Title: ontogeny of 3D rib curvature and its importance for the understanding of human thorax development

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ABSTRACT

Objectives: Sagittal and axial rib orientation relative to the spine are two factors that modify rib cage morphology during ontogeny. Some studies suggest that these factors do not operate in the same way at the upper (ribs 1-5) and lower thorax (ribs 6-10) during postnatal growth, but it is unknown if the ontogenetic thoracic changes are produced by morphological changes of the ribs (intrinsic rib factors) or by external factors related to costal joints (extrinsic rib factors).

Material and methods: To clarify these questions, we applied 3D geometric morphometrics of landmarks and sliding semilandmarks (N=20/rib) to 280 individual ribs (1-10) of *Homo sapiens* comprising the entire human ontogeny and growth simulations.

Results: PCA shows that intrinsic rib factors (rib torsion and axial rib curvature) are ontogenetic factors of variability that contribute to configuring the adult thorax shape. Moreover, growth simulations and regression slopes suggest that the upper thorax unit is comprised by ribs 1-7 and the lower unit at least by ribs 8-10.

Discussion: These results suggest anatomical constraints for ontogenetic rib variation, since ribs 1-7 (true ribs) are directly linked to the sternum. Moreover, these results are supported by functional anatomy because pulmonary kinematics would influence the upper unit and diaphragmatic kinematics would influence the lower one. Our findings are relevant not only to understanding how changes at individual ribs contribute to the adult thorax morphology, but also to the development and evolution of the modern human rib cage.

INTRODUCTION

The understanding of the 3D morphological changes that occur in the rib cage during the ontogeny (from newborns to adults) of *H. sapiens* has changed little since the early 80's (Openshaw, 1984). Nevertheless, in recent years it has received special attention in different fields of study such as biomechanics or evolutionary biology (Gayzik et al., 2008; Bastir et al., 2013a, b; Shi et al., 2014; Weaver et al., 2014). This is in part due to methodological advances and improvements in morphometric quantification due to the use of semilandmark methods (García-Martínez, 2013a, b; Bastir et al., 2013a, b; García-Martínez et al., 2014a; Shi et al., 2014; Weaver et al., 2014; Bastir et al., 2015). However, it should be noted that this research has mainly focused on the variability of thorax morphology in anatomical connection.

Some authors have addressed ribcage ontogeny considering it as a whole (Openshaw, 1984; Gayzik et al., 2008; Shi et al., 2014; Weaver et al., 2014) but there is evidence that morphological growth changes at the upper rib cage could be different from those at the lower (Bastir et al., 2013). Different morphometric patterns of the upper and lower thorax have been observed not only during human ontogeny but also in the sexual dimorphism of the thoracic vertebrae or even throughout the evolution of the thorax shape in different hominin lineages (Bastir et al., 2013a; Schmid et al., 2013; Bastir et al., 2014a; Bastir et al., 2015; García-Martínez et al., 2014; García-Martínez and Bastir, 2015).

Ontogenetic implications

Specifically, some authors (Openshaw et al., 1984; Bastir et al., 2013; Weaver et al., 2014) have found that from newborns to young adults (not the elderly), lowering of the ribs relative to the spine into the sagittal plane and medio-lateral expansion of the ribcage in the axial plane are the main factors which configure the shape of the adult rib cage. However, Bastir et al. (2013a) have suggested that these factors do not occur in the same way at the upper and lower thorax, suggesting modular growth. According to these last authors, there is an increase in the relative medio-lateral

dimensions of the upper thorax compared to a relative narrowing of the lower thorax throughout ontogeny, which transforms the pyramidal infant thorax into the barrel-shaped thorax of adults. These authors related this transition to morpho-functional interactions with the shoulder or pelvic girdle as well as insertions of the diaphragm in ribs 7-12 (Spaltzehloltz, 1970; De Troyer et al., 2005).

Intrinsic and extrinsic rib factors modulating thorax development

Because the ribcage is an anatomical composite structure, the adult thorax shape is configured not only by the ontogenetic development of the ribs, but also by the ontogenetic development of their costal joints at the sternal and vertebral ends. Therefore, in order to study the factors that contribute to variability in the construction of the adult rib cage, it is important to separate the intrinsic rib factors (change of the proper rib morphology) from the extrinsic ones (change at the costal joints without change of rib morphology).

