Developmental changes of the facial skeleton from birth to 18 years within a South African cohort (A Computed Tomography study)

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Preface

This study is a representation of original work by the author and has not been submitted to other universities in any form. Acknowledgement in the text has been made where the work of others was used.

The research described in this project was supervised by Dr C.O. Rennie and Prof. L. Lazarus (Discipline of Clinical Anatomy, School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, South Africa), and was conducted in the above mentioned Institution (on the Nelson R. Mandela School of Medicine campus and a private medical centre in Durban).

Declaration

I, Miss Kristen Niemann, declare as follows:

1) That the work described in this thesis has not been submitted to the University of KwaZulu-Natal or other tertiary institution for purposes of obtaining an academic qualification, whether by myself or any other party.

2) That my contribution to the project as primary author and principal investigator was as follows:

   - Collection of data needed for literature review;
   - Collection, analysis and interpretation of data;
   - Formulation of manuscript and compiled the research dissertation.

3) That the contributions of others to the project were as follows: Dr C.O. Rennie acted as the main supervisor, aiding in the formulation of the research idea and study design, reviewing all work before submission, giving corrections and feedback on work done. Prof. L. Lazarus acted as co-supervisor, aiding in the formulation of the research idea and study design, reviewing all work before submission, giving corrections and feedback on work done.

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Prof. L. Lazarus

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List of Acronyms and Abbreviations

AID	Anterior interorbital distance
ANS	Anterior nasal spine
CT	Computed tomography
ICC	Inter-class correlation
LOD	Lateral orbital wall distance
PNS	Posterior nasal spine
ZAD	Zygomatic arch distance
ZAL	Zygomatic arch length

Abstract

Introduction: The facial skeleton or viscerocranium has been recently noted as a method for age estimation as its development is influenced not only by the developing paranasal air sinuses and tooth eruption, but also the individual's ancestry particularly population specific normative data. This study aimed to investigate the developmental changes of the facial skeleton in males and females from birth to 18 years within the South African population with African ancestry to estimate age. The facial skeleton was assessed according to five regions *viz:* - orbital, nasal, midfacial, maxillary and mandibular.

Methods and materials: A retrospective study which consisted of 239 computed tomography (CT) scans of subadult individuals (0–18 years of age) of African ancestry (128 males; 111 females) was conducted. The scans were obtained from an online server utilised by a private medical facility in the eThekwini Muncipality. The DICOM images were viewed from an online Picture Archiving and Communication Systems server using Infinitt software (version 5.0.1.1) which is the standard software used by the practitioners. Linear parameters in the horizontal, sagittal, and vertical planes assessed the development of the viscerocranial regions.

Results:

a) *Development*: Most of the viscerocranial regions experienced a rapid increase in growth between 0–5 years of age viz: orbital (orbital height and width: 0–5 years; lateral orbital wall distance: 0–3.75 years), midfacial (zygomatic arch distance: 0–3.75 years), nasal (aperture height and width: 0–5 years); maxilla (length: 0–3.75 years), mandibular (mandible width: 0–5 years). Thereafter growth continued to increase at a slower rate in the orbital width (0.61–0.8 mm/year in females; 0.56–0.76 mm/year in males), lateral orbital wall distance (1.1 mm/year in females; 1 mm/year in males), zygomatic arch distance (1.6 mm/year in females; 1.8 mm/year in males), nasal aperture width (0.45 mm/year in females; 0.4 mm/year in males) and height (0.63 mm/year in females; 0.77 mm/year in males), maxillary length (0.7 mm/year in females; 0.81 mm/year in males), mandible width (1.1 mm/year in females; 1.5 mm/year in males) and mandible head widths (right: 0.42 mm/year in females, 0.49 mm/year in males; left: 0.52 mm/year in females, 0.68 mm/year in males). Additionally, in the orbital region, the orbital width underwent two periods of rapid growth i.e., 0–5 and 10–18 years of age, whilst the anterior interorbital distance noted no significant increase after 7.5 years of age.

b) *Sexual dimorphism*: Males displayed overall larger measurements than females in all the parameters, except for the anterior interorbital distance and the zygomatic arch lengths (ZAL) on the right and left, as females displayed larger measurements. Although these differences were not statistically significant ($p>0.05$). The only measurements which displayed statistically significant differences between males and females were the left orbital height ($p = 0.048$), nasal aperture height ($p = 0.048$) and the mandible width ($p = 0.05$), in which males displayed larger measurements than females.

c) *Age estimation*: The measurements which displayed the strongest correlation to age were the ZAD ($r = 0.8842$, $p<0.001$), ZAL (right: $r = 0.8929$, $p<0.001$; left: $r = 0.8656$, $p<0.001$) and the mandible width ($r = 0.8444$, $p<0.001$). Formulas were derived for the measurements which could be used to estimate age.

Discussion and conclusion:

The findings from this study have outlined the development of the viscerocranium in subadult individuals with African ancestry. This study discussed the correlation between the development patterns of each viscerocranial region with age. The data from this study can be a useful addition to the existing data on the skeletal developments of subadult South African individuals. Forensically the development of formulas for subadult individuals could be utilised in the age estimation of skeletal remains.

Chapter 1
Introduction

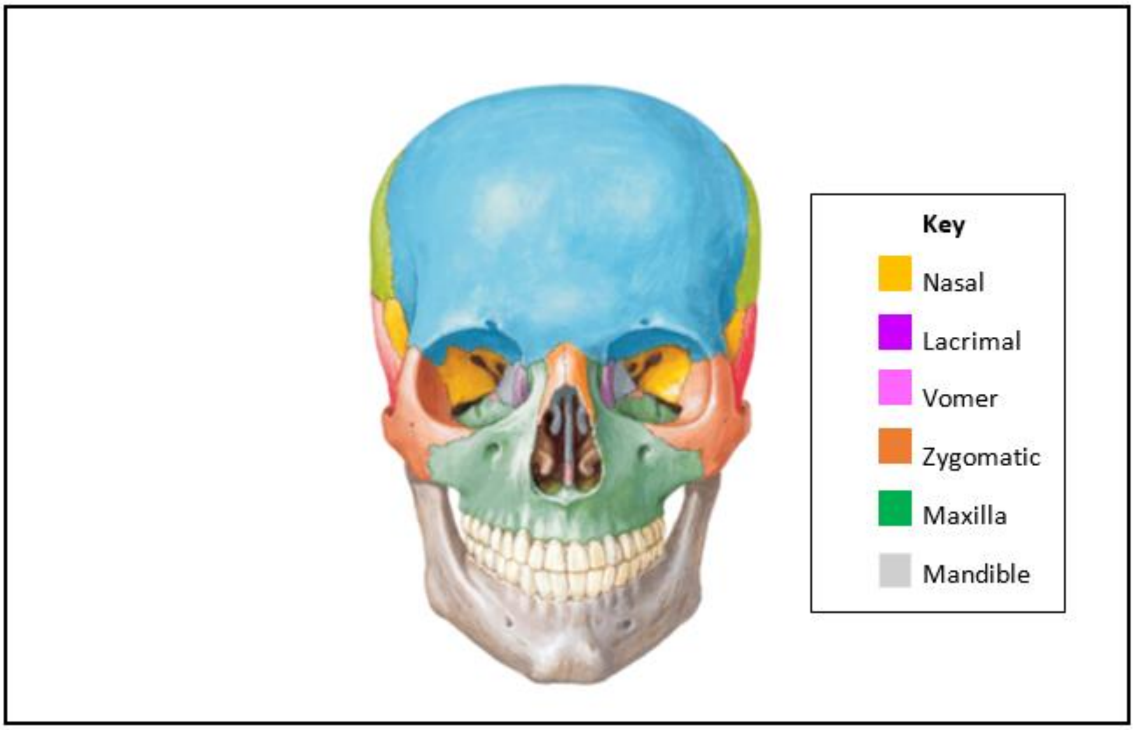
1.1 Introduction

The official statistics on the number of missing children in South Africa is difficult to find, as the South African Police Service does not make any distinction between adults and children in the reported numbers (Briers 2015). Although it has been reported that a child goes missing every five hours in South Africa, 23% of these missing children being either trafficked, found deceased, or never recovered (Missing Children South Africa n.d.). There were 943 child murders reported between April 2019 and March 2020 in South Africa (Mahlakoana 2020). When the remains of an unknown individual are found at a crime scene, such an individual must be identified. In South Africa however, the identification of unknown remains can be challenging due to lack of dental records and comparable DNA in individuals with low socioeconomic standing (Dorfling *et al.*, 2018). A forensic anthropologist is brought in where skeletal remains are found at a crime scene. A biological profile must then be created, which involves age, sex, stature, and ancestry estimations (İşcan and Steyn 1999; Mustafa *et al.*, 2019; Ubelaker and Khosrowshahi 2019). Currently, in South Africa there are limited methods of age estimations for subadult remains in advanced stages of decomposition (Krüger *et al.*, 2017).

Subadult individuals are defined as individuals who are still in the development and growth phase of life, usually below 18 years of age (Christensen *et al.*, 2014). Age estimation is a very important aspect of the biological profile as it can lead to the identification of unknown remains (Krüger *et al.*, 2017; Mustafa *et al.*, 2019; Ubelaker and Khosrowshahi 2019). The method of age estimation which is selected is reliant upon the skeletal remains present at the crime scene, and they differ from those methods used on immature and adult remains (Milner and Boldsen 2012; Kumagai *et al.*, 2018; Ubelaker and Khosrowshahi 2019). The current age estimation methods conducted using bones (such as long bone lengths) are far from perfect despite the many years of work that has gone into the available research, as sample differences in ancestry and health status can result in bias (Milner and Boldsen 2012). Although when compared to other identification methods, osteometric measurements done with radiological methods were seen to be more efficient (Mustafa *et al.*, 2019) and is a method that plays an important role in an anthropological investigation (Buyuk *et al.*, 2017).

The facial skeleton or viscerocranium has been known to play an important role in the formation of biological profiling during anthropological studies (Mustafa *et al.*, 2019) and has piqued the interest of researchers in many fields, especially in biological anthropology (Machado *et al.*, 2017). To utilise the viscerocranium for age estimation purposes a detailed knowledge of viscerocranial development is required (Briers 2015). Ageing of the face is a complex process that includes both the soft tissues and the viscerocranium (Mendelson *et al.*, 2007; Kahn and Shaw 2008) and this process is not clearly understood (Mendelson *et al.*,2007). Specific regions of the viscerocranium have been studied in South African populations by authors such as Hutchinson *et al.* (2012) who reported on the development of the mandible from 31 gestational weeks – 36 months of age. Although after a thorough review of the available literature, it can be noted that there are limited studies to our knowledge that outline the development of the viscerocranium as a whole in subadult South African individuals.

The viscerocranium is made up of the nasal, lacrimal, vomer, palatine, zygomatic bones as well as the maxilla and mandibular bones (Sadler and Langman 2011; Moore *et al.*, 2014) (Figure 1.1). The viscerocranium can be divided into five regions, namely: orbital, nasal, midface, maxilla, and mandible (Jacob and Buschang 2011; Buschang *et al.,* 2013; Mellion *et al.,* 2013; Buyuk *et al.,* 2017; Al-Jewair *et al.*, 2018).



Source: Adapted from Netter (2005)

Figure 1.1: Viscerocranial bones

These regions develop at different rates to one another (Mendelson *et al.*, 2007; Ross and Williams 2010; Bastir and Rosas 2013; Machado *et al.*, 2017) as well as at different rates to the rest of the body (Al-Jewair *et al.*, 2018). Viscerocranium growth is regulated by sensory organs, paranasal air sinus development and tooth eruptions (Ross and Williams 2010). The cranial bones also become thicker during growth (Cattaneo 2009). Previous studies have looked at the development of the separate regions of the face, but there is a lack of studies on the development of the entire subadult facial skeleton from birth to adulthood (Albert *et al.*, 2019).

Furthermore, knowing the normal growth patterns of the viscerocranium will allow physicians to identify anomalous growth (Palanisamy *et al.*, 2016) such as in cases where teratogen exposure or genetic conditions are present (Gondré-Lewis *et al.*, 2015). The data from this study could benefit orthodontists clinically as the understanding of the growth and development rates of the viscerocranium will aid in diagnosis as well as treatment planning (Palanisamy *et al.*, 2016). Knowing the timing of the maxillary and mandibular growth rates would help the orthodontist time procedures and treatments for maximum opportunity of success (Palanisamy *et al.*, 2016).

1.1.1 Research questions

1) What is the facial skeleton morphometry within a South African Black population?

2) Are growth patterns different for males and females?

3) Which region of the facial skeleton is more accurate in estimating age?

1.1.2 Aim

The study aimed to investigate the developmental changes of the facial skeleton in males and females from birth to 18 years within the South African population with African ancestry to estimate age.

1.1.3 Objectives

1) Identify the growth patterns of the different regions (orbital, nasal, midfacial, maxillary and mandibular) of the facial skeleton using linear measurements.

2) Compare the growth patterns from these linear measurements of the different regions according to age.

3) Correlate these growth patterns to age.

4) Compare and identify the differences in the overall growth patterns and measurements between males and females from birth to 18 years.

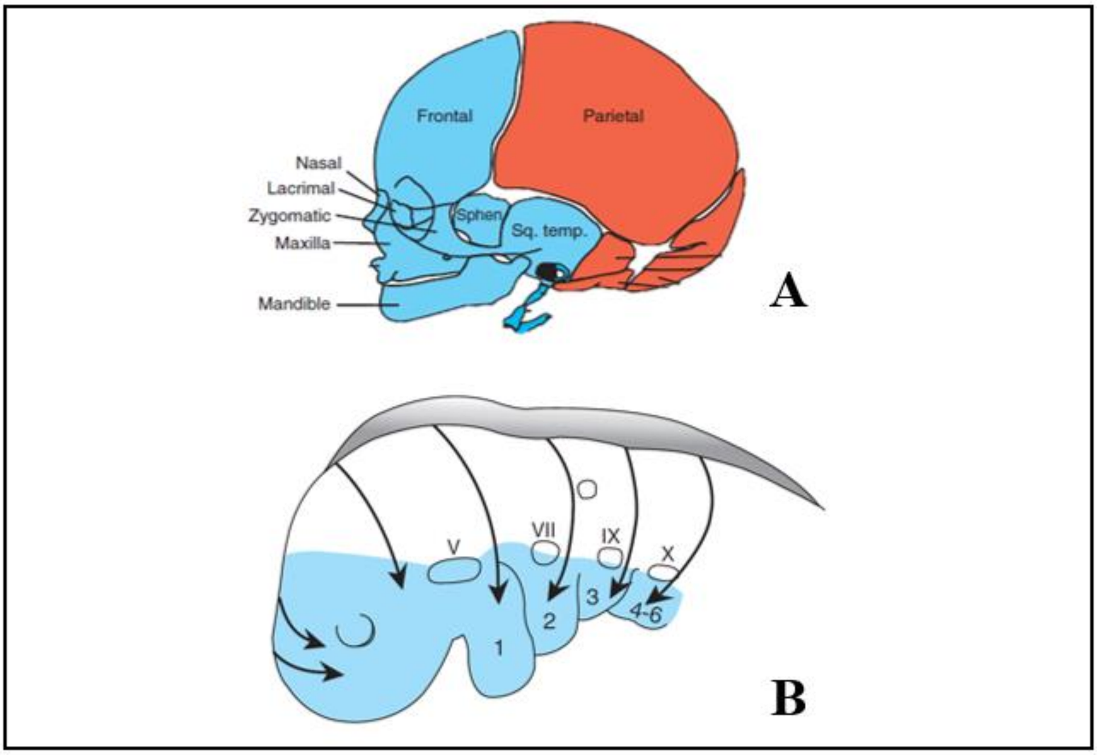
5) Compare and determine which region of the facial skeleton will be more reliable in age estimation.

6) Derive a formula for age estimation utilising facial morphometric data.

1.2 Literature review

1.2.1 Development of the facial skeleton

Embryologically the viscerocranium (nasalis, maxillae, premaxillae, zygomatic and mandible) and neurocranium (frontal, parietal, squamous and temporal bones) originate from neural crest cells and somites and develop through the process of membranous ossification (Figure 1.2.a). During the fourth and fifth week of embryological development, the neural crest cells migrate from their origin in the neuroectoderm to form pharyngeal arches (Figure 1.2.b); and these contribute to the unique external appearance of the developing embryo. Towards the end of the fourth week of embryological development, the centre of the face is formed by the stomodeum (embryological precursor to the mouth) and the first pair of pharyngeal arches. The bony structures that will arise from the first pharyngeal arch are the premaxilla, zygomatic bone, temporal bone, and mandible. (Sadler 2011)

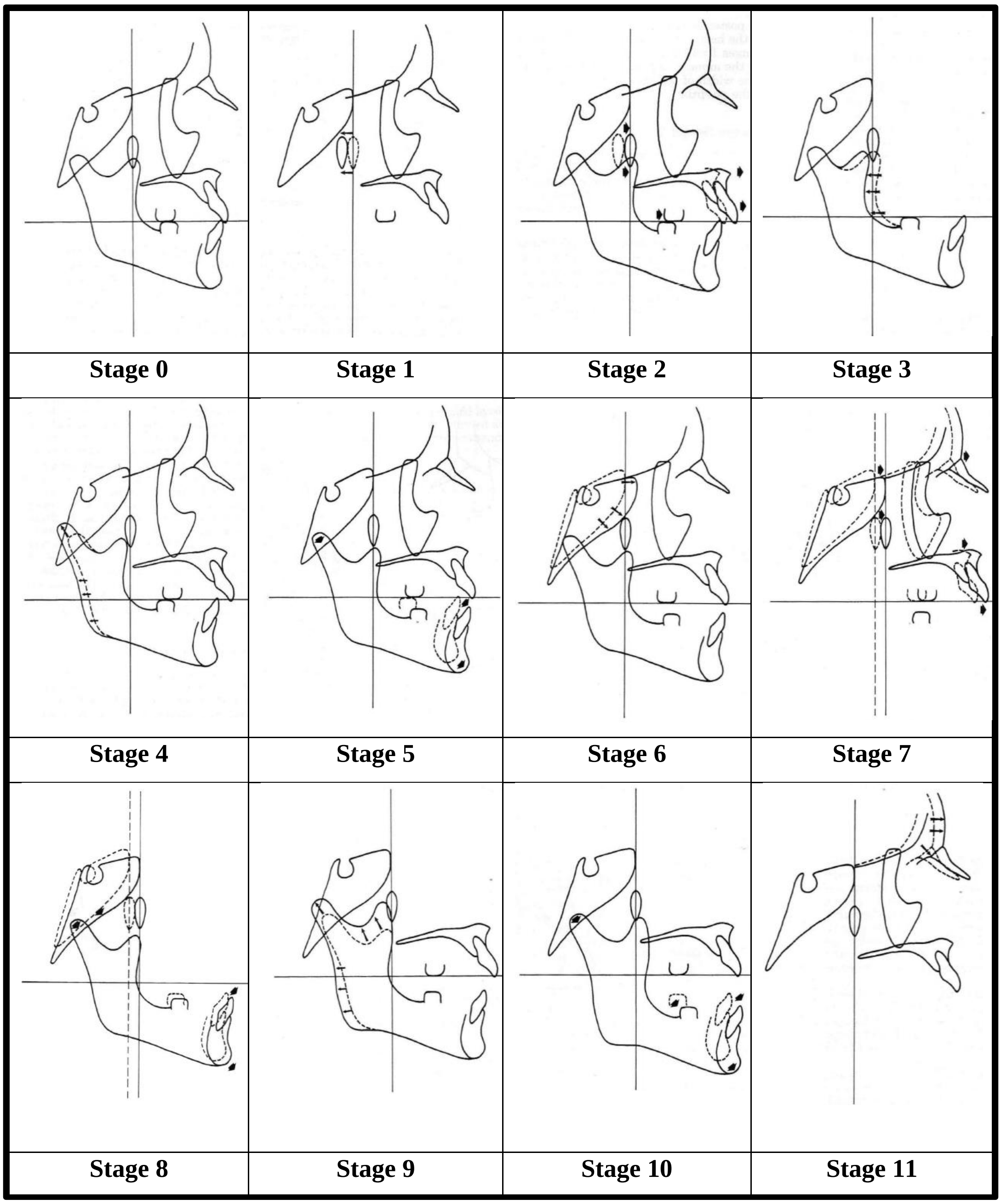


Source: Adapted from Sadler (2011)

Figure 1.2: A) Embryological origins of the skull which develop from neural crest cells (blue)
B) Migration of the neural crest cells from the neuroectoderm to the pharyngeal arches

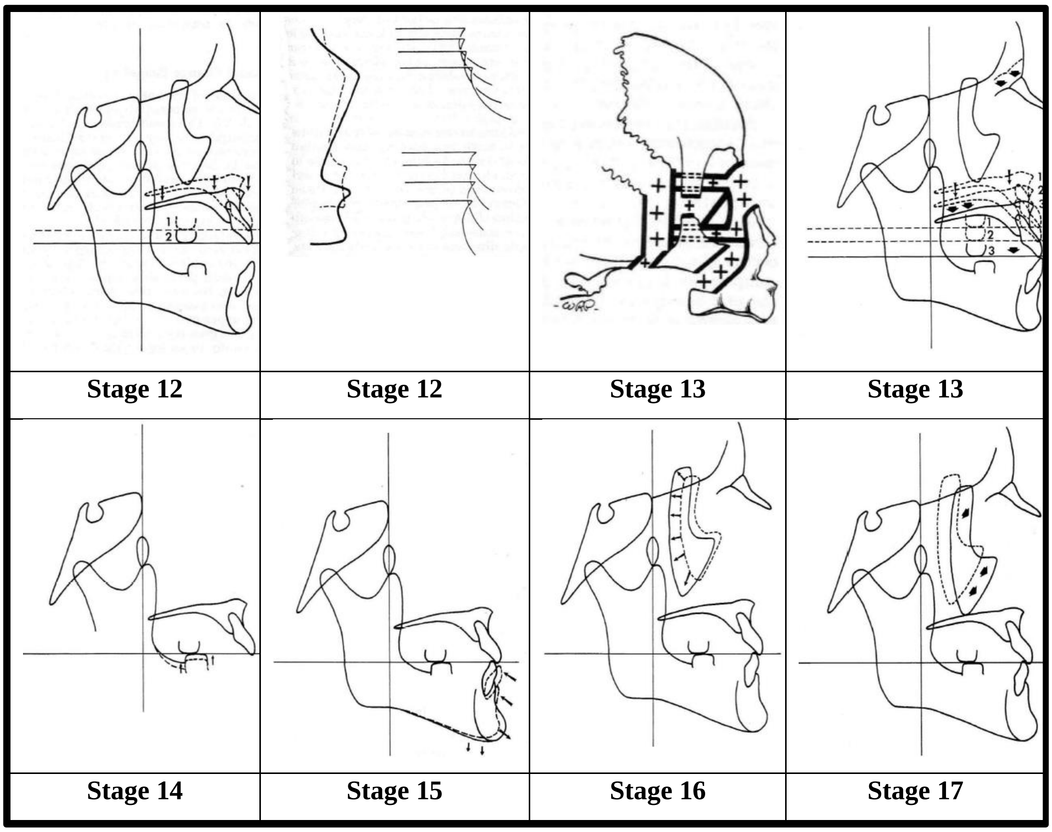
In the postnatal period of growth in subadults Enlow and Hans (1996) noted that the viscerocranial regions was separated into 17 stages, some of which occur simultaneously during cranial development (Figure 1.3 and Figure 1.4). Anatomical texts or literature does not define very well at which ages these stages occur. Stages one and two reports on maxillary arch lengthening posteriorly which results in the maxilla being carried anteriorly and the arch protruding forward. Simultaneously, stages three to six are occurring in the mandible, where equivalent changes take place for structural balance to be maintained. The mandible experiences posterior lengthening and anterior displacement. Stages seven and eight deal with the midfacial region being anteriorly displaced because of the middle cranial fossa expansion. Stages nine and ten occur simultaneously in the mandible. The mandible undergoes horizontal growth and anterior displacement to match the expanding midfacial region. Stage eleven describes the horizontal growth of the overall facial area, as well as the anterior displacement of the nasal bones. This anterior displacement is due to the anterior cranial fossa floor and frontal bone growth (Enlow and Hans 1996).

Stages twelve and thirteen describes the nasomaxillary complex undergoing vertical lengthening, which results in a vertical displacement of the maxilla causing dental eruption. Stages fourteen and fifteen describes alveolar bone growth and development. The final stages of sixteen and seventeen describe the vertical lengthening of the orbital rim, zygomatic arch enlargement, and lateral remodelling. (Enlow and Hans 1996)



Source: Adapted from Enlow and Hans (1996)

Figure 1.3: Schematic diagram of the developmental stages 1–11 of the viscerocranium



Source: Adapted from Enlow and Hans (1996)

Figure 1.4: Schematic diagram of the developmental stages 12–17 of the viscerocranium

1.2.2 Age estimation in forensic anthropology

Conducting measurements of the human body can be traced as far back as ancient Egypt (Utkualp and Ercan 2015). During ancient times measurements of the body were mainly used for artistic purposes, the practice was later taken up by those studying the medical and biological anthropology fields (Pool *et al.*, 2016; Utkualp and Ercan 2015). Forensic anthropology is a branch of biological anthropology, involving the study of skeletal remains (Márquez-Grant 2015). A biological profile is defined as the estimation of age, sex, stature, and ancestry (İşcan and Steyn 1999; Mustafa *et al.*, 2019; Ubelaker and Khosrowshahi 2019). These biological profiles help with the identification of skeletal remains when the remains are too badly decomposed to conduct fingerprint or DNA analysis (Franklin 2010; Buyuk *et al.*, 2017; Ubelaker and Khosrowshahi 2019).

Many methods can be used when it comes to age estimations on subadult remains (Franklin 2010), such as diaphyseal growth and dental eruption patterns (Christensen *et al.*, 2014). Dentition and skeletal growth are the most common forms of age estimation in subadults (Franklin 2010). It has become clear that to get a more accurate estimation of age from skeletal remains in subadults one should use a combination of multiple age estimation methods, such as dentition and skeletal methods and not just a single method (Austin and King 2016; Kumagai *et al.,* 2018; Ubelaker and Khosrowshahi 2019). Another factor that must be taken into consideration when doing age estimations on skeletal remains is the ancestry of the remains as variations exist between different ancestral groups (McDowell *et al.,* 2015; Ubelaker *et al.,* 2019).

Dentition is seen as the most reliable method of age estimation, as the eruption and root development of teeth are well documented (Franklin 2010) and tooth eruption follows a specific pattern which is correlated to age as dental development is under stronger genetic control (Christensen *et al.,* 2014). The most favoured methods of dental age estimation are the Moorrees, Fanning and Hunt and Demirjian, Goldstein and Tanner methods (Christensen*et al.,* 2014). Phillips and Van Wyk Kotze (2009) conducted a study whereby they compared the Moorrees, Fanning and Hunt and Demirjian, Goldstein and Tanner methods of age estimation using dentition in three different South African subadult samples. It was noted that the age of the South African subadult samples was underestimated when using the Morrees, Fanning and Hunt method, while the Demirjian, Goldstein and Tanner method overestimated the individual's ages. This illustrates that some dental age estimation methods are not reliable for the South African samples (Phillips and Van Wyk Kotze 2009). More recently Esan and Schepartz (2018) have developed dental standards specifically for black South African individuals between 5 – 20 years of age. Skeletal growth generally looks at the calcification of various ossification centres in long bones (Franklin 2010). Skeletal growth is favoured as the linear relationship between age and the diaphyseal length during development is strong and can be used to approximately 10 years of age which is when the epiphysis begins to fuse with the diaphysis (Christensen *et al.,* 2014). Although more recently there have been studies that have looked at regions of the facial skeleton development as a possible method of age estimation (Braga and Treil 2007; Özer *et al.,* 2016). Braga and Treil (2007) found that the growth of the centroid size of the facial skeleton could be used for age estimation. A study by Palanisamy *et al.* (2016) noted that the growth of the viscerocranium is related to the maturity of long bones. Although there is a lack of literature showing how the entire viscerocranium can be used for age estimation purposes in subadult samples, most subadult studies focus on the teeth (Milner and Boldsen 2012) or the neurocranium (Kumagai *et al.,* 2018) for age estimation. The South African literature that focuses on the viscerocranium looks at it from either a sex estimation (Franklin *et al.,* 2006) or an ancestry estimation (İşcan and Steyn 1999; Dinkele 2018) standpoint, but not age estimation, especially in subadults.

The age of the remains at the time of death is important as sex, ancestry and stature estimations vary among subadults and adults (Christensen *et al.,* 2014; Ubelaker and Khosrowshahi 2019). In cases involving subadult individuals, age is a parameter that can be used to reliably reduce the number of possible matches on a missing persons list, as sex estimations are not always accurate on individuals until puberty (Wood 2015). Age estimation in post-pubescent individuals has proven more complex than sex estimation, as age estimations involve considerations for the continual growth and developmental changes taking place (White and Folkens 2005). The age estimation techniques used in the identification of unknown remains will vary depending on whether the skeletal remains are that of an adult or a subadult (Ubelaker and Khosrowshahi 2019). There is rapid growth of the viscerocranium within the first year of life, with development being almost complete at the age of five years (Waitzman *et al.,* 1992; Albert *et al.,* 2019; Manlove *et al.,* 2020). There are many studies on the development of the facial skeleton in subadult individuals with European ancestry (Waitzman *et al.*1992; Snodell *et al.,* 1993; Grymer and Bosch 1997; Thordarson *et al.,* 2006; Nanda *et al.,* 2012; Bastir and Rosas 2013; Prystanska *et al.,* 2018), but there is however a lack of literature on subadult South African individuals of African ancestry. It is known that ancestry does play a role in growth and development (İşcan and Steyn 1999), which is why ancestry specific standards are required for accurate age estimations (Christensen *et al.,* 2014). To our knowledge there is currently limited research focussing on the development of the entire viscerocranium and elaborating on its development comprehensively. Most research focuses on the development of a single facial region (Albert *et al.,* 2019).

1.2.3 Defining ancestry versus population

The difference between the terms, race, ethnicity, and ancestry, can often be misunderstood, which can lead to prejudice and bias (Wade *et al.,* 2020). The term 'race' is a subjective social construct of identity, which places an individual into a racial grouping based on phenotypic traits, such as skin colour, hair type, body type, rather than biological ancestry (Wade *et al.,* 2020).

The social construct of race is used throughout the world by individuals to group themselves and others (Wade *et al.,* 2020). Many countries, including South Africa, previously used racial categories to justify discrimination and bias (Wade *et al.,* 2020). Thus, the scientific community has moved away from separating individuals into racial categories (Stull *et al.,* 2014; Wade *et al.*2020). Ancestry is the only scientifically accepted method of grouping individuals by morphological variations (L'Abbe *et al.*.2013; Stull *et al.,* 2014).

According to Statistics South Africa (2019), the four main ancestry groups in South Africa are 'Black Africans' (South Africans with African ancestry, 80.7%); 'Coloured' (8.8%); 'White'

(South Africans with European ancestry, 7.9%); and 'Indian/Asian' (South Africans with Asian ancestry, 2.6%).

