within one block most relevant for predicting the variables in the other block and vice versa. These new paired latent variables, or singular warps (SW) (one

per block) account for as much as possible of the covariation between the two original sets of variables. The singular warps display the maximal covariance between both the within-block shape variables and the shape variables of the other block (Rohlf & Corti, 2000).

The amount of covariance explained by each pair of latent variables and the cross-set correlations '*r*' for paired variables (singular wrap scores of individuals) determine the biological significance of each observation – covariation detected in each dimension and the level of integration between blocks. Consequently, these values also determine the dimension(s) that might be meaningful when interpreting the results (Rohlf & Corti, 2000). In the present study, we evaluated the first two dimensions, which represented approximately 80% of the total covariance. A permutation test (9999 permutations) was used to assess whether the covariation in the first two dimensions was statistically significant (Rohlf & Corti, 2000).

Two-block PLS and singular warp analysis were also performed with PLSMAKER6G (Sheets, 2006) to confirm results and obtain transformation grids. Only the statistically significant ($P < 0.05$) or marginally significant ($P \leq 0.10$) singular warps are presented.

Results

Sexual dimorphism and size differences

Regarding sexual dimorphism, no statistically significant separation was found between the sexes in the young group. The adult group showed sexual dimorphism for the Allpoints landmark set ($P = 0.00$), the Face ($P = 0.02$) and the Latbase set ($P = 0.01$). However, sexual dimorphism and its potential effect on morphological integration were not directly investigated in the present study because of inadequate size of the sex subgroups (but see Discussion for female results). Thus, subjects of both sexes were pooled in each age group. Although sex is not expected to influence patterns of integration in adults, this remains to be tested.

Size (lnCS) differed significantly between children and adults ($P < 0.00$) for all landmark sets (Allpoints, Midbase, Latbase, Face). Within groups, size differences between males and females were also evident for all landmark sets, except for Midbase in children (Table 1). Thus, the test for allometry within groups is justified to control another potentially confounding factor for studying patterns of morphological integration.

Principal components analysis

Concerning the configuration of all the landmarks (Allpoints), the first five PCs, accounting for 59.2% of the total variance, were considered meaningful, based on inspection of the scree plot. The subjects were graphed along the PC1 and PC2 axes, which accounted for 37.9% of the total variance (21.7 and 16.2%, respectively) (Fig. 2). TPS grids show the wide range of skeletal configurations included in the sample, in the anteroposterior and vertical dimensions. Regarding age-related differences, separation between children and adults was evident along an oblique direction between PC1 and PC2, but mainly along PC2. It seems that PC1 mainly describes variation in basicranial flexion and divergency of skeletal planes, whereas PC2 describes the anteroposterior intermaxillary relationship. The main characteristic that differentiated children from adults was a tendency for facial convexity for children (Fig. 2).

Allometry – regression analysis

Multivariate regression of shape (dependent variables) on size (lnCS – independent variable) demonstrated the significant presence of allometry in both groups and in all landmark configurations examined (Allpoints, Latbase, Face), except for Midbase in children. In adults, Midbase showed marginally significant allometry (Table 2). The shape variance that was explained by allometry ranged from 2.2 to 13.0% for Midbase and Latbase in adults, respectively. For all the remaining landmark configurations that showed significant allometry, the variance explained by the regression model was approximately 4%, which is considered a rather small value (Table 2).

PLS and singular warp analysis

Results obtained from 2-block PLS analysis, with and without removing the effect of allometry, are shown in Table 3.