As an example, lowering of the ribs has been identified as an important factor of variability during human ontogeny. Such lowering, which has been observed previously (Openshaw, 1984; Bastir et al., 2013a, b; Weaver et al., 2014), could be a mechanical rotation at the costo-vertebral joint (extrinsic rib factor). In fact, a classic text book on human anatomy (Gray, 1918) suggests that this lowering is caused by rotation of the ribs because of gravitational effects related to posture. However, other authors have observed that rib torsion in adults (understood as the three-dimensional spiraling of the rib shaft independent of the costal joints, and therefore an intrinsic rib factor) is an important factor of variability which could contribute to lowering of the ribs relative to the sagittal plane (Schmid, 1991; Mann, 1993; Dudar, 1993; Bastir et al., 2015). However, since there is a lack of knowledge about the individual rib ontogeny, we do not have information about the possibility of rib torsion contributing to the lowering of the ribs observed in the human ontogenetic process. If lowering is produced only by declination (an extrinsic rib factor), we should expect that postnatal ontogeny does not alter the degree of rib torsion (an intrinsic rib factor). It is also possible

that both intrinsic and extrinsic factors interplay together in order to produce morphological variation in rib plane orientation relative to the sagittal plane during ontogeny.

Regarding medio-lateral expansion of the thorax, some authors (Openshaw et al., 1984; Bastir et al., 2013) have argued that this feature is produced by changes in axial rib curvature (an intrinsic factor) throughout ontogeny. However, extrinsic factors, such as the orientation of the transverse processes of the thoracic vertebrae, could also contribute to medio-lateral expansion of the thorax at least in adults (Bastir et al., 2014a), and there is evidence for ontogenetic changes in this orientation (Latimer and Ward, 1993). Because no 3D rib growth study is currently available, it is not clear how the axial rib curvature contributes (intrinsically) to medio-lateral expansion of the thorax.

If medio-lateral expansion of the thorax is caused by the orientation of the transverse processes (an extrinsic rib factor), we should expect no changes in axial rib curvature during ontogeny (an intrinsic rib factor). It is also possible that both intrinsic and extrinsic factors interact to produce morphological variation in the medio-lateral expansion of the thorax during ontogeny.

Functional implications of morphological costal changes

Morphological changes in rib orientation linked to kinematic function have been reported in several studies. On one hand, lowering of the ribs observed from newborns to young adults makes elevation by intercostal muscle action possible (De Troyer et al., 2005; Ratnovsky et al., 2008). However, it is still unknown whether this lowering is caused by a rotation of the ribs at the costal joints (an extrinsic rib factor) or by an increase of rib torsion (an intrinsic rib factor).

On the other hand, medio-lateral expansion of the lower thorax makes diaphragmatic action possible (De Troyer et al., 2005; Ratnovsky et al., 2008; García-Martínez et al., 2014). However, it is still unknown whether medio-lateral expansion of the thorax is caused by a different orientation of

the transverse processes (an extrinsic rib factor) or by differences in the axial rib curvature (intrinsic rib factor).

Breathing in new-borns is essentially diaphragmatic because the ribs are more horizontal than in adults, so the respiratory muscles cannot raise them effectively. Since the lower thorax is medio-laterally expanded, the diaphragm will also be broader and will allow for more efficient diaphragmatic functioning. However, because of changes in the 3D configuration of the ribcage caused by changes in rib-orientation during the first three years, the intercostal muscles are able to raise the ribs more efficiently and thoracic breathing also becomes more effective (De Troyer et al., 2005; Bastir et al., 2013).

Thorax modularity

Openshaw et al. (1984) hypothesized that medio-lateral expansion of the thorax occurs in the whole thorax during postnatal ontogeny. However, Bastir et al. (2013a) were more specific, suggesting that this medio-lateral expansion is more pronounced in the upper than the lower thorax. Thus, if medio-lateral widening of the rib cage is observable at the individual costal level, we should expect a different growth pattern in ribs belonging to the upper (ribs 1-5) and lower thorax (ribs 6-10).