Bernitz *et al.* (2014) published an article on the past and current status of forensic science in South Africa. In this paper, they use the terms ancestry and population affinity interchangeably. Population affinity can be defined as the degree of similarities an individual shares with a reference sample (Winburn and Algee-Hewitt 2021). For the sake of consistency, this study will refer to ancestry as seen in recent South African forensic anthropology studies (L'Abbe *et al.*, 2013; Stull *et al.*, 2014).

1.3 Morphometry of the viscerocranium

The facial skeleton can be divided into different regions namely: orbital, midfacial, maxillary and mandibular (Enlow and Hans 1996; Jacob and Buschang 2011; Buschang *et al.*, 2013; Mellion *et al.*, 2013; Buyuk *et al.*, 2017; Al-Jewair *et al.*, 2018; Manlove *et al.*, 2020). These may develop at different rates and in different directions to one another (Mendelson *et al.*, 2007; Ross and Williams 2010; Bastir and Rosas 2013; Machado *et al.*, 2017). The phenomenon of different facial regions having different growth patterns is known as allometry (Machado *et al.*, 2017). Allometry is the reason why a child's face does not resemble a smaller version of the adult face (Machado *et al.*, 2017) (Figure 1.5).

Source: Adapted from Manlove *et al.* (2020)

Figure 1.5: Viscerocranium showing developmental changes from newborn to adult

Bastir *et al.* (2006) separated the skull into morphological and functional regions (midline cranial base; lateral cranial floor; midline neurocranium; facial and mandibular structures) to study the growth differences. Braga and Treil (2007) separated the skull into sections such as the viscerocranium and basicranium, to study the sequences of growth in size and shape of each section individually. Various studies describe regions of the viscerocranium as orbital (bony orbit height and width, anterior interorbital distance, lateral orbital wall distance), midfacial (nasal aperture height and width, maxillary width and length, zygomatic arch distances and bizygomatic distance), and mandibular (mandible width, ramus height, mandible length) (Waitzman *et al.,* 1992; Snodell *et al.,* 1993; Nanda *et al.,* 2012). More recent studies define the midfacial region as being the zygoma measurements, whereas the nasal and maxillary measurements are divided into their own facial regions (Albert *et al.,* 2019; Manlove *et al.,* 2020).

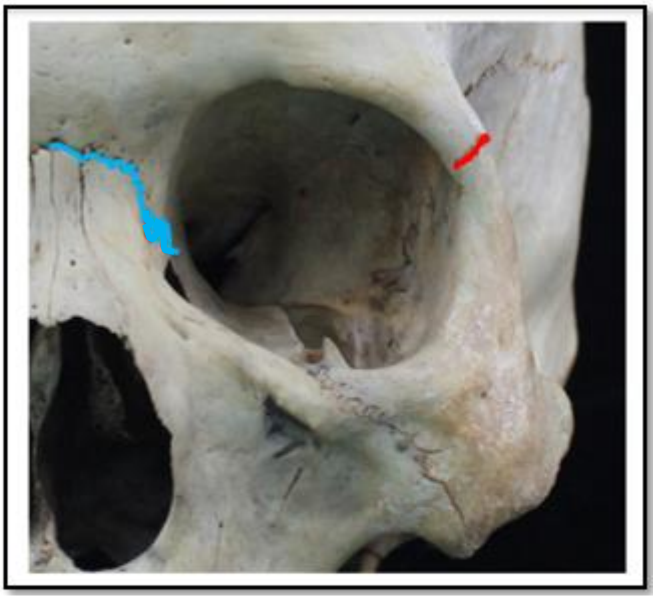
Most studies analyse the morphometry of the viscerocranium by subdividing it into regions namely: orbit; midfacial; nasal; maxillary and mandibular (Jacob and Buschang 2011; Buschang *et al.,* 2013; Mellion *et al.,* 2013; Buyuk *et al.* 2017; Al-Jewair *et al.,* 2018; Manlove *et al.,* 2020). These regions will be discussed in detail under the following sections: orbit under 1.3.1, midfacial under 1.3.2, nasal under 1.3.3, maxillary under 1.3.4 and mandibular under 1.3.5.

1.3.1 Orbit

The bony orbital aperture plays an important role in the recognition and assessment of the face (Özer *et al.,* 2016). It is the link between the outside world and the brain as it is the opening to the orbit which contains the eyeball and the optic nerve (Costello *et al.,* 2012; Özer *et al.,* 2016). Previous literature noted that the orbit displays differences between varying ancestries (Rossi *et al.,* 2012; Özer *et al.,* 2016). The orbital region in adults was studied in detail by authors such as Mendelson *et al.* (2007); Kahn and Shaw (2008); Richard *et al.* (2009); Rossi *et al.* (2012); Xing *et al.* (2013); Fetouh and Mandour (2014); Sing *et al.* (2017); Dorfling *et al.* (2018); and Mustafa *et al.* (2019), although there is a lack of literature on the development of the orbital region in subadults.

A South African study conducted by Dorfling *et al.* (2018) looked at the eye as well as soft tissue landmarks in the orbital region on both adult cadaveric specimens and computed tomography (CT) scans in individuals with African ancestry. This study assessed the location of the soft tissue structures such as the eye and the canthi, in relation to the facial approximation standards that are currently used. Age estimations were not included in this study. The study concluded that individuals of African ancestry displayed a more rectangular orbit shape. Xing *et al.* (2013) conducted a geometric morphometric study (analysis of shape changes in biological structures using Cartesian coordinates (Slice 2007) on photographs of male dry bone skulls. The shapes of the orbital aperture were compared between Asian, European, and South African skulls. The shape analysis technique in this study correctly identified the African samples in only 41% of the sample, which is significantly lower than the Asian and European samples that were correctly identified in 60% of the sample. However, the linear discriminant analysis was more accurate in ancestry estimation, with 74.4% of the African sample correctly identified. It was noted that the African orbit was not as rectangular as that of the European samples studied, and not as rounded as the Asian sample. This study found that the most variable parts of the orbits between the samples analysed in the study were the internal and lateral aspects of the upper orbit formed by the frontal bone and the internal aspects of the lower orbit formed by the zygomas and maxilla (Xing *et al.*, 2013).

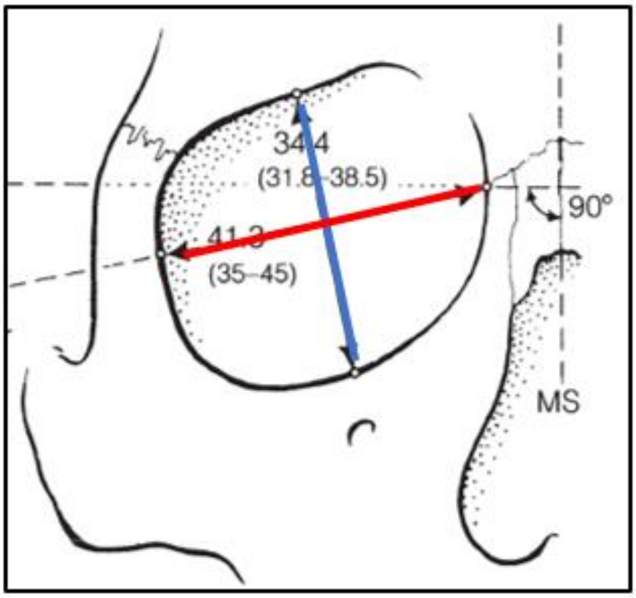
Small *et al.* (2018) conducted a South African study looking at inter-landmark distances on various parts of the human crania, to determine if sex could be accurately estimated using cranial measurements. The study looked at crania of adult individuals with European ancestry and included parameters of the orbital aperture. The distances measured in the orbital region were interorbital breadth and dacryon (suture between the lacrimal bone, the frontal bone, and the maxilla) to frontomalare temporale (suture on the lateral border of the orbital rim between the frontal bone and the zygomatic bone) (Figure 1.6). This study developed new discriminant functions for sex estimation using inter-landmark distances that were on average 88.2% accurate. Although the orbital region displayed the lowest discriminant accuracy at 71.8%, they concluded that further analysis in a South African setting is required (Small *et al.*2018). Few studies, such as those done by Lang *et al.* (1983), Waitzman *et al.* (1992), Kaya *et al.* (2014), Özer *et al.* (2016) and Pool *et al.* (2016) looked at the bony orbit in the subadult population. These studies looked at individuals of European (Waitzman *et al.*, 1992) and Turkish (Kaya *et al.*, 2014; Özer *et al.*, 2016) ancestries.



Source: Adapted from Michigan State University (n.d.)

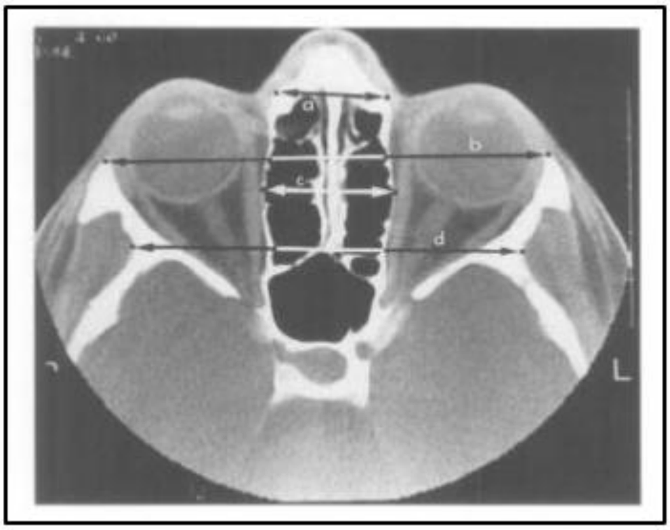
Figure 1.6: Image of the skull indicating the dacryon (blue) and zygomalare temporale (red)

Lang *et al.* (1983) and Waitzman *et al.* (1992) provided normative data for the development of the subadult orbital region. The morphological parameters measured by Lang *et al.* (1983) in the orbital region included the orbital width, orbital height, and interorbital distance (Figure 1.7). Waitzman *et al.* (1992) measured the anterior interorbital distance (AID) and lateral orbital wall distance (LOD) (Figure 1.8).



Source: Adapted from Lang *et al.* (1983)

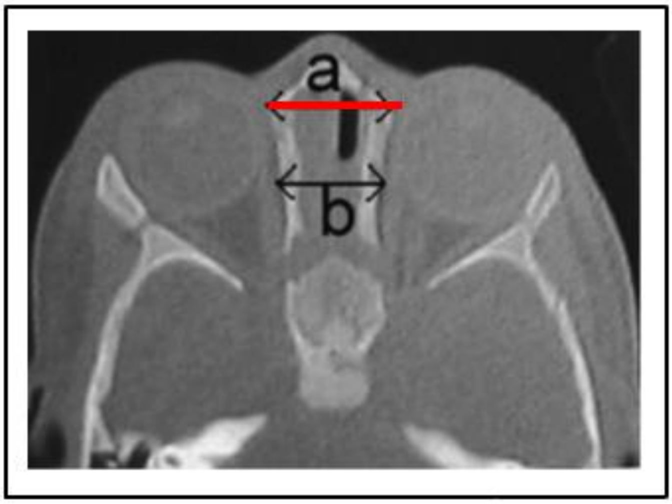
Figure 1.7: Schematic of the orbital aperture measurements orbital width (red) and orbital height (blue)



Source: Waitzman *et al.* (1992)

Figure 1.8: Orbital region measurements done by Waitzman displaying the (a) anterior interorbital distance; (b) lateral orbital wall distance

Waitzman *et al.* (1992) found that there was rapid growth in the first five years of development with most of the adult size attained during these periods and there was great variability in the growth rates of the orbital region. The AID displayed small growth after birth, unlike the LOD that showed a substantial size increase during the first year of development with continued growth during childhood.

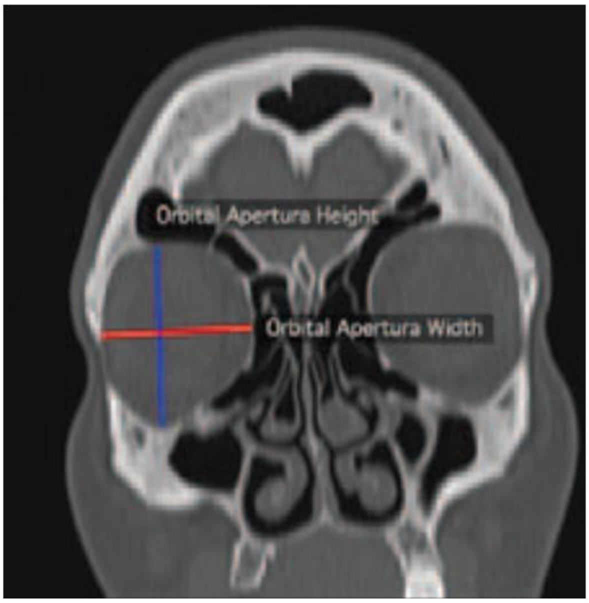


Source: Adapted from Aslan *et al.* (2009)

Figure 1.9: Horizontal section displaying the anterior interorbital distance (red) in individuals 4 days to 1 year of age

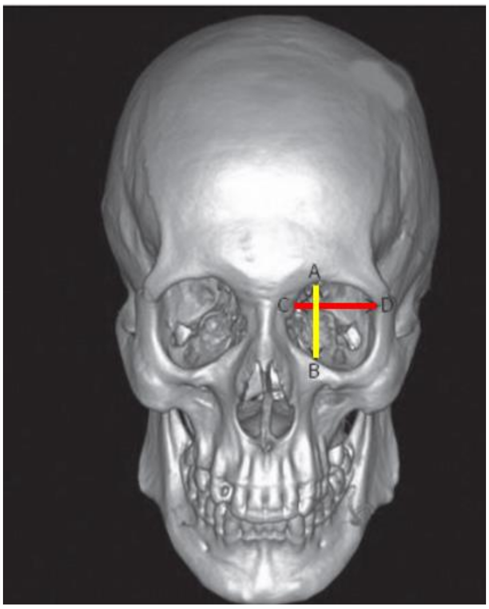
Waitzman *et al.* (1992) looked at the orbital region and noted that the mean overall values were greater in males than in the female subjects studied. Aslan *et al.* (2009) identified the mean anterior interorbital distance in a Turkish population (Figure 1.9) as being 17.8±1.4 mm between birth and one year of age. Costello *et al.* (2012) noted that the orbital region undergoes rapid growth rates in the first year of life, with most of the growth being complete by five years of age, which is in agreement with the earlier findings of Waitzman *et al.* (1992). This rapid growth rate of the orbital region is a result of the eye and the optic neurological structures developing during this stage of life (Costello *et al.*, 2012).

Kaya *et al.* (2014) and Özer *et al.* (2016) conducted studies on the orbital region in Turkish populations. The ages ranged between 13–86 (Kaya *et al.*, 2014) and 5–74 years (Özer *et al.*, 2016). These studies looked at the differences between the male and female orbital height and width (Figure 1.10 and Figure 1.11). These studies did not provide data for the individual ages, although the orbital width and height was noted to be larger in males than in females. Table 1.1 displays the mean measurements for the males and females in these studies. In European samples, the orbital width average was 30 mm at five years of age, which was 93% of the adult value, reaching full adult size by eleven years of age in males and eight years of age in females (Costello *et al.*, 2012). Orbital height, however, had a slower growth rate than the other orbital measurements (Costello *et al.*, 2012).



Source: Ozer et al. (2016)

Figure 1.11: Orbital region measurements done by Ozer et al. displaying the orbital height (blue) and orbital width (red)



Source: Adapted from Kaya et al. (2014)

Figure 1.10: Orbital region measurements done by Kaya et al. displaying the orbital height (yellow) and orbital width (red)

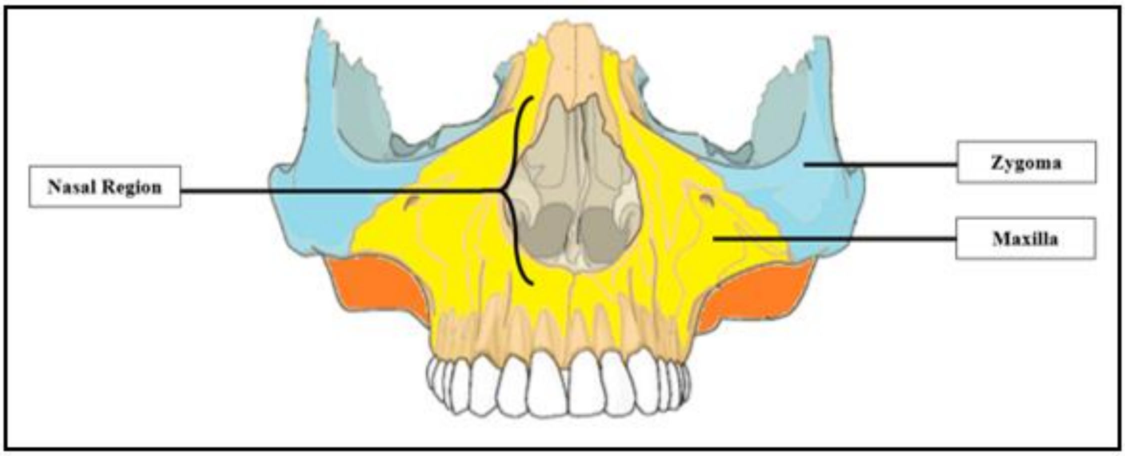
Table 1.1: Morphometric orbital parameters

Morphometric orbital parameters						
Author (year) population	**Age (years) (sample size)**	**Sex**	**Orbital width (mm) ±SD**		**Orbital height (mm) ±SD**	
			Right	**Left**	**Right**	**Left**
Kaya *et al.*(2014) Turkish	13–86 (112)	Male	37.04 (±1.79)	36.86 (±1.57)	33.9 (±2.27)	34.5 (±2.2)
		Female	35.78 (±1.5)	35.39 (±1.58)	32.6 (±2.4)	33.16 (±2.19)
Özer *et al.*(2016) Turkish	5–74 (198)	Male	33.99 (±1.85)	34.17 (±2.1)	37.7 (±2.42)	37.77 (±2.48)
		Female	33.07 (±1.65)	33.27 (±1.77)	36.55 (±2.29)	36.97 (±2.19)

Pool *et al.* (2016) conducted a CT study on 204 subadults ranging from birth to 36 months of age. The ancestry of the patients studied was not noted in this study and was listed as a limitation as there have been differences according to ancestry noted in the orbital region (Pool *et al.*, 2016). The parameters included the bony anterior interorbital distance (AID) and bony lateral orbital wall distances (LOD) (Figure 1.8). It was noted that the AID increased from 14.16±0.74 mm in the 0–3 month age group up to 16.21±0.75 mm in the 12–18 month age group. After which the LOD remained steady with an average measurement between 15.7–16.8 mm. Furthermore, the bony LOD was noted to increase throughout the age groups studied. The 0–3 month averaged 65.56±1.76 mm, increasing to 77.98±1.57 mm by the 12–18 month age group. The final age group (18–36 months) saw a gradual increase from 79–80.5 mm. The substantial increase in the LOD in this study is consistent with the findings from Waitzman *et al.* (1992).

1.3.2 Midfacial

The midfacial region has been described as the area of the facial region including the zygoma, the nasal region and the maxilla (Aktop *et al.*, 2013; Kim *et al*, .2018) (Figure 1.12). Although Waitzman *et al.* (1992) considered the midfacial part to be focused around the zygoma parameters and some recent studies have divided the midfacial region into the nasal, maxilla, and zygomatic regions as well (Albert *et al.*, 2019; Manlove *et al.*, 2020). Zygomatic parameters will be utilised in the current study review to ascertain the midfacial development; nasal and maxillary parameters will be discussed under 1.3.3 and 1.3.4 respectively. Sex differences have been noted in the midfacial region with males having overall larger measurements than females (Waitzman *et al.*, 1992; Snodell *et al.,* 1993).



Source: Adapted from Aktop *et al.* (2013)

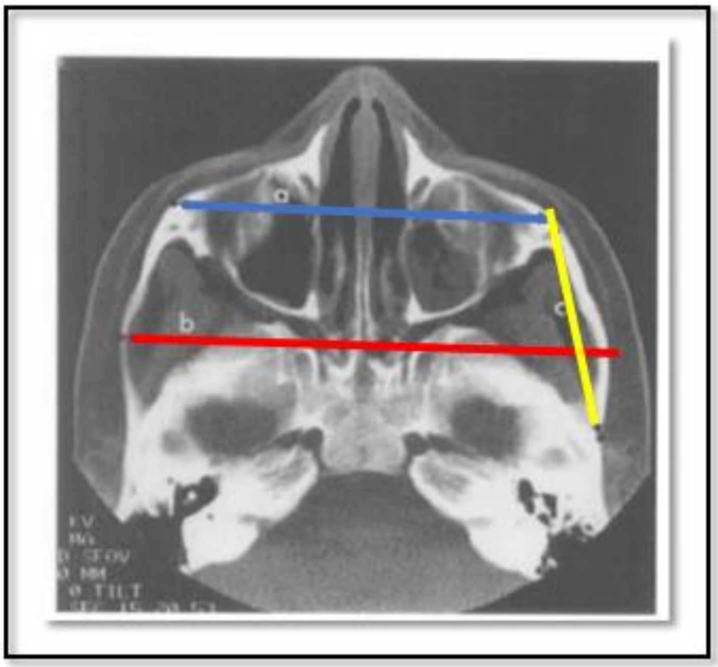
Figure 1.12: Bones making up the midfacial region

The midfacial region in adults was studied in detail by authors such as İşcan and Steyn (1999), Bastir and Rosas (2013), Small *et al.* (2018), Mustafa *et al.* (2019) and Tawha *et al.* (2020), although there is a lack of literature on the development of the midfacial region in subadults. A South African study conducted by İşcan and Steyn (1999) looked at adult skulls of both African and European ancestries. This study aimed to develop standards for estimating ancestry from adult crania. Small *et al.* (2018) conducted a South African study on adult skulls of European ancestry to derive a unique discriminant function for sex estimation. This study looked at the zygomatic region with relation to accurate sex estimation. A recent South African study done by Small *et al.* (2018) investigated inter-landmark distances on the adult skull for sex estimation purposes in multiple ancestry groups. This study concluded that the zygomas could be used to estimate sex with high accuracy (Small *et al.*, 2018). Tawha *et al.* (2020) conducted a South African study on 158 adult crania and observed that the zygomatic bones could be used to better estimate ancestry.

Few studies, such as those done by Waitzman *et al.* (1992), Snodell *et al.* (1993), Ross and Williams (2010), and Machado *et al.* (2017) looked at the midfacial region in the subadult population. These studies looked at individuals of European (Waitzman *et al.,* 1992), African (Ross and Williams 2010), and Brazilian (Machado *et al.,* 2017) ancestries.

Review papers written by Costello *et al.* (2012) and Manlove *et al.* (2020) refer to the study by Waitzman *et al.* (1992). Both review papers stated that the midfacial region displayed a rapid growth within the first year of life, after which it begins to plateau until the ages of approximately

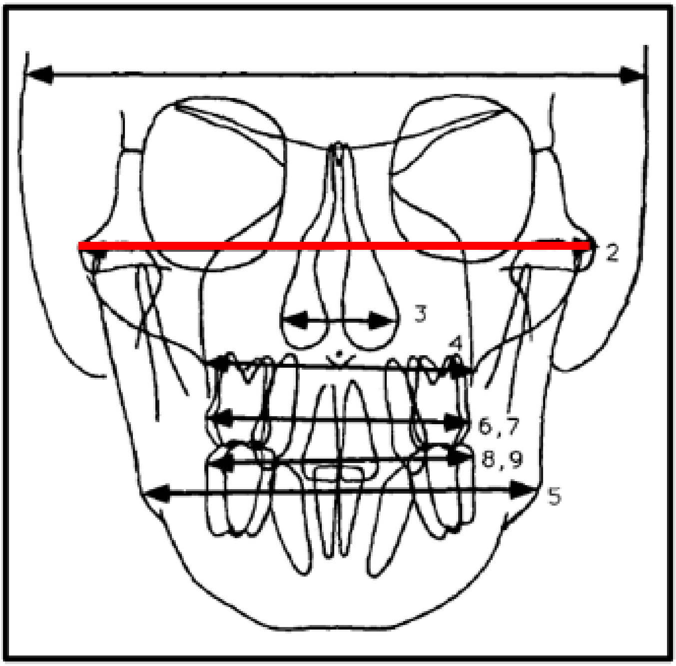
6–8 years of age. However, when the growth of the midfacial region is compared to that of the orbit and the cranium, the midface develops at a more gradual rate. Furthermore, the Waitzman *et al.* (1992) study assessed the midfacial region by utilising the following measurements: interzygomatic-buttress distance, interzygomatic-arch distance, and zygomatic arch length (Figure 1.13) providing normative data for the midfacial region on CT scans of European individuals from birth to 17 years of age. The midfacial region in comparison did not experience the same significant size increase as the rest of the cranium in the first year of development. The midfacial region instead, continued to grow during the later stages of development (6–16 years of age), more so than the neurocranium, which could be contributed to the paranasal air sinuses, which grew between these stages (Bastir and Rosas 2013). Waitzman *et al.* (1992) noted that the zygomatic arch length and the interzygomatic-arch distance growths are highly correlated with one another (r = 0.88).



Source: Waitzman *et al.* (1992)

Figure 1.13: Midfacial region measurements done by Waitzman *et al.*displaying the interzygomatic-buttress distance (blue); interzygomatic-arch distance (red) and zygomatic-arch distance (yellow)

Snodell *et al.* (1993) and Nanda *et al.* (2012) conducted studies on 50 cephalometric radiographs of individuals with European ancestry between the ages of 4–25 years of age and 6–18 years respectively. Their studies produced detailed summaries of the horizontal changes that take place in the viscerocranium. The bizygomatic distance (Figure 1.14) had the greatest increase between 6–18 years of age in the Snodell *et al.* (1993) study, with the mean bizygomatic distance of six years of age being 110.82±3.45 mm for males and 108.22±3.27 mm for females. While at 18 years of age the mean bizygomatic distance was 134.06±4.8 mm for males and 126.03±5.68 mm for females. The rate of growth between 6–18 years was 0.2–1.4 mm per year in the Snodell *et al.* (1993) study. Nanda *et al.* (2012) noted that the bizygomatic distance increased by 1.5–2 mm per year between 6–11 years of age for females and 6–13 years of age for males. In females, the growth of the bizygomatic distance was complete by the age of 17.5 years, whereas males had not completed growth by 18 years of age.



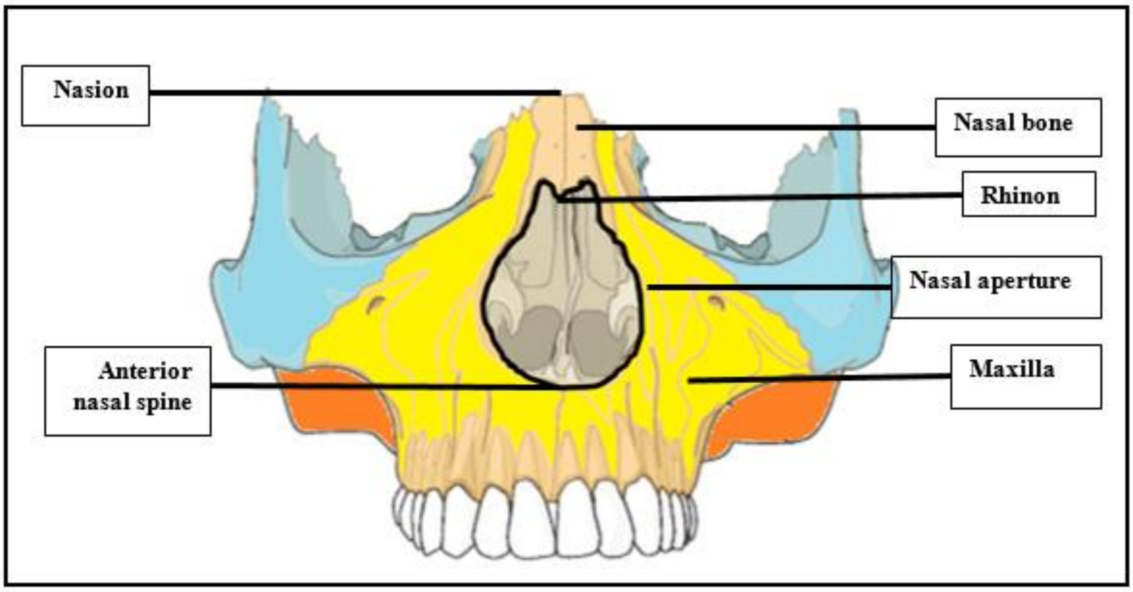
Source: Adapted from Snodell *et al.* (1993)

Figure 1.14: Schematic of the bizygomatic distance (red)

Machado *et al.* (2017) conducted a study on 1000 photographs of individuals between the ages of 6–22 years of age with Brazilian ancestry. This study calculated the average relative growth of the face by using multiple facial measurements, one of which being the bizygomatic distance. The measurements done on these images were in pixels and therefore are not included, as they cannot be compared to the measurements done in millimetres. This study observed that there was significant growth between the groups 6–10 years of age and 10–14 years of age.

1.3.3 Nasal

The nose is mainly composed of cartilage in the early years of an infant (Manlove *et al.*, 2020). The nasal aperture, also known as the pyriform aperture, is the pear-shaped boundary between the nasal vestibule and the nasal cavity proper (Papesch and Papesch 2016). It is formed laterally and inferiorly by the maxilla which fuses anteriorly, this fusion is known as the anterior nasal spine (ANS) and superiorly by the nasal bones that fuse together at the midline (Figure 1.15) (Papesch and Papesch 2016). The nasal aperture height, width, and shape differs between individuals of different ancestries (McDowell *et al.*, 2015; Cunningham *et al.*, 2016). The nose undergoes two important periods of growth, the first being between 2–5 years of age; the second period is when the individual undergoes puberty (Manlove *et al.*, 2020). The nasal expansion in childhood is substantial, due to the enlargement of the lungs during childhood (Enlow and Hans 1996).



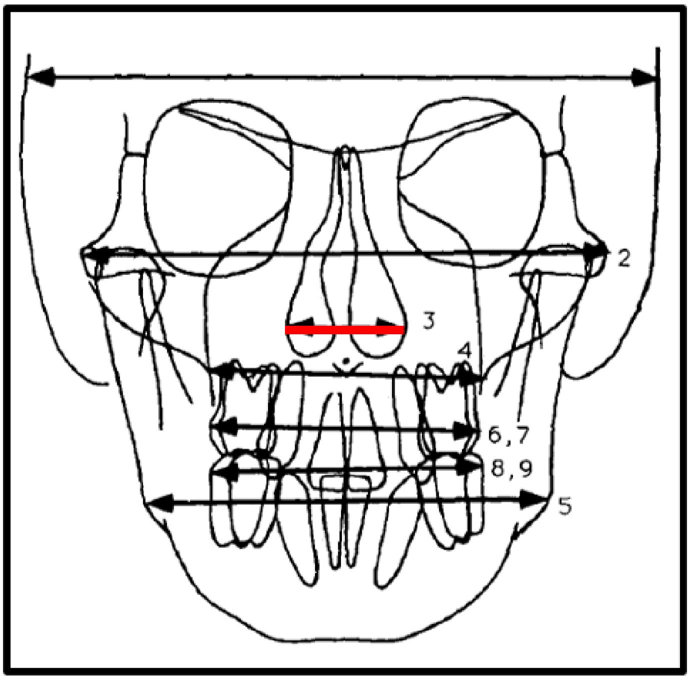
Source: Adapted from Aktop *et al.* (2013)

Figure 1.15: Viscerocranium with the nasal aperture outlined in black

The nasal region in adults was studied in detail by authors such as Grymer *et al.* (1991); Bastir and Rosas (2013); Chinas *et al.* (2017); De Araujo *et al.* (2018) and Utsuno *et al.* (2018), although there is a lack of literature on the development and ancestry standards of the nasal region in subadults. A South African study conducted by McDowell *et al.* (2015) observed the nasal aperture shape variation in three adult South African ancestry groups, namely: African, European, and mixed ancestries. This study aimed to identify sexual dimorphism and ancestry specific standards using the nasal aperture with linear measurements and geometric morphometrics of adult crania. This study observed that the nasal aperture in both male and female individuals with African ancestry was shorter and rounder when compared to their European counterparts. The individuals with mixed ancestry displayed differences in nasal aperture shape between males and females; males had nasal aperture shapes which lay between the African and European shapes, whereas female individuals nasal aperture shape was similar to individuals with African ancestry.