Table 1 Mean of logarithm of centroid size (standard deviation in parentheses) by age group and sex. Unpaired *t*-tests comparing male and female subjects within age groups.

	lnCS – children 6–10 years			lnCS – adults 20–35 years		
	Males	Females	*P*-value	Males	Females	*P*-value
All points	5.543 (0.037)	5.516 (0.038)	0.00	5.690 (0.031)	5.614 (0.047)	0.00
Face	5.196 (0.040)	5.167 (0.043)	0.00	5.347 (0.034)	5.270 (0.049)	0.00
Midbase	4.238 (0.033)	4.222 (0.040)	0.07	4.319 (0.058)	4.274 (0.057)	0.00
Latbase	4.461 (0.051)	4.436 (0.050)	0.04	4.549 (0.063)	4.497 (0.063)	0.00

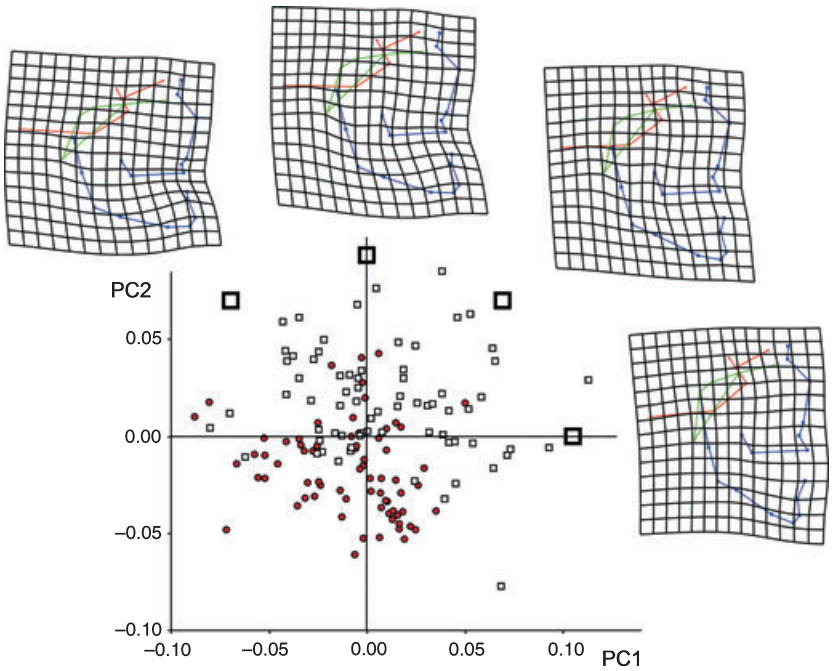

Fig. 2 Scatter plot of the PC scores of the 142 specimens. The *x*-axis is the first PC axis, explaining 21.7% of the variance, the *y*-axis is the second PC axis, explaining 16.2% of the variance. Red circles: children, black squares: adults. The deformed grids illustrate the thin-plate spline interpolation of the entire form showing the transformations implied by changes along the PC axis 1 and 2 scores (right and middle top), as well as the combination of the axes (top left and top right). The large squares show the position of each specimen that corresponds to the deformation showed by each nearby TPS grid.

Table 2 Multivariate regression of shape variables on lnCS, percentage of the variance explained by the model and *P*-value provided by Wilks' Lambda.

	Children 6–10 years		Adults 20–35 years	
	Variance explained (%)	*P*-value	Variance explained (%)	*P*-value
All points	3.5	0.05	4.5	0.00
Face	4.1	0.01	4.4	0.00
Midbase	0.7	0.37	2.2	0.05
Latbase	4.6	0.02	13.0	0.00

The null hypothesis of no difference in morphological integration between the lateral basicranium and the face compared to the midline basicranium and the face at the two developmental stages (childhood and adult life) was rejected, supporting the idea that common developmental timing is an important factor that influences patterns of integration between craniofacial structures. When only female subjects were analyzed, the results indicated the same patterns of integration as those presented for our original mixed sex sample. These data are not presented or analyzed here due to space considerations.

The presence of allometry influenced the strength of morphological covariation in specific cases (mainly in covariation between Latbase and Face, at dimension 2) in children and adults. However, this did not substantially affect the patterns of integration and the sequence of changes through the development and maturation of the organism (Table 3). Thus, for reasons of clarity, we mention here only significant ($P < 0.05$) or marginally significant ($P \leq 0.10$) results that were obtained after removing the effect of allometry (see Materials and methods section). Regarding statistical significance, one exception is made for adults, in the case of covariation between Latbase and Face, at dimension 2, where allometry expressed the most extensive influence in terms of strength of integration ($r = 0.64$, $P = 0.00$ with allometry, and $r = 0.44$, $P = 0.13$ after removing the effects of allometry), reducing covariation below statistical significance. However, because covariation patterns, as evaluated by singular warp analysis, were similar in both circumstances, the findings are nevertheless analyzed.