Hypotheses that explain the modularity of the thorax include association with the sternum, kinematics of respiration, and integration with adjacent anatomical regions. First of all, this fact could be attributed to thorax anatomy, since the ribs belonging to the upper unit (true ribs) have the anatomical constraint of the direct link to the sternum, while the lower ribs (mainly false and floating ribs) do not (Spalteholtz, 1970). Secondly, functional anatomy also supports this separation since the development of the upper thorax could be influenced mainly by pulmonary kinematics while the growth of the lower thorax could be influenced by diaphragmatic kinematics (De Troyer et al., 2005; Bastir et al., 2013a; García-Martínez et al., 2014a, b). Finally, anatomical integration could

also account for this separation (Bastir, 2008). This is because upper thorax development could be influenced by that of the shoulder girdle (Schmid et al., 2013; Roach and Richmond, 2015) whereas lower thorax development would be influenced by spine lordosis related to posture (Slijper, 1942; Bastir et al., 2013a; Bastir et al., 2014a), thoraco-pelvic integration (Jellema et al., 1993; Bastir et al., 2014b) or even by sub-thoracic systems such as guts (Aiello, 1997).

Aims of this study

The aim of this study is to fill the gap of knowledge about the ontogenetic growth of individual ribs in modern humans. In this context, we test the following hypotheses:

Hypothesis 1: If rib lowering is only caused by declination (mechanical rotation, so an extrinsic rib factor), we should expect that postnatal ontogeny will not alter the degree of rib torsion (an intrinsic rib factor).

Hypothesis 2: If medio-lateral expansion of the thorax is only caused by orientation of the transverse processes (an extrinsic rib factor), we should expect no changes in axial rib curvature (an intrinsic rib factor) throughout the ontogeny.

Hypothesis 3: If the modularity of the thorax is also observable at costal level, we should not expect the same growth pattern in ribs belonging to the upper (ribs 1-5) and lower thorax (ribs 6-10).

MATERIALS AND METHODS

Materials

In order to test the hypotheses, we used 280 computed tomography (CT) reconstructions of costal elements (ribs 1-10) that belong to 28 rib cages comprising the entire human postnatal ontogeny (from newborn to adults). The data were obtained from hospital subjects at the Medizinischen Universität of Innsbruck (Austria). The subjects, except for three newborns that were scanned in France for virtual autopsy post-mortem, were scanned previously for medical reasons not

related to this study. In none of the cases could any pathologies affecting skeletal rib form be appreciated. The age and sex composition are detailed in Table 1 in Supplementary Information.

The approval to use these pre-existing CT scans for our research was obtained in writing from the *Comite consultatif pour la protection des personnes dans la recherche biomédicale Bordeaux A* and from the *Ethikkommission der Medizinischen Universität Innsbruck* (AN5025, 323/4.24) and prior to analysis all CT-data were anonymized to comply with the Helsinki Declaration (Goodyear et al., 2007).

Methods

Modern human ribs were isolated from each thorax in anatomical connection throughout a semi-automatic segmentation of DICOM images employing the software Mimics 8.0 (<http://biomedical.materialise.com/mimics>) and the “full width half maximum” approach (Spoor et al., 1993). In order to reduce possible errors related to left-right laterality, only ribs from the left side were segmented from each thorax.

The post-processing of 3D surface models of skeletal elements (cleaning, smoothing, and mesh hole-filling) was carried out by Artec Studio software (www.Artec3D.com) and the final 3D surface models of ribs 1-10 of the left side were imported into Viewbox4 software (www.dhal.com). Then, landmarks and semilandmarks for sliding (Gunz et al., 2005) were located on the rib models in order to describe the external curvature of each individual rib.

Landmarks were placed on the ribs following the protocol of Bastir et al. (2013a). Twenty 3D landmarks thus described the morphology of the curvature of each rib. Because of their uncertain location along the ribs, semilandmarks were then slid along their corresponding curves with respect to the fixed landmarks to minimize bending energy also following the protocol of Bastir et al. (2013a): first to the template and then to the consensus of the full sample.