Ridel *et al.* (2018) investigated the nasal region using cone beam CT in a South African study where the individuals were adults of African and European ancestries. They investigated the nasal aperture to predict where landmarks should be placed on the bone for accurate soft-tissue reconstruction. All the bony measurements conducted in this study displayed statistically significances between individuals of African and European ancestry. The males of African ancestry had statistically significant different nasal heights when compared to the females with African ancestry. Small *et al.* (2018) conducted a South African study on adult skulls of European ancestry to derive a function for sex estimation that is 88.2% accurate when estimating sex. The function which they derived was: DS = -25.24 + 0.008x1 + 0.119x 2 + 0.145x3 + 0.358x4 + 0.128x5 + (-0.214)x6 + (-0.158)x7, where DS = discriminant score and x1-7 = measured variables. The nasal aperture was looked at in this study with relation to accurate sex estimation. This study found that sex could be more accurately estimated in males than in females using the nasomaxillary measurements (Small *et al.*, 2018). Few studies, such as those done by Shah *et al.* (1991); Snodell *et al.* (1993); Grymer and Bosch (1997); Thordarson *et al.* (2006); Kim *et al.* (2008); Nanda *et al.* (2012); Buyuk *et al.* (2017); Noble *et al.* (2019) looked at the nasal region in the subadult population. These studies looked at individuals of Asian (Shah *et al.*1991; Kim *et al.*2008); European (Snodell *et al.*, 1993; Grymer and Bosch 1997; Thordarson *et al.*, 2006); Turkish (Buyuk *et al.*, 2017) and Australian (Noble *et al.*, 2019) populations. There is poor representation of the subadult development of the nasal region in the South African population of African ancestry.

Snodell *et al.* (1993) conducted a study on 50 cephalometric radiographs of individuals with European ancestry between the ages of 4–25 years of age. This study aimed to identify growth patterns for craniofacial parameters as well as the differences between males and females. There were many vertical and horizontal measurements done in this study such as the cranial width, bizygomatic width, nasal width, maxillary width, mandibular width, maxillary intermolar width, and mandibular intermolar width. The linear measurement, which aligns with this part of the review, is the nasal width (Figure 1.16). The results of the nasal width include the mean and standard deviation of the individuals at 6 years of age and the individuals at 18 years of age (Table 1.2). The nasal width had the greatest increase between the ages of 6–18 years when compared to all other horizontal measurements done (0.2–1.4 mm a year) (Snodell *et al.*, 1993). The limitations noted in this study include the distortion that may occur on the cephalometric radiograph images due to patients moving their heads.

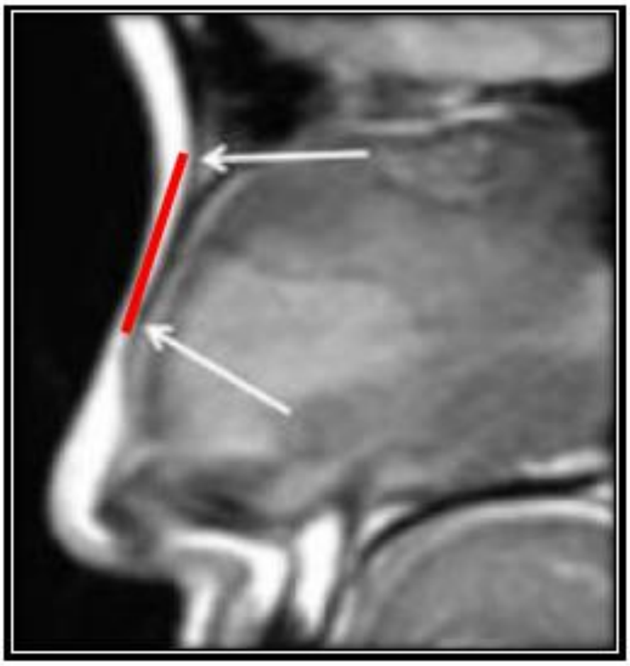


Source: Adapted from Snodell *et al.* (1993)

Figure 1.16: Schematic representation of the nasal aperture width (red)

Thordarson *et al.* (2006) conducted a longitudinal study on 396 individuals of European ancestry. Cephalometric radiographs were taken at 6 years of age and again at 16 years of age. This study aimed to investigate the changes that occur between 6 and 16 years of age, as well as to compare the growth differences between males and females. The nasal region measurements conducted in this study included the nasal bone length (N–RH) (Figure 1.17) (Table 1.2). Males displayed larger nasal bone length measurements than females at 16 years of age; however, there was no statistical significance between the male and female nasal bone lengths at 6 years of age.

Kim *et al.* (2008) analysed the development of the nasal septum with regards to age and sex on Magnetic Resonance Imaging images of 280 individuals with Asian ancestry between 1–70 years of age. They also measured the soft tissue and nasal bone length on the Magnetic Resonance Imaging images (Figure 1.17). The individual measurements were not provided in the study; however, it was noted that in both males and females the nasal bone length experienced an increase from birth to teenage years (16.9±2.7 mm) and during their twenties (19.3±2.9 mm). This study excluded individuals who presented with deviated septa.



Source: Adapted from Kim *et al.* (2008)

Figure 1.17: Sagittal section of the nasal bone length (red) in individuals of Asian ancestry between 1–70 years of age

Cunningham *et al.* (2016) referenced data on the nasal aperture done by Lang (1989), which aimed at noting normal measurements of the nasal aperture in subadult individuals of European ancestry. The morphometric parameters that were noted were the nasal aperture height, superior width, and maximum width. The reported values for nasal aperture height and maximum widths are in Table 1.2.

Nanda *et al.* (2012) provided a detailed summary of the horizontal changes that take place in the viscerocranium. The longitudinal study composed of 50 craniometric radiograph records of individuals between 6–18 years of age with European ancestry. The nasal measurement done in this study was the nasal aperture width (Figure 1.16) (Table 1.2). The total size increase of the nasal aperture width between 6–18 years of age was 5.8 mm in females and 7.5 mm in males. The maximum nasal aperture width growth was between 10 years of age and 11 years of age in females, and between 8 years of age and 10 years of age in males, with a second rapid increase occurring at 15 years of age. This study comprised of the same sample as that used by Snodell *et al.* (1993), therefore the limitations would be consistent with those mentioned in the Snodell *et al.* (1993) study.

Table 1.2: Morphometric nasal parameters in subadult samples

Morphometric nasal parameters in subadult samples								
Author (year)	**Ancestry (sample size)**	**Age**	**Nasal aperture width**		**Nasal height**		**Nasal bone length**	
			Male	**Female**	**Male**	**Female**	**Male**	**Female**
Lang (1989)	Not disclosed	Neonate	12.4		11.3		-	
		1	16.5		17.4		-	
		5	18.2		22.6		-	
		13	19.7		26.0		-	
		Adult	23.6		29.1		-	
Snodell *et al.* (1993)	European (50)	6	22.93±1.92	22.88±1.66	-	-	-	-
		18	30.48±2.07	28.64±2.49	-	-	-	-
Thordarson et al. (2006)	European (396)	6	-	-	-	-	18.2±2.5	17.8±2.4
		16	-	-	-	-	22.2±3.5	20.9±3.0
Nanda et al. (2012)	European (50)	6	22.93±1.92	22.88±1.66	-	-	-	-
		7	23.48±2.03	23.17±2.1	-	-	-	-
		8	24.56±1.87	24.09±2.16	-	-	-	-
		9	24.7±2.06	24.58±2.42	-	-	-	-
		10	26.12±2.11	24.94±2.55	-	-	-	-
		11	26.49±1.77	26.14±2.23	-	-	-	-
		12	27.39±2.54	26.55±2.45	-	-	-	-
		13	27.84±2.44	27.14±2.6	-	-	-	-
		14	27.81±2.64	27.1±2.15	-	-	-	-
		15	28.98±2.66	28.12±2.5	-	-	-	-
		16	29.1±2.42	28.32±3.13	-	-	-	-
		17	29.88±2.4	28.76±3.12	-	-	-	-
		18	30.48±2.07	28.64±2.49	-	-	-	-
Buyuk (2017)	Turkish (148)	14–15	32.05±3.06	31.37±2.75	-	-	-	-
Key: (-) Represents parameters which were not analysed in the respective studies								

Buyuk *et al.* (2017) conducted a retrospective study on cephalic radiographs of 148 individuals of Turkish ancestry with a mean age of 14.55±1.42 for males and 14.95±1.8 years for females. This study aimed to investigate if there is an association between the morphology of the frontal air sinus and the viscerocranium. The nasal region measurement done on the cephalometric radiographs was the nasal width (Figure 1.18) (Table 1.2). They found that the nasal width correlated significantly to maxilla width and mandibular width in both males and females, with female nasal width showing significant correlations to the right and left frontal air sinus widths.

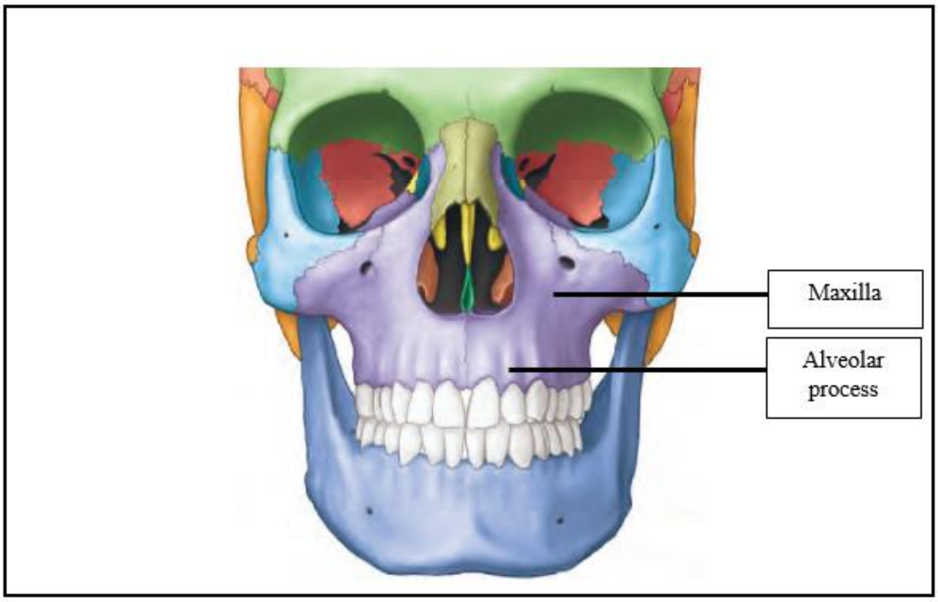
Source: Adapted from Buyuk *et al.* (2017)

Figure 1.18: Cephalometric radiograph of the skull with the nasal width measurement indicated (red)

It can be seen from the studies listed above, that the nasal region of subadult individuals with European ancestry has been studied in detail, whereas there is a lack of literature on the nasal region of subadult individuals with African ancestry. There is also a lack of literature that uses CT scans to evaluate the nasal region, with a majority of the studies utilising cephalometric radiographs to conduct linear measurements (Shah *et al.*, 1991; Snodell *et al.*, 1993; Thordarson *et al.*, 2006; Nanda *et al.*, 2012; Buyuk *et al.*, 2017).

1.3.4 Maxillary

The maxillae, more commonly known as the upper jaw, makes up a large portion of the lower viscerocranium which holds the maxillary teeth in the alveolar processes (Figure 1.19) (Moore *et al.,* 2014; Cunningham *et al.,* 2016). The maxilla forms part of the lateral and inferior part of the nasal aperture, it extends inwards forming part of the floor and lateral walls of the nasal cavity as well as the floor of the orbit and the anterior section of the oral cavity roof (Moore *et al.*, 2014; Cunningham *et al.*, 2016). The maxilla articulates with the zygomatic bones bilaterally, the ethmoid and frontal bones superiorly, the nasal, lacrimal and inferior conchae medially and the palatine bones posteriorly (Moore *et al.*, 2014; Cunningham *et al.*, 2016).



Source: Adapted from Moore *et al.* (2014)

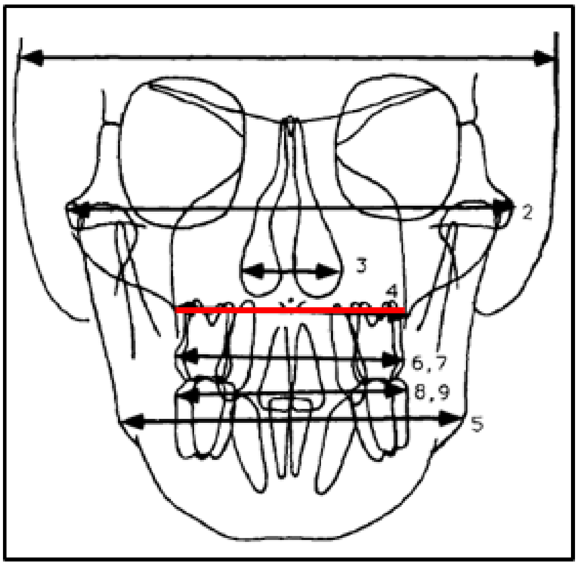
Figure 1.19: Image illustrating the parts of the maxilla

The maxilla undergoes its most rapid periods of growth between 1–2 years of age and again between 3–5 years of age (Costello *et al.*, 2012; Manlove *et al.*, 2020). The maximum maxillary height is reached at approximately 12 years of age in females and approximately 15 years of age in males (Costello *et al.*, 2012; Manlove *et al.*, 2020). The maxillary width displays rapid growth rates after 7 years of age and has reached over 90% of its adult size by 12 years of age, after which growth is seen to plateau (Albert *et al.*, 2019; Nanda *et al.*, 2012). Mellion *et al.* (2013) found that the onset of the maxillary pubertal growth spurt was at 9.8 years of age in females and 12 years of age in males, while the peak ages of the growth spurt were at 11.5 years of age in females and 14.4 years of age in males. Maxillary width and length were significantly larger in males throughout development (Buyuk *et al.*, 2017; Al-Jewair *et al.*, 2018).

There have been multiple geometric morphometric studies done on the crania, such as those by Ross and Williams (2010), Bastir and Rosas (2013), Stull *et al.* (2014) and Noble *et al.*(2019) which looked at the growth and development in different regions of the skull. These studies were conducted to understand not only how the skull changes as the individuals age (Ross and Williams 2010; Noble *et al.*2019), but also to identify sex variations (Noble *et al.*2019) and the correlations which exist between the inner and outer facial skeleton (Bastir and Rosas 2013).

The subadult maxillary region was studied extensively in previous literature, although there is a lack of literature for the development of the maxilla in a South African setting. Subadult studies on the maxilla have done on individuals with European (Snodell *et al.,* 1993; Grymer and Bosch 1997; Nanda *et al.,* 2012; Buschang *et al.,* 2013; Prystanska *et al.,* 2018), Turkish (Buyuk *et al.,* 2017) and African (Al-Jewair *et al.,* 2018) ancestries.

Snodell *et al.* (1993) and Nanda *et al.* (2012) illustrated growth patterns of the viscerocranium (Figure 1.20). The mean and standard deviation data of the maxillary of these studies are noted in Table 1.3. The most rapid period of growth was 7–11 years of age in both the male and female samples studied (Snodell *et al.,* 1993). The growth in females being completed at 15 years of age and 17 years of age in males (Snodell *et al.,* 1993). Females experienced 0.5–1.5 mm of growth a year, while males had a 0.5–1.7 mm per year growth increase (Snodell *et al.*1993). The maxillary width was noted to increase by 10.1 mm and 7.4 mm in males and females respectively at the ages of 6 – 18 years (Nanda *et al.,* 2012). This study observed accelerated growth 8–12 years old (Nanda *et al.,* 2012).



Source: Adapted from Snodell *et al.* (1993)

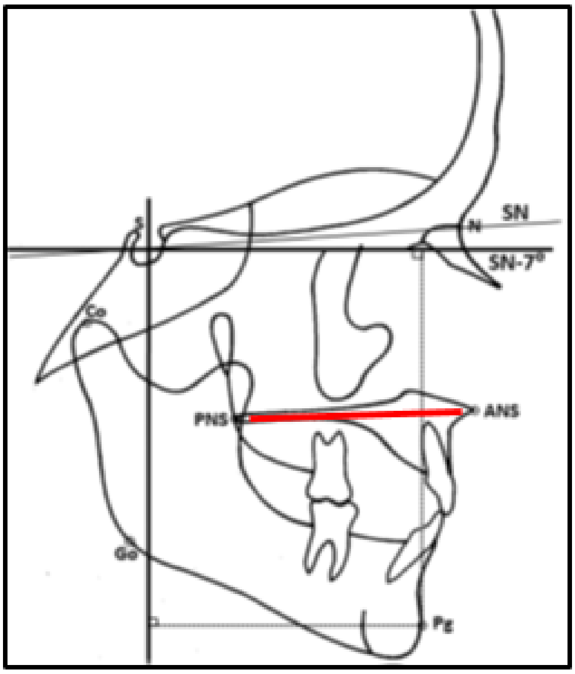
Figure 1.20: Schematic illustration of the maxillary width (red)

Table 1.3: Morphometric maxillary parameters in subadult samples

Morphometric maxillary parameters in subadult samples				
Author (Year)	**Ancestry**	**Age**	**Maxillary width**	
			Male	**Female**
Snodell *et al.* (1993)	European	6	56.12±2.34	54.44±1.86
		18	66.24±3.12	61.8±2.97
Nanda *et al.* (2012)	European	6	56.17±2.34	54.44±1.86
		7	57.67±2.23	55.52±2.1
		8	58.63±2.16	56.71±2.23
		9	60.04±2.53	58.06±2.39
		10	61.37±2.88	58.86±2.34
		11	62.81±2.81	59.73±2.68
		12	63.03±2.99	60.26±2.79
		13	63.51±2.99	60.83±2.57
		14	64.16±3.2	61.42±3.19
		15	65.81±3.17	62.09±3.06
		16	66.02±3.56	61.96±2.49
		17	66.17±3.34	61.88±2.54
		18	66.24±3.12	61.8±2.97
Buyuk (2017)	Turkish	14–15	65.25±3.85	63.09±3.36

Buyuk *et al.* (2017) investigated the correlation between the frontal paranasal air sinus and the morphometry of the viscerocranium in a Turkish population from 148 cephalometric radiographs. The sample consisted of females with an average age of 14.95±1.8 years of age and males with an average of 14.55±1.42 years of age. The maxillary width measurements are listed in Table 1.3. This study showed that the maxillary width was significantly correlated to cranial, nasal, and mandibular widths in both males and females, although it displayed no significant correlation to the frontal air sinus.

Buschang *et al.* (2013) conducted a longitudinal study on 111 female subadult individuals of European ancestry, where annual cephalic radiographs were taken to determine if there is a period of significant growth of the viscerocranium in females between the ages of 10–15 years of age. The cephalic radiographs recorded three linear measurements: two in the mandibular region, and one maxillary measurement from the anterior nasal spine (ANS) to the posterior nasal spine (PNS): ANS–PNS (Figure 1.21). The author did not provide the measurements done on the cephalometric radiographs; instead, they described the growth velocity (mm/year) of each measurement, which is shown in Table 1.4.



Source: Adapted from Buschang *et al.* (2013)

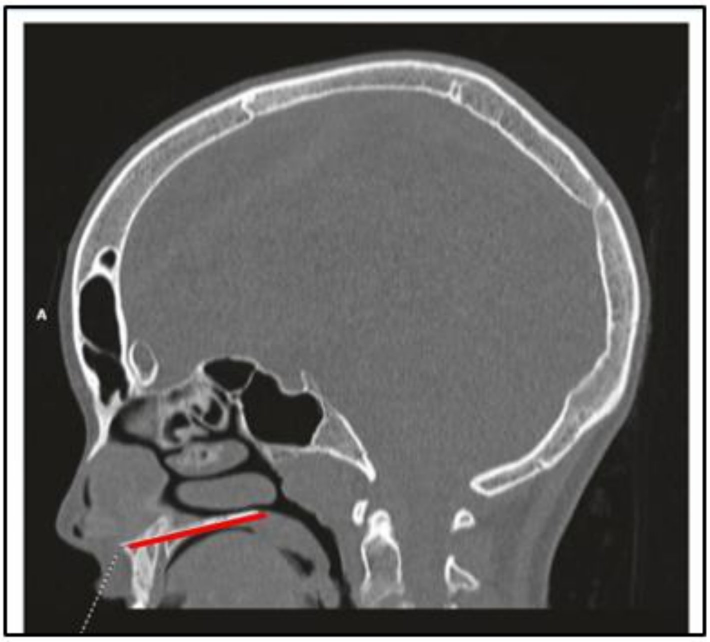
Figure 1.21: Schematic of ANS–PNS measurement (red) done by Buschang

Table 1.4: Maxilla growth velocities in subadult samples

Maxilla growth velocities in a subadult sample as adapted from Buschang et al.	
Age	**ANS–PNS**
10	0.82 mm/year
11	0.75 mm/year
12	0.68 mm/year
13	0.61 mm/year
14	0.55 mm/year

Source: Adapted from Buschang *et al.* (2013)

Prystanska *et al.* (2018) investigated the correlation between the growth of the maxillary air sinus and the viscerocranium. One of the linear measurements reported in this study was the ANS–PNS measurement (Figure 1.22). They did not provide measurements, but the ANS–PNS measurement showed the weakest correlation to the growth of the maxillary air sinus.



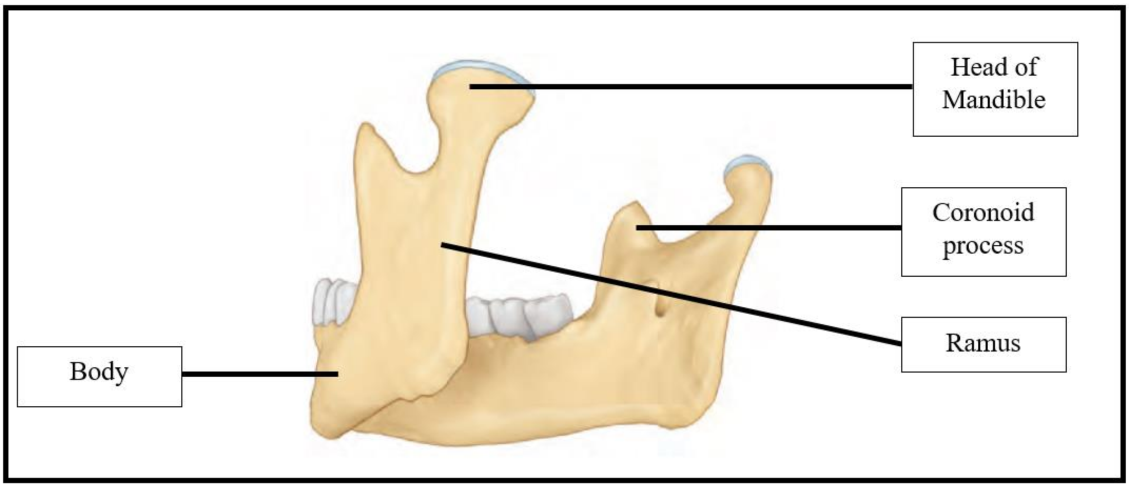
Source: Adapted from Prystanska *et al.* (2018)

Figure 1.22: CT image displaying ANS–PNS measurement (red)

1.3.5 Mandibular

The mandible, also known as the lower jaw, is a u-shaped bone that holds the mandibular teeth in its alveolar processes (Figure 1.23) (Moore *et al.*, 2014). The mandible has a body, ramus, coronoid process and a head (Figure 1.23) which articulates with the temporal bone at the temporomandibular joint (Moore *et al.*, 2014; Cunningham *et al.*, 2016).

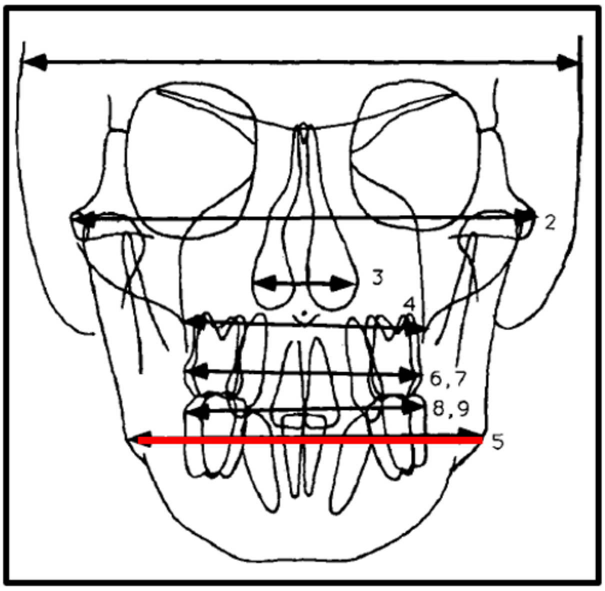
The development of the mandible has been well-documented using linear measurements in subadult individuals with European ancestry (Snodell *et al.*, 1993; Thordarson *et al.*, 2006; Jacob *et al.*, 2011; Nanda *et al.*, 2012; Buschang *et al.*, 2013). There have also been geometric morphometric studies conducted on the French population to assess subadult growth of the mandible (Braga and Treil 2007) and Australian (Fan *et al.*, 2019) population groups. The mandible has completed most of its growth by five years of age (Manlove *et al.*, 2020) with 60–70% of its adult size reached by one year of age (Albert *et al.*, 2019). These studies show the peak growth of the mandible around 13.6–14.5 years of age in males (Mellion *et al.*, 2013; Albert *et al.*, 2019) and 10–12 years of age in females (Buschang *et al.*, 2013; Mellion *et al.*, 2013; Albert *et al.*, 2019).



Source: Adapted from Moore *et al.* (2014)

Figure 1.23: Image illustrating the parts of the mandible

Studies by İşcan and Steyn (1998) on the mandible in adults within South African populations identified parameters that could be useful for sex and ancestry estimations. Whilst an older study by Jacobson (1978) observed the pattern of prognathism, prominence of the facial profile in adult South African individuals with African ancestry. South Africans with African ancestry had a shorter ramus height when compared to their European counterparts (Jacobson 1978).



Source: Adapted from Snodell *et al.* (1993)

Figure 1.24: Schematic illustration of the mandibular width (red)

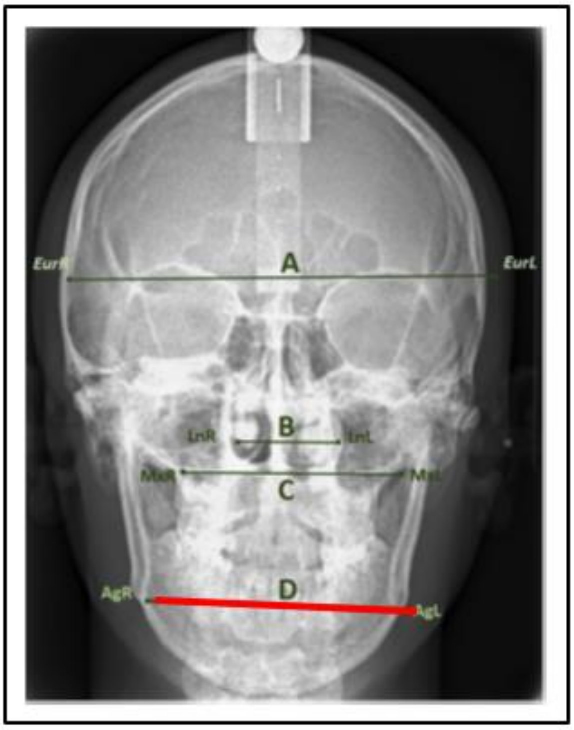
Snodell *et al.* (1993) and Nanda *et al.* (2012) conducted a study that looked at the mandibular width (Figure 1.24). The reported results include the mean and standard deviation of the individuals of 6 years of age and 18 years of age (Table 1.5). The most rapid period of growth was between 7–10 years of age (Snodell *et al.*, 1993). The mandibular width in males at 6–7 years of age was the most reliable measurement when it came to age prediction; this was not true in females between these ages. However, the predictability between the ages of 11–12 years of age was high in both sexes. Females experienced 0.5–2.0 mm of growth a year, whilst males had a 1.5–3.0 mm per year growth increase. The mandible increased more than the maxilla (Table 1.5) (Nanda *et al.*, 2012). The mandibular width continued to increase after 18 years of age in females, which could be due to the muscles of mastication that attach in this area (Snodell *et al.*, 1993). A South African study by Hutchinson *et al.* (2012) utilising specimens from 31 weeks gestational age – 36 months after birth, noted that the morphology of the mandible was more prominent when the muscles of mastication were in use. They also noted that tongue growth and deciduous tooth eruption influenced changes in the mandible (Hutchinson *et al.*, 2012).

Table 1.5: Morphometric parameters in the mandibular region

Morphometric parameters in the mandibular region					
Author (Year)	**Age**	**Mandible width**		**Mandibular length**	
		Male	**Female**	**Male**	**Female**
Snodell et al. (1993)	6	78.43±4.42	76.33±2.77		
	18	99.36±5.17	92.17±3.96		
Thordarson et al. (2006)	6	-	-	92.6±3.8	90.8±3.9
	16	-	-	117.3±5.2	109.5±5.2
Nanda et al. (2012)	6	78.43±4.42	76.33±2.77	-	-
	7	80.99±4.92	78.56±3.4	-	-
	8	83.17±5.07	80.72±3.22	-	-
	9	85.15±4.85	82.67±3.68	-	-
	10	86.65±5.61	84.16±3.21	-	-
	11	88.43±5.11	85.51±3.84	-	-
	12	89.66±5.27	87.03±3.89	-	-
	13	91.2±5.25	88.29±4.2	-	-
	14	92.81±5.25	90.21±4.06	-	-
	15	95.71±6.36	90.94±3.87	-	-
	16	97.24±6.2	91.8±5.06	-	-
	17	98.47±6.46	91.86±4.9	-	-
	18	99.36±5.17	92.17±3.96	-	-
Buyuk et al. (2017)	14–15	85.28±4.37	82.55±4.37	-	-
Key: (-) Represents parameters which were not analysed in the respective studies					

Buyuk *et al.* (2017) assessed the correlation between the frontal air sinus and the viscerocranium from a forensic perspective. This study consisted of 148 cephalometric radiographs; the mean age of the males studied was 14.55±1.42 years of age and the females mean age was 14.95±1.80 years of age. The authors conducted vertical measurements of the viscerocranium including the mandibular width (Figure 1.25). The mean measurements for the males and females are given in Table 1.5. They noted that the mandibular width displayed significant correlations with the right frontal air sinus height and width in males, although in females the mandibular width displayed significant correlations with only the maxillary width.