In children, mainly the midline basicranium, but also the lateral cranial base structures, showed covariation with the face (Midbase: Dimension 1, $r = 0.48$, $P = 0.10$, Latbase: Dimension 2, $r = 0.47$, $P = 0.02$). As midline cranial base attains adult shape early during ontogeny (Bastir et al. 2006), the morphological integration with the face was restricted to Dimension 2 ($r = 0.46$, $P = 0.07$) in the mature organism. However, the lateral cranial base structures strengthened their integration with the face in adulthood ($r = 0.56$, $P = 0.00$ and $r = 0.44$, $P = 0.13$, for the first two dimensions, respectively). These findings indicate that developmental processes, studied in terms of common developmental timing, have a significant influence on morphological integration and are in some degree responsible for the covariation patterns observed in adults. This influence is further explored by singular warp analysis, which is described below.

Results of the singular warp analysis are presented only for the statistically significant or marginally significant

Table 3 Two-block PLS analysis results based on 9999 permutations.

Age group	Blocks of data	Dimension	Correlation *r*	*P*-value	Covariance explained %
6–10 years	Midbase – Face	1	**0.49**/**0.48**	0.08/0.10	44.0/43.5
		2	0.36/0.38	0.52/0.42	27.8/26.0
	Latbase – Face	1	0.43/0.46	0.21/0.15	47.7/52.6
		2	0.41/**0.47**	0.17/0.02	29.9/30.5
20–35 years	Midbase – Face	1	0.43/0.40	0.17/0.32	60.2/50.4
		2	0.44/**0.46**	0.14/0.07	16.6/21.1
	Latbase – Face	1	**0.56**/**0.56**	0.00/0.00	61.2/50.5
		2	**0.64**/0.44	0.00/0.13	23.2/31.5

First value is without removing allometry and second value is after regressing out allometry. Dimensions represent SW axes, correlations (*r*) represent the strength of integration between blocks, *P*-value shows the statistical significance (permutation test) of the correlation coefficient (*r*), and the last column presents the percentage of covariance explained by each dimension. Numbers in bold signify statistical significance at $P \leq 0.10$.

correlations, with the exception of covariation between Latbase and Face, at dimension 2 for adults, for reasons explained earlier. We did not detect appreciable differences between TPS grids obtained with and without removing the effect of allometry. It seems that allometry exerts an influence only on the strength of morphological covariation between structures, but does not affect the way structures are morphologically integrated. For consistency, we present the TPS grids that resulted after regressing out allometry (Figs 3–7).

Concerning singular warp analysis, one important finding is that the main characteristics of the morphological covariation patterns between cranial base structures and the face remain stable through ontogeny, even though the strength and amount of integration between structures change.

SW1 explained 43.5% of the covariance of the midline cranial base with the face in children (Table 3). The correlation observed was moderate ($r = 0.48$) and close to the upper limit of marginal significance ($P = 0.10$). TPS deformation grids showed that a more flexed midline cranial base and a posteriorly positioned cribriform plate were associated with a Class III skeletal pattern (i.e. relatively retruded maxilla and protruded mandible) and an increased lower anterior facial height (Fig. 3).

Concerning covariation patterns between lateral cranial base shape and facial shape in children, only SW2 was significant ($P = 0.02$) and revealed a moderate correlation ($r = 0.47$), explaining 30.5% of the covariance (Table 3). It seems that a relatively flat and more anteriorly positioned middle cranial fossa was associated with a Class II skeletal pattern (protruded maxilla and slightly retruded mandible with decreased ramus and corpus flexion) with relatively increased lower facial height (Fig. 4).

