3D atlas of human embryology

New insights in human development

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“The less general structural relations are formed after the more general, and so on until the most special appear. The embryo of any given form, instead of passing through the state of other definite forms, on the contrary separates itself from them. Fundamentally the embryo of a higher animal form never resembles the adult of another animal form, but only its embryo.”

*Karl Ernst von Baer, 1828*
Abstract

Introduction: The hyoid-larynx complex is highly prone to anatomical variation. The etiology of anatomical variants like Eagle’s syndrome and the aberrant hyoid apparatus can be explained from embryonic development. Modern textbooks state that the hyoid bone body develops from second and third pharyngeal arch cartilages and that thyroid cartilage derives from fourth and sixth arch cartilages. This description, however, is incompatible with various anatomical variants and it is unclear whether it was based on observations in human embryos or on comparative embryology.

Material and Methods: Histological sections of 14 human embryos from the Carnegie collection between Carnegie stage 17 and 23 (42-60 days) were examined. Three-dimensional models were prepared in an interactive format. These anatomical models provide crucial spatial information and facilitate interpretation.

Results: We observed a less complicated development of the hyoid-larynx complex than as it is currently described in textbooks. The body of the hyoid bone originates from a single growth center, without overt contributions from second and third pharyngeal arch cartilages. The fourth and sixth arch cartilages were not detected in human embryos; the thyroid and cricoid cartilages develop as mesenchymal condensations in the neck region.

Discussion: Despite new research techniques, theories about hyoid-larynx complex development from the beginning of the twentieth century have not been refuted properly and can still be found in modern literature. Based on observations in human embryos we propose a new and relatively simpler description of the development of the hyoid-larynx complex, to facilitate better understanding of the etiology of anatomical variants.

Introduction

Anatomical variants of the hyoid-larynx complex have an incidence of 4-30% in the general population (De Paz et al. 2012; de Santana Jr et al. 2006; Petrovic et al. 2008), with Eagle’s syndrome and the aberrant hyoid apparatus as most diagnosed variants. Hyoid-larynx anomalies are of great importance for radiological examination and surgery of the neck region. Because fractures in this region are important indicators for strangulation and blunt trauma on the neck, significance of this complex has been well recognized in forensic sciences. The embryonic development of this complex, however, has been poorly described, and it is unclear whether current descriptions, which form the basis for the explanation of the development of these variants, are based on observations of original human embryos or on comparative embryology.

Dwight stated already in 1907 that the process of hyoid-larynx complex development provides a great diversity in shapes of the hyoid bone (Pollanen and Ubelaker 1997). Therefore, it is of both clinical and forensic relevance to describe normal embryonic development of the hyoid-larynx complex in an understandable fashion. Descriptions of the development of this complex in human embryos, however, are lacking. Modern textbooks provide conceptual descriptions that are historically based on the comparative embryology of this complex in other animals, instead of being traceable to observations in real human embryos (Fig. 1B) (de Bakker et al. 2016). Furthermore, current theories about the development of the hyoid-larynx complex from the second, third, fourth and sixth pharyngeal arch cartilages remain inadequate in elucidating some of the observed anatomical variants (de Bakker et al. submitted). Therefore, we initiated this study based upon the analysis of sections of original human embryos.
The development of the human hyoid-larynx complex revisited

We studied the embryonic development of the hyoid-larynx complex in human embryos from the Carnegie Collection in Silver Spring, MD, USA, to verify the established scheme of the development of this complex from second, third, fourth and sixth pharyngeal arch cartilages (Fig. 1B). Our data suggest a new and less complex process (Fig. 1C), in which the body of the hyoid bone originates from a single growth center, without overt contributions from second and third pharyngeal arch cartilages. Furthermore, the fourth and sixth pharyngeal arch cartilages were not detected in human embryos; the thyroid and cricoid cartilages develop as mesenchymal condensations in the neck region.