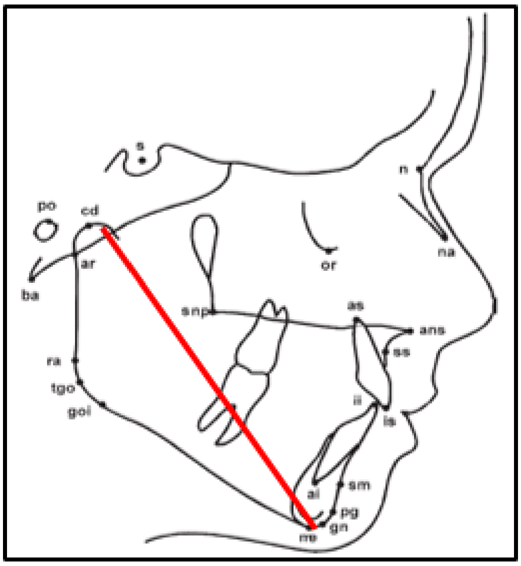
Kumagai *et al.* (2018) combined multiple age prediction methods in subadults to assess whether if combining age estimation methods would lead to better estimations of age. The authors looked at two dental and four skeletal age estimation methods. One of the skeletal measurements that was assessed was the mandibular width (Figure 1.25). The measurements were not provided, although it was noted that the mandibular width showed a strong correlation to age in both males and females.



Source: Adapted from Buyuk *et al.* (2017)

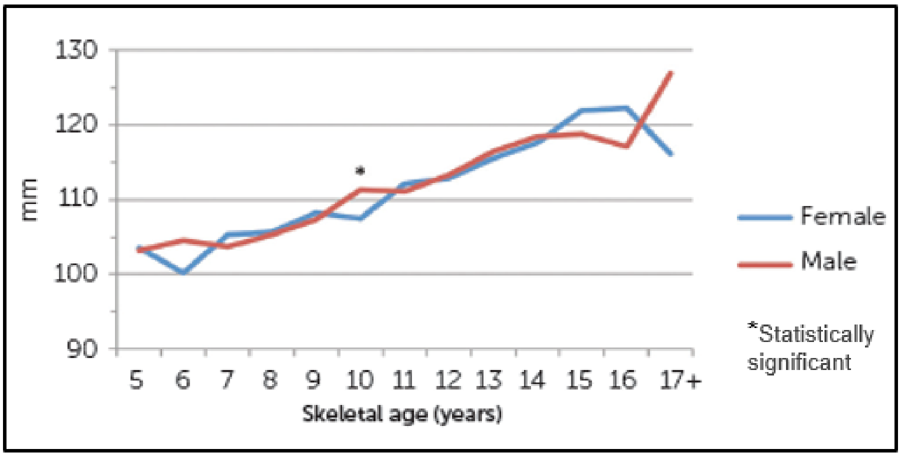
Figure 1.25: Cephalometric radiograph of the skull with the mandibular width measurement indicated (red)

Thordarson *et al.* (2006) conducted a longitudinal study on 396 individuals of European ancestry. Cephalometric radiographs were taken at 6 years of age and again at 16 years of age. This study investigated the changes in the mandible length (Figure 1.26) (Table 1.5) which occur from 6–16 years of age and compared the growth differences between males and females. Al-Jewair *et al.* (2018) conducted a subadult study on individuals of African ancestry. They noted that the mandibular length experiences a gradual increase as the age increased in both the sexes, with large contrasts between the male and female measurements 5–7 years of age. At 10 years of age, there were statistically significant differences in mandible length in males and females (Figure 1.27).



Source: Adapted from Thordarson *et al.* (2006)

Figure 1.26: Schematic illustration of the mandibular length (red)



Source: Adapted from Al-Jewair *et al.* (2018)

Figure 1.27: Mandibular length growth

1.4 Forensic significance

According to the South African Police Service presentation on the crime situation, the murders in South Africa have seen an increase in recent years with an average of 58 people a day being murdered (https://www.saps.gov.za/services/crimestats.php). The reported murder cases in South Africa for 2019/20 being 21 325, while 943 child murders were reported between April 2019 and March 2020 (Mahlakoana 2020). This illustrates an increase in child murders when compared to a post written by Delany and Mathews (2018) stating that the number of reported child murders in the year 2016/17 was 803. These numbers reflect only the reported murder cases. According to Missing Children South Africa (n.d.) a child goes missing every five hours in South Africa with only 77% of children being found. The remaining 23% being either desceased, trafficked or never found. A forensic anthropologist can assist with the identification of skeletal remains in situations where the soft tissue of the individual is compromised or in advanced stages of decomposition to the point where DNA analysis cannot be used for identification purposes (Buyuk *et al.*, 2017; Krüger *et al.*, 2017; Ubelaker and Khosrowshahi 2019). Identification of unknown remains is of high importance to medico-legal forensic practitioners during an investigation (Brough *et al.*, 2012; Noble *et al.*, 2019). For unknown skeletal remains to be identified a biological profile has to be created (Noble *et al.*, 2019). The most reliable parameter of a subadult biological profile is age estimation as it is based on the analysis of morphological and morphometric parameters which indicate growth and development (Krüger *et al.*, 2017). Age estimations prove very important in a criminal investigation, as the age of an individual will impact how their court case is dealt with as well as the protection and support the individual will receive (Franklin 2010; Tiemensma and Phillips 2016; Ubelaker and Khosrowshahi 2019). It is important to have an accurate age estimation as a child who is incorrectly deemed an adult would not receive all the legal protection and support that they are entitled to (Tiemensma and Phillips 2016).

1.5 Clinical significance

Knowledge of the normal growth patterns will allow physicians to identify unusual or pathogenic growth (Palanisamy *et al.*, 2016) such as in cases of foetal alcohol syndrome that is prevalent in South African populations (Briers 2015). The data from the proposed study would benefit clinicians as the understanding of the growth and development rates of the viscerocranium will aid in diagnosis as well as treatment planning (Nanda *et al.*, 2012; Palanisamy *et al.*, 2016). If the treatments are not done at the correct stage the outcomes of the procedures would not be successful (Snodell *et al.*, 1993). Therefore, detailed knowledge on the timing of the viscerocranial growth rates would help orthodontist, maxillofacial and plastic surgeons time procedures for maximum success.

1.6 General methodology

1.6.1 Study design

The development of the facial skeleton from birth to 18 years of age within a South African cohort using CT scans was investigated. The linear measurements were correlated to age to derive a formula that could be used for age estimation using the facial skeleton.

1.6.2 Setting

The study was a retrospective study, on a subset of a population between birth and 18 years of age. In this study, the subset is individuals of African ancestry from the South African population. The study examined CT scans obtained from a private medical centre within the eThekwini Region. The CT scans included those of the facial skeleton. Permission to access CT data was obtained from the private medical clinic that used a Siemens Biograph mCT 64 flow (PET-CT) manufactured in Germany (Appendix A). Laser guiding in the orbitomeatal plane was used to ensure that scans were taken in the correct plane. The digital imaging and communications in medicine (DICOM) images were viewed from an online Picture Archiving and Communication Systems server on a personal computer (HP laptop 15-bs003ni, Intel core i3, 4GB RAM) using Infinitt software (version 5.0.1.1) (Figure 1.28) which is the standard software used by the practitioners. The scans which were used in this study were all 1 mm thick. Approval was obtained via Biomedical Research Ethics Committee at UKZN (BREC/00001011/2020) (Appendix B).

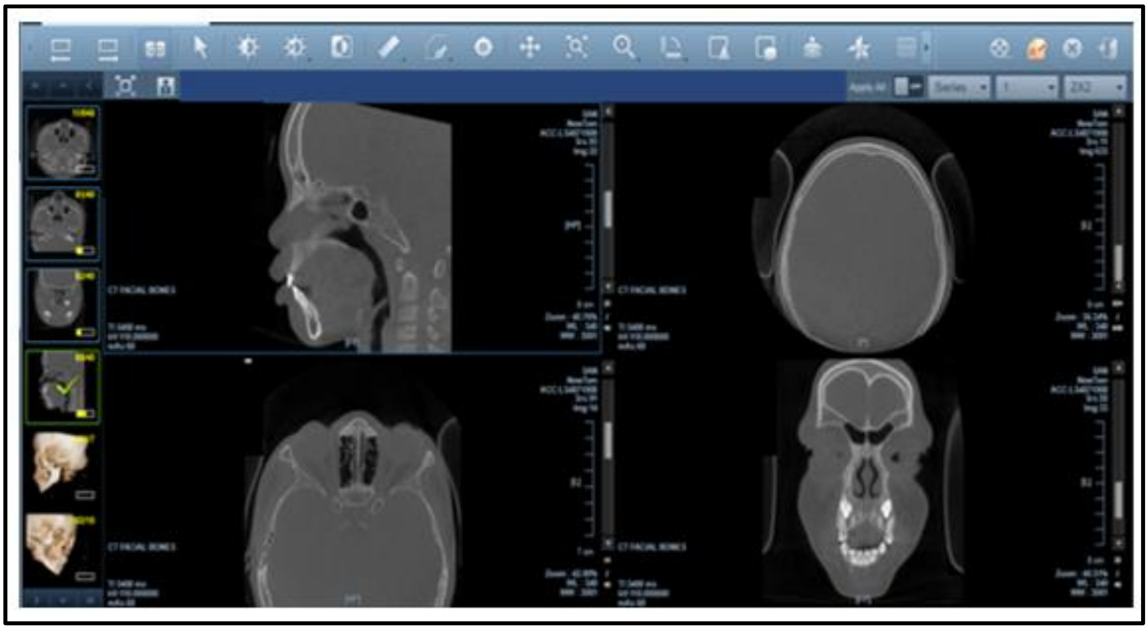


Figure 1.28: Image of the Infinitt software (version 5.0.1.1) used to analyse CT scans

1.6.3 Participant selection and sampling strategy

The study was a retrospective review of CT scans done on South African patients of African descent between birth to 18 years of age, which are considered subadults as they are still in the growth and development phase of life, which is usually below 18 years of age (Christensen *et al.*, 2014).

A biostatistician from the University of KwaZulu-Natal determined that a sample size of 262 was required to estimate the correlation between skeletal measurements and age to within ±0.1 with power of 80% and probability of 95% and assuming a baseline correlation of 0.7. Approximately 500 CT scans were reviewed, and the final study consisted of 239 CT scans that were selected which fit into the inclusion criteria. The distribution between males and females was as evenly distributed as the data available would allow. Kail (2012) stated that infants are below one year of age, toddlers are between 1–2 years of age, pre-schoolers are between 2–5 years of age, school-aged children are between 6–13 years of age and adolescents are between 14–19 years of age. The sample of 239 scans between 0–18 years of age was divided according to the grouping above, but then further divided into five categories for more even data distribution. The final five age categories used in this study was below 1 year of age, 1–5 years of age, >5–10 years of age, >10–15 years of age and over 15 years of age.

Inclusion criteria

- CT scans of individuals with African ancestry who were between birth and 18 years of age with no history of trauma or surgery to the viscerocranium.
- Brain scans with normal anatomy, no damage to the skull or facial skeleton.
- CT scans should be 1 mm thickness and should be clear.

Exclusion criteria

- CT scans of individuals over 18 years of age.
- Brain CTs with abnormal anatomy, injury or damage to the skull or facial skeleton.
- CT scans thicker than 1 mm.
- CT scans where the images were not clear.

1.6.4 Data collection and statistical analysis

This study consisted of a sample size of 239 CT scans that fit into the inclusion criteria. All data was analysed using R Statistical Computing Software of the R Core Team version 3.6.3. A p-value of less than 0.05 was considered statistically significant. The linear measurements were conducted three times and an average was found. The statistical analysis was done using the average measurements. The median values between males and females were compared using Ranksum test. Chi-square and Fischer's Exact tests were done to detect large and small expected frequencies in the data respectively, between males and females, and the age categories. A paired t test was done to compare the linear measurements conducted on the right and left sides. To determine the correlations between the linear measurements and age for both males and females scatter plots were made with p-values and regression lines. Scatterplot gam graphs were made to assess the growth patterns of the viscerocranial regions over time. Inter-class correlation (ICC) coefficient tests were conducted to assess the level of agreement between the Intra- and Inter-observer measurements.

1.7 Measurements

In this study the facial skeleton was be subdivided down into regions, namely: orbital, nasal, midfacial, maxillary and mandibular as per previous CT studies (Waitzman *et al.,* 1992; Enlow and Hans 1996; Rossi and Williams 2010; Manlove *et al.,* 2020). Measurements were taken between the various bony and sutural landmarks in each of these regions (Tables 1.6 – 1.10; Appendix C). The CT scans were viewed in the horizontal, sagittal, and coronal views using Infinitt software (version 5.0.1.1) and measurements were recorded in millimetres. The measurements were compared to identify any growth patterns between the age groups as well as the differences between developmental rates between males and females.

Table 1.6: Orbital region parameters

Orbital region parameters		
Measurement	**Landmark 1**	**Landmark 2**
Orbital width	Zygomaticofrontal suture	Dacryon
Orbital height	Zygomaticomaxillary sutures	Perpendicular line drawn from the inferior landmark to the superior orbital rim
Anterior interorbital distance (AID)	Dacryon on right	Dacryon on left
Lateral orbital distances (LOD)	Most anterior aspect of lateral orbital wall on right	Most anterior aspect of lateral orbital wall on left

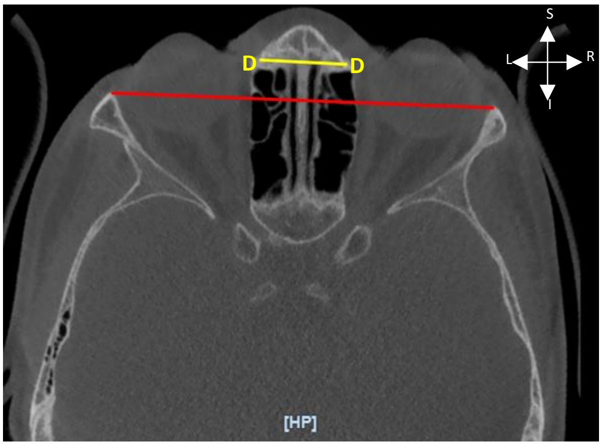


Figure 1.29: Horizontal CT scan in the orbital region displaying the AID (Yellow) and LOD (Red) (Superior view)

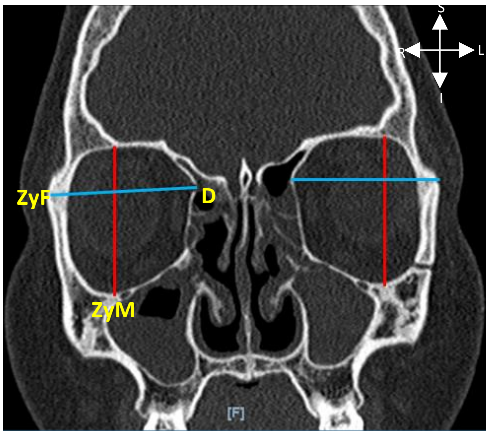


Figure 1.30: CT scan in the orbital region displaying the orbital height (red) and orbital width (blue) (Coronal view)

Key: D – Dacryon; ZyF – Zygomaticofrontal suture; ZyM – Zygomaticomaxillary suture

Table 1.7: Nasal region parameters

Nasal region parameters		
Measurement	**Landmark 1**	**Landmark 2**
Maximum aperture width	Most lateral part of the nasal aperture on right	Most lateral part of the nasal aperture on left
Aperture height	Rhinion (Rh)	Anterior nasal spine (ANS)

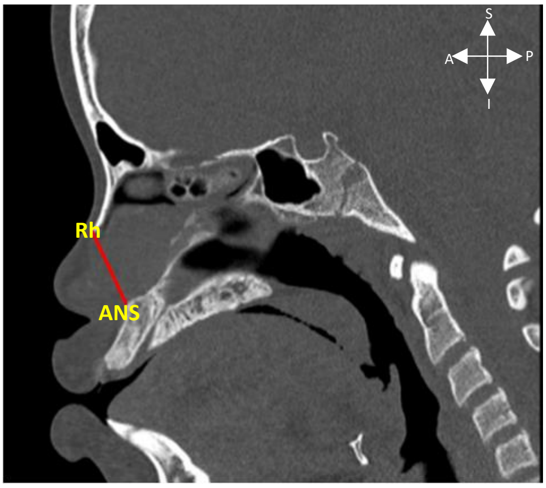


Figure 1.31: CT scan in the nasal region displaying the nasal aperture height (red) (sagittal view)

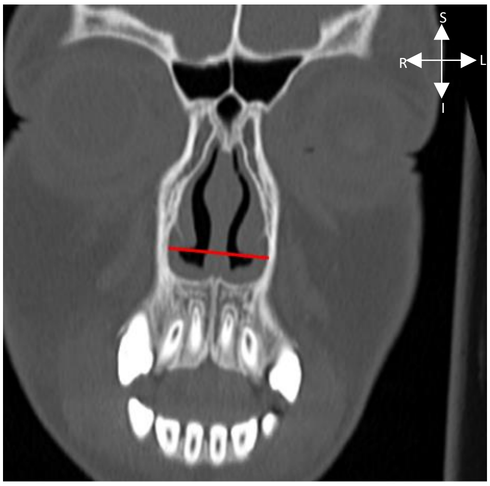


Figure 1.32: CT scan in the nasal region displaying the nasal aperture width (red) (coronal view)

Key: Rh – Rhinion; ANS – Anterior nasal spine

**Table 1.8:	Midfacial region parameters**

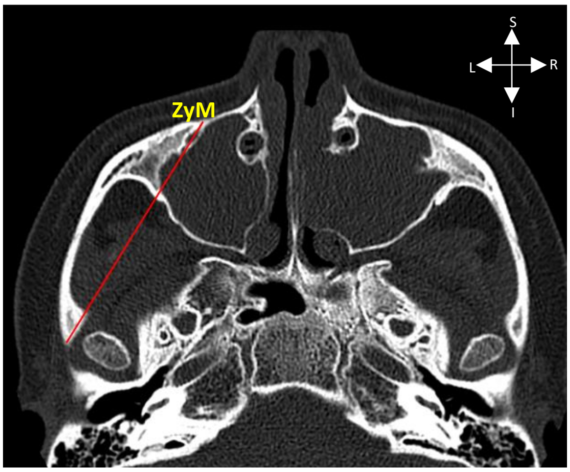
<table>
<tr><th colspan="3">Midfacial region parameters</th></tr>
<tr><th>Measurement</th><th>Landmark 1</th><th>Landmark 2</th></tr>
<tr><td>Zygomatic arch length (ZAL)</td><td>Zygomaticomaxillary suture</td><td>The point where the zygomatic arch enters the temporal bone</td></tr>
<tr><td>Zygomatic arch distance (ZAD)</td><td>Most lateral part of the zygomatic arch on the right</td><td>Most lateral part of the zygomatic arch on the left</td></tr>
<tr><td colspan="2">

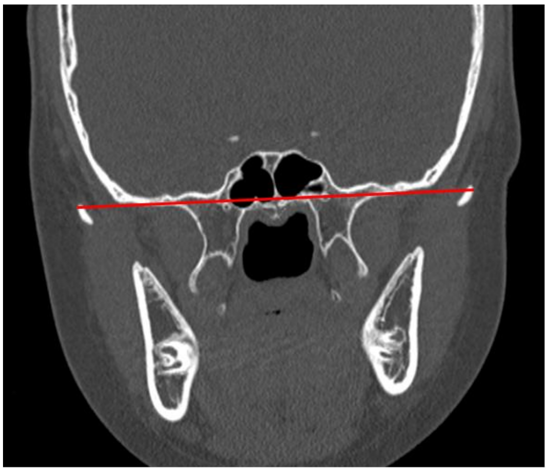
Figure 1.33: Horizontal CT scan in the midfacial region displaying the Zygomatic arch length (red) (superior view)</td><td>
Figure 1.34: CT scan in the midfacial region displaying the Zygomatic arch distance (red) (coronal view)</td></tr>
<tr><td colspan="3">Key: ZyM – Zygomaticomaxillary suture</td></tr>
</table>

Table 1.9: Maxillary region parameters

Maxillary region parameters		
Measurement	**Landmark 1**	**Landmark 2**
Maxillary length	Anterior nasal spine (ANS)	Posterior nasal spine (PNS)

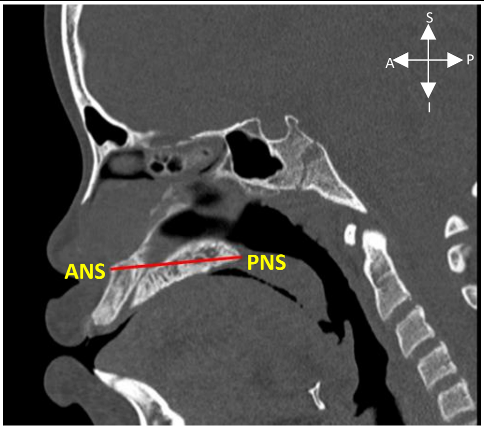


Figure 1.35: CT scan of the maxillary region displaying the ANS–PNS measurement (red) (sagittal view)

Key: ANS – Anterior nasal spine; PNS – Posterior nasal spine

Table 1.10: Mandibular region parameters

Mandibular region parameters		
Measurement	**Landmark 1**	**Landmark 2**
Mandible head width	Medial point of mandible head	Lateral point of mandible head
Mandibular width	Most lateral point on the mandible head on the right	Most lateral point on the mandible head on the left

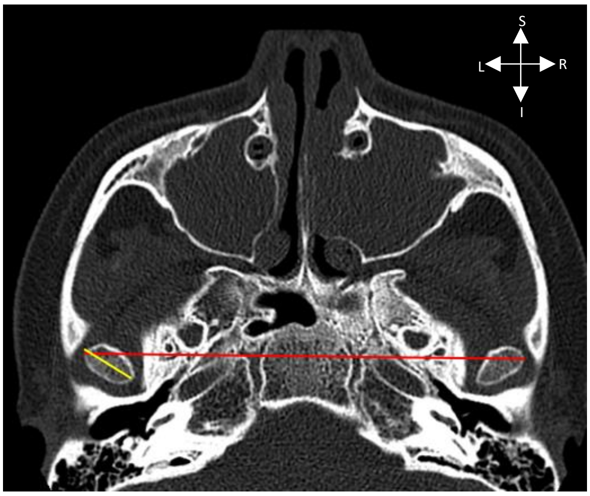


Figure 1.36: Horizontal CT of the mandibular region in the superior view displaying the mandiblewidth (red) and the mandible head width (yellow)

1.8 Layout of thesis

This Master's dissertation by research was prepared in the manuscript format following the guidelines laid out by the College of Health Sciences, University of KwaZulu-Natal. The structural outline is as follows:

1.8.1 Chapter One: Introduction

This chapter presented a background and an extensive review of literature on the development of the viscerocranial regions (orbital, nasal, midfacial, maxillary and mandibular) as reported by existing studies. Included in this chapter are the aims, research objectives and an overview of the methodology of this study.

1.8.2 Chapter Two: Scientific manuscript

This chapter constitutes an original research manuscript entitled: Developmental changes of the facial skeleton from birth to 18 years within a South African cohort (A Computed Tomography study), which addresses the research aims and objectives in relation to the development of the different regions of the facial skeleton and was subsequently published in the Journal of Forensic and Legal Medicine (https://doi.org/10.1016/j.jflm.2021.102243).

1.8.3 Chapter Three: Synthesis

This chapter concludes the development of the viscerocranium from 0–18 years of age. The developmental growth patterns of the viscerocranial regions (orbital, midfacial, nasal, maxillary and mandibular) was provided. Correlations of the viscerocranial regions to age were made and utilised to derive age estimation formulas based on linear measurements. Furthermore, the limitations and future recommendations were highlighted in this chapter.

1.9 References

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Chapter 2
Scientific Manuscript

Title: Developmental changes of the facial skeleton from birth to 18 years within a South African cohort (A computed tomography study)

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2.1 Abstract

Skeletal remains are often found on a crime scene in which a forensic anthropologist is then consulted to create a biological profile, which includes the estimation of age, sex, ancestry and stature. The viscerocranium plays an important role in the formation of a biological profile. However, to utilise the viscerocranium for age estimation, population specific normative data and knowledge of the development of the viscerocranium is required. Therefore, this study aimed to investigate the developmental changes from birth to 18 years of age of the facial skeleton of individuals from a South African cohort. This study comprised of 239 computed tomography (CT) scans (128 males;111 females). The viscerocranium was subdivided into five regions viz.:- orbital, nasal, midfacial, maxillary and mandibular. The linear parameters in each region were correlated to age to identify the developmental growth patterns of the viscerocranial regions according to male and female. The measurements which displayed the highest correlations with age were used to develop formulas which could be used for age estimation. The results of this study showed that the measurements in the orbital, midfacial, maxillary and mandibular regions experienced rapid growth between 0–5 years of age, with the nasal region increasing steadily over time. It was noted that males displayed overall larger measurements than females except for the anterior interorbital distance and both right and left zygomatic arch lengths (ZAL). Although only the left orbital height, nasal aperture height and mandible width displayed statistically significant size differences according to sex ($p \leq 0.05$). The measurements which showed the highest correlations to age were the zygomatic arch distance ($r = 0.8842$, $p<0.001$), ZAL (right: $r = 0.8929$, $p<0.001$; left: $r = 0.8656$, $p<0.001$) and the mandible width ($r = 0.8444$, $p<0.001$). Formulas were derived for the measurements that could be used to forensically estimate age within a subadult cohort.

Keywords: Viscerocranium, Facial skeleton, Age estimation, Development, Morphometry, Subadult

2.2 Introduction

South Africa is known to have a high rate of violent crimes, which results in many unidentified skeletal remains (Lottering 2020). When unidentified skeletal remains are discovered at a crime scene, a biological profile needs to be created, which involves age, sex, stature, and ancestry estimations (İşcan and Steyn 1999; Franklin 2010; Machado *et al.*, 2017; Noble *et al.,* 2019; Mustafa *et al.,* 2019; Ubelaker and Khosrowshahi 2019). In South Africa, the identification of unknown remains can be challenging due to lack of dental records and comparable DNA in individuals with low socioeconomic standing (Dorfling *et al.,* 2018). Currently, there are limited methods of age estimations for subadult remains in advanced stages of decomposition (Krüger *et al.,* 2017). The facial skeleton or viscerocranium plays an important role in the formation of a biological profile during anthropological studies (Mustafa *et al.,* 2019). The developmental changes of the viscerocranium have piqued the interest of researchers in many fields, especially in biological anthropology (Machado *et al.,* 2017).

The ageing of the face is a complex process that is not clearly understood (Mendelson *et al.,* 2007) and includes both the soft tissues and the viscerocranium (Mendelson *et al.,* 2007; Kahn and Shaw 2008). To utilise the viscerocranium for age estimation purposes, detailed knowledge of viscerocranial development is required (Briers 2015). The current age estimation methods done using bones are far from perfect despite the many years of research (Milner and Boldsen 2012). Although, when compared to other identification methods, osteometric measurements done with radiological methods were seen to be more efficient (Mustafa *et al.,* 2019). Additionally, radiological methods are a non-invasive method of viewing skeletal structures, which plays an important role in anthropological investigation as CT scans allow for size and shape analysis of skeletal structures to be done without an autopsy (Franklin *et al.,* 2016).

Previous studies have looked at the development of the separate regions of the face, but there is a lack of studies which analyse the development of the entire subadult facial skeleton (Albert *et al.,* 2019). The viscerocranium can be divided into five regions, *viz.*: orbital, nasal, midface, maxillary and mandibular (Jacob and Buschang 2011; Buyuk *et al.,* 2017; Al-Jewair *et al.,*2018; Manlove *et al.,* 2020). These regions develop at different rates to one another (Mendelson *et al.,*2007; Ross and Williams 2010; Bastir and Rosas 2013; Machado *et al.,* 2017) as well as at different rates to the rest of the body (Al-Jewair *et al.,* 2018), as the viscerocranium growth is regulated by the developing paranasal air sinuses, the sensory organs and tooth eruptions (Ross and Williams 2010). The major studies which report on the viscerocranium as a whole focus mainly on the shape differences in adult samples (Chovalopoulou *et al.,* 2017; Dereli *et al.,* 2018). A review of the available literature revealed that there is a paucity of knowledge on the development of the entire viscerocranium in a subadult South African cohort of African ancestry.

The data from this study could aid in the understanding of the normal developmental patterns of the viscerocranium, in addition to population specific normative data, that could aid forensic anthropologists with age estimations. Furthermore, understanding would also allow physicians to identify unusual or pathogenic growth as well as assist in treatment planning (Palanisamy *et al.*, 2016).

This study aimed to investigate the developmental changes of the facial skeleton in males and females from birth to 18 years within a cohort of the South African population to estimate age.

2.3 Materials and methods

This was a retrospective study which consisted of 239 CT scans of subadult individuals of African ancestry (125 males; 111 females). The scans were obtained from an online server utilised by a private medical facility in the eThekwini Municipality (CT scanner Siemens Biograph mCT 64 flow (PET-CT), manufactured in Germany). Ethical clearance for this study was obtained from the Biomedical Research Ethical Committee at the University of KwaZulu-Natal (BREC/00001011/2020). The inclusion criteria for selecting scans were as follows: a) individuals should be of African ancestry, b) individuals should be 0–18 years of age, c) individuals should have normal anatomy, no pathologies or trauma to the viscerocranium, d) CT scans could not be thicker than 1 mm slices and e) the scan had to include all planes. Laser guiding in the orbitomeatal plane was used to ensure that scans were taken in the correct plane. The digital imaging and communications in medicine (DICOM) images were viewed from an online Picture Archiving and Communication Systems server on a personal computer (HP laptop 15-bs003ni, Intel core i3, 4GB RAM) using Infinitt software (version 5.0.1.1) which is the standard software used by the practitioners. The same software was used to conduct the measurements in the horizontal, sagittal, and vertical planes. The parameters of each viscerocranial region were measured three times using the sutural and bony landmarks outlined in Table 2.1.