In adults, midline base structures were moderately correlated with the face ($r = 0.46$, $P = 0.07$), but only in SW2,

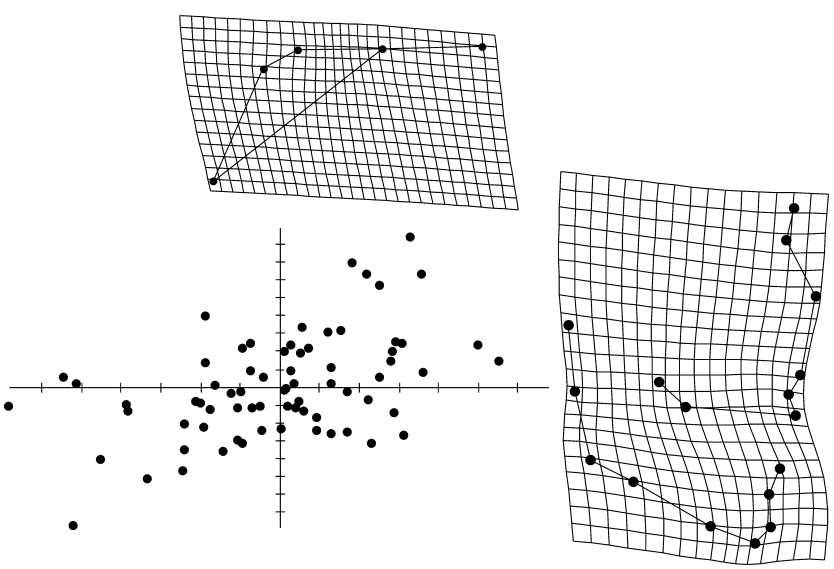

Fig. 3 Plot of singular axis 1 scores for the face (*x*-axis) and the midline cranial base (*y*-axis) in children that explains 43.5% of total covariance, after removing allometry. The associated TPS transformation grids show the pattern of covariance between these structures.

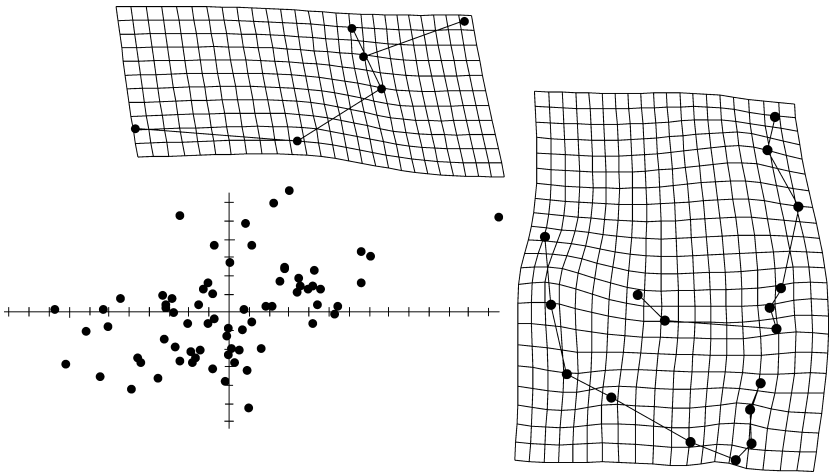

Fig. 4 Plot of singular axis 2 scores for the face (*x*-axis) and the lateral cranial base (*y*-axis) in children, explaining 30.5% of total covariance, after removing allometry. The associated TPS transformation grids show the pattern of covariance between these structures.