The hyoid-larynx complex

The adult hyoid-larynx complex is described as the combination of the hyoid apparatus or stylohyoid chain - consisting of the styloid processes of the temporal bones of the skull, the stylohyoid ligaments and the lesser horns of the hyoid bone, the body and greater horns of the hyoid bone, the thyroid cartilage including its superior and inferior horns, cricoid cartilage, arytenoid cartilages and their ligaments. A schematic overview of the normal anatomy of the hyoid-larynx complex is shown in figure 1A.

As part of the viscerocranium, the hyoid bone is a solitary horseshoe-shaped bone in the anterior midline of the neck, just above the thyroid cartilage, to which it is connected with the medial and lateral thyrohyoid ligaments (D’Souza, Harish, and Kiran 2010; Gok, Kafa, and Fedakar 2012; Leksan et al. 2005). The hyoid bone consists of a body and two paired processes, the lesser and greater horns (Gok, Kafa, and Fedakar 2012; Porrath 1969). The lesser and greater horns are normally united to the quadrilateral shaped hyoid bone body by fibrous tissue or a true joint, which may become ankylosed later in life (D’Souza, Harish, and Kiran 2010; Gok, Kafa, and Fedakar 2012; Gupta et al. 2008; Hänsch 1977; Miller, Walker, and O’Halloran 1998; Pollanen and Chiasson 1996; Porrath 1969; Soerdjbalie-Maikoe and van Rijn 2008; Ubelaker 1992). The lesser horns are also known in literature as cornu minus, epihyal, hypo-hyal or apohyal (Dwight 1907; Lesoine 1966) and the greater horn as cornu majus.

Functionally, the hyoid bone aids in tongue movement and swallowing and it maintains patency of the pharynx. It therefore serves as an anchor for the suprahyoid muscles (digastric, stylohyoid, mylohyoid, geniohyoid and the middle pharyngeal constrictor muscle) and the infrahyoid muscles (sternohyoid, omohyoid and thyrohyoid) (Gray 2010; Urbanova et al. 2013; Schünke et al. 2006; Lesoine 1966).

The three auditory ossicles (i.e. malleus, incus and stapes) are not part of the hyoid-larynx complex, but since they develop from first and second pharyngeal arch cartilages they were also incorporated in this research.

Anatomical variants

The hyoid-larynx complex is one of the most polymorphic regions in the human body. The hyoid bone reveals a large variance, most often due to the lack of symmetry in the greater and lesser horns (Di et al. 2004; Papadopoulos, Lykaki-Anastopoulou, and Álvanidou 1989; Pollanen and Ubelaker 1997). Common anatomical variations of the hyoid bone can be found in the process of ankylosis of the joints between the hyoid body with the greater and/or lesser horns. Ankylosis is a physiological process that progresses with age (D’Souza, Harish, and Kiran 2010; Gok, Kafa, and Fedakar 2012; Gupta et al. 2008; Hänsch 1977; Miller, Walker, and O’Halloran 1998; Pollanen and Chiasson 1996; Porrath 1969; Soerdjbalie-Maikoe and van Rijn 2008; Ubelaker 1992). Morphological variations of the hyoid bone are closely related to sex (Gok, Kafa, and Fedakar 2012; Kindschuh, Dupras, and Cowgill 2012; Miller, Walker, and O’Halloran 1998; Urbanova et al. 2013), race (Gok, Kafa, and Fedakar 2012; Kindschuh, Dupras,
and Cowgill 2010, 2012), body proportions (weight and length) (Pollard et al. 2011; Urbanova et al. 2013) and age (D’Souza, Harish, and Kiran 2010; Gok, Kafa, and Fedakar 2012; Gupta et al. 2008; Hänsch 1977; Miller, Walker, and O’Halloran 1998; Pollanen and Chiasson 1996; Porrath 1969; Soerdjbalie-Maikoe and van Rijn 2008; Ubelaker 1992; Urbanova et al. 2013). Multiple variations can be found in the same person and variations can occur either unilaterally or bilaterally and even absence of the hyoid bone has been reported (de Santana Jr et al. 2006; Di et al. 2004; Eagle 1958; Vougiouklakis 2006). Hyoid abnormalities have been related to other clinical conditions such as Pierre Robin Sequence, micrognatia and cleft lip and palate cases (El Amm and Denny 2008; Gok, Kafa, and Fedakar 2012).