Table 2.1: Viscerocranial region parameters

Viscerocranial region parameters			
Region	**Measurement**	**Landmark 1**	**Landmark 2**
Orbital	Orbital width	Zygomaticofrontal suture	Dacryon
	Orbital height	Zygomaticomaxillary sutures	Perpendicular line drawn from the inferior landmark to the superior orbital rim
	Anterior interorbital distance	Dacryon on right	Dacryon on left
	Lateral orbital distances	Most anterior aspect of lateral orbital wall on right	Most anterior aspect of lateral orbital wall on left
Nasal	Maximum aperture width	Most lateral part of the nasal aperture on right	Most lateral part of the nasal aperture on left
	Aperture height	Rhinion	Anterior nasal spine
Midfacial	Zygomatic arch length	Zygomaticomaxillary suture	The point where the zygomatic arch enters the temporal bone
	Zygomatic arch distance	Most lateral part of the zygomatic arch on the right	Most lateral part of the zygomatic arch on the left
Maxillary	Maxillary length	Anterior nasal spine	Posterior nasal spine
Mandibular	Mandible head width	Medial point of mandible head	Lateral point of mandible head
	Mandibular width	Most lateral point on the mandible head on the right	Most lateral point on the mandible head on the left

2.4 Statistical analysis

Approximately 500 CT scans were reviewed, and the final study consisted of 239 CT scans that were selected which fit into the inclusion criteria. The distribution between males and females was as evenly distributed as the data available would allow. During data analysis, age was categorised into five age groups (<1 year of age; 1–5 years of age; >5–10 years of age; >10–15 years of age; >15 years of age). All data was analysed using R Statistical Computing Software of the R Core Team version 3.6.3. A p-value of less than 0.05 was considered statistically significant. The linear measurements of the viscerocranial regions were compared according to age and sex. The Kruskal Wallis and ANOVA tests were used to compare the medians and means of the linear measurements between the age categories respectively. The Ranksum test was used to compare the means between the medians between the males and females. A paired t-test was conducted to compare the overall right and left side for the orbit and mandible measurements. Chi-square and Fischer's Exact tests were done to detect large and small expected frequencies in the data respectively, between males and females, and the age categories. Scatter plots for the linear correlations with p-values and regression lines were created to determine the correlations between the linear measurements and age for both sexes. Scatterplot gam graphs were made to assess the growth patterns of the viscerocranial regions over time.

2.5 Results

2.5.1 Morphometric results of the viscerocranial regions

Analysis of the data collected from 239 CT scans revealed that the growth between the age groups for each viscerocranial region displayed statistically significant increases ($p<0.001$) (Table 2.2). Although overall, the only measurements which showed significant differences between males and females (Table 2.3) was the orbital height left ($p = 0.048$), nasal aperture height ($p = 0.048$) and mandible width ($p = 0.05$). It was noted that the overall measurements done between the left and right, orbital heights, orbital widths, zygomatic arch lengths, and mandible head widths displayed no statistically significant differences ($p>0.05$).

Table 2.2: Morphometric measurements of the viscerocranial regions according to the age categories

Morphometric measurements of the viscerocranial regions according to the age categories							
Age groups	**<1yr (N=16)**	**1–5yrs (N=30)**	**>5–10yrs (N=69)**	**>10–15yrs (N=71)**	**>15yrs (N=53)**	**p-value**	**Overall (N=239)**
Anterior interorbital distance						<0.001	
Mean±SD (CV%)	16.3±2.24(13.8)	22.5±5.66(25.2)	24.6±3.77(15.4)	23.2±3.39(14.7)	23.8±2.93(12.3)		23.2±4.18(18.1)
Median (Q1–Q3)	16.5(15.2–18.1)	21.2(19.5–24.1)	23.9(22.0–27.0)	23.2(20.5–25.3)	23.5(21.7–25.5)	Kruskal	23.1(20.4–25.5)
Lateral orbital wall distance						<0.001	
Mean±SD (CV%)	71.7±6.02(8.4)	84.7±5.64(6.7)	91.8±4.31(4.7)	98.5±5.28(5.4)	100±3.13(3.1)		93.4±9.06(9.7)
Median (Q1–Q3)	70.8(68.8–74.3)	84.9(81.8–89.3)	91.4(88.1–95.1)	98.4(94.6–101)	100(98.1–103)	Kruskal	95.3(88.5–99.5)
Orbital height right						<0.001	
Mean±SD (CV%)	29.2±3.31(11.3)	34.7±2.40(6.9)	36.6±2.52(6.9)	37.1±2.42(6.5)	36.2±3.14(8.7)	ANOVA	35.9±3.27(9.1)
Median (Q1–Q3)	28.6(27.0–32.0)	35.1(33.6–36.1)	36.2(35.1–38.4)	37.1(35.5–38.8)	35.6(33.7–38.6)		36.0(34.3–38.3)
Orbital height left						<0.001	
Mean±SD (CV%)	29.9±3.23(10.8)	34.9±2.28(6.5)	36.3±2.24(6.2)	36.9±2.60(7.0)	36.5±2.91(8.0)	ANOVA	35.9±3.07(8.5)
Median (Q1–Q3)	29.6(28.0–33.3)	34.9(33.1–36.7)	36.1(35.0–37.7)	37.1(35.6–38.4)	36.2(34.4–37.8)		36.1(34.4–37.8)
Orbital width right						<0.001	
Mean±SD (CV%)	27.5±2.41(8.7)	32.5±2.14(6.6)	34.4±2.14(6.2)	37.6±2.95(7.8)	39.9±1.34(3.3)		35.9±4.01(11.2)
Median (Q1–Q3)	27.6(26.0–28.4)	32.8(31.2–33.8)	34.4(33.3–35.7)	37.2(35.4–39.9)	39.8(38.9–40.9)	Kruskal	35.7(33.3–39.1)
Orbital width left						<0.001	
Mean±SD (CV%)	27.6±2.09(7.6)	32.7±2.07(6.3)	35.2±7.58(21.5)	37.1±4.99(13.5)	39.7±1.28(3.2)		36.0±5.88(16.3)
Median (Q1–Q3)	27.4(26.3–28.8)	32.9(31.4–33.8)	34.5(33.3–35.6)	36.7(35.7–39.5)	39.8(38.6–40.6)	Kruskal	35.8(33.5–39.0)
Aperture height						<0.001	
Mean±SD (CV%)	14.2±1.87(13.2)	21.1±4.31(20.4)	24.1±2.19(9.1)	27.4±2.77(10.1)	28.5±2.56(9.0)		25.0±4.63(18.5)
Median (Q1–Q3)	14.2(13.5–15.0)	21.1(18.3–23.5)	24.0(22.5–25.3)	27.1(25.6–29.1)	28.6(26.6–30.0)	Kruskal	25.5(22.7–28.3)
Aperture width						<0.001	
Mean±SD (CV%)	16.8±2.65(15.8)	20.8±1.91(9.2)	23.0±1.85(8.0)	25.0±2.30(9.2)	25.3±2.28(9.0)		23.5±3.12(13.3)
Median (Q1–Q3)	16.9(16.2–18.1)	20.9(20.1–22.0)	22.9(22.1–24.6)	24.9(23.5-26.4)	25.6(24.3-26.6)	Kruskal	23.8(22.0-25.5)

Table 2.2 continued							
Age groups	**<1yr (N=16)**	**1–5yrs (N=30)**	**>5–10yrs (N=69)**	**>10–15yrs (N=71)**	**>15yrs (N=53)**	**p-value**	**Overall (N=239)**
Zygomatic arch distance						<0.001	
Mean±SD (CV%)	81.3±6.75(8.3)	103±8.38(8.2)	115±5.13(4.5)	124±6.17(5.0)	128±5.03(3.9)		117±13.9(11.9)
Median (Q1–Q3)	80.8(75.8–84.8)	105(98.8–109)	115(111–118)	123(120–127)	128(125–132)	Kruskal	120(110–126)
Zygomatic arch length right						<0.001	
Mean±SD (CV%)	37.6±4.82(12.8)	47.1±4.00(8.5)	57.3±7.19(12.5)	67.7±5.12(7.6)	70.9±3.43(4.8)		59.8±11.9(19.9)
Median (Q1–Q3)	38.1(33.8–40.2)	46.7(43.4–49.3)	59.4(53.9–62.7)	68.1(65.2–71.0)	70.8(68.8–74.0)	Kruskal	63.3(49.3–69.4)
Zygomatic arch length left						<0.001	
Mean±SD (CV%)	38.4±5.40(14.1)	48.6±4.52(9.3)	58.6±7.32(12.5)	68.8±6.12(8.9)	71.2±3.16(4.4)		60.7±12.0(19.7)
Median (Q1–Q3)	37.8(35.0–41.8)	48.3(44.5–52.1)	60.4(53.0–62.8)	68.8(65.3–72.2)	71.5(69.2–73.2)	Kruskal	62.4(51.4–70.9)
ANS–PNS						<0.001	
Mean±SD (CV%)	34.4±3.10(9.0)	40.6±4.83(11.9)	44.9±3.28(7.3)	49.3±3.27(6.6)	50.8±3.49(6.9)	ANOVA	46.3±5.74(12.4)
Median (Q1–Q3)	34.2(32.0–36.1)	40.2(37.4–43.2)	44.5(42.5–46.7)	49.1(46.8–51.6)	51.1(48.2–52.8)		46.8(43.0–50.4)
Mandible width						<0.001	
Mean±SD (CV%)	73.3±3.77(5.1)	92.7±8.60(9.3)	104±5.70(5.5)	112±5.99(5.4)	115±6.29(5.5)	ANOVA	106±12.2(11.6)
Median (Q1–Q3)	72.1(70.4–75.6)	94.8(87.6–98.4)	103(99.9–107)	111(108–116)	115(111–118)		108(100–114)
Mandible head right						<0.001	
Mean±SD (CV%)	10.2±1.60(15.7)	13.3±2.38(17.9)	15.4±1.88(12.2)	17.7±1.83(10.4)	18.5±2.02(10.9)	ANOVA	16.2±3.05(18.8)
Median (Q1–Q3)	10.1(9.52–10.5)	13.4(11.4–14.8)	15.3(14.0–17.0)	17.7(16.5–19.0)	18.4(17.4–20.0)		16.6(14.3–18.3)
Mandible head left						<0.001	
Mean±SD (CV%)	10.1±1.44(14.2)	13.2±1.88(14.2)	15.4±2.01(13.0)	18.1±1.76(9.7)	18.7±2.13(11.4)	ANOVA	16.3±3.15(19.3)
Median (Q1–Q3)	9.96(9.39–11.0)	13.3(11.9–14.3)	15.4(14.0–17.0)	18.0(17.0–19.3)	18.6(17.2–20.3)		16.8(14.2–18.7)
Key: CV – Coefficient of variation; Q1 – first quartile, Q3 – third quartile							

Table 2.3: Overall differences of the linear parameters between females and males from birth to 18 years of age

The overall differences of the linear parameters according to sex from birth to 18 years of age				
Sex	**Female (N=111)**	**Male (N=128)**	**p-value**	**Overall (N=239)**
Anterior interorbital distance			0.845	
Mean±SD (CV%)	23.2±4.16(17.9)	23.1±4.21(18.2)		23.2±4.18(18.1)
Median (Q1–Q3)	23.2(20.4–25.4)	23.0(20.3–25.6)	Ranksum	23.1(20.4–25.5)
Lateral orbital wall distance			0.913	
Mean±SD (CV%)	93.3±8.57(9.2)	93.5±9.49(10.2)		93.4±9.06(9.7)
Median (Q1–Q3)	96.7(88.1–99.4)	94.1(89.5–100)	Ranksum	95.3(88.5–99.5)
Orbital height right			0.055	
Mean±SD (CV%)	35.5±3.17(8.9)	36.3±3.32(9.1)		35.9±3.27(9.1)
Median (Q1–Q3)	35.7(33.9–37.8)	36.2(34.6–38.9)	Ranksum	36.0(34.3–38.3)
Orbital height left			0.048	
Mean±SD (CV%)	35.5±2.85(8.0)	36.3±3.22(8.9)		35.9±3.07(8.5)
Median (Q1–Q3)	36.0(33.8–37.1)	36.3(34.8–38.0)	Ranksum	36.1(34.4–37.8)
Orbital width right			0.689	
Mean±SD (CV%)	35.8±3.88(10.8)	36.0±4.13(11.5)		35.9±4.01(11.2)
Median (Q1–Q3)	35.7(33.3–39.1)	35.9(33.5–39.0)	Ranksum	35.7(33.3–39.1)
Orbital width left			0.646	
Mean±SD (CV%)	35.8±3.65(10.2)	36.1±7.29(20.2)		36.0±5.88(16.3)
Median (Q1–Q3)	35.7(33.5–38.8)	35.9(33.6–39.0)	Ranksum	35.8(33.5–39.0)
Aperture height			0.048	
Mean±SD (CV%)	24.5±4.06(16.6)	25.5±5.04(19.7)		25.0±4.63(18.5)
Median (Q1–Q3)	25.3(22.3–27.0)	26.2(23.0–28.7)	Ranksum	25.5(22.7–28.3)
Aperture width			0.814	
Mean±SD (CV%)	23.5±3.34(14.2)	23.5±2.92(12.4)		23.5±3.12(13.3)
Median (Q1–Q3)	23.6(22.0–25.7)	23.9(21.9–25.2)	Ranksum	23.8(22.0–25.5)
Zygomatic arch distance			0.146	
Mean±SD (CV%)	116±12.0(10.4)	117±15.4(13.1)		117±13.9(11.9)
Median (Q1–Q3)	119(110–124)	120(111–128)	Ranksum	120(110–126)
Zygomatic arch length right			0.477	
Mean±SD (CV%)	60.9±10.9(17.9)	58.9±12.6(21.4)		59.8±11.9(19.9)
Median (Q1–Q3)	64.6(53.0–69.4)	60.4(49.2–69.4)	Ranksum	63.3(49.3–69.4)
Zygomatic arch length left			0.898	
Mean±SD (CV%)	61.6±10.3(16.7)	60.0±13.2(22.0)		60.7±12.0(19.7)
Median (Q1–Q3)	65.6(55.7–69.5)	62.1(50.4–71.7)	Ranksum	62.4(51.4–70.9)
ANS–PNS			0.126	
Mean±SD (CV%)	45.7±5.34(11.7)	46.9±6.04(12.9)		46.3±5.74(12.4)
Median (Q1–Q3)	46.8(43.7–49.6)	47.4(43.0–51.6)	Ranksum	46.8(43.0–50.4)
Mandible width			0.050	
Mean±SD (CV%)	105±10.4(9.9)	107±13.6(12.8)		106±12.2(11.6)
Median (Q1–Q3)	106(98.9–112)	109(101–117)	Ranksum	108(100–114)
Mandible head right			0.468	
Mean±SD (CV%)	16.0±2.84(17.7)	16.3±3.22(19.7)		16.2±3.05(18.8)
Median (Q1–Q3)	16.5(14.1–18.0)	16.7(14.6–18.6)	Ranksum	16.6(14.3–18.3)
Mandible head left			0.213	
Mean±SD (CV%)	16.0±2.82(17.6)	16.6±3.40(20.5)		16.3±3.15(19.3)
Median (Q1–Q3)	16.8(14.2–18.2)	16.9(14.2–19.4)	Ranksum	16.8(14.2–18.7)
Key: CV – Coefficient of variation; Q1 – first quartile, Q3 – third quartile				

2.5.2 Growth patterns of the morphometric parameters according to age and sex

2.5.2.1 Orbital

Analysis of the growth patterns of the orbital parameters revealed that the males and females grew in unison with a rapid rate from 0–5 years of age. Thereafter, the orbital width had a second peak growth from 10–15 years of age. The orbital height however, displayed no significant growth after 5 years of age, which means the orbital height attains maximum growth between 0–5 years of age. The lateral orbital wall distance (LOD) experienced peak growth from 0–3.75 years of age and continued to increase at a slower rate throughout childhood. The anterior interorbital distance (AID) attained maximum growth at 7.5 years of age, with no significant growth thereafter. Males and females displayed similar growth patterns for all the orbital region parameters, with males appearing to display higher values than females for the orbital height and width and lateral orbital wall distance. (Figure 2.1)

The orbital height increased between 2–3.8 mm/year in females and 2.1–3.1 mm/year in males, with no statistically significant growth increases after 2.5 years of age on the right side and 5 years of age on the left (Figure 2.2).

The orbital width increased between 1.8–2.8 mm/year in females and 2–3.7 mm/year in males. After 5 years of age the orbital width's growth decreased to 0.61–0.8 mm/year in females and 0.56–0.76 mm/year in males (Figure 2.2).

The LOD increased 6.5 mm/year in females and 6.1 mm/year in males below 3.75 years of age. The LOD continued to increase throughout childhood at a rate of 1.1 mm/year in females and 1 mm/year in males (Figure 2.2).

The AID increased at a rate of 1.3 mm/year in females and males below 7.5 years of age. No statistically significant increases were noted after 7.5 years of age (Figure 2.2).

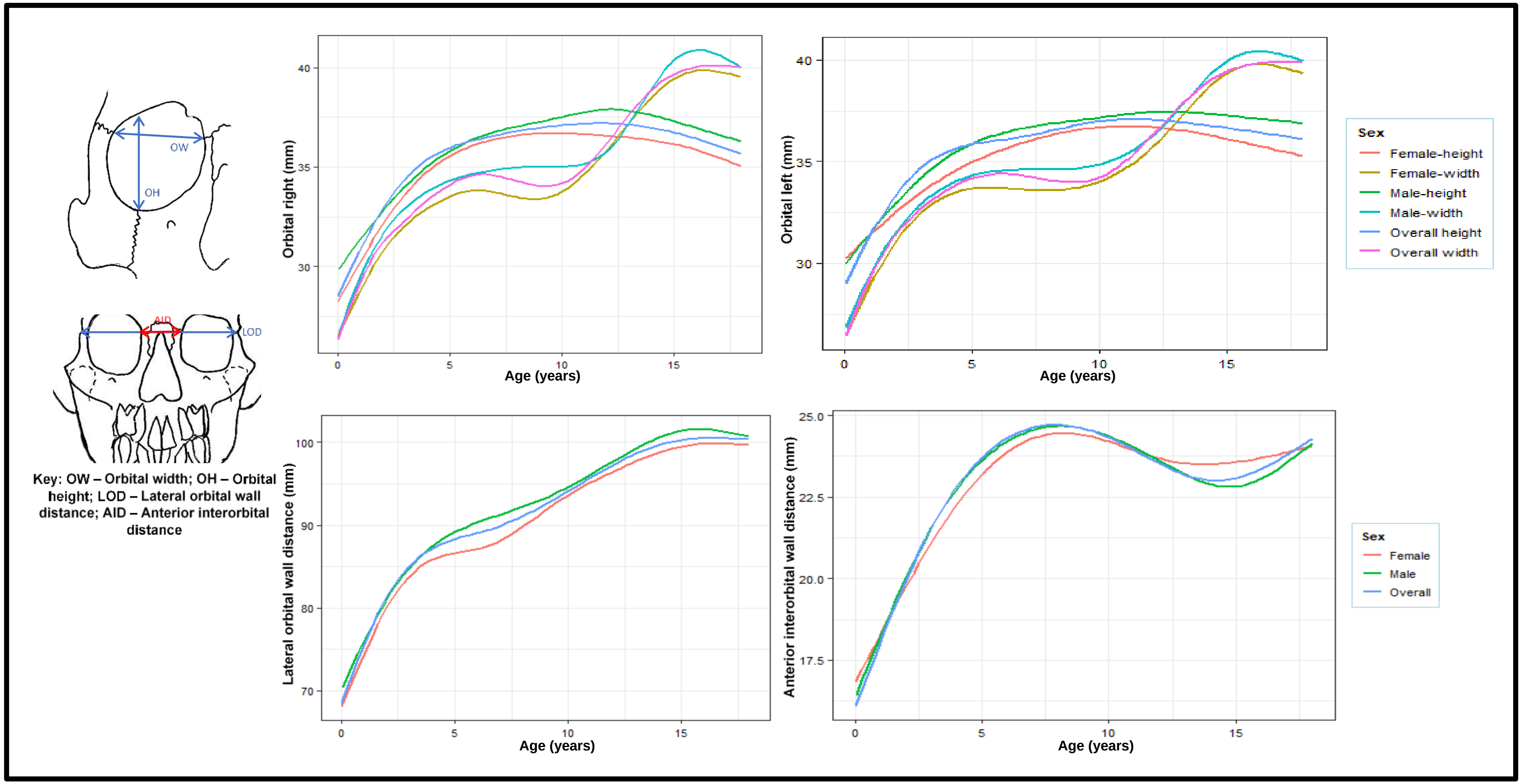

Figure 2.1: Graphs indicating growth of the orbital region over time

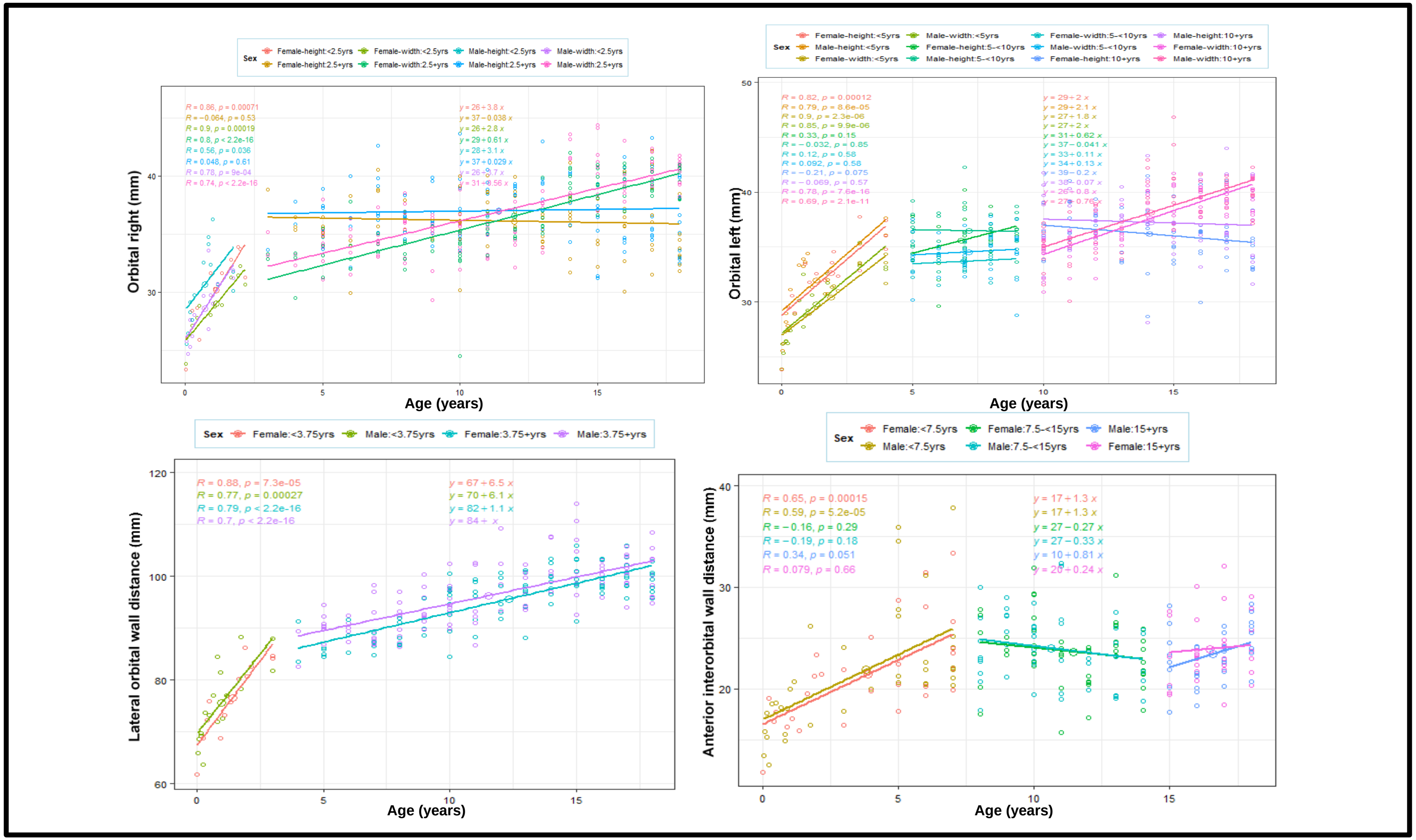

Figure 2.2: Graphs indicating growth rate of the orbital region over time

2.5.2.2 Midfacial

The zygomatic arch distance (ZAD) displayed little difference between males and female's growth patterns below 5 years of age. Although over 5 years of age the males displayed larger measurements (Figure 2.3). At 0–5 years of age, the ZAD displayed a rapid increase in size, growth continued throughout childhood. The ZAD increased at a rate of 10 mm/year for both males and females below 3.75 years of age. Whereas, above 3.75 years of age the ZAD growth rate was 1.6 mm/year in females and 1.8 mm/year in males (Figure 2.3).

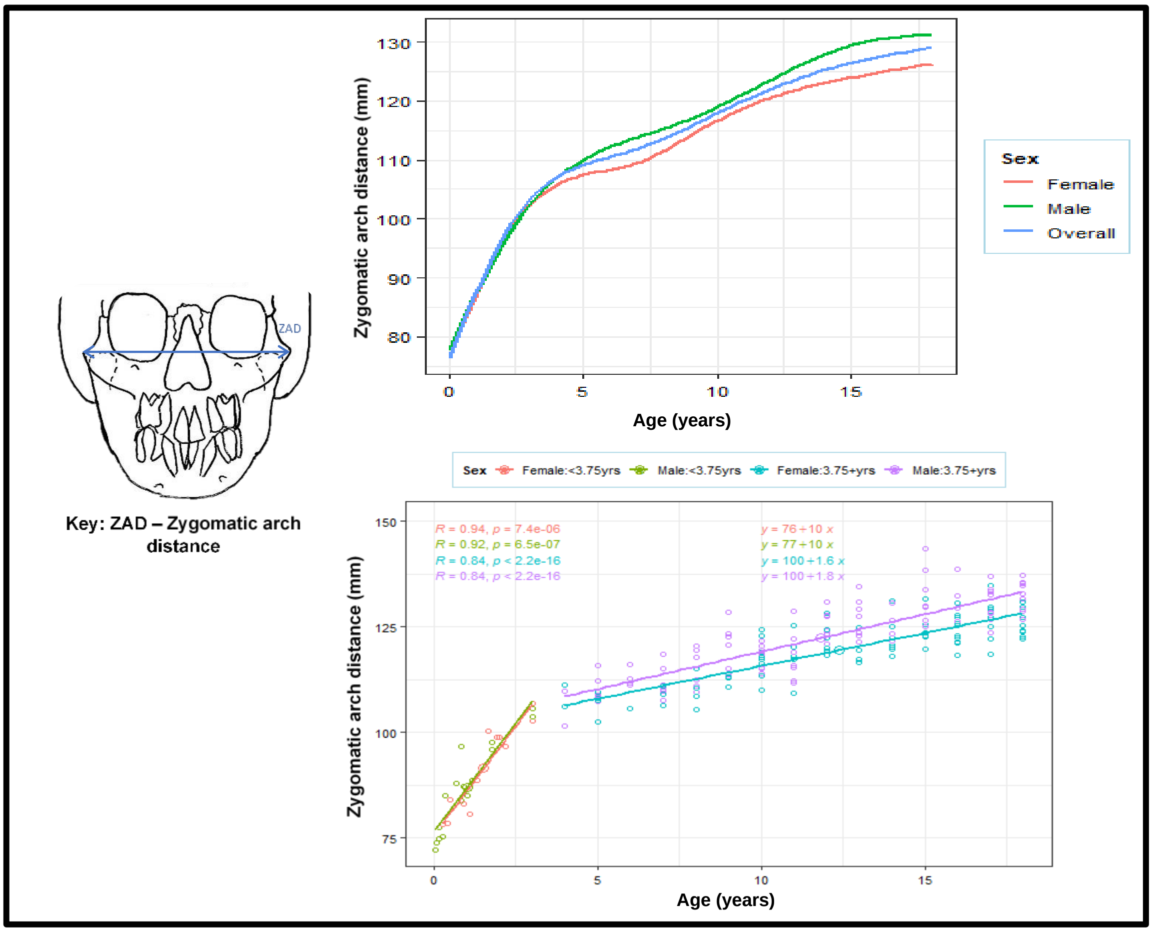

Figure 2.3: Graph indicating growth of the midfacial region over time

2.5.2.3 Nasal

The nasal aperture height displayed overall larger measurements in males than females, although the nasal aperture height was the only nasal parameter to have statistically significant differences between the sexes (p = 0.048). Males and females displayed similar growth patterns, although males had higher values than females. The nasal aperture parameters were noted to increase consistently over time. The nasal aperture height grew 0.63 mm/year in females and 0.77 mm/year in males (Figure 2.4). The nasal aperture width grew 0.45 mm/year in females and 0.4 mm/year in males (Figure 2.4).

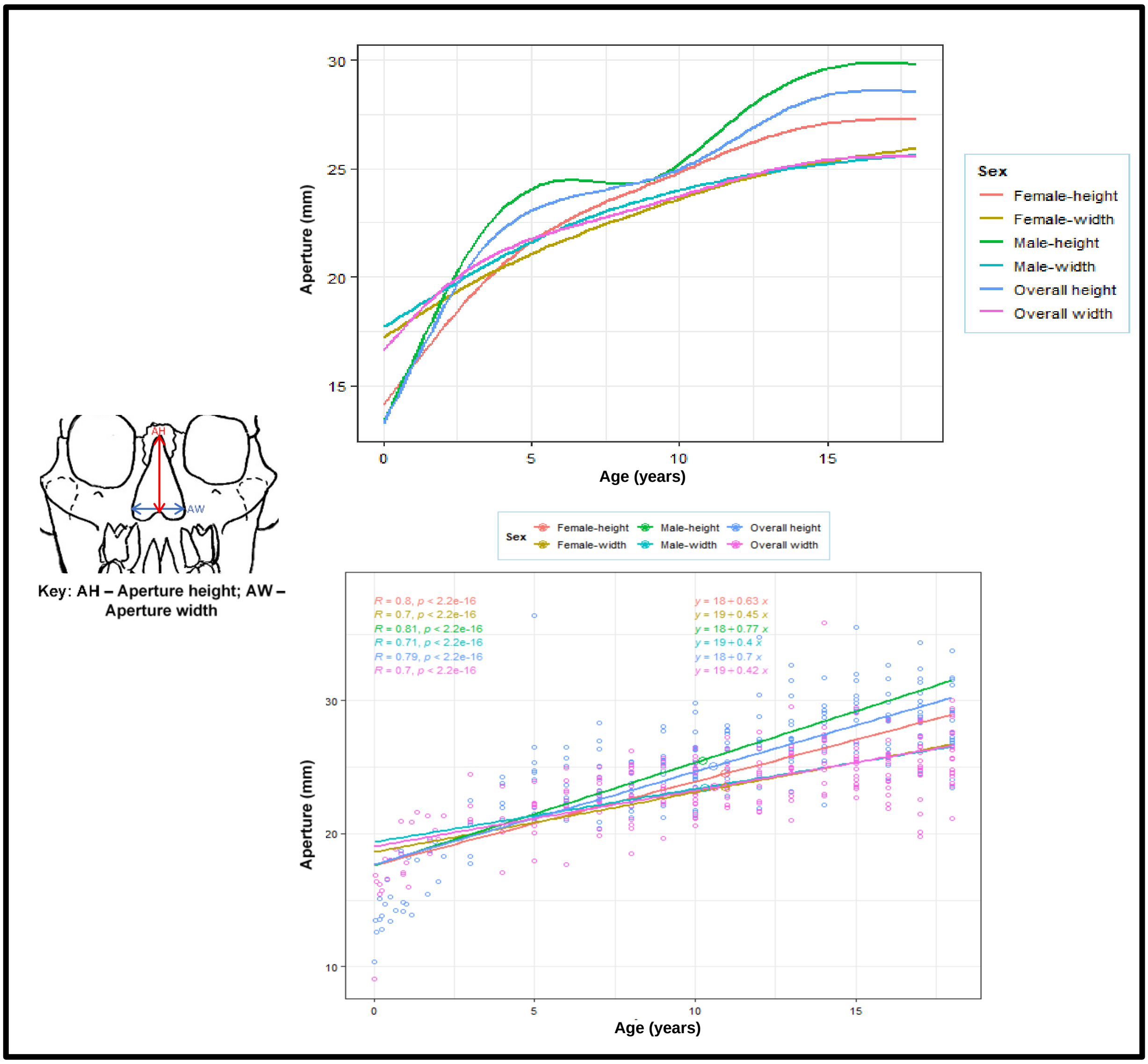

Figure 2.4: Graph indicating growth of the nasal region over time

2.5.2.4 Maxillary

The distance between the ANS and PNS had a consistent size increase over time, with the males displaying overall larger measurements than the females, although the differences were not significant. Rapid growth was seen from 0–3.75 years of age with the growth rate being 3.7 mm/year in females and 4.1 mm/year in males below. The maxilla continued to grow throughout childhood, but at a slower rate of 0.7 mm/year in females and 0.81 mm/year in males (Figure 2.5).