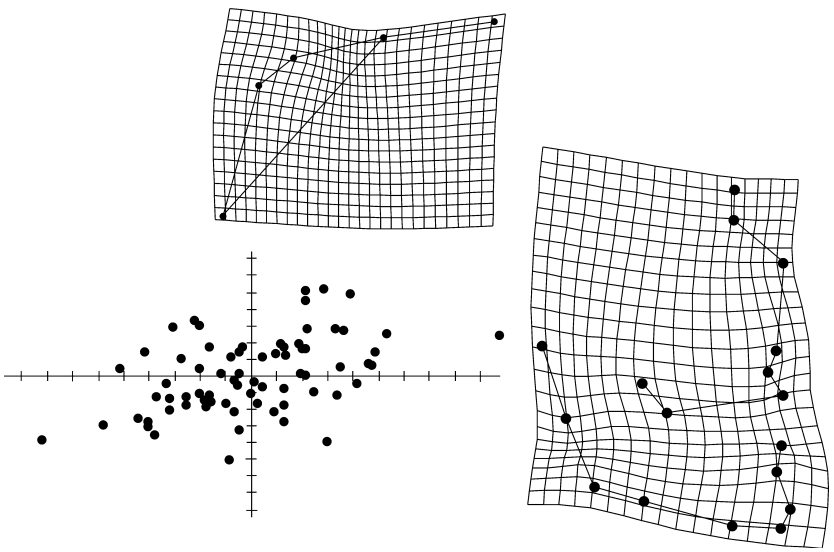

Fig. 5 Plot of singular axis 2 scores for the face (*x*-axis) and the midline cranial base (*y*-axis) in adults, explaining 21.1% of total covariance, after removing allometry. The associated TPS transformation grids show the pattern of covariance between these structures.

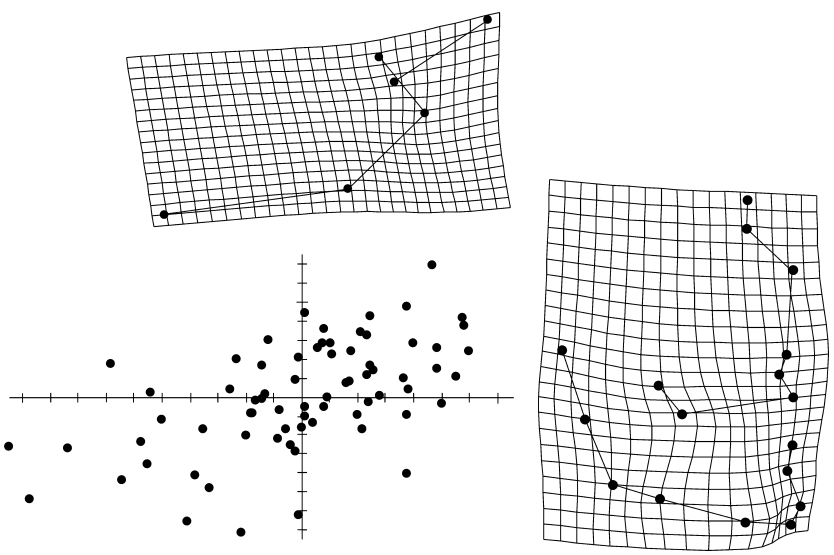

Fig. 6 Plot of singular axis 1 scores for the face (*x*-axis) and the lateral cranial base (*y*-axis) in adults, explaining 50.5% of total covariance, after removing allometry. The associated TPS transformation grids show the pattern of covariance between these structures.