Significant anatomical variants can cause symptoms like foreign body sensation in the throat (de Santana Jr et al. 2006; Eagle 1948, 1949; Lesoine 1966; Shul’ga, Zaitsev, and Zaitseva 2006) and dysphagia (de Santana Jr et al. 2006; Lesoine 1966; Shul’ga, Zaitsev, and Zaitseva 2006) in Eagle’s syndrome for example, or restriction of neck movement (Lykaki and Papadopoulos 1988) and dysphagia (Dwight 1907) in the aberrant hyoid apparatus. The main cause for Eagle’s syndrome can be found in partial ossification of the stylohyoid ligament on the cranial side, providing elongation of the styloid process (Shul’ga, Zaitsev, and Zaitseva 2006). Since there is a continuum between Eagle’s syndrome and aberrant hyoid apparatus these variants are often intermingled in literature, but Eagle’s syndrome proper only describes symptoms due to anatomical variation of the styloid process, whereas in the aberrant hyoid apparatus the complete stylohyoid chain may be affected.

Fig. 1. Conventional versus proposed view on the embryonic development of the hyoid-larynx complex.

A: Anatomy of the hyoid-larynx complex

B: Conventional theory on development

C: Proposed theory on development

Fig. 1. Conventional versus proposed view on the embryonic development of the hyoid-larynx complex. A: Lateral view of a schematic representation of the normal anatomy of the adult neck region including anatomical annotations. B: The current opinion of the division of the neck region in 5 pharyngeal arch zones, depicting the origin of each component of the hyoid-larynx complex (Cochard 2012; Moore, Persaud, and Torchia 2016; Sadler 2015; Carlson 2014; Gok, Kafa, and Fedakar 2012; Lesoine 1966; Soerdjbalie-Maikoe and van Rijn 2008; Gray 2010; Hamilton, Boyd, and Mossman 1972; Schünke et al. 2006). C: Proposed view on the development of the hyoid-larynx complex. Only the first three pharyngeal arch cartilages develop as pharyngeal arch cartilages, the thyroid and cricoid cartilages develop separately from each other from mesenchymal condensations. The body of the hyoid bone develops from one growth center (anlage), without the contribution from the second and third pharyngeal arch cartilages (Dwight 1907; Rodriguez-Vazquez et al. 2011).
The development of the human hyoid-larynx complex revisited

Materials and Methods

Fourteen human embryos of the Carnegie Collection, ranging in age from 42 to 60 days of development, corresponding to Carnegie stages 17 to 23 (O'Rahilly and Müller 1987; Streeter 1942), were used to study the development of the hyoid-larynx complex. Histological sections of two specimens per stage were analyzed. Details about the specimens can be found in Table 1.

Detailed three-dimensional (3D) reconstructions of the hyoid-larynx complex were made by manually segmenting each structure in histologically stained sections, using the software package Amira (version 5.4, http://www.amira.com). The segmented structures were: first pharyngeal arch cartilages (Meckel’s), second pharyngeal arch cartilages (Reichert’s), greater horns of hyoid bone, the auditory ossicles (i.e. malleus, incus and stapes), body of hyoid bone, thyroid cartilages, cricoid cartilage, arytenoid cartilages and tracheal rings. Each structure was bilaterally segmented in each embryo if present in the studied specimen. In the framework of the making of an interactive three-dimensional digital atlas and quantitative database of human development, all other organs and structures were reconstructed as well, permitting reliable positioning of the hyoid-larynx complex relative to other structures (de Bakker et al. 2016).