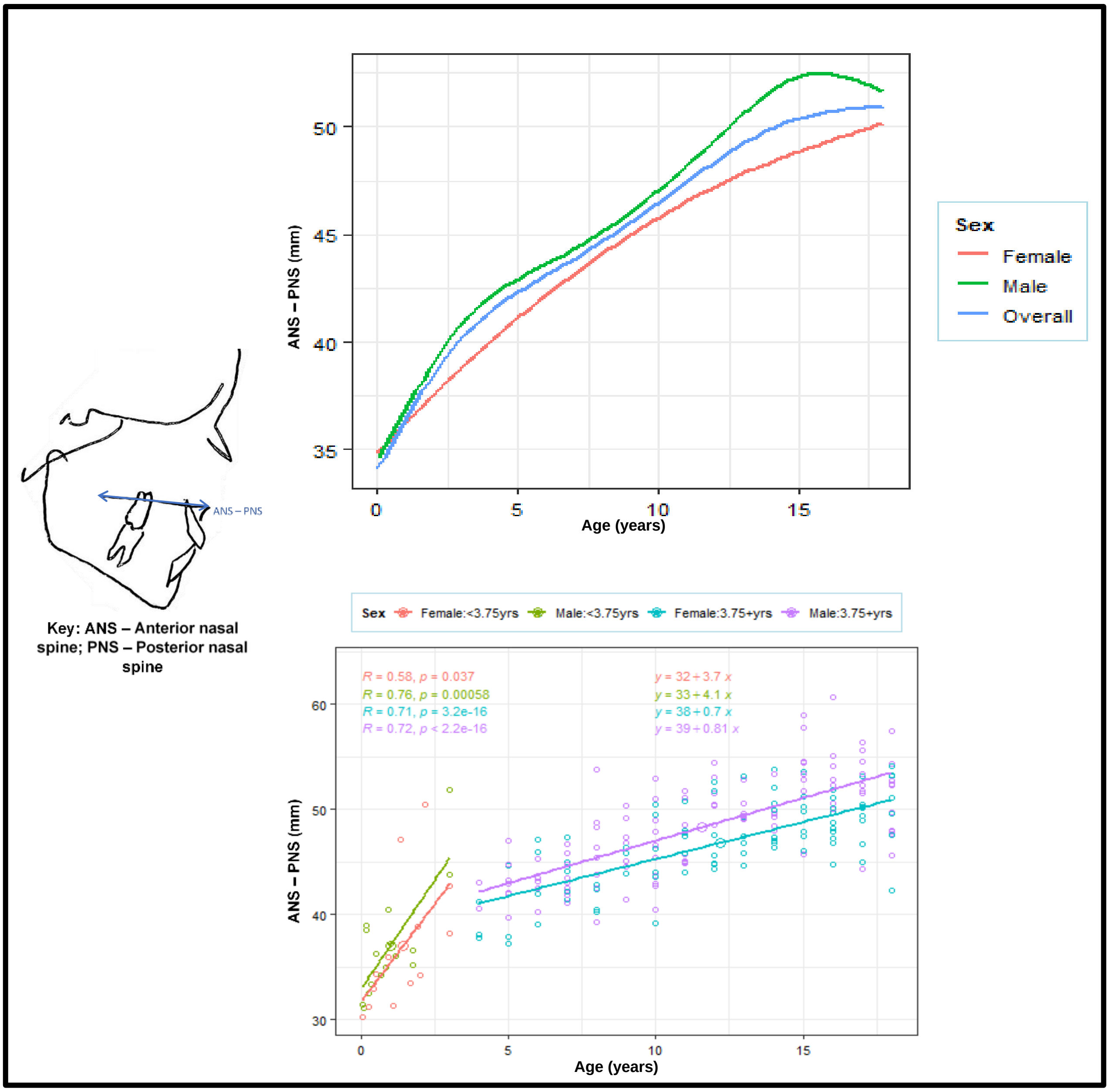

Figure 2.5: Graph indicating growth of the maxillary region over time

2.5.2.5 Mandibular

The mandibular region of males and females grew rapidly in unison from 0–5 years of age. After which males displayed overall larger values than females, although the mandible width was the only parameter in which this difference was statistically significant (p = 0.05).

The mandibular width grew rapidly from 0–5 years of age at a rate of 7 mm/year in females and 6.7 mm/year in males. The mandible width continued to grow at a slower rate of 1.1 mm/year in females and 1.5 mm/year in males after 5 years of age (Figure 2.6).

The mandible head widths were noted to increase consistently at a rate of 0.42–0.52 mm/year in females and 0.49–0.63 mm/year in males. The left mandible head width was seen to attain maximum size at between 10–15 years of age, although the right mandible head width had not attained maximum size by 18 years of age. As the age limit of this study was 18 years of age, this study cannot comment on any growth of the right mandible head width after 18 years of age.

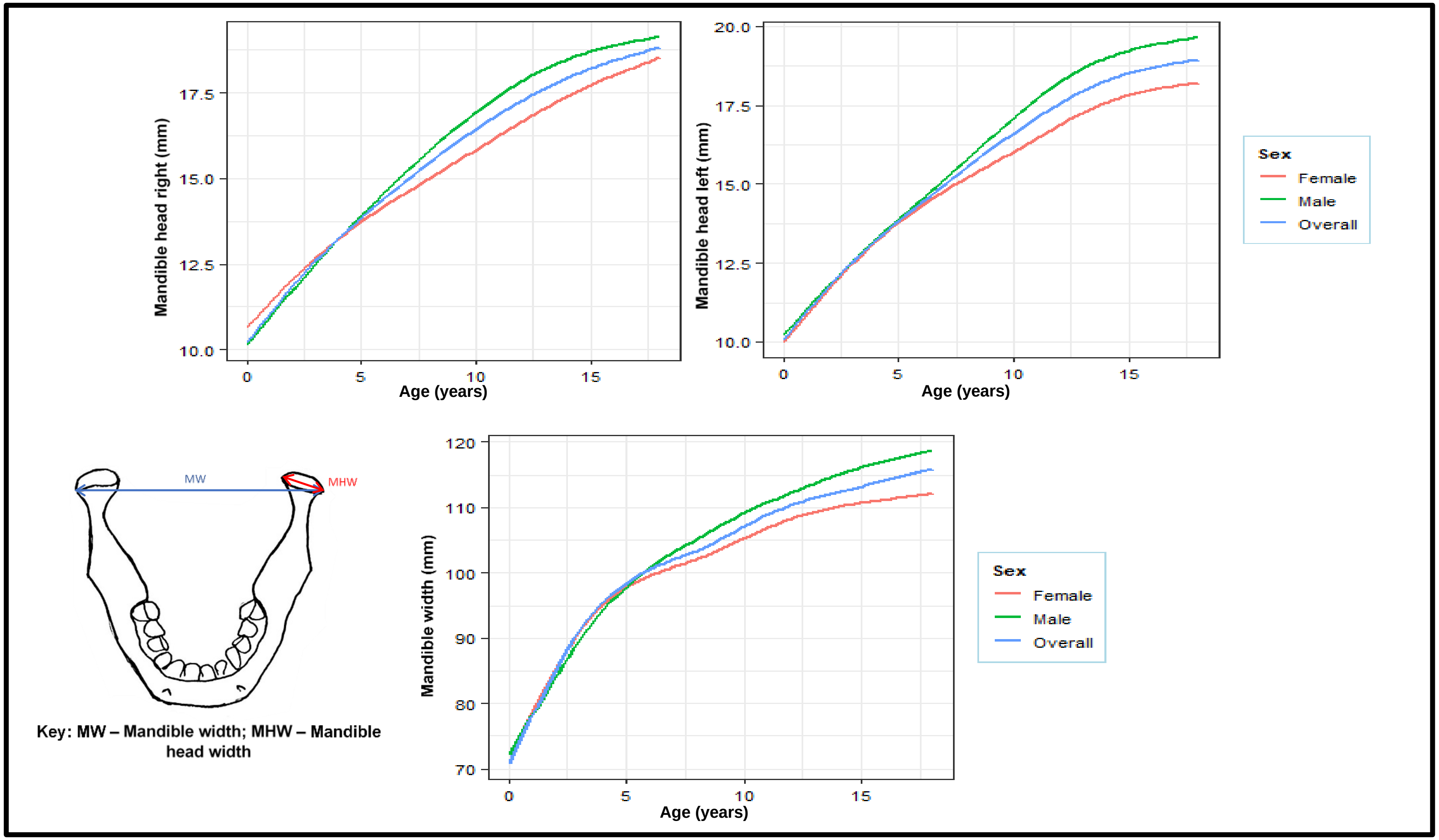

Figure 2.6: Graphs indicating growth of the mandibular region over time

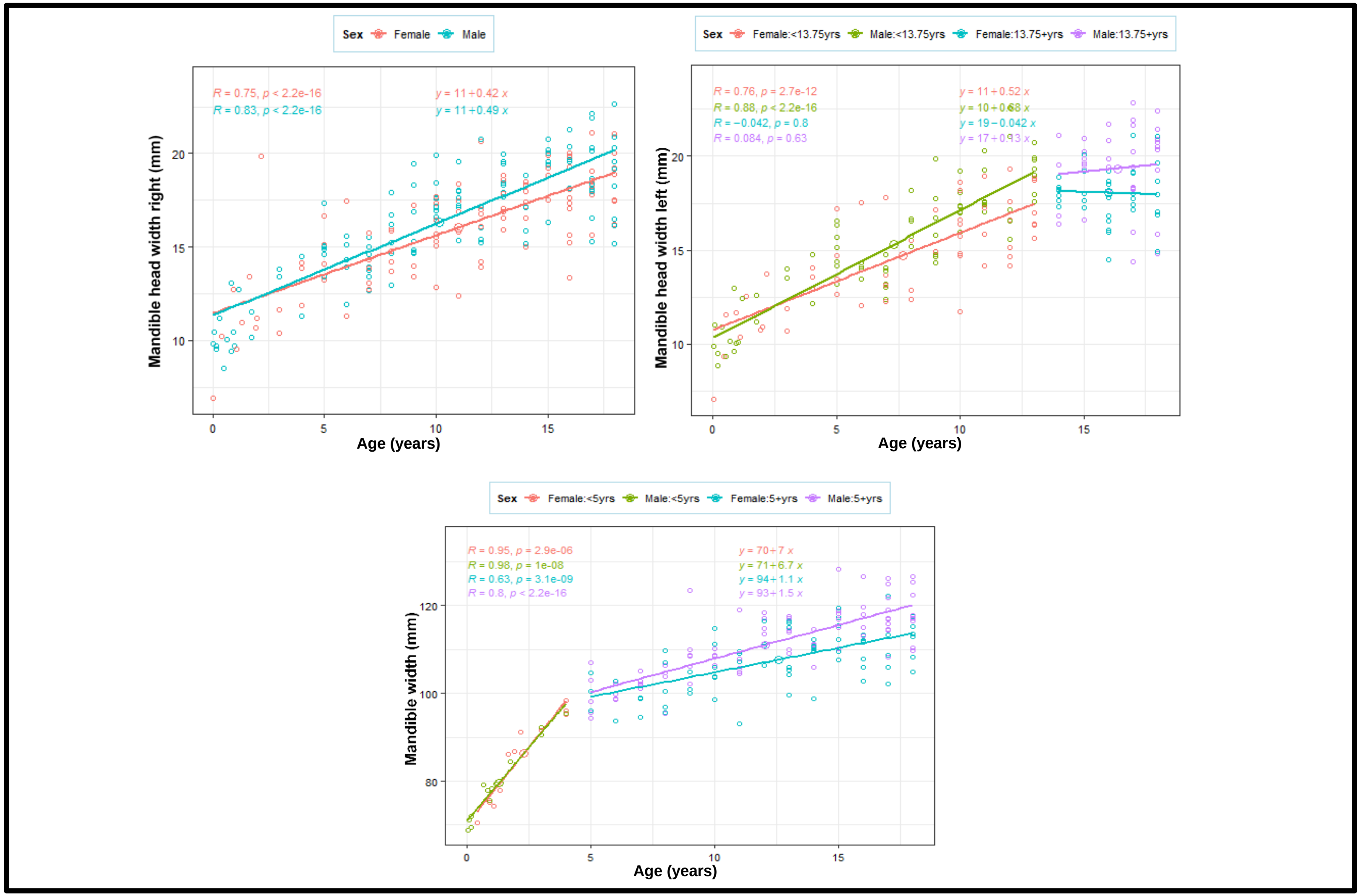

Figure 2.7: Graphs indicating growth of the mandibular region over time

2.5.3 Age estimation using the viscerocranial regions

A correlation scatterplot was drawn up with a regression line for the measurements which displayed the highest correlations to age. The measurements with the strongest correlation to age which can be used to accurately estimate age are the zygomatic arch length (ZAL) (right: $r = 0.8929$, $p<0.001$ and left: $r = 0.8656$, $p<0.001$), ZAD ($r = 0.8842$, $p<0.001$) and mandibular width ($r = 0.8444$, $p<0.001$). The right and left ZALs are very strongly correlated ($r = 0.9689$, $p<0.001$) with one another as well as the ZAD and the mandibular width ($r = 0.9599$, $p<0.001$).

Equations for age estimation were determined using the regression line on the scatter plots. These equations are listed in Table 2.4. With regards to the measurements which displayed the strongest correlations with age, sex was seen to be significant for only the mandibular width ($p = 0.05$). Therefore, sex should be noted, as there are sex-specific equations that can be utilised. (Figure 2.8)

Although it was difficult to measure the ZAL, the values were strongly correlated to age. Therefore, an age estimation equation could be formulated.

Table 2.4: Age estimation equation

Age estimation equations			
Measurement	**Sex unknown**	**Male**	**Female**
ZAD	y = -29 + 0.34x	y = -27 + 0.32x	y = -34 + 0.39x
ZAL (right)	y = -15 + 0.42x	y = -14 + 0.4x	y = -17 + 0.45x
ZAL (left)	y = -15 + 0.4x	y = -13 + 0.38x	y = -17 + 0.46x
Mandible width	y = -28 + 0.36x	y = -27 + 0.36x	y = -30 + 0.4x
Key: y = age estimation; x = measurement in mm			

2.6 Inter- and intra-observer reliability

Inter-class correlation (ICC) coefficient tests for the parameters indicated a high degree of agreement between the intra- and inter-observer measurements when a sample of 21 CT scans was randomly selected (ICC varied between 0.82 and 1.00) (Table 2.5). The ICC coefficient between the measurements done by the same investigator also showed a high degree of agreement (ICC varied between 0.88 and 1.00) (Table 2.6).

Table 2.5: The inter-class correlation of the intra- and inter-observer error

The inter-class correlation of the intra- and inter-observer error			
Parameter	**n**	**Judges**	**ICC (95% CI, p-value)**
Anterior interorbital distance	21	2	0.91 (0.8–0.96, p<0.001)
Lateral orbital wall distance	21	2	0.98 (0.96–0.99, p<0.001)
Orbital height (right)	21	2	0.82 (0.61–0.91, p<0.001)
Orbital height (left)	21	2	0.92 (0.84–0.96, p<0.001)
Orbital width (right)	21	2	0.92 (0.82–0.96, p<0.001)
Orbital width (left)	21	2	0.96 (0.9–0.98, p<0.001)
Aperture height	21	2	0.93 (0.85–0.97, p<0.001)
Aperture width	21	2	0.92 (0.84–0.96, p<0.001)
Zygomatic arch distance	21	2	1 (1.00–1, p<0.001)
Zygomatic arch length (right)	21	2	0.94 (0.88–0.97, p<0.001)
Zygomatic arch length (left)	21	2	0.97 (0.93–0.98, p<0.001)
ANS–PNS	21	2	0.82 (0.62–0.92, p<0.001)
Mandible width	21	2	1.00 (1.00–1, p<0.001)
Mandible head width (right)	21	2	0.97 (0.93–0.98, p<0.001)
Mandible head width (left)	21	2	0.98 (0.957–0.99, p<0.001)

Table 2.6: The inter-class correlation of the intra-observer error

The inter-class correlation of the intra-observer error			
Parameter	**n**	**Judges**	**ICC (95% CI, p-value)**
Anterior interorbital distance	236	3	0.99 (0.98–0.99, p<0.001)
Lateral orbital wall distance	236	3	1 (1–1, p<0.001)
Orbital height right	235	3	0.88 (0.86–0.90, p<0.001)
Orbital height left	235	3	0.97 (0.97–0.98, p<0.001)
Orbital width right	237	3	0.93 (0.92–0.94, p<0.001)
Orbital width left	237	3	0.99 (0.99–1, p<0.001)
Nasal aperture height	238	3	0.97 (0.96–0.97, p<0.001)
Nasal aperture width	237	3	0.98 (0.97–0.98, p<0.001)
Zygomatic arch distance	213	3	1 (1–1, p<0.001)
Zygomatic arch length right	110	3	0.93 (0.91–0.94, p<0.001)
Zygomatic arch length left	111	3	1 (1–1, p<0.001)
ANS–PNS	238	3	0.99 (0.99–0.99, p<0.001)
Mandibular width	179	3	1 (1–1, p<0.001)
Mandible head width right	212	3	0.99 (0.99–0.99, p<0.001)
Mandible head width left	212	3	0.99 (0.99–0.99, p<0.001)

2.7 Discussion

While it is known that the viscerocranial regions (orbital, nasal, midfacial, maxillary and mandibular) develop at different rates, literature which illustrates the overall growth of the viscerocranium is still required (Albert *et al.,* 2019). The overall measurements conducted in each region of the viscerocranium displayed a linear relationship with age, which is consistent with previous studies (Lang *et al.,* 1983; Waitzman *et al.,* 1992, Snodell, Nanda and Currier 1993; Thordarson *et al.,* 2006; Albert *et al.,* 2019; Manlove *et al.,* 2020). However, there is no consistent method of age categorisation in previous literature which studied the viscerocranial regions, for example, Waitzman *et al.* (1992) categorised the ages per year, whereas Jacob and Buschang (2011) compared individuals 10 years of age with individuals 15 years of age.

2.7.1 Development

2.7.1.1 Orbital

The orbital aperture is the link between the outside world and the brain (Costello *et al.,* 2012; Özer *et al.,* 2016). There are differences in orbital apertures between individuals with different ancestries (Rossi *et al.,* 2012; Özer *et al.,* 2016). South African studies which have reported on the orbital region of the viscerocranium have been conducted on adult specimens (Xing *et al.,* 2013; Dorfling *et al.,*2018; Small *et al.,* 2018). Studies that looked at the orbital region of the viscerocranium in subadult specimens, noted that the orbital width, orbital height, AID and LOD measurements increased as the individuals aged (Lang *et al.,* 1983; Waitzman *et al.,* 1992; Pool *et al.,* 2016). It was noted that the orbital region reaches 93% of its adult size by 5 years of age (Waitzman *et al.,* 1992; Costello *et al.,* 2012). Overall, it was noted that males displayed larger measurements than females (Waitzman *et al.,* 1992; Özer *et al.,* 2016).

Manlove *et al.* (2020) reported that the orbit grows at a rapid pace for the first year of life with most of the growth being complete by 5 years of age. The orbital height undergoes a more gradual growth when compared to the orbital width (Costello *et al.,* 2012). The present study found that the orbital height, displayed no significant growth increases after 5 years of age, with the orbital width displaying very small growth after 5 years of age, which is consistent with earlier studies (Waitzman *et al.,* 1992, Costello *et al.,* 2012). The right orbital width increased by only 0.61 mm/year in females and by 0.56 mm/year in males after 2.5 years of age and the left orbital width increased by only 0.8 mm/year in females and by 0.76 mm/year in males after 10 years of age. The literature which was reviewed reported the mean values of the orbital height and width in the age ranges studied [Kaya *et al.*, 2014 (13–86 years of age), Özer *et al.*, 2016 (5–74 years of age)]. Whereas Lang *et al.* (1983) provided normative mean values of the orbital height and width for ages 0–11 years of age.

The growth patterns in the present study for the orbital height and width were similar, with the males displaying larger measurements than females, the differences were however not significant except for the left orbital height ($p = 0.048$). The LOD growth below 3.75 years of age was 6.5 mm/year in females and 6.1 mm/year in males. In the sample group above 3.75 years of age the LOD growth rate slowed to 1.1 mm/year and 1 mm/year in females and males, respectively. These findings agree with previous studies that found that the LOD displays a substantial size increase during the first year of development, with continued growth throughout childhood (Waitzman *et al.*1992; Pool *et al.*2016). The AID increased by only 1.3 mm/year in both sexes 0–7.5 years of age and displayed no statistically significant growth changes after 7.5 years of age. Concurring with previous studies showing that the AID displays little growth after birth (Waitzman *et al.*, 1992). The present study found that the mean AID <1 year of age was 16.3±2.24 mm which is smaller than the measurements recorded by Aslan *et al.* (2009) who found the AID for Turkish individuals <1 year of age to be 17.8±1.4 mm. The sample of the study conducted by Aslan *et al.* (2009) only included individuals <1 year of age; therefore, comparisons of the measurement differences between the other age groups in the current study could not be done.

2.7.1.2 Midfacial

The midfacial region of the viscerocranium is made up of the zygoma, the nasal region and the maxilla (Aktop *et al.*, 2013; Kim *et al.*, 2018). Many studies have looked at the zygoma in adults in detail (İşcan and Steyn 1999; Bastir and Rosas 2013; Small *et al.*, 2018; Mustafa *et al.*, 2019; Tawha *et al.*, 2020), with South African adult studies looking at the midfacial region for the estimation of sex (İşcan and Steyn 1999) and ancestry (Tawha *et al.*, 2020) in adult samples. The subadult studies which have been done have noted an increase in ZAD or bizygomatic distance (Waitzman *et al.*, 1992; Snodell *et al.*, 1993; Nanda *et al.*, 2012; Machado *et al.*, 2017), which was also noted in the present study. In previous studies over 80% of the adult bizygomatic size was reached by 6 years of age (Nanda *et al.*, 2012).

The present study noted that the ZAD increased rapidly from 0–3.75 years of age, with growth increasing at a rate of 10 mm/year in both males and females <3.75 years of age, growth continued after 3.75 years of age, but at a much slower rate (1.6 mm/year in females and 1.8 mm/year in males). These findings are consistent with what was noted in the Nanda *et al.* (2012) study which stated that a 1.5–2 mm/year increase was noted between 6–11 years of age in females and 6–13 years of age in males. Furthermore, the findings of Snodell *et al.* (1993) which noted the ZAD to increase by 0.2-1.4 mm/year from 6–18 years of age. The present study findings agree with Waitzman *et al.* (1992) study which noted that the growth of the midfacial region continues during the later stages of development (6–>15 years of age).

The present study utilised bony and sutural landmarks, this was especially essential when taking the ZAL parameters. However, due to the difficulty in utilising retrospective scans where the patient did not keep their head fixed on the orbitomeatal plane during the course of the CT scan being taken, this influenced whether both landmarks required to take the measurement could be identified in the same slice. Therefore, the ZAL is missing many values, which explains the lack of growth pattern analysis. It was, however, noted that the ZAL were overall slightly larger in the >10–15 and >15-year age categories in the present study when compared to the findings reported by Waitzman *et al.* (1992).

2.7.1.3 Nasal

The nasal aperture is the boundary between the nasal vestibule and the nasal cavity formed by the maxillae and the nasal bones (Papesch and Papesch 2016). Differences in the nasal aperture shape have been seen between individuals with different ancestries (McDowell *et al.,* 2015; Cunningham *et al.,* 2016). The South African studies which have been done have looked at adult individuals (McDowell *et al.,* 2015; Ridel *et al.,* 2018; Small *et al.,* 2018). This present study found that the nasal aperture width increased over time, which confirms studies done by Snodell *et al.* (1993) and Nanda *et al.* (2012). This present study found that the nasal aperture width increased by 0.45 mm/year in females and 0.4 mm/year in males, which is in agreement with the Snodell *et al.* (1993) study which stated that the nasal aperture width increased between 0.2–1.4 mm/year. Initially, males displayed larger measurements than females, which is consistent with previous studies (Thordarson *et al.,* 2006), although, the present study noted that after approximately 12 years of age females displayed slightly larger nasal aperture width measurements than males. The size difference was not statistically significant and was not reported in the literature reviewed. The nasal aperture height was noted to increase over time which is concurs with the measurements reported by Lang *et al.* (1983).

2.7.1.4 Maxillary

The maxillae make up a large portion of the viscerocranium (Moore *et al.,* 2014; Cunningham *et al.*2016). They articulate with the zygomas, nasal bones, frontal bone, and lacrimal bones in the viscerocranium (Moore *et al.,* 2014; Cunningham *et al.,* 2016). The maxillary length increased over time, which is consistent with previous literature done on samples with African ancestry (Al-Jewair *et al.,* 2018). The ANS–PNS had a rapid growth rate from 0–3.75 years of age, with a growth rate of 3.7 mm/year in females and 4.1 mm/year in males, which is consistent with previous studies which have stated that the maxilla undergoes rapid periods of growth at 1–2 and 3–5 years of age (Costello *et al.*, 2012; Manlove *et al.*, 2020).

2.7.1.5 Mandibular

South African studies on adult mandibles have noted that it can be useful in sex (Steyn and İşcan 1998) and ancestry estimations (İşcan and Steyn 1999). Literature has noted that the mandible has almost completed growth by 5 years of age (Manlove *et al.*, 2020). While many studies have looked at the mandible growth in subadult samples, most have looked at the mandible width between the gonions of the mandible (Snodell *et al.,* 1993; Thorsdarson *et al.,* 2006; Nanda *et al.,* 2012; Buyuk *et al.,* 2017; Albert *et al.,* 2019). The present study, however, measured the mandible width from the most lateral point of the head of the mandible on the right to the most lateral point of the head of the mandible on the left. This measurement was utilised, as the available CT scans did not include the entire mandible with only the mandible heads being found consistently. This measurement was not reported in the reviewed literature. The present study noted that males displayed overall larger measurements than females in the mandibular region which is consistent with the previous literature (Buyuk *et al.,* 2017). Growth in the mandibular region increased over time, which has been noted in previous literature (Snodell *et al.,* 1993; Nanda *et al.,* 2012; Al-Jewair *et al.,* 2018). Mandibular width experienced a rapid increase at 0–<5 years of age (7 mm/year in females and 6.7 mm/year in males) which is not in agreement with the study by Snodell *et al.* (1993) which noted the rapid period of growth to be from 7–10 years of age. The mandible width was found to be significantly larger in males than in females (p = 0.05). The mandible head width on both the right- and left-hand side was noted to be larger in males than females which is consistent with the mandibular region measurements reported in previous studies (Buyuk *et al.,* 2017). The present study found that the mandible head width on the left reached peak growth between >10 – 15 years of age, whereas the right mandible head width had not reached peak growth at 18 years of age. Normal asymmetry of the face, between the right and left sides of the face, is common, with the right side usually being larger than the left (Haraguchi *et al.,* 2008).

2.7.2 Sexual dimorphism

Previous literature has noted that males display overall larger viscerocranial measurements than females (Waitzman *et al.,* 1992). The present study found this to be true in all regions of the viscerocranium, apart from the AID and ZAL (left and right) measurements. Although the only measurements which displayed statistically significant differences between males and females were the left orbital height ($p = 0.048$), nasal aperture height ($p = 0.048$) and the mandibular width ($p = 0.05$).

2.7.3 Age estimation

The linear viscerocranial measurements were correlated to age and the measurements which illustrated the strongest correlations with age were used to derive age estimation formulas. The measurements in the present study which showed a strong correlation to age were the ZAD ($r = 0.8842$. $p>0.001$), ZAL (left: $r = 0.8656$, $p< 0.001$; right: $r = 0.8929$, $p<0.001$) and the mandibular width ($R = 0.8444$, $p<0.001$). The mandible width has been noted to show a strong correlation to age in a study conducted by Kumagai *et al.* (2018) ($r = 0.68$, $p<0.001$). The ZAD and ZAL measurements, however, have not been correlated to age in any of the literature reviewed. A novel finding of this study is the formulas which can be used to estimate age using the ZAD, ZALs and mandible width (Table 2.6). Of the literature reviewed there have been no other authors who have presented formulas which can be used for age estimation using viscerocranial measurements. The ZAD was measured in the coronal plane, while the ZALs and the mandibular width was measured in the transverse at the point where both landmarks could be identified. To test the reliability of the measurements 21 scans were randomly selected by an inter-observer and the measurements were replicated, inter-rater analysis was done by ICC. This showed a high degree of agreement between the intra- and inter-observers in this study for all the measurements, ZAD [1 (1–1, $p<0.001$)], ZAL [right: 0.94 (0.88–0.97, $p<0.001$); left: 0.97 (0.93-0.98, $p<0.001$)] and mandible width [1 (1–1, $p<0.001$)].

2.8 Conclusion

This study presented data of the viscerocranium in a South African cohort. The findings of this study highlighted the development and growth patterns of the viscerocranial regions (orbital, midfacial, nasal, maxillary and mandibular) according to age and sex using CT scans. This study identified which viscerocranial measurement has the strongest correlation to age and can be used for age estimation purposes utilising the formulas derived. The data from this study can be a useful addition to existing data on the skeletal development in South African subadults with African ancestry. Additionally, the findings from this study could be applied to viscerocranial surgery or forensic anthropology.

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Chapter 3
Synthesis

This study aimed to investigate the development of the viscerocranium in a South African cohort from 0–18 years of age. The viscerocranium was divided into five regions *viz* - orbital, midfacial, nasal, maxillary and mandibular. The linear measurements done in each region were correlated with age to observe the growth patterns of each region. Furthermore, the measurements which displayed the highest correlation to age were used to develop formulas which could aid in age estimation.

3.1 Development

The measurements which were conducted in each viscerocranial region displayed a linear relationship with age, which has been reported in previous literature (Lang 1983; Waitzman *et al.*, 1992; Snodell *et al.*, 1993; Thordarson *et al.*, 2006; Albert *et al.*, 2019; Manlove *et al.*, 2020).