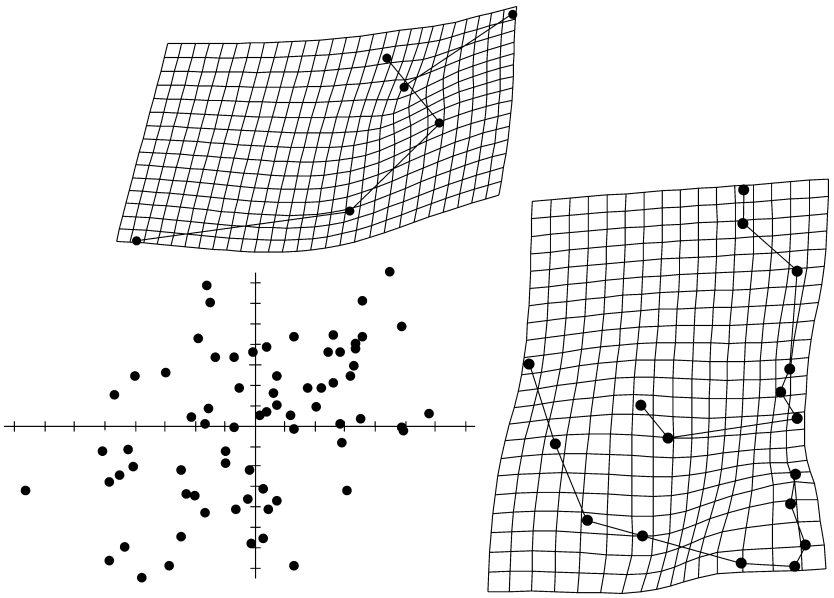

Fig. 7 Plot of singular axis 2 scores for the face (*x*-axis) and the lateral cranial base (*y*-axis) in adults, explaining 31.5% of total covariance, after removing allometry. The associated TPS transformation grids show the pattern of covariance between these structures.

which explained 21.1% of the total covariance. As shown by the transformation grids, a more inclined midline base with an upward rotated cribriform plate is associated with a prognathic mandible (Class III pattern), and an increased lower anterior facial height (Fig. 5). At the anteroposterior level, this covariation pattern was similar to that observed for children (Fig. 3).

Correlation between the lateral cranial base shape and the face was relatively strong, ($r = 0.56$, $P = 0.00$) for SW1 in adults, and explained a large amount of the covariance (50.5%) between the two structures. A relatively flat middle cranial fossa and slightly shortened lateral cranial base, with the frontal structures more upwardly positioned, was associated with a slight Class II tendency and a reduced anterior facial height (Fig. 6). SW2 also revealed a relatively strong ($r = 0.64$) and statistically significant ($P = 0.00$) correlation between the lateral cranial base and the face, but only when allometry was included in the analysis. The removal of the effect of allometry weakened the existing correlation ($r = 0.44$), which also lost statistical significance ($P = 0.13$), although it increased the amount of covariance explained from 23.2 to 31.5%. This was the greatest influence of allometry on the strength of morphological integration observed in the present study. However, the pattern of integration is presented and analyzed here, as it was found unaltered whether allometry was present or not. A deeper, shorter and more posteriorly positioned middle cranial fossa was associated with a retruded maxilla and a protruded mandible (Class III pattern) with increased corpus length, and decreased lower anterior facial height (Fig. 7). As was the case for Midbase and Face, the covariation pattern between the Latbase and Face in adults was similar to the one observed for children.

Discussion

The present study was conducted on subjects that presented a wide range of dental and skeletal patterns. A matter of concern was whether the sample included subjects with extreme morphological patterns, resulting perhaps from undiagnosed pathologies that would skew the results. We sought these potential outliers by performing PCA analyses on the four landmarks sets, separately for each age group. After removing those outliers identified by visual inspection of the PCA plots and equalizing the number of subjects between groups, we arrived at an alternative study sample of 65 children and 65 adults. This produced similar results to those obtained from the original sample (71 children, 71 adults), so it will not be discussed further.

Concerning the variation present in the sample, PCA clearly demonstrated the wide range of skeletal malocclusion patterns included in the sample, in the anteroposterior and vertical dimension. The first two PCs described divergency of skeletal planes and anteroposterior intermaxillary relationship, in accord with previous findings from a different orthodontic sample (Halazonetis, 2004). TPS grids showing variation in overall shape revealed that children, on average, had a relatively more retruded mandible and protruded maxilla (Class II pattern) than adults (Fig. 2). These findings are consistent with present knowledge regarding normal growth and development of the human craniofacial complex (Björk & Skieller, 1983; Enlow & Hans, 1996). Individuals with different levels of jaw discrepancies are demonstrated along PC1 axis, but this is expected as the shape variation of the sample according to skeletal relations is considerable.

The different male/female ratio between the two groups, the size differences between sexes, and the detected sexual

dimorphism in the adult sample raise the question of the presence of ontogenetic or static allometry in the sample. The influence of allometry on morphological integration was restricted to the strength of integration (Table 3), whereas covariation patterns remained unaltered. In general, allometry accounted for only a small percentage of variation in the present sample (approximately 4%). In the case of Latbase in adults, where allometry explained 13% of total variance (Table 2), allometry exerted the greatest influence on the strength of the detected covariation (SW2 for Face to Latbase).