Interactive 3D models (3D-PDFs) of one specimen per stage were created in order to clarify the three-dimensional appearance of the developing hyoid-larynx complex. To this end, the Amira reconstructions were first processed in the open source software package Blender version 2.68a (https://www.blender.org) to reduce the number of polygons, which gives a reduction in file size and a smoother appearance of the 3D model, without loss of significant detail. Thereafter, the 3D model was exported into Adobe Acrobat 9 Pro Extended (http://www.adobe.com) to create 3D-PDF files, containing all reconstructed structures per stage (de Boer et al. 2011). The 3D-PDF with all stages was added to this manuscript as supplementary data and can be viewed with a recent version of Adobe Reader (freeware, http://www.adobe.com).

Results

The reader is encouraged to read the results along with the interactive 3D-PDF file in the online data supplement (accessible online).

An overview of the reconstructed specimens is given in figure 2. Stage 17 (42-44 days) showed only the first faint signs of mesenchymal condensations in the neck region. The first clearly distinguishable structures were the first and second pharyngeal arch cartilages and the hyoid body anlage in stage 18 (44-48 days). In stage 18, this cylindrical shaped growth center is located ventrally along the cranio-caudal axis in-between left and right second pharyngeal arch cartilage. In subsequent stages, more mesenchymal derived cartilaginous tissue was added to this anlage until the adult shape of the hyoid bone body was reached, as shown in figure 3. This anlage was described by Rodríguez-Vázquez et al. as a triangular and plate-like condensation, but the 3D reconstruction of this anlage in stage 18 reveals a mere cylindrical appearance (Rodriguez-Vazquez et al. 2011). In the stage 18 embryo the auditory ossicles (i.e. malleus, incus and stapes) could not be sufficiently separated yet and therefore this region of mesenchymal condensation was referred to as ‘Bone-forming Region’. From stage 19 (48-51 days) onwards, malleus, incus and stapes could be morphologically clearly separated. In stage 19 the cartilaginous bars of the greater horns were identified as third pharyngeal arch cartilages. Presence of a fourth and sixth
cartilaginous bar as was expected from literature (Hamilton, Boyd, and Mossman 1972; Soerdjbalie-Maikoe and van Rijn 2008; Gray 2010; Cochard 2012; Gok, Kafa, and Fedakar 2012; Carlson 2014; Sadler 2015; Moore, Persaud, and Torchia 2016) was not confirmed. Instead, the shape of the thyroid cartilage was immediately discernable as condensed mesenchyme in stage 19, without being preceded by a cartilaginous bar of pharyngeal arch cartilage. In stage 20 (51-53 days) the shape of the cricoid cartilage also became discernable as mesenchymal packaging. The first tracheal rings and the arytenoid cartilages could first be distinguished in stage 22 (54-58 days). The hyoid-larynx complex in stage 23 (56-60 days) resembled that of stage 22.

Table 1. Overview of embryonic specimens used to examine the development of the hyoid-larynx complex