3.1.1 Orbital region

Previous literature indicates that the orbital region undergoes substantial growth in the first five years of life (Waitzman *et al.*, 1992; Manlove *et al.*, 2020). This study found this to be true as the orbital region parameters displayed rapid a growth increase between 0–5 years of age. The right and left orbital heights and anterior interorbital distances noted no significant size increases after 5 and 7.5 years of age respectively. Whilst the orbital width underwent a second period of growth after 5 years of age and the lateral orbital wall distance increased throughout childhood. The orbital height and width measurements differed slightly in the present study when compared to previous studies done on the Turkish population by Kaya *et al.* (2014) (Table 3.1). These differences could be attributed to the samples analysed as Kaya *et al.* (2014) sample included adult individuals.

Table 3.1: Comparisons of the orbital region parameters

Comparisons of orbital region parameters						
Author (year) population	**Age (years) (sample size)**	**Sex**	**Orbital width (mm) ±SD**		**Orbital height (mm) ±SD**	
			Right	**Left**	**Right**	**Left**
Kaya *et al.* (2014) Turkish	13–86 (112)	Male	37.04 (±1.79)	36.86 (±1.57)	33.9 (±2.27)	34.5 (±2.2)
		Female	35.78 (±1.5)	35.39 (±1.58)	32.6 (±2.4)	33.16 (±2.19)
Niemann *et al.* (2021) South African	0–18 (239)	Male	36.0 (±4.13)	36.1 (±7.29)	35.5 (±3.17)	36.3 (±3.22)
		Female	35.8 (±3.88)	35.8 (±3.65)	36.3 (±3.32)	35.5 (±2.85)

3.1.2 Midfacial region

The zygomatic arch distance experienced a rapid increase when growth of the midfacial region increased throughout childhood, which is consistent with earlier findings (Waitzman *et al.*1992; Nanda *et al.*, 2012). The present study found the ZAD and ZAL measurements were similar to the results reported by Waitzman *et al.* (1992) (Table 3.2). The age groups which displayed the biggest differences between the present study and Waitzman *et al.* (1992) were >10–15 and >15 years of age. In these age groups the present study displayed larger values. The ZAD increased at a rate of 1.6 mm/year in females and 1.8 mm/year in males after 3.75 years of age in the present study, which is consistent with the findings of Snodell *et al.* (1993) which noted the ZAD increased at a rate of 0.2–1.4 mm/year.

Table 3.2: Comparisons of the midfacial region parameters

Comparisons of midfacial region parameters				
Author (year) population	**Sample size**	**Age**	**ZAD (mm)**	**ZAL (mm)**
Waitzman *et al.* (1992) European	542	>1	73.5±5.6–85.9±4.2	33.3±3.4–43.3±3.3
		1–5	87.3±5.6–101.9±44	44.2±3.3–49.3±3.8
		>5–10	105.5±5.5–110.7±5.7	50.2±4.2–54.1±2.2
		>10–15	110.7±4.7–118.9±7.3	54.1±2.9–58.0±3.4
		>15	118.7±9.4–120.1±6.4	58.4±3.3–59.3±3.0
Niemann *et al.* (2021) South African	239	<1	81.3±6.75	37.6±4.82
		1–5	103±8.38	47.1±4.0
		>5–10	115±5.13	57.3±7.19
		>10–15	124±6.17	67.7±5.12
		>15	128±5.03	70.9±3.43

3.1.3 Nasal region

The present study noted that the nasal aperture width increased consistently over time at a rate of 0.45 mm/year in females and 0.4 mm/year in males, which is consistent with earlier findings by Snodell *et al.* (1993), who reported that the nasal aperture width increases between 0.2-1.4 mm/year. The nasal aperture width measurements were larger in the present study when compared to previous studies done by Lang (1989). The nasal aperture height increased over time as seen previously, although, the nasal aperture height measurements for individuals <1 year of age in the present study were larger than the neonate reported measurements by Lang (1989) (Table 3.3).

Table 3.3: Comparisons of the nasal region parameters

Comparisons of nasal region parameters				
Author (year) population	**Sample size**	**Age**	**Aperture width (mm)**	**Aperture height (mm)**
Lang (1989)	-	neonate	9.8	11.3
		1–5	16.5–18.2	17.4–22.6
Niemann *et al.* (2021) South African	239	<1	16.8±2.65	14.2±1.87
		1–5	20.8±1.91	21.1±4.3

3.1.4 Maxillary region

The maxillary length (anterior nasal spine–posterior nasal spine) measurement increased over time, which is consistent with previous findings (Al-Jewair *et al*,.2018). Rapid growth between 0–3.75 years of age was noted (3.7 mm/year in females and 4.1 mm/year in males), which is consistent with studies by Costello *et al.* (2012) and Manlove *et al.* (2020), which state that the maxilla undergoes rapid growth at 1–2 and 3–5 years of age. After 3.75 years of age, the maxillary length increased by only 0.7 mm/year in females and 0.81 mm/year in males. These findings substantiate the findings of Buschang *et al.* (2013) which states that the maxillary length grows at 0.82–0.55 mm/year in individuals from 10–14 years of age.

3.1.5 Mandibular region

The data from this study noted the mandibular region parameters increased over time, which is consistent with previous studies (Snodell *et al.*1993; Nanda *et al.*2012; Al-Jewair *et al.*2018). Manlove *et al.* (2020) noted that growth occurs in every mandibular region (condyles, rami, and body). Previous studies have assessed the mandible width between the gonions of the mandible (Snodell *et al.,* 1993; Nanda *et al.,* 2012; Buyuk *et al.,* 2017) as well as the mandibular length from the condyles to the gnathion (Thordarson *et al.,* 2006; Al-Jewair *et al.,* 2018). Although, in the present study the mandible width was measured between the mandible heads as these were the bony landmarks which were found consistently. The mandible head width measurements which were done in the present study were not noted in the literature which was reviewed. The left mandible head width was noted to reach peak growth between >10 – 15 years of age, whereas the right mandible head width had not yet reached peak growth by 18 years of age. Asymmetry of the face is normal, with the right side usually being larger than the left (Haraguchi *et al.,* 2008).

3.2 Sexual dimorphism

Previous literature indicates that viscerocranial measurements are overall larger in males than in females (Waitzman *et al.,* 1992; Snodell *et al.,* 1993; Thordarson *et al.,* 2006; Nanda *et al.,* 2012; Kaya *et al.,* 2014). This study found this to be true for the viscerocranial measurements, except for the AID and zygomatic arch lengths. Statistically significant differences between the sexes were found in the left orbital height ($p = 0.048$), nasal aperture height ($p = 0.048$) and mandible width ($p = 0.05$).

3.3 Age estimation

All the measurements displayed a linear correlation to age. The measurements which displayed the highest correlations to age were the ZAD ($r = 0.8842$, $p<0.001$), ZALs (Right $r = 0.8929$, $p<0.001$; Left: $r = 0.8656$, $p = <0.001$) and mandible width ($r = 0.8444$, $p<0.001$). By using these highly correlated measurements, a formula for each parameter was created which can be used for age estimation (Table 3.4).

Table 3.4: Age estimation equations

Age estimation equations			
Measurement	**Sex unknown**	**Male**	**Female**
ZAD	y = -29 + 0.34x	y = -27 + 0.32x	y = -34 + 0.39x
ZAL (right)	y = -15 + 0.42x	y = -14 + 0.4x	y = -17 + 0.45x
ZAL (left)	y = -15 + 0.4x	y = -13 + 0.38x	y = -17 + 0.46x
Mandible width	y = -28 + 0.36x	y = -27 + 0.36x	y = -30 + 0.4x
Key: y = age estimation; x = measurement in mm			

3.4 Viscerocranial growth and significance of the results

The viscerocranium growth occurs in many stages as outlined by Enlow and Hans (1996). This growth is known to be influenced by the eruption of teeth and the development of the paranasal air sinuses and sensory organs (Ross and Williams 2010). Additionally, it is known from previous research that the viscerocranial growth is also influenced by the base of the cranium, specifically the anterior cranial base, which has direct connections to the upper-middle face (Nie 2005). The posterior cranial base has been noted to influence the position and prognathism of the mandible (Nie 2005). Hutchinson *et al.* (2012) noted that the eruption of deciduous teeth as well as the growth of the tongue and the use of the muscles of mastication had an influence on the mandible development. The current study provided information on the growth rates of the viscerocranial regions which could be a useful addition to the existing data on the skeletal development in South African subadults with African ancestry. The results from the current study displayed the growth of the viscerocranium, although the direction of growth could not be assessed from the linear measurements conducted. Regression formulas were formulated for age estimation purposes using these linear measurements when ancestry is a known variable between birth – 18 years of age. Should sex be an unknown variable, there are formulas which can be used with these linear measurements. Although, sex specific formulas were also generated, should sex be known. Additionally, the findings from this study could be applied to viscerocranial surgery for treatment planning or forensic anthropology for age estimation of both living and deceased individuals.

3.5 Limitations

A limitation of this study was the availability of CT scans which fit the inclusion criteria below 5 years of age. The CT scans utilised in this study were brain scans and did not always include the entire mandible, therefore measurements which have displayed significant age-related changes specifically for this bone in previous studies could not be replicated (Snodell *et al.,* 1993; Thordarson *et al.,* 2006; Hutchinson *et al.,* 2012; Nanda *et al.,* 2012).

3.6 Recommendations for future research

This study assessed the relationship between the growth of the viscerocranial regions and age to understand the development in individuals of African ancestry. Previous literature indicates that ancestry plays an important role in growth and development (İşcan and Steyn 1999). This study compared data from an African ancestry with previous literature and found that some viscerocranial parameters (such as the orbital height, nasal aperture height and width, ZAD and

ZAL) assessed in the present study displayed larger measurements than the European studies. However, it is recommended that future research compares the development of the viscerocranium in different ancestry groups within the South African population in order to develop population specific normative data. It would also benefit future researchers to increase the sample size to have smaller age categories. To enhance the age estimation formulas within the subadult group utilising viscerocranial measurements future research could expand the study to include the use of Bayesian statistics and a multivariate approach. As the measurements conducted in this study can be replicated on dry bones, future research could compare the findings of this study with dry bone specimens. As this study could not compare the linear measurements and growth patterns to the overall direction of growth, particularly the cranial base, it is recommended that future studies should look at this, as well as the shape of the skull and include measurements of full 3D reconstructions.

A thorough review of the literature has made it apparent that the current study reports on the development of the viscerocranium in South African subadults of African ancestry that has not been fully described in the literature. Additionally, the present study outlined the normal mean values of the viscerocranial regions *viz:* orbital, midfacial, nasal, maxillary and mandibular, in the five age groups (<1, 1–5, >5–10, >10–15, >15). Lastly, the growth of the viscerocranial regions was correlated to age and the measurements, which showed the highest correlations, were used in the derivation of formulas that could be used for age estimation.

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Appendix A
Permission Letter

compass
HEALTH CENTRE

Tel: (031) 563 1314
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E-mail: reception@compasshc.co.za

75 Kensington Drive
(Adelaide Tambo Drive)
Durban North,
4051

PERMISSION TO CONDUCT RESEARCH

To Whom It May Concern,

I Dr Bruce Grant give consent to Miss Kristen Niemann [Developmental changes of the facial skeleton from birth to 18 years within a South African cohort (A Computed Tomography study)] (218007650) to access computed tomographical (CT) scan images of patients within the requested age and population groups.

All identifying information on patients is to be kept anonymous; only age, sex and population group is to be recorded in the study.

Kindly also note that the name of the practice should not be revealed nor reported in any scientific fora.

Miss Niemann will be granted access to software and records to obtain the requested information.

[redacted]

DR BRUCE GRANT
M.Tech.Chiropractic. D.U.T.
Practice No: 0238708

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Appendix B
Final Ethical Approval

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

25 June 2020

Miss Kristen Niemann (218007650)
School of Lab Med & Medical Sc
Westville

Dear Miss Niemann,

Protocol reference number: BREC/00001011/2020
Project title: Developmental changes of the facial skeleton from birth to 18 years within a South African cohort (A Computed Tomography study)
Degree Purposes: MMedSci

EXPEDITED APPLICATION: APPROVAL LETTER

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application.

The conditions have been met and the study is given full ethics approval and may begin as from 25 June 2020. Please ensure that outstanding site permissions are obtained and forwarded to BREC for approval before commencing research at a site.

This approval is subject to national and UKZN lockdown regulations dated 5th June 2020 which is available on the BREC website (http://research.ukzn.ac.za/Libraries/BREC/Proposed_UKZN_BREC_revision_to_research_constraints_anticipating_change_to_Level_3_lockdown.sflb.ashx).

This approval is valid for one year from 25 June 2020. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be noted by a full Committee at its next meeting taking place on 14 July 2020.

Yours sincerely

[redacted]

Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

Biomedical Research Ethics Committee
Chair: Professor D R Wassenaar
UKZN Research Ethics Office Westville Campus, Govan Mbeki Building
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Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

Founding Campuses: Edgewood · Howard College · Medical School · Pietermaritzburg · Westville

INSPIRING GREATNESS

Appendix C
Data Sheet Sample (data available on request)

Age: ______________________CT:______________________ Sex:____ No. ______

Orbit								
							Avg.	
AID								
LOD								
ITD								
	Right				**Left**			
OH								
OW								

Nasal				
				Avg.
AH				
AW				

Midfacial								
							Avg.	
ZAD								
	Right				**Left**			
ZAL								

Maxilla				
				Avg.
ANS – PNS				

Mandible								
	Right				**Left**			
Head width								
Width								

Appendix D
Conference Attendance, Funding and Publications

- *School of Laboratory Medicine and Medical Sciences Virtual Research Symposium, University of KwaZulu-Natal, Durban, 18 September 2020.*

  Niemann, K., Lazarus, L., Rennie. C.O. Preliminary results of the developmental changes of the facial skeleton from birth to 10 years within a South African cohort (a computed tomography study).

- *The international association for craniofacial identification, Virtual Short Program, 28 August 2021.*

  Niemann, K., Lazarus, L., Rennie. C.O. Developmental changes of the facial skeleton from birth to 18 years within a South African cohort (A computed tomography study).

- Niemann, K., Lazarus, L., Rennie. C.O. Developmental changes of the facial skeleton from birth to 18 years within a South African cohort (A computed tomography study). *Journal of Forensic and Legal Medicine*, 83(2021) https://doi.org/10.1016/j.jflm.2021.102243

- NRF Grantholder-linked Student Support 2019.
- NRF Grantholder-linked Student Support 2020.

Appendix E
Manuscript Publication

Journal of Forensic and Legal Medicine 83 (2021) 102243

Contents lists available at ScienceDirect

Journal of Forensic and Legal Medicine

journal homepage: www.elsevier.com/locate/yjflm

Research Paper

Developmental changes of the facial skeleton from birth to 18 years within a South African cohort (A computed tomography study)

Kristen Niemann, Lelika Lazarus, Carmen O. Rennie*

Discipline of Clinical Anatomy, School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, South Africa

ARTICLE INFO

Keywords:
Viscerocranium
Facial skeleton
Age estimation
Development
Morphometry
Subadult

ABSTRACT

Skeletal remains are often found on a crime scene in which a forensic anthropologist is then consulted to create a biological profile, which includes the estimation of age, sex, ancestry and stature. The viscerocranium plays an important role in the formation of a biological profile. However, to utilise the viscerocranium for age estimation, population specific normative data and knowledge of the development of the viscerocranium is required. Therefore, this study aimed to investigate the developmental changes from birth to 18 years of age of the facial skeleton of individuals from a South African cohort. This study comprised of 239 computed tomography (CT) scans (128 males; 111 females). The viscerocranium was subdivided into five regions viz.: orbital, nasal, midfacial, maxillary and mandibular. The linear parameters in each region were correlated to age to identify the developmental growth patterns of the viscerocranial regions according to male and female. The measurements which displayed the highest correlations with age were used to develop formulas which could be used for age estimation. The results of this study showed that the measurements in the orbital, midfacial, maxillary and mandibular regions experienced rapid growth between 0 and 5 years of age, with the nasal region increasing steadily over time. It was noted that males displayed overall larger measurements than females except for the anterior interorbital distance and both right and left zygomatic arch lengths (ZAL). Although only the left orbital height, nasal aperture height and mandible width displayed statistically significant size differences according to sex ($p \leq 0.05$). The measurements which showed the highest correlations to age were the zygomatic arch distance ($r = 0.8842$, $p < 0.001$), ZAL (right: $r = 0.8929$, $p < 0.001$; left: $r = 0.8656$, $p < 0.001$) and the mandible width ($r = 0.8444$, $p < 0.001$). Formulas were derived for the measurements that could be used to forensically estimate age within a subadult cohort.

1. Introduction

South Africa is known to have a high rate of violent crimes, which results in many unidentified skeletal remains.[1] When unidentified skeletal remains are discovered at a crime scene, a biological profile needs to be created, which involves age, sex, stature, and ancestry estimations.[2–7] In South Africa, the identification of unknown remains can be challenging due to lack of dental records and comparable DNA in individuals with low socioeconomic standing.[8] Currently, there are limited methods of age estimations for subadult remains in advanced stages of decomposition.[9] The facial skeleton or viscerocranium plays an important role in the formation of a biological profile during anthropological studies.[6] The developmental changes of the viscerocranium have piqued the interest of researchers in many fields, especially in physical anthropology.[4]

The ageing of the face is a complex process that is not clearly understood[10] and includes both the soft tissues and the viscerocranium.[10,11] To utilise the viscerocranium for age estimation purposes, detailed knowledge of viscerocranial development is required.[12] The current age estimation methods done using bones are far from perfect despite the many years of research.[13] Although, when compared to other identification methods, osteometric measurements done with radiological methods were seen to be more efficient.[6] Additionally, radiological methods are a non-invasive method of viewing skeletal structures, which plays an important role in anthropological investigation as CT scans allow for size and shape analysis of skeletal structures to be done without an autopsy.[14]

Previous studies have looked at the development of the separate

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regions of the face, but there is a lack of studies which analyse the development of the entire subadult facial skeleton.[15] The viscerocranium can be divided into five regions, viz.: orbital, nasal, midface, maxillary and mandibular.[16–19] These regions develop at different rates to one another,[4,10,20,21] as well as at different rates to the rest of the body,[14] as the viscerocranium growth is regulated by the developing paranasal air sinuses, the sensory organs and teeth eruptions.[20] The major studies which report on the viscerocranium as a whole focus mainly on the shape differences in adult samples.[22,23] A review of the available literature revealed that there is a paucity of knowledge on the development of the entire viscerocranium in a subadult South African cohort of African ancestry.

The data from this study could aid in the understanding of the normal developmental patterns of the viscerocranium, in addition to population specific normative data, that could aid forensic anthropologists with age estimations. Furthermore, understanding would also allow physicians to identify unusual or pathogenic growth as well as assist in treatment planning.[24]

This study aimed to investigate the developmental changes of the facial skeleton in males and females from birth to 18 years within a cohort of the South African population to estimate age.

2. Materials and methods

This was a retrospective study which consisted of 239 CT scans of subadult individuals of African ancestry (128 males; 111 females). The scans were obtained from an online server utilized by a private medical facility in the eThekwini Municipality (CT scanner Siemens Biograph mCT 64 flow (PET-CT), manufactured in Germany). Ethical clearance for this study was obtained from the Biomedical Research Ethics Committee at the University of KwaZulu-Natal (BREC/00001011/2020). The inclusion criteria for selecting scans were as follows: a) individuals should be of African ancestry, b) individuals should be 0–18 years of age, c) individuals should have normal anatomy, no pathologies or trauma to the viscerocranium, d) CT scans could not be thicker than 1 mm slices and e) the scan had to include all planes. Laser guiding in the orbitomeatal plane was used to ensure that scans were taken in the correct plane. The digital imaging and communications in medicine (DICOM) images were viewed from an online Picture Archiving and Communication Systems server on a personal computer (HP laptop 15-bs000nl, Intel core i3, 4 GB RAM) using Infinitt software (version 5.0.1.1) which is the standard software used by the practitioners. The same software was used to conduct the measurements in the horizontal, sagittal, and vertical planes. The parameters of each viscerocranial region were measured three times using the sutural and bony landmarks outlined in Table 1.

3. Statistical analysis

Approximately 300 CT scans were reviewed, and the final study consisted of 239 CT scans that were selected which fit into the inclusion criteria. The distribution between males and females was as evenly distributed as the data available would allow. During data analysis, age was categorized into five age groups (<1 year of age; 1–5 years of age; >5–10 years of age; >10–15 years of age; >15 years of age). All data was analysed using R Statistical Computing Software of the R Core Team version 3.6.3. A p-value of less than 0.05 was considered statistically significant. The linear measurements of the viscerocranial regions were compared according to age and sex. The Kruskal Wallis and ANOVA tests were used to compare the medians and means of the linear measurements between the age categories respectively. The Bonferroni test was used to compare the means between the medians between the males and females. Chi-square and Fischer's Exact tests were done to detect large and small expected frequencies in the data respectively, between males and females, and the age categories. Scatter plots for the linear correlations with p-values and regression lines were created to determine the correlations between the linear measurements and age for both sexes. Scatterplot gam graphs were made to assess the growth patterns of the viscerocranial regions over time.

Table 1
Viscerocranial region parameters.

Region	Measurement	Landmark 1	Landmark 2
Orbital	Orbital width	Zygomaticofrontal suture	Dacryon
	Orbital height	Zygomaticomaxillary suture	Perpendicular line drawn from the inferior landmark to the superior orbital rim
	Anterior interorbital distance	Dacryon on right	Dacryon on left
	Lateral orbital distance	Most anterior aspect of lateral orbital wall on right	Most anterior aspect of lateral orbital wall on left
Nasal	Maximum aperture width	Most lateral part of the nasal aperture on right	Most lateral part of the nasal aperture on left
	Aperture height	Rhinion	Anterior nasal spine
Midfacial	Zygomatic arch length	Zygomaticomaxillary suture	The point where the zygomatic arch meets the temporal bone
	Zygomatic arch distance	Most lateral part of the zygomatic arch on the right	Most lateral part of the zygomatic arch on the left
Maxillary	Maxillary length	Anterior nasal spine	Posterior nasal spine
Mandibular	Mandible head width	Medial point of mandible head	Lateral point of mandible head
	Mandibular width	Most lateral point on the mandible head on the right	Most lateral point on the mandible head on the left

4. Results

4.1. *Morphometric results of the viscerocranial regions*

Analysis of the data collected from 239 CT scans revealed that the growth between the age groups for each viscerocranial region displayed statistically significant increases ($p < 0.001$) (Table 2). Although overall, the only measurements which showed significant differences between males and females (Table 3) was the orbital height left ($p = 0.040$), nasal aperture height ($p = 0.048$) and mandible width ($p = 0.03$).

4.2. *Growth patterns of the morphometric parameters according to age and sex*

4.2.1. Orbital

Analysis of the growth patterns of the orbital parameters revealed that the males and females grew in unison with a rapid rate from 0 to 5 years of age. Thereafter, the orbital width had a second peak growth from 10 to 15 years of age. The orbital height however, displayed no significant growth after 5 years of age, which means the orbital height attains maximum growth between 0 and 5 years of age. The lateral orbital wall distance (LOD) experienced peak growth from 0 to 3.75 years of age and continued to increase at a slower rate throughout childhood. The anterior interorbital distance (AID) attained maximum growth at 7.5 years of age, with no significant growth thereafter. Males and females displayed similar growth patterns for all the orbital region parameters, with males appearing to display higher values than females for the orbital height and width and lateral orbital wall distance (Fig. 1).

Table 2
Morphometric measurements of the viscerocranial regions according to the age categories.

Age groups	<1yr (N = [illegible])	1–5yrs (N = [illegible])	>5–10yrs (N = [illegible])	>10–15yrs (N = 71)	>15yrs (N = [illegible])	p-value	Overall (N = [illegible])
Anterior interorbital distance						<0.001	
Mean ± SD (CV%)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]
Median (Q1–Q3)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Kruskal	[illegible]
Lateral orbital wall distance						<0.001	
Mean ± SD (CV%)	[illegible]	[illegible]	[illegible]	[illegible]	100 ± [illegible]		[illegible]
Median (Q1–Q3)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Kruskal	[illegible]
Orbital height right						<0.001	
Mean ± SD (CV%)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	ANOVA	[illegible]
Median (Q1–Q3)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]
Orbital height left						<0.001	
Mean ± SD (CV%)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	ANOVA	[illegible]
Median (Q1–Q3)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]
Orbital width right						<0.001	
Mean ± SD (CV%)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]
Median (Q1–Q3)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Kruskal	[illegible]
Orbital width left						<0.001	
Mean ± SD (CV%)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]
Median (Q1–Q3)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Kruskal	[illegible]
Aperture height						<0.001	
Mean ± SD (CV%)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]
Median (Q1–Q3)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Kruskal	[illegible]
Aperture width						<0.001	
Mean ± SD (CV%)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]
Median (Q1–Q3)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Kruskal	[illegible]

Table 2 continued

Age groups	<1yr (N = [illegible])	1–5yrs (N = [illegible])	> 5–10yrs (N = [illegible])	> 10–15yrs (N = 71)	> 15yrs (N = [illegible])	p-value	Overall (N = [illegible])
Zygomatic arch distance						<0.001	
Mean ± SD (CV%)	[illegible]	[illegible]	118 ± [illegible]	124 ± [illegible]	[illegible]		117 ± [illegible]
Median (Q1–Q3)	[illegible]	[illegible]	118(111–118)	[illegible]	[illegible]	Kruskal	[illegible]
Zygomatic arch length right						<0.001	
Mean ± SD (CV%)	37.4 ± [illegible]	47.1 ± [illegible]	57.3 ± 7.[illegible]	67.7 ± [illegible]	[illegible]		[illegible]
Median (Q1–Q3)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Kruskal	[illegible]
Zygomatic arch length left						<0.001	
Mean ± SD (CV%)	[illegible]	[illegible]	[illegible]	[illegible]	71.3 ± [illegible]		[illegible]
Median (Q1–Q3)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Kruskal	[illegible]
[illegible]						<0.001	
Mean ± SD (CV%)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	ANOVA	[illegible]
Median (Q1–Q3)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]
Mandible width						<0.001	
Mean ± SD (CV%)	[illegible]	[illegible]	104 ± [illegible]	[illegible]	[illegible]	ANOVA	[illegible]
Median (Q1–Q3)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]
Mandible head right						<0.001	
Mean ± SD (CV%)	[illegible]	[illegible]	15.4 ± [illegible]	17.7 ± [illegible]	18.5 ± [illegible]	ANOVA	[illegible]
Median (Q1–Q3)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]
Mandible head left						<0.001	
Mean ± SD (CV%)	10.1 ± 1.44([illegible])	[illegible]	[illegible]	[illegible]	[illegible]	ANOVA	[illegible]
Median (Q1–Q3)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]

Key: CV – Coefficient of variation; Q1 – first quartile, Q3 – third quartile

The orbital height increased between 2 and 2.8 mm/year in females and 2.1–3.1 mm/year in males, with no statistically significant growth increases after 2.5 years of age on the right side and 5 years of age on the left (Fig. 2).

The orbital width increased between 1.8 and 2.8 mm/year in females and 2–3.7 mm/year in males. After 5 years of age the orbital width's growth decreased to 0.61–0.8 mm/year in females and 0.56–0.75 mm/year in males (Fig. 2).

The LOD increased 6.8 mm/year in females and 6.1 mm/year in males below 3.75 years of age. The LOD continued to increase throughout childhood at a rate of 1.1 mm/year in females and 1 mm/year in males (Fig. 2).

The AID increased at a rate of 1.3 mm/year in females and males below 7.5 years of age. No statistically significant increases were noted after 7.5 years of age (Fig. 2).

4.2.2. Midfacial

The zygomatic arch distance (ZAD) displayed little difference between males and female's growth patterns below 5 years of age. Although over 5 years of age the males displayed larger measurements (Fig. 3). At 0–5 years of age, the ZAD displayed a rapid increase in size, growth continued throughout childhood. The ZAD increased at a rate of 10 mm/year for both males and females below 3.75 years of age. Whereas, above 3.75 years of age the ZAD growth rate was 1.6 mm/year in females and 1.8 mm/year in males (Fig. 3).

4.2.3. Nasal

The nasal aperture height displayed overall larger measurements in males than females, although the nasal aperture height was the only nasal parameter to have statistically significant differences between the sexes (p = 0.040). Males and females displayed similar growth patterns, although males had higher values than females. The nasal aperture parameters were noted to increase consistently over time. The nasal

Table 3
Overall differences of the linear parameters between females and males from birth to 18 years of age.

Sex	Female (N = 111)	Male (N = 129)	p-value	Overall (N = 240)
Anterior interorbital distance			0.549	
Mean ± SD (CV%)	23.2 ± 4.16 (17.9)	23.1 ± 4.21 (18.2)		23.2 ± 4.18 (18.1)
Median (Q1–Q3)	23.2 (20.4–25.4)	23.0 (20.2–26.9)	Unknown	23.1 (20.4–25.5)
Lateral orbital wall distance			0.919	
Mean ± SD (CV%)	93.3 ± 8.87 (9.5)	93.5 ± 9.49 (10.2)		93.4 ± 9.08 (9.7)
Median (Q1–Q3)	95.7 (89.1–99.4)	94.1 (88.5–100)	Unknown	95.3 (88.5–99.5)
Orbital height right			0.068	
Mean ± SD (CV%)	35.5 ± 3.17 (8.9)	36.5 ± 3.33 (9.1)		36.0 ± 3.27 (9.1)
Median (Q1–Q3)	35.7 (33.9–37.8)	36.2 (34.5–38.5)	Unknown	36.0 (34.3–38.0)
Orbital height left			0.045	
Mean ± SD (CV%)	35.5 ± 3.13 (8.9)	36.3 ± 3.30 (9.0)		35.9 ± 3.27 (9.1)
Median (Q1–Q3)	36.0 (33.8–37.1)	36.5 (34.8–38.5)	Unknown	36.1 (34.4–37.8)
Orbital width right			0.089	
Mean ± SD (CV%)	36.8 ± 3.86 (10.5)	36.0 ± 4.19 (11.6)		36.9 ± 4.01 (11.3)
Median (Q1–Q3)	36.7 (33.8–39.1)	35.9 (33.5–38.5)	Unknown	36.7 (33.3–39.1)
Orbital width left			0.548	
Mean ± SD (CV%)	35.9 ± 3.65 (10.2)	36.1 ± 7.39 (20.5)		36.0 ± 5.86 (16.3)
Median (Q1–Q3)	36.7 (33.5–38.8)	35.9 (33.4–38.5)	Unknown	35.8 (33.5–39.0)
Aperture height			0.049	
Mean ± SD (CV%)	24.5 ± 4.05 (16.4)	25.5 ± 5.04 (19.7)		25.0 ± 4.63 (18.5)
Median (Q1–Q3)	25.3 (22.3–27.8)	26.2 (22.8–28.7)	Unknown	25.8 (22.7–28.0)
Aperture width			0.814	
Mean ± SD (CV%)	22.5 ± 3.34 (14.8)	22.8 ± 2.92 (12.8)		22.6 ± 3.12 (13.8)
Median (Q1–Q3)	22.8 (22.0–24.7)	22.9 (21.8–24.2)	Unknown	22.8 (22.0–24.5)
Zygomatic arch distance			0.146	
Mean ± SD (CV%)	116 ± 12.0 (10.4)	117 ± 13.4 (11.1)		117 ± 12.9 (11.0)
Median (Q1–Q3)	119 (110–124)	120 (111–128)	Unknown	120 (110–125)
Zygomatic arch length right			0.477	
Mean ± SD (CV%)	60.9 ± 10.9 (17.9)	58.9 ± 11.9 (20.2)		59.8 ± 11.9 (19.9)
Median (Q1–Q3)	61.8 (53.0–68.4)	59.4 (48.2–66.4)	Unknown	60.2 (48.3–66.4)
Zygomatic arch length left			[illegible]	
Mean ± SD (CV%)	61.8 ± 10.2 (16.7)	58.0 ± 11.5 (19.8)		59.7 ± 10.9 (18.2)
Median (Q1–Q3)	62.5 (53.7–68.5)	58.1 (50.4–71.7)	Unknown	60.4 (51.4–70.9)
ANS–PNS			0.125	
Mean ± SD (CV%)	45.7 ± 5.34 (11.7)	45.9 ± 6.04 (12.9)		46.3 ± 5.74 (12.4)
Median (Q1–Q3)	46.8 (43.7–48.8)	47.4 (43.8–51.6)	Unknown	46.6 (43.8–50.4)
Mandible width			0.037	
Mean ± SD (CV%)	105 ± 10.4 (9.9)	107 ± 13.8 (12.8)		106 ± 12.3 (11.6)
Median (Q1–Q3)	106 (98.9–113)	109 (101–117)	Unknown	108 (100–114)
Mandible head right			0.488	
Mean ± SD (CV%)	15.6 ± 2.84 (17.5)	15.8 ± 2.33 (14.7)		15.2 ± 2.05 (13.6)
Median (Q1–Q3)	15.5 (14.1–18.0)	15.7 (14.4–18.0)	Unknown	15.6 (14.3–18.3)
Mandible head left			0.212	
Mean ± SD (CV%)	16.0 ± 2.80 (17.8)	16.6 ± 2.46 (15.0)		16.3 ± 2.18 (13.3)
Median (Q1–Q3)	16.8 (14.2–18.2)	16.8 (14.3–19.4)	Unknown	16.8 (14.2–18.7)

Key: CV – Coefficient of variation; Q1 – first quartile, Q3 – third quartile

aperture height grew 0.43 mm/year in females and 0.77 mm/year in males (Fig. 4). The nasal aperture width grew 0.45 mm/year in females and 0.4 mm/year in males (Fig. 4).