Considering the possible mixed sex effects on the results, PLS and singular warp analysis only in females indicated the same patterns of integration as those presented for the mixed sex sample. Thus, this potential confounding factor was excluded. However, a direct comparison of the magnitude of integration is not possible, as sample size considerably affects the strength of morphological covariation between structures (Mitteroecker & Bookstein, 2007).

As patterns of integration were not significantly influenced by allometry, singular warp analysis is only discussed without including the effect of size on shape. The findings in children (Figs 3 and 4) indicate specific roles of each basicranial element in the development of malocclusions (Enlow et al. 1969), already present at least before puberty. The almost constant relationships between cranial base and facial structures from childhood to adulthood, despite the change in primary roles from Midbase to Latbase observed through ontogeny and developmental maturation, reveal a potentially strong genetic background that determines the craniofacial shape configuration from early stages. The genetic control of certain craniofacial traits was also identified by heritability studies (Sherwood et al. 2008). It is possible that this genetic influence dominates morphogenesis, setting specific constrains to functional demands that may only exert secondary influences on that basis. This speculation is further strengthened by the findings of Jeffery & Spoor (2004) that demonstrated the association of maxillary protrusion with cranial base retroflexion in the prenatal period; a pattern also observed in our data for children (SW1, Fig. 3) and adults (SW2, Fig. 5). The findings concerning the strength of integration in adults are supported by the study of Bastir & Rosas (2006), who analyzed 2D cephalometric data from 144 adult human skulls using the same landmark configurations. Their subjects were from geographically distinct regions and were characterized by acceptable occlusion.

A principal mechanism that results in phylogenetic changes is the accumulation of variations in growth and development (Arthur, 2002). Thus, an additional reason for investigating ontogenetic changes of the craniofacial complex is to explore processes that underlie cranial evolution. It is known that the angle of the midline cranial base is established early in ontogeny, but that the face is developing for much longer. As the midline basicranium grows, it elongates and flexes in the synchondroses (Scott, 1958). After the eruption of M1, there are no significant increases in any measure of cranial base flexion in *Homo sapiens*, which is consistent with the neural growth trajectory – expansion of the brain (Lieberman & McCarthy, 1999). On the other hand, the lateral basicranium matures until later in puberty (Sgouros et al. 1999; Goodrich, 2005) and thus shares a longer ontogenetic trajectory in common with the face (Buschang et al. 1983; Bastir et al. 2006). Increases in basicranial breadth and length also occur in sutures (e.g. the occipito-mastoid), and the endocranial fossa of the basicranium deepens through drift, in which resorption and deposition occur along the superior and inferior surfaces, respectively (Enlow, 1990; Bastir & Rosas, 2009). Data from the present study suggest that patterns of integration remain to some degree constant through ontogeny even though there is a positional change in the primary roles of covariation patterns from midline base to lateral elements, which is at least partially explained by the duration of common developmental timing between structures.

From an ontogenetic point of view, the basicranium and neurocranium grow in tandem in a rapid neural growth trajectory, forming a highly integrated morphological unit, the neuro-basicranial complex (Duterloo & Enlow, 1970; Lieberman et al. 2000a). In contrast, the maxilla and mandible mostly follow the skeletal growth curve (Buschang et al. 1983). Thus, on one hand, because of spatial and temporal reasons, the basicranium may set some preconditions on the development of the face. On the other hand, it is widely supported that facial growth is partially independent of the neuro-basicranial complex because it occurs along a skeletal growth trajectory that, to a large extent, continues after the completion of neural growth (Moss & Young, 1960; Watts, 1985; Farkas et al. 1992). The findings of the present study bring into agreement both viewpoints by presupposing the dissociation of the cranial base into midline and lateral structures. The midline cranial base, accompanied by the lateral base to a lesser degree, seems to be associated with the development of facial morphology in children, whereas in adults, it is the lateral cranial base structures that dominate the integration patterns with the face (Table 3).