<table>
<thead>
<tr>
<th>CS</th>
<th>Origin*</th>
<th>Specimen #</th>
<th>Year</th>
<th>CRL (mm)</th>
<th>Sex</th>
<th>Fixation medium</th>
<th>Staining</th>
<th>Z-res (μm)</th>
</tr>
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<tr>
<td>17</td>
<td>CC</td>
<td>6521</td>
<td>1933</td>
<td>10.6</td>
<td></td>
<td>Corrosive Acetic Acid</td>
<td>Alum cochineal (i.e. carmine)</td>
<td>10.0</td>
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<tr>
<td>17</td>
<td>CC</td>
<td>6520</td>
<td>1932</td>
<td>12.2</td>
<td></td>
<td>Corrosive Acetic Acid</td>
<td>Alum cochineal (i.e. carmine)</td>
<td>17.9</td>
</tr>
<tr>
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<td>CC</td>
<td>6524</td>
<td>1933</td>
<td>9.7</td>
<td></td>
<td>Corrosive Acetic Acid</td>
<td>Aluminum Cochineal</td>
<td>10.2</td>
</tr>
<tr>
<td>18</td>
<td>CC</td>
<td>4430</td>
<td>1923</td>
<td>15.9</td>
<td>F</td>
<td>Corrosive Acetic Acid</td>
<td>Alum cochineal (i.e. carmine)</td>
<td>37.2</td>
</tr>
<tr>
<td>19*</td>
<td>CC</td>
<td>2114</td>
<td>1918</td>
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<td>Formalin</td>
<td>Aluminum Cochineal</td>
<td>40.8</td>
</tr>
<tr>
<td>19</td>
<td>CC</td>
<td>8965</td>
<td>1952</td>
<td>17.7</td>
<td></td>
<td>Zenker's Formol</td>
<td>Borax Carmine - Orange G</td>
<td>60.7</td>
</tr>
<tr>
<td>20*</td>
<td>CC</td>
<td>462</td>
<td>1910</td>
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<td>M</td>
<td>Formalin</td>
<td>Aluminum Cochineal</td>
<td>42.4</td>
</tr>
<tr>
<td>20</td>
<td>AMC</td>
<td>52025</td>
<td>~1975</td>
<td>19.8</td>
<td>M</td>
<td>Bouin</td>
<td>Haematoxylin-azophloxine</td>
<td>30.5</td>
</tr>
<tr>
<td>21*</td>
<td>CC</td>
<td>7254</td>
<td>1936</td>
<td>17.4</td>
<td>M</td>
<td>Bouin</td>
<td>Hematoxylin and Eosin</td>
<td>60.1</td>
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<tr>
<td>21</td>
<td>CC</td>
<td>4090</td>
<td>1922</td>
<td>19.4</td>
<td>F</td>
<td>Formalin</td>
<td>Alum cochineal (i.e. carmine)</td>
<td>99.6</td>
</tr>
<tr>
<td>22*</td>
<td>CU</td>
<td>895</td>
<td>1914</td>
<td>21.2</td>
<td>F</td>
<td>Formalin</td>
<td>Aluminum Cochineal</td>
<td>50.5</td>
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<tr>
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<td>H983</td>
<td>1962</td>
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<td>M</td>
<td>Formalin</td>
<td>HE / trichrome / silver</td>
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<tr>
<td>23*</td>
<td>CC</td>
<td>950</td>
<td>1914</td>
<td>23.8</td>
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<td>Aluminum Cochineal</td>
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<tr>
<td>23</td>
<td>CC</td>
<td>9226</td>
<td>1954</td>
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<td>M</td>
<td>Formalin</td>
<td>Azan</td>
<td>144.3</td>
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</table>

CS: Carnegie stage * Origin of the specimen: CC, Carnegie Collection: National Museum of Health and Medicine, US Army, Silver Spring, MD, USA; AMC, Academic Medical Center, University of Amsterdam, The Netherlands; CU, Cambridge University, United Kingdom. CRL: crown-rump length in mm. Z-res: Calculated Z-resolution in μm

* A three-dimensional reconstruction of this specimen is included in the supplementary 3D-PDF.
The development of the human hyoid-larynx complex revisited

Discussion

A new description for hyoid-larynx development

As compared with current descriptions in literature (Fig. 1B) (Hamilton, Boyd, and Mossman 1972; Soerdjbalie-Maikoe and van Rijn 2008; Gray 2010; Cochard 2012; Gok, Kafa, and Fedakar 2012; Carlson 2014; Sadler 2015; Moore, Persaud, and Torchia 2016), we observed a less complicated development of the hyoid-larynx complex from only the second and third pharyngeal arch cartilage, in combination with a single hyoid body anlage (Dwight 1907; Rodriguez-Vazquez et al. 2011). The fourth and sixth pharyngeal arch cartilages that were expected to give rise to thyroid and cricoid cartilages were not observed as cartilaginous bars. Instead, the thyroid and cricoid were immediately discernable as mesenchymal condensations in the neck region, without being preceded in their development by a cartilaginous bar (Fig. 1C).