Finally, the whole costal sample of landmarks and semilandmarks (x, y, z coordinates) was submitted to Procrustes superimposition and then separated in 10 subsets of 3D rib shape coordinates (according the rib sequence 1-10) and analyzed separately.

Patterns of size and shape variation during ontogeny were studied through Principal Component Analysis (PCA) in Procrustes form space (Mitteroecker et al., 2004) in order to test changes in rib torsion and axial rib curvature (hypotheses 1 and 2). This kind of PCA reduces shape variation in a few dimensions by retaining the size information through the inclusion of centroid size information after Procrustes superimposition (Dryden and Mardia, 1998; Mitteroecker et al., 2004).

These analyses were computed for each rib subset, so 10 Procrustes form spaces were studied. To assess the pattern of morphological change during ontogeny, the first three components were plotted for each subset. Ordinations were calculated and shape differences of the surface associated with variations along the PC axes were visualized using EVAN Toolkit (version 1.63; <http://www.evan-society.org/>). In this way, we explored the morphological changes along the PC1 warps (which order in size and thus in ontogeny) in relation to sagittal and axial rib curvatures, relevant to the adult thorax configuration.

In order to test if modularity of the thorax is also observable at costal level (hypothesis 3), we conducted a series of growth simulation experiments (Cobb and O'Higgins, 2004, McNulty et al., 2006; Neubauer et al., 2010). Interchanging the ontogenetic trajectories between different elements inside of a "hypothetical unit" (elements – ribs; unit – upper or lower thorax) will allow us to test whether its elements share the same growth pattern. Elements that share it would belong to the same unit, while elements that do not belong to the same unit would not share a common growth pattern (Rosas and Bastir, 2004; Bastir et al., 2006, Esteve-Altava et al. 2013).

To observe differences in growth vectors we carried out a series of growth simulations. These analyses test whether an ontogenetic allometric pattern is characteristic of the upper ribs

(upper thorax growth pattern) and another for the lower ribs (lower thorax growth pattern). If upper and lower thorax growth modularity is the consequence of upper and lower rib growth differences, then these differences should be reflected in the variation of the individual rib growth trajectories. Consequently, applying these different trajectories to individual ribs should lead to differences in the estimated adult morphologies. On the other hand, if no differences are found among individual rib ontogenies, upper and lower thorax modularity is likely a consequence of factors unrelated to rib ontogeny. Applying different ontogenies to different ribs should not lead to (statistical) differences in the simulated adults. Differences between simulated and true adults are tested by mean shape permutations.

To analyze this, we calculated 10 allometric growth vectors in form space (one for each rib subset) taking the mean infant form as a starting point of the vectors and the mean adult form as the vectors' target. Then, we applied each vector (10 vectors in total) to grow the mean infant of the remaining rib subsets, thus creating nine simulations and one control specimen (the mean infant rib growth with its proper ontogenetic vector) for each rib subset.

In order to observe whether simulations fall inside the range of the true adult ribs of each subset and since the ontogenetic trajectory of each rib subset is mainly linear, we have computed 10 regressions of Procrustes shape coordinates on size (one for each rib subset) in MorphoJ 1.05f (Klingenberg, 2011). Then, we traced a 95% confidence ellipse around the true adult sample and observed whether the nine adult simulations for each mean infant rib fall inside of the true adult range.

Elements that do not share common growth trajectories show different slopes in their growth curves (Cobb and O'Higgins, Bastir and Rosas, 2004, Mitteroecker et al., 2005). Therefore, in order to reinforce our results, we have calculated the slope of each regression with the 95% confidence interval, observing whether the slopes of upper ribs fall inside of the interval of lower ribs. These analyses were carried out in PAST software (Hammer et al., 2001).

RESULTS

Form spaces

10 PCA analyses in form space showing PC1, PC2 and PC3 projection suggest a very close link between size and shape (Figure S1, Supplementary Information) thus reflecting a mainly ontogenetic change and explaining a reasonable amount of variance for each rib subset (Table 1). The linearity of each plot suggests linear growth during ontogeny (at least using this set of landmarks) [Table 1 here].