Our data indicate that whereas middle cranial base structures are related to facial patterns in children, lateral cranial base elements assume the primary role later in life, possibly through developmental and/or functional mechanisms during maturation of the human craniofacial complex. However, although the specific covariation patterns were identified and remained relatively stable throughout ontogeny, the determination of the exact role of the cranial base structures on the development and establishment of skeletal jaw discrepancies may require more specific, and maybe larger, longitudinal samples. Furthermore, the direct evaluation of the impact of function through a more experimental design would be really informative. However, this is

quite difficult if not impossible for human subjects because of ethical constraints.

Apart from the common developmental timing hypothesis, the strong integration of the lateral cranial base and the face in adults may also be attributed to the direct connections between these structures through the masticatory apparatus. Muscles of mastication grow throughout later ontogeny under the influence of the growth hormone, and features affected by this growth might become integrated through development and function (Marroig & Cheverud, 2001). The role of masticatory muscle function on craniofacial growth and development has been emphasized by several authors (Kiliaridis, 1995, 2006; Raadsheer et al. 1996). Animal studies demonstrated the influence of masticatory muscles on bone remodeling and condylar and sutural growth, whereas human studies have connected the capacity of the masticatory apparatus, and especially of the masseter muscle, with incidences and types of malocclusions at the vertical, sagittal and transverse planes (Kiliaridis & Kalebo, 1991; Kiliaridis, 1995, 2006; Raadsheer et al. 1996).

An additional reason for the existing differences in strength and patterns of morphological integration of the face with midline and lateral aspects of the basicranium may be the type of growth of the basicranial structures. The midline basicranium grows mostly via endochondral ossification at synchondroses. In contrast, the lateral basicranium, along with the face and neurocranium, grow via intramembranous ossification in sutures. Evidence from recent studies suggests that endochondral ossification may be less subject to epigenetic interactions (such as relative brain size) with nearby organs compared with intramembranous ossification. Intramembranous ossification seems to be influenced by organ growth through mechanical forces which upregulate transcription factors in sutures to induce osteogenesis (Opperman, 2000; Wilkie & Morriss-Kay, 2001; Yu et al. 2001; Spector et al. 2002), but synchondroses elongate much like endochondral growth plates incorporating some intrinsic growth potential (Cohen et al. 1985; Kreiborg et al. 1993; Jeffery & Spoor, 2002). However, human and animal studies have suggested that growth of the face and, mainly, the brain also influences, to some respect, endochondral growth of the cranial base (Lieberman & McCarthy, 1999; Hallgrímsson et al. 2007; Lieberman et al. 2008; Bastir et al. 2010; Holton et al. 2010). This supports the hypothesis that variations in neural and facial growth patterns express notable influences on the whole craniofacial morphology.

The processes that underlie integration are a key to understanding the mechanisms of normal or pathological craniofacial development and evolutionary morphology (Boughner & Hallgrímsson, 2008). From the present study, it is evident that lateral cranial base structures consolidate their role regarding facial morphology in adults through developmental and maybe functional maturation. On the other hand, midline cranial base has a primary role in this field in early developmental stages, possibly setting some constraints and general directions for further development. The null hypothesis of no difference in the strength of morphological integration between the face and the lateral basicranium compared to the face and the midline cranial base in two developmental stages (childhood and adulthood) was rejected. The face and the lateral basicranium, which comprise structures with more common developmental timing, presented increased morphological integration in adults. At present it is not clear whether and to what degree the processes that produce adult integration are developmental vs. functional in origin. However, the results of this study indicate that developmental mechanisms, acting during periods of common developmental timing, are a key factor in shaping morphological integration.

Future research regarding the role of cranial base structures in facial morphology and malocclusion patterns should take into account the developmental stage of subjects studied, as well as the dissociation of the cranial base in middle and lateral structures. Studies of morphological variation, modularity and patterns of integration between cranial base structures through ontogeny would also offer further insights into these issues. Finally, investigation of 3D data might enhance our knowledge about the developmental mechanisms that lead to the establishment of adult craniofacial morphology in humans.

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