The textbook view on the development of the hyoid-larynx complex (Fig. 1B) states that the body of the hyoid bone derives from second and third pharyngeal arch cartilages, contributing to the upper and lower part of the hyoid bone body, respectively (Hamilton, Boyd, and Mossman 1972; Soerdjbalie-Maikoe and van Rijn 2008; Gray 2010; Cochard 2012; Gok, Kafa, and Fedakar 2012; Carlson 2014; Sadler 2015; Moore, Persaud, and Torchia 2016). Anatomic variations that match this notion of a dual origin of the body of the hyoid bone have not been reported. Also, the development of the thyroid cartilage is described as a joint contribution of fourth and sixth pharyngeal arch cartilages in the same fashion as the hyoid bone body, with an upper part derived from the fourth pharyngeal arch cartilage and a lower part derived from the sixth pharyngeal arch cartilage (Hamilton, Boyd, and Mossman 1972; Tuchmann-Duplessis and Haegel 1974; Cochard 2012; Carlson 2014; Sadler 2015). Again, variants which fit in such a description of the dual origin of the thyroid cartilage have not been reported and it remains ambiguous why the fifth pharyngeal arch cartilage is not mentioned in human development of this complex.

The evolutionary development of the hyoid-larynx complex can be deduced from comparative anatomy between animal species (Romer 1962). The hyoid-larynx complex is thought to derive from a series of arcuate structures, i.e. the pharyngeal arch cartilages, that develop into the branchial arches or gill arches in fish. Some of the most primitive chordates have over 50 pairs of gills. The more basic vertebrates such as jawless fish usually have seven pairs of cartilaginous gill arches. The more advanced vertebrates as cartilaginous fish display five to seven pairs of gill arches and bony fish only have three pairs. It is therefore to be expected that further advanced vertebrates like humans do not display more than three pairs of pharyngeal arch cartilages during embryonic development. In human embryos, we indeed observed only the first three cartilaginous bars of the pharyngeal arches, whereas the presence of a fourth and a sixth cartilaginous bar was expected based on the established literature concerning human development (Hamilton, Boyd, and Mossman 1972; Soerdjbalie-Maikoe and van Rijn 2008; Gray 2010; Cochard 2012; Gok, Kafa, and Fedakar 2012; Carlson 2014; Sadler 2015; Moore, Persaud, and Torchia 2016). We therefore propose that the cartilaginous structures caudal of the third pharyngeal arch cartilage, i.e. the thyroid, cricoid and arytenoid cartilages, develop directly from pharyngeal arch derived mesenchyme, instead of being preceded in their development by a cartilaginous bar.

The rationale behind the textbook view on development of the hyoid-larynx complex (Fig. 1B) can be explained by the strong influence of the recapitulation theory by Ernest Haeckel (Haeckel 1891). This theory describes that animals go through the evolutionary stages of their remote ancestors during embryonic development. In this conventional view, segmented pharyngeal arch cartilages join together to form a hyoid-larynx complex. Authors were, particularly before the 1930s,
often inspired by Haeckels recapitulation theory and tried to incorporate the anatomy of more basic vertebrates in their view on human development (Haeckel 1891). From that time on, modern laboratory animals and lab techniques became available for researchers, but despite these new techniques, theories from the beginning of the twentieth century have not been refuted properly and can still be found in modern papers and textbooks. We propose a new and simpler theory on the development of the hyoid-larynx complex based on findings in human embryos (Fig. 1C), hoping that in future literature it will replace the ancient animal-based schemes.