The warps associated with PC1 in form space of each rib subset can be observed in Figure 1 and Figure 2. Different growth patterns at the upper and lower ribs occur in regard to axial rib curvature but not in rib torsion. Regarding rib torsion, in newborns the upper and lower ribs have less torsion than those of the central thorax. After growth, all adult ribs show an increase in torsion compared to that observed in newborns, but the pattern of torsion along the rib sequence in adults is still very similar to that of newborns: ribs of the central thorax have more torsion than those of the upper and lower thorax. Due to this increase in torsion during ontogeny, non-adult ribs are generally less helicoidal than their adult counterparts.

[insert Figure 1 here]

As for axial rib curvature, we can observe that infant ribs are straighter (less curved) in the axial view than their adult counterparts (Fig.2). A different growth pattern can be observed in the upper and lower ribs. After growth, all adult ribs are more curved than those belonging to infants, but the upper ribs present their point of maximum curvature at the mid-shaft while in the lower ribs this point is observed more dorsally, closer to the *angulus costae*.

[insert Figure 2 here]

The warps associated with PC1 in form space (Figures 1 and 2) show that ribs undergo several complex changes in their 3D curvature throughout ontogeny due to intrinsic rib factors. All ribs of the sequence undergo an increase in rib torsion (sagittal curve) and we also observe changes in axial curvature of the ribs, which is different in the upper and lower ribs. These results suggest that hypothesis 1 (we should expect that postnatal ontogeny will not alter the degree of rib torsion) and hypothesis 2 (we should expect no changes in axial rib curvature throughout the ontogeny) should be rejected and hypothesis 3 (we should not expect the same growth pattern in ribs belonging to the upper and lower thorax) should be accepted.

Ontogenetic growth vectors and regression analysis

Figures 2a-j from Supplementary Information show a close link between size and shape, which explains a reasonable amount of the variance for each rib subset (at least >7.13%; at least $p < 0.05$; Table 2). Moreover, it is important to note that a larger amount of variance is collected in the central rib subsets compared to upper and lower rib subsets (Table 2) [Table 2 here].

Growth trajectories of ribs 8-10 are not useful for simulating the true adult morphologies of the rest of the ribs (ribs 1-7) because when these vectors are used for simulation, the predictions fall outside the 95% confidence ellipse of the true adults (this is because the point of maximum curvature is more dorsal than it really is in true ribs) (Figure 2a-g from Supplementary Information). However, when we predict ribs 8-10, we observe that any trajectory can be used to predict a true adult rib since all the predictions fall inside the 95% confidence ellipse of real adults (Figure 2h-j from Supplementary Information).

This suggests that adult ribs 8-10 have a more variable morphology than ribs in the remaining thorax since they are more widely spread out along the ordinate axis of the regression. This fact is also reflected by the low percentage of variance explained by the lower rib regressions.

Finally, Table 3 shows regression slopes of each rib subset regression with 95% confidence interval. The slopes of ribs 1-7 are outside of the interval of ribs 8-10 and vice versa [table 3 here].

DISCUSSION

This study has examined how intrinsic rib factors, such as rib torsion and axial rib curvature, are important to understanding rib growth during postnatal ontogeny. In addition, we tested whether ontogenetic modularity observed at the thorax level could also be recognized in individual ribs, or whether thorax modularity is an emergent phenomenon (novelty) of an anatomical composite structure, a phenomenon imposed by mutual anatomical constraints of vertebrae and ribs.

Implications of the ontogenetic variability of the ribs during human ontogeny

This study indicates that postnatal ontogenetic shape changes observed in rib features such as sagittal or axial curvatures are more complex than previously thought. Our data show that the lowering of the ribs within the thorax is caused by an increase in rib torsion during ontogeny, a phenomenon observed to occur in a similar way at every rib level. This result does not contradict the possibility that extrinsic rib factors at the costal joints (rib declination) could also be involved in the lowering of the ribs. Nonetheless, our results demonstrate that rib torsion is an important intrinsic rib factor during postnatal ontogeny.

In accordance with our results, we can reject hypothesis 1 (we should expect that postnatal ontogeny will not alter the degree of rib torsion) because we observe changes in the torsion pattern of ribs (an intrinsic rib factor) during ontogeny. Instead, we believe that rib declination and rib torsion are two related phenomena that occur together and modify the thoracic configuration throughout ontogeny. However, it is important to note that extrinsic rib factors (rib declination) become apparent only when looking at the thorax in anatomical connection; intrinsic rib factors (rib torsion), on the other hand, become more evident when we look at individual ribs.

Regarding medio-lateral expansion, our results show that axial rib curvature is an important intrinsic rib factor that causes adult ribs to be more axially curved than their non-adult counterparts. Therefore, we can also reject hypothesis 2 (we should expect no changes in axial rib curvature throughout the ontogeny). Instead, we believe that axial rib curvature and transverse processes orientation could be two related phenomena that modify the medio-lateral dimension of the thorax during ontogeny. However, the ontogeny of the transverse processes is not addressed in this study, and should be investigated in future research in order to know how vertebral ontogeny contributes to the medio-lateral expansion of the rib cage.

The information provided in this study could be important not only for understanding the generation of variation among adult *Homo sapiens*, for example, in sexual dimorphism (Bellemare et al., 2003; 2006) or in specific climatic adaptation (Weinstein, 2007), and also in the generation of different thorax morphologies proposed for other hominin species such as Neanderthals (Franciscus and Churchill, 2002; Weinstein, 2008; Gómez-Olivencia et al., 2009; García-Martínez et al., 2014; Bastir et al., 2015). In addition, the understanding of rib ontogeny could help us to understand the thorax morphology of important sub-adult fossil specimens such as the *Homo ergaster* KNM-WT 15000 (Jellema et al., 1993; Roach and Richmond, 2015) or the *Australopithecus sediba* MH1 (Schmid et al., 2013).

Functional implications of rib changes for understanding thorax ontogeny

Anatomy textbooks (e.g., Gray, 1918) show newborns' ribs to be more horizontal than those of adults, and also show that they become more caudally oriented during early ontogeny because of mechanical rotation (declination) due to gravitational processes caused by bipedal posture. This phenomenon has also been found in several recent studies (Openshaw et al., 1984; Bastir et al., 2013a; Shi et al., 2014; Weaver et al., 2014). However, since all of these works studied the thorax in anatomical connection, it was not clear if this rib lowering was caused solely by declination, or if intrinsic rib factors were also involved.

Our regression results demonstrate for the first time that this lowering of the ribs, accounting for important functional changes from diaphragmatic breathing to intercostal muscle breathing, could be caused by changes of rib torsion. This is an intrinsic rib factor that cannot be easily determined by looking at the thorax in anatomical connection, and thus requires exclusive analysis of individual ribs. Our results do not exclude some degree of rib declination (an extrinsic rib factor) as a factor that contributes to rib lowering.

Biomechanical action of intercostal muscles elevates ribs from a declined state that cause thoracic inspiration (De Troyer et al., 2005). When ribs are more horizontal (in newborns, for instance) the respiratory muscles cannot raise the ribs effectively. This breathing mode becomes insufficient for respiration and must be compensated by diaphragmatic breathing. However, the increase in rib torsion observed during ontogeny makes thoracic breathing more efficient, because when ribs are more declined, the intercostal muscles can raise them more effectively (De Troyer et al., 2005; Bastir et al., 2013). This is important for understanding functional changes of the rib cage during ontogeny, but is also important for understanding human evolution, since some studies have observed that changes in rib torsion could affect the functional anatomy of respiratory system in other hominin species such as *Australopithecus* or Neanderthals (Schmid et al., 1991; Bastir et al., 2015).

It should be noted that since there is some percentage of variance not covered (see Table 2) by our regressions, our landmark protocol doesn't collect all morphological variance. Future research is needed in order to address this question and cover a larger amount of the variance.