Fig. 2. Developmental series of embryonic development of the hyoid-larynx complex. A: Lateral view on the six reconstructed stages of embryonic development. From left to right stage 18 (44-48 days), 19 (48-51 days), 20 (51-53 days), 21 (53-54 days), 22 (54-58 days) and 23 (56-60 days) are shown. An interactive **three-dimensional PDF**, containing these reconstructions, is incorporated in the supplementary data. Cr: cranial, Ca: caudal, V: ventral, D: dorsal. B: Detailed ventral view on the developing hyoid-larynx complex. The colors are explained in the legend. Cr: cranial, Ca: caudal, L: left, R: right. Note how the hyoid body anlage develops in stage 18 (44-48 days), the greater horns (third pharyngeal arch cartilages) and thyroid cartilages develop in stage 19 (48-51 days) and the cricoid cartilage first arises in stage 20 (51-53 days). It takes approximately 55 days for the tracheal rings to develop.
Clinical and Forensic relevance

The hyoid-larynx complex is one of the most polymorphic regions in the human body. Fractures in this complex occur often in a forensic setting and are one of the best indicators of strangulation or other instances of blunt trauma on the neck (Gok, Kafa, and Fedakar 2012; Kindschuh, Dupras, and Cowgill 2010; Urbanova et al. 2013). However, a fracture of these structures is not necessary to conclude these types of trauma as presence of a hemorrhage in the neck region is a strong indicator for these diagnoses. In forensic examination of the hyoid-larynx complex, great care should be taken since some anatomical variants mimic fractures in the hyoid-larynx complex (de Bakker et al. submitted). Furthermore, symptoms of anatomical variants in the hyoid-larynx complex are often not recognized by clinicians (Shul’ga, Zaitsev, and Zaitseva 2006). It is therefore advisable that clinicians involved in the examination of the neck (e.g. otolaryngologists, surgeons, radiologists and dentists) and forensic experts in particular take note of the development and anatomical variety of the hyoid-larynx complex.

Fig. 3. Histological sections of six human embryonic specimens between Carnegie stage 18 and 23. The arrow indicates the hyoid body anlage in stages 18 to 21, which becomes fully incorporated in the hyoid bone in stages 22 and 23. The plane and level of sectioning is shown in every right upper corner. A: stage 18 (44-48 days) specimen 6524. B: stage 19 (48-51 days) specimen 2114. C: stage 20 (51-53 days) specimen 462. D: stage 21 (53-54 days) specimen 7254. E: stage 22 (54-58 days) specimen 895. F: stage 23 (56-60 days) specimen 950.
Conclusion

A solid basic knowledge of the embryonic development of the hyoid-larynx complex enables understanding of the etiology of various anatomical variants. It is therefore of utmost importance that embryonic development of the hyoid-larynx complex is known by clinicians and forensic experts. We propose that the development of the hyoid-larynx complex is less complicated than as it is described in modern textbooks. The second and third pharyngeal arch cartilages contribute to the formation of the styloid process and lesser and greater horns of the hyoid bone. The hyoid bone body however, derives from the hyoid body anlage without overt contributions from second and third pharyngeal arch cartilages. The fourth and sixth pharyngeal arch cartilages were not observed as cartilaginous bars. So instead of being preceded in their development by the fourth and sixth pharyngeal arch cartilages, the thyroid and cricoid were immediately discernable as mesenchymal condensations in the neck region.

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The development of the human hyoid-larynx complex revisited

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**Supplementary 3D-PDF (available online)**

Interactive 3D developmental series of embryonic development of the hyoid-larynx complex Stage 18 (44-48 days) specimen 6524, stage 19 (48-51 days) specimen 2114, stage 20 (51-53 days) specimen 462, stage 21 (53-54 days) specimen 7254, stage 22 (54-58 days) specimen 895 and stage 23 (56-60 days) specimen 950 are incorporated in the subsequent pages of the 3D-PDF. The reconstructions on which the 3D-PDF is based, are generated from histological sections of human embryos, as described in the Materials and Methods section. All structures were manually outlined in Amira, after which artifacts were corrected by knowledge-driven modeling in Blender. The 3D-PDF should be viewed in Adobe Reader® X or higher, available from http://www.adobe.com/downloads/. 3D interaction is only possible on MS Windows or Mac OS. Javascript must be enabled. For more advanced selection options, right-click on the 3D model and choose: “Show Model Tree”.