Implications of the ontogenetic variability of ribs for understanding thoracic units

Our results show that different changes in axial rib curvature (but not in rib torsion) are observed in the growth of upper and lower thorax units. After growth, the upper ribs present the point of maximum curvature at the mid-shaft while in the lower ribs this point is observed more

dorsally, closer to the *angulus costae*. This different growth seen in lower ribs could be related to the invagination of the spine (thoraco-lumbar lordosis) that has been observed in the ontogenetic process by different authors (Gayzik et al., 2008; Bastir et al., 2013a; Shi et al., 2014; Weaver et al., 2014). This phenomenon, together with the previously observed increase in rib torsion, would cause lower rib cages in adults to be narrower than those of non-adults. For that reason, our results support hypothesis 3 (we should not expect the same growth pattern in ribs belonging to the upper and lower thorax) since we observe that the modular pattern of the thorax is also observable at costal level.

However, this hypothesis can only be partially accepted. Our results allow us to be more specific in establishing this separation than previously (Bastir et al., 2013) since the upper unit is comprised of true ribs (ribs 1-7) while the lower thorax is comprised (at least) of ribs 8-10. This modular pattern is observable at the confidence intervals of the regression slopes (Table 3) because the lower rib slopes (ribs 8-10) are outside the interval of that of the upper ribs. However, it is important to note that this is not as clear when observing predicted adult individuals through the use of growth trajectories. This is because upper ribs predicted by lower rib growth vectors fall outside the modern human adult range, while the lower ones predicted with the upper rib vectors fall inside the confidence range of true adult individuals (Figures 2a-j from Supplementary Information). The confidence ellipses for adult lower ribs are wider than those of upper ribs because adult ribs are more spread out along the ordinate axis (shape). This observation suggests that adult lower rib morphology is more variable than morphology of adult upper ribs, a fact also observable because lower rib regressions are less explanatory than regressions of upper ribs.

It is interesting to note that the morphological architecture of the first rib is very different from that of the rest of the ribs, and that its muscle attachments are also very specific (Spaltzehloltz, 1970). Anatomical and functional integration could support specific integration of the first ribs with the neck or shoulder girdle not observed in other ribs (Schmid et al., 2013; Roach and Richmond,

2015). However, our results (see Table 3) show that the first rib regression slope (shape on size) falls between the confidence interval of ribs 1-7 but is outside of the interval of the rest of the ribs. Finally, regarding floating ribs, here we hypothesize that the eleventh and twelfth ribs share a common growth pattern with ribs 8-10. However, this issue should be addressed in future studies because in the present study we have not quantified them because of the lack of homology in the landmarks of the rib head.

The modular model proposed in this work (upper unit – ribs 1-7; lower unit – at least ribs 8-10) is coherent with thorax anatomy. This is because development of ribs belonging to the upper unit (so-called true ribs), are the only ones which have the anatomical constraint of a direct link to the sternum, while ribs belonging to the lower unit (at least false and probably floating ribs, as well) do not present this constraint (Spaltzehloltz, 1970). Functional anatomy also supports this separation since the development of the upper unit could be influenced by pulmonary kinematics while lower unit growth could be influenced by diaphragmatic kinematics (De Troyer et al., 2005; Bastir et al., 2013a; García-Martínez et al., 2014a, b).

Finally, anatomical integration (Bastir, 2008) could also account for this separation. This is because upper thorax development may be influenced by the shoulder girdle (Schmid et al., 2013; Roach and Richmond, 2015) and lower thorax development may be influenced by spine lordosis related to posture (Slijper, 1942; Bastir et al., 2013a; Bastir et al., 2014a), thoraco-pelvic integration (Jellema et al., 1993; Bastir et al., 2014b) or even by sub-thoracic systems such as guts (Aiello, 1997). This is also reinforced by the mosaic features found in the thorax of fossil species like *Australopithecus sediba* (Schmid et al., 2013), since the upper thorax shows archaic features (probably related to an adaptation to arboreal locomotion of the scapular girdle, or a retention of primitive characters) whereas the lower thorax presents a more modern-like morphology, probably also linked to a more modern-like pelvis.

Limitations of the study

Although this paper greatly expands our knowledge of individual rib ontogeny, more research is needed in order to clarify some questions, for example, the growth trajectories of floating ribs. Methodological improvements in the landmark quantification protocol or a larger sample of ontogenetic data could also help us confirm the results presented here.

Conclusions of the study

This study has shown for the first time that intrinsic rib factors, such as rib torsion and axial rib curvature, are important factors during postnatal ontogeny which contribute to establishing the 3D configuration of the adult thorax. However, our results do not exclude the possibility that ontogeny of extrinsic rib factors, such as rib declination or the orientation of transverse processes, are also involved in configuring the morphology of the adult thorax. These problems should be addressed in future research.

Our results make evident the difference between rib torsion and rib declination, and, because extrinsic rib factors such as rib declination are not observable at costal level, suggest that rib torsion should be focused on in studies on individual ribs. In addition, this study has allowed us to be more specific in the assessment of thoracic units. Growth simulations and individual rib ontogenetic trajectories suggest an upper thoracic unit comprised of ribs 1-7 and a lower unit comprised of ribs 8-10 (at least), a result that is consistent with aspects of functional and anatomical integration.

Our findings are relevant to understanding how intrinsic rib factors cause changes in individual ribs that contribute to configuring the 3D morphology of the adult thorax. This information could also be important for understanding the development and evolution of the modern human rib cage and the thoracic morphology of other hominin species.

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Author contribution

Conceived and designed the experiments: DGM, MB. Performed the experiments: DGM MB. Analyzed the data: DGM MB. Contributed reagents/materials/analysis tools: DGM MB WR. Wrote the manuscript: DGM MB.

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Captions to the figures

Figure 1: 3D warps showing morphological changes undergone along the ontogeny at every rib level observed in the axial view. The ribs are displayed in anatomical sequence 1-10 from the left to the right side of the figure. The arrow shows the direction of the ontogenetic change.

Figure 2: 3D warps showing morphological changes undergone along the ontogeny at every rib level observed in interior lateral view. The ribs are displayed in anatomical sequence 1-10 from the top to the bottom of the figure. The arrow shows the direction of the ontogenetic change.

Ontogenetic change

Table 1: Percentage of variance explained by PC1-PC3 of the principal component analysis (PCA) in form space of each subset of ribs.

	PC1 (%)	PC2 (%)	PC3 (%)	PC1-PC3 (%)
1st rib PCA	95.91	1.73	0.90	98.54
2nd rib PCA	97.95	0.92	0.43	99.30
3rd rib PCA	98.03	0.95	0.40	99.38
4th rib PCA	97.92	1.20	0.41	99.53
5th rib PCA	98.43	0.79	0.41	99.63
6th rib PCA	98.49	0.81	0.30	99.60
7th rib PCA	98.48	0.83	0.29	99.60
8th rib PCA	98.48	0.83	0.30	99.61
9th rib PCA	98.69	0.65	0.31	99.65
10th rib PCA	98.46	0.76	0.32	99.54

Table 2: Percentage of variance explained for each regression analyses of the Procrustes coordinates on size of each subset of ribs employed in the study as well as their statistic p-value.

	% of variance	p-value
1st rib regression	10.86	0.01
2nd rib regression	22.55	<0.01
3rd rib regression	21.18	<0.01
4th rib regression	19.09	<0.01
5th rib regression	19.91	<0.01
6th rib regression	20.08	<0.01
7th rib regression	17.75	<0.01
8th rib regression	8.54	0.02
9th rib regression	8.03	0.04
10th rib regression	7.13	0.04

Table 3: slope (a) of the multivariate regression analysis of each subset of ribs, as well as their 95% confidence intervals in parentheses.

	Slope (CI)
1st rib slope (a)	8.52 (6.80 - 10.11)
2nd rib slope (a)	7.97 (6.00 - 9.62)
3rd rib slope (a)	7.93 (5.72 - 9.54)
4th rib slope (a)	7.68 (5.55 - 9.46)
5th rib slope (a)	8.49 (6.41 - 10.30)
6th rib slope (a)	8.20 (6.19 - 10.00)
7th rib slope (a)	8.19 (5.90 - 9.98)
8th rib slope (a)	12.36 (8.56 - 15.23)
9th rib slope (a)	14.66 (10.26 - 18.21)
10th rib slope (a)	14.97 (9.16 - 19.03)