4.2.4. *Maxillary*

The distance between the ANS and PNS had a consistent size increase over time, with the males displaying overall larger measurements than the females, although the differences were not significant. Rapid growth was seen from 0 to 3.75 years of age with the growth rate being 3.7 mm/year in females and 4.1 mm/year in males before. The maxilla continued to grow throughout childhood, but at a slower rate of 0.7 mm/year in females and 0.81 mm/year in males (Fig. 5).

4.2.5. *Mandibular*

The mandibular region of males and females grew rapidly in unison from 0 to 5 years of age. After which males displayed overall larger values than females, although the mandible width was the only parameter in which this difference was statistically significant ($p = 0.05$).

The mandibular width grew rapidly from 0 to 5 years of age at a rate of 7 mm/year in females and 6.7 mm/year in males. The mandible width continued to grow at a slower rate of 1.1 mm/year in females and 1.5 mm/year in males after 5 years of age (Fig. 6).

The mandible head widths were noted to increase consistently at a rate of 0.42–0.52 mm/year in females and 0.49–0.53 mm/year in males (Fig. 7). The left mandible head width was seen to attain maximum size at between 10 and 15 years of age, although the right mandible head width had not attained maximum size by 18 years of age.

4.3. *Age estimation using the viscerocranial region*

A correlation scatterplot was drawn up with a regression line for the measurements which displayed the highest correlations to age. The measurements with the strongest correlation to age which can be used to accurately estimate age are the zygomatic arch length (ZAL) (right: $r = 0.8929$, $p < 0.001$ and left: $r = 0.8686$, $p < 0.001$), ZAD ($r = 0.8848$, $p < 0.001$) and mandibular width ($r = 0.8444$, $p < 0.001$). The right and left ZALs are very strongly correlated ($r = 0.9889$, $p < 0.001$) with one another as well as the ZAD and the mandibular width ($r = 0.9699$, $p < 0.001$).

Equations for age estimation were determined using the regression line on the scatter plots. These equations are listed in Table 4. With regards to the measurements which displayed the strongest correlations with age, sex was seen to be significant for only the mandibular width ($p = 0.05$). Therefore, sex should be noted, as there are sex-specific equations that can be utilized.

Although it was difficult to measure the ZAL, the values were

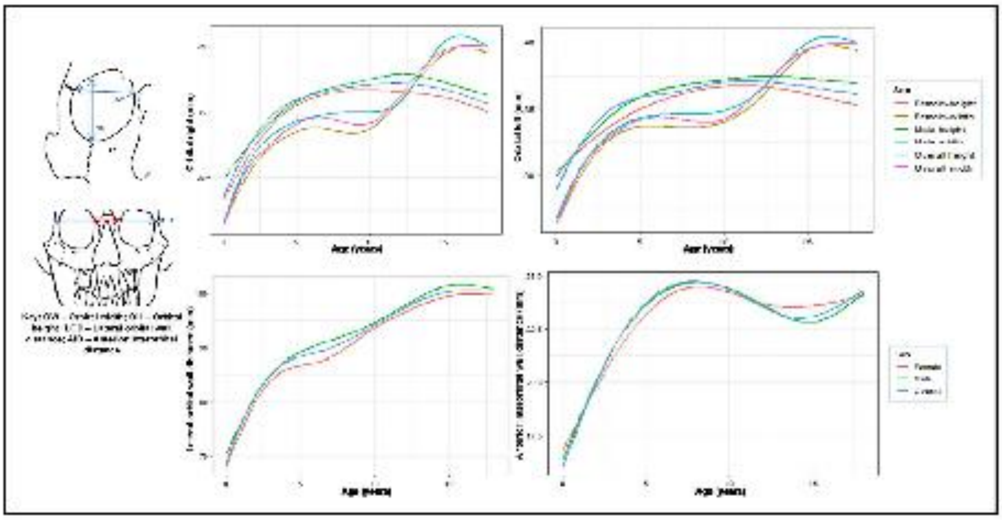

Fig. 1. Graphs indicating growth of the orbital region over time.

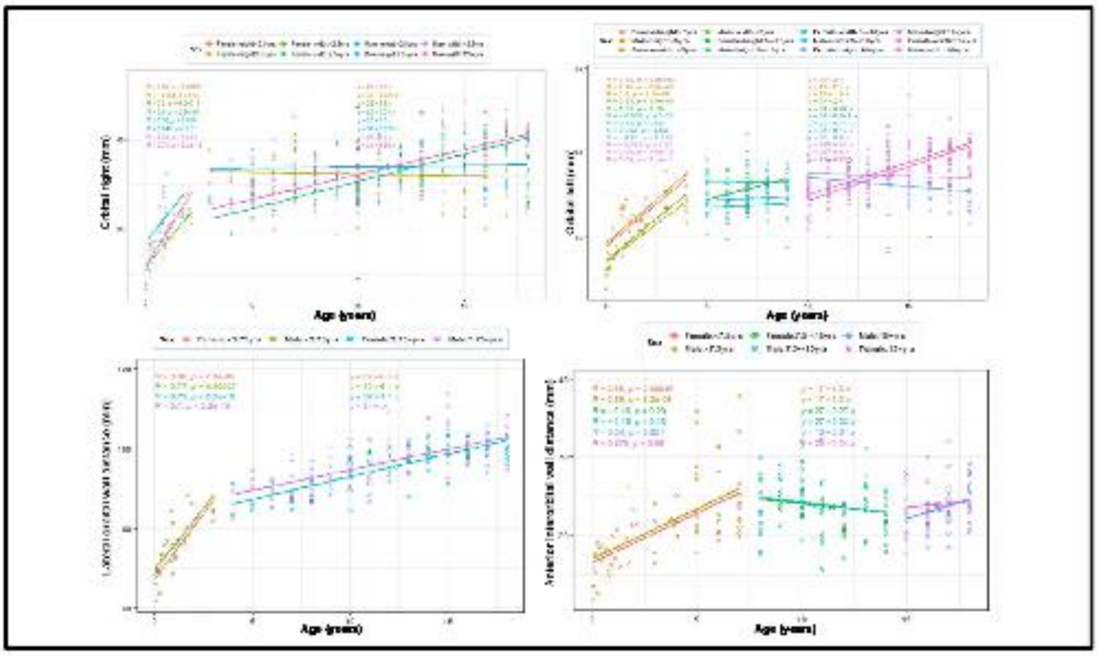

Fig. 2. Graphs indicating growth rate of the orbital region over time.

strongly correlated to age. Therefore, an age estimation equation could be formulated.

4.4. Inter- and intra-observer reliability

Inter-class correlation (ICC) coefficient tests for the parameters indicated a high degree of agreement between the intra- and inter-observer measurements when a sample of 21 CT scans was randomly selected (ICC varied between 0.82 and 1.00) (Table 5). The ICC coefficient between the measurements done by the same investigator also showed a high degree of agreement (ICC varied between 0.88 and 1.00) (Table 6).

5. Discussion

While it is known that the viscerocranial regions (orbital), nasal,

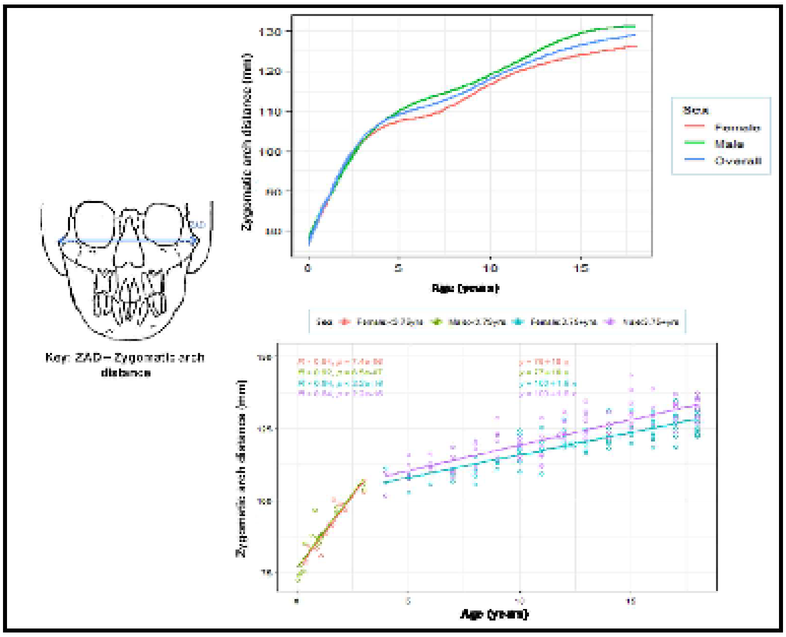

Fig. 3. Graph indicating growth of the midfacial region over time.

midfacial, maxillary and mandibular) develop at different rates, literature which illustrates the overall growth of the viscerocranium is still required.[15] The overall measurements conducted in each region of the viscerocranium displayed a linear relationship with age, which is consistent with previous studies.[15,19,26–28] However, there is no consistent method of age categorisation in previous literature which studied the viscerocranial regions, for example, Waitzman et al.[25] categorised the ages per year, whereas Jacob and Buschang[16] compared individuals 10 years of age with individuals 15 years of age.

5.1. Development

5.1.1. Orbital

The orbital aperture is the link between the outside world and the brain.[19,20] There are differences in orbital apertures between individuals with different ancestries.[30,31] South African studies which have reported on the orbital region of the viscerocranium have been conducted on adult specimens.[4,17,32] Studies that looked at the orbital region of the viscerocranium in subadult specimens, noted that the orbital width, orbital height, AID and LOD measurements increased as the individuals aged.[25,26,34] It was noted that the orbital region reaches 95% of its adult size by 5 years of age.[26,29] Overall, it was noted that males displayed larger measurements than females.[26,29]

Minkova et al.[29] reported that the orbit grows at a rapid pace for the first year of life with most of the growth being complete by 8 years of age. The orbital height undergoes a more gradual growth when compared to the orbital width.[29] The present study found that the orbital height, displayed no significant growth increases after 8 years of age, with the orbital width displaying very small growth after 5 years of age, which is consistent with earlier studies.[26,29] The right orbital width increased by only 0.51 mm/year in females and by 0.56 mm/year in males after 2.5 years of age and the left orbital width increased by only 0.8 mm/year in females and by 0.76 mm/year in males after 10 years of age. The literature which was reviewed reported the mean values of the orbital height and width in the age ranges studied [Kaya et al.[35] (18–86 years of age), Özer et al.[30] (5–74 years of age)]. Whereas Lang et al.[45] provided normative mean values of the orbital height and width for ages 0–11 years of age.

The growth patterns in the present study for the orbital height and width were similar, with the males displaying larger measurements than females, the differences were however not significant except for the left orbital height (p = 0.048). The LOD growth below 3.75 years of age was 6.5 mm/year in females and 6.1 mm/year in males. In the sample group above 3.75 years of age the LOD growth rate slowed to 1.1 mm/year and 1 mm/year in females and males, respectively. These findings agree with previous studies that found that the LOD displays a substantial size increase during the first year of development, with continued growth throughout childhood.[25,34] The AID increased by only 1.3 mm/year in both sexes 0–7.5 years of age and displayed no statistically significant growth changes after 7.5 years of age. Concurring with previous studies showing that the AID displays little growth after birth.[26] The present study found that the mean AID <1 year of age was 15.8 ± 2.24 mm which is smaller than the measurements recorded by Aslan et al.[36] who found the AID for Turkish individuals <1 year of age to be 17.8 ± 1.4 mm. The sample of the study conducted by Aslan et al.[36] only included individuals <1 year of age; therefore, comparisons of the measurement differences between the other age groups in the current study could not be done.

5.1.2. Midfacial

The midfacial region of the viscerocranium is made up of the zygoma, the nasal region and the maxilla.[37,38] Many studies have looked at the zygoma in adults in detail,[1,4,21,25,39] with South African adult studies looking at the midfacial region for the estimation of sex[2] and

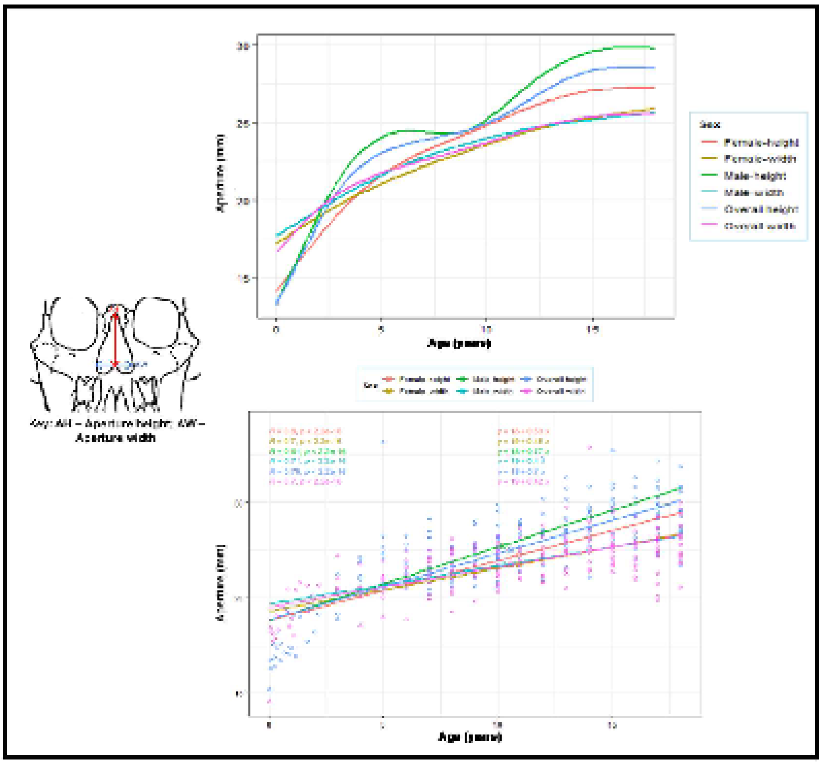

Fig. 4. Graph indicating growth of the nasal region over time.

ancestry[26] in adult samples. The subadult studies which have been done have noted an increase in ZAD or bizygomatic distance,[4,25,27,40] which was also noted in the present study. In previous studies over 80% of the adult bizygomatic size was reached by 6 years of age.[40]

The present study noted that the ZAD increased rapidly from 0 to 3.75 years of age, with growth increasing at a rate of 10 mm/year in both males and females <3.75 years of age, growth continued after 3.75 years of age, but at a much slower rate (1.6 mm/year in females and 1.8 mm/year in males). These findings are consistent with what was noted in the Nanda et al.[40] study which stated that a 1.5–2 mm/year increase was noted between 6 and 11 years of age in females and 6–15 years of age in males. Furthermore, the findings of Sandall et al.[27] which noted the ZAD to increase by 0.2–1.4 mm/year from 6 to 18 years of age. The present study findings agree with Waitzman et al.[25] study which noted that the growth of the midfacial region continues during the later stages of development (6–>18 years of age).

The present study utilized bony and sutural landmarks, this was especially essential when taking the ZAL parameters. However, due to the difficulty in utilizing retrospective scans where the patient did not keep their head fixed on the orbitomeatal plane during the course of the CT scan being taken, this influenced whether both landmarks required to take the measurement could be identified in the same slice. Therefore, the ZAL is missing many values, which explains the lack of growth pattern analysis. It was, however, noted that the ZAL were overall slightly larger in the >10–15 and > 15-year age categories in the present study when compared to the findings reported by Waitzman et al.[25]

5.1.3. *Nasal*

The nasal aperture is the boundary between the nasal vestibule and the nasal cavity formed by the maxillae and the nasal bones.[41] Differences in the nasal aperture shape have been seen between individuals with different ancestries.[42,43] The South African studies which have been done have looked at adult individuals.[39,42,44] This present study found that the nasal aperture width increased over time, which confirms studies done by Sandall et al.[27] and Nanda et al.[40] This present study found that the nasal aperture width increased by 0.45 mm/year in females and 0.4 mm/year in males, which is in agreement with the Sandall et al.[27] study which stated that the nasal aperture width increased between 0.2 and 1.4 mm/year. Initially, males displayed larger measurements than females, which is consistent with previous studies,[40] although, the present study noted that after approximately 12 years of age females displayed slightly larger nasal aperture width measurements than males. The size difference was not statistically significant and was not reported in the literature reviewed. The nasal aperture height was noted to increase over time which is concurs with the measurements reported by Lang et al.[28]

5.1.4. *Maxillary*

The maxillae make up a large portion of the viscerocranium.[40,45] They articulate with the zygomas, nasal bones, frontal bone, and lacrimal bones in the viscerocranium.[40,45] The maxillary length increased over time, which is consistent with previous literature done on samples with African ancestry.[13] The AN3–PNS had a rapid growth rate from 0 to 3.75 years of age, with a growth rate of 3.7 mm/year in

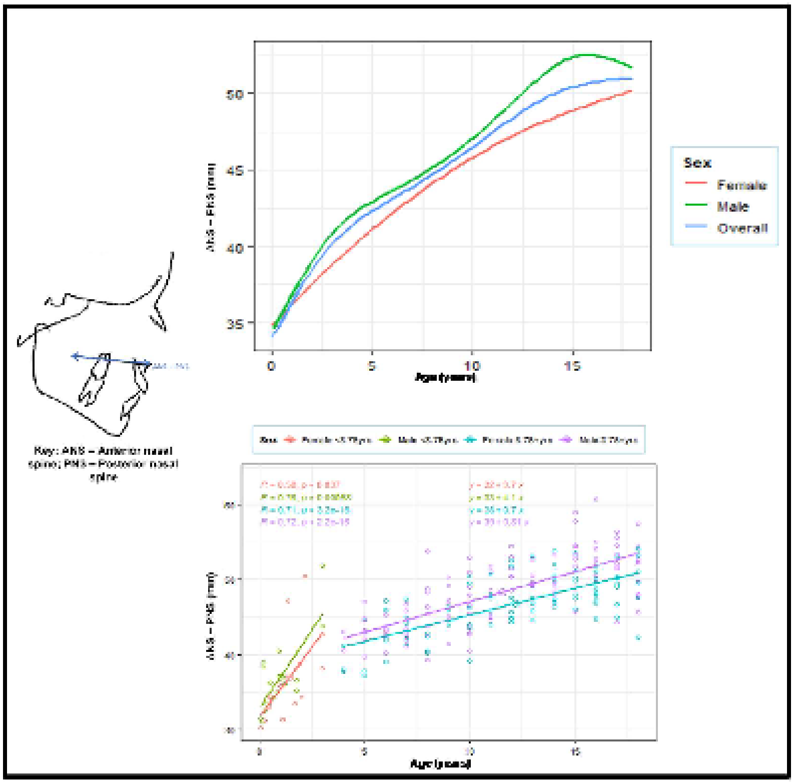

Fig. 8. Graph indicating growth of the maxillary region over time.

females and 4.1 mm/year in males, which is consistent with previous studies which have stated that the maxilla undergoes rapid periods of growth at 1–2 and 3–5 years of age.[18,20]

5.1.5. Mandibular

South African studies on adult mandibles have noted that it can be useful in sex[46] and ancestry estimations.[3] Literature has noted that the mandible has almost completed growth by 5 years of age.[19] While many studies have looked at the mandible growth in subadult samples, most have looked at the mandible width between the gonions of the mandible.[18,17,27,28,40] The present study, however, measured the mandible width from the most lateral point of the head of the mandible on the right to the most lateral point of the head of the mandible on the left. This measurement was utilized, as the available CT scans did not include the entire mandible with only the mandible heads being found consistently. This measurement was not reported in the reviewed literature. The present study noted that males displayed overall larger measurements than females in the mandibular region which is consistent with the previous literature.[17] Growth in the mandibular region increased over time, which has been noted in previous literature.[18,27,40] Mandibular width experienced a rapid increase at 0–<5 years of age (7 mm/year in females and 6.7 mm/year in males) which is not in agreement with the study by Smadell et al.[27] which noted the rapid period of growth to be from 7 to 10 years of age. The mandible width was found to be significantly larger in males than in females (p = 0.05). The mandible head width on both the right- and left-hand side was noted to be larger in males than females which is consistent with the mandibular region measurements reported in previous studies.[17] The present study found that the mandible head width on the left reached peak growth between >10 and 15 years of age, whereas the right mandible head width had not reached peak growth at 18 years of age. Normal asymmetry of the face, between the right and left sides of the face, is common, with the right side usually being larger than the left.[47]

5.2. Sexual dimorphism

Previous literature has noted that males display overall larger viscerocranial measurements than females.[24] The present study found this to be true in all regions of the viscerocranium, apart from the AID and ZAL (left and right) measurements. Although the only measurements which displayed statistically significant differences between males and females were the left orbital height (p = 0.048), nasal aperture height (p = 0.048) and the mandibular width (p = 0.05).

5.3. Age estimation

The linear viscerocranial measurements were correlated to age and the measurements which illustrated the strongest correlations with age were used to derive age estimation formulae. The measurements in the present study which showed a strong correlation to age were the ZAD (r

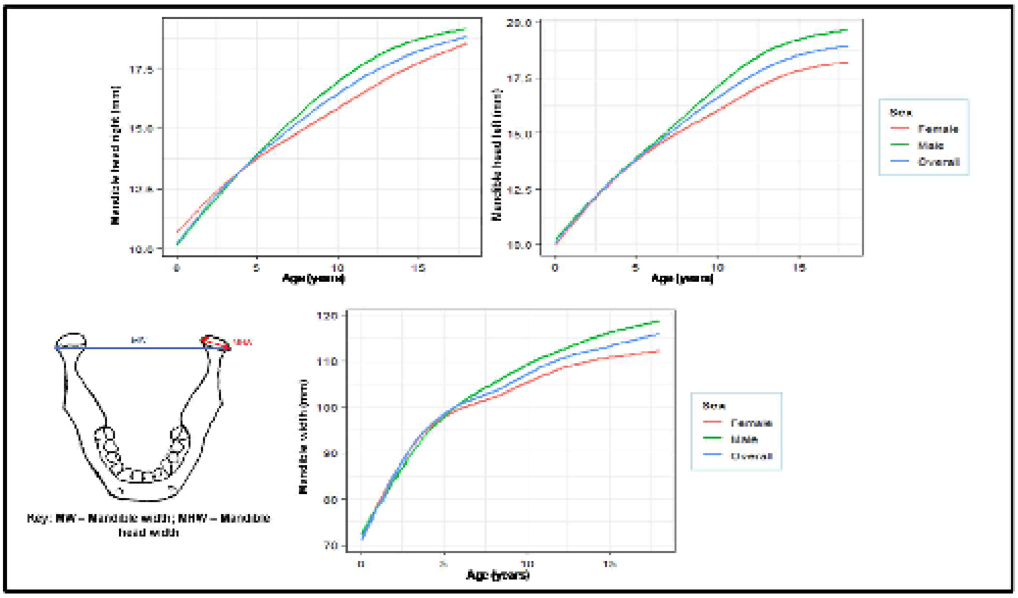

Fig. 6. Graphs indicating growth of the mandibular region over time.

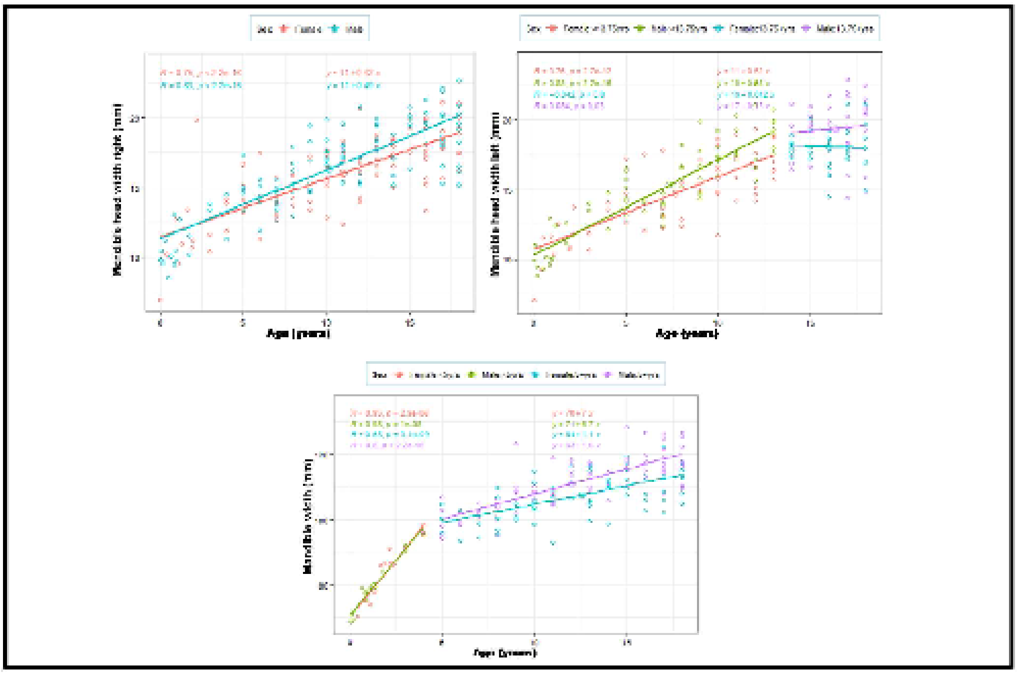

Fig. 7. Graphs indicating growth of the mandibular region over time.

Table 4
Age estimation equation.

Measurement	Sex unknown	Male	Female
ZAD	y = −39 + 0.34x	y = −37 + 0.32x	y = −34 + 0.[illegible]x
ZAL (right)	y = −13 + 0.42x	y = −14 + 0.4x	y = −17 + 0.46x
ZAL (left)	y = −15 + 0.4x	y = −13 + 0.38x	y = −17 + 0.46x
Mandible width	y = −35 + 0.38x	y = −27 + 0.3[illegible]x	y = −30 + 0.4x
Key: y = age estimation; x = measurement in mm.			

Table 5
The inter-class correlation of the intra- and inter-observer error.

Parameter	n	Judges	ICC (95% CI, p-value)
Anterior interorbital distance	31	3	0.93 (0.8–0.96, p < 0.001)
Lateral orbital wall distance	31	3	0.95 (0.86–0.98, p < 0.001)
Orbital height (right)	31	3	0.82 (0.51–0.91, p < 0.001)
Orbital height (left)	31	3	0.93 (0.84–0.96, p < 0.001)
Orbital width (right)	31	3	0.92 (0.82–0.96, p < 0.001)
Orbital width (left)	31	3	0.95 (0.9–0.98, p < 0.001)
Aperture height	31	3	0.93 (0.88–0.97, p < 0.001)
Aperture width	31	3	0.92 (0.84–0.96, p < 0.001)
Zygomatic arch distance	31	3	1 (1.00–1, p < 0.001)
Zygomatic arch length (right)	31	3	0.94 (0.88–0.97, p < 0.001)
Zygomatic arch length (left)	31	3	0.97 (0.95–0.98, p < 0.001)
ANS–PNS	31	3	0.82 (0.63–0.91, p < 0.001)
Mandible width	31	3	1.00 (1.00–1, p < 0.001)
Mandible head width (right)	31	3	0.97 (0.95–0.98, p < 0.001)
Mandible head width (left)	31	3	0.98 (0.957–0.99, p < 0.001)

Table 6
The inter-class correlation of the intra-observer error.

Parameter	n	Judges	ICC (95% CI, p-value)
Anterior interorbital distance	236	3	0.99 (0.98–0.99, p < 0.001)
Lateral orbital wall distance	235	3	1 (1–1, p < 0.001)
Orbital height right	236	3	0.98 (0.98–0.99, p < 0.001)
Orbital height left	235	3	0.97 (0.97–0.98, p < 0.001)
Orbital width right	237	3	0.98 (0.98–0.94, p < 0.001)
Orbital width left	237	3	0.98 (0.98–1, p < 0.001)
Nasal aperture height	236	3	0.97 (0.96–0.97, p < 0.001)
Nasal aperture width	237	3	0.98 (0.97–0.98, p < 0.001)
Zygomatic arch distance	218	3	1 (1–1, p < 0.001)
Zygomatic arch length right	130	3	0.93 (0.93–0.94, p < 0.001)
Zygomatic arch length left	131	3	1 (1–1, p < 0.001)
ANS–PNS	236	3	0.99 (0.99–0.99, p < 0.001)
Mandibular width	179	3	1 (1–1, p < 0.001)
Mandible head width right	218	3	0.99 (0.99–0.99, p < 0.001)
Mandible head width left	212	3	0.98 (0.98–0.98, p < 0.001)

$= 0.5542$, $p > 0.001$), ZAL (left: $r = 0.8585$, $p < 0.001$; right: $r = 0.8929$, $p < 0.001$) and the mandibular width ($R = 0.8444$, $p < 0.001$). The mandible width has been noted to show a strong correlation to age in a study conducted by Kamagai et al.[illegible] ($r = 0.68$, $p < 0.001$). The ZAD and ZAL measurements, however, have not been correlated to age in any of the literature reviewed. A novel finding of this study is the formulas which can be used to estimate age using the ZAD, ZALs and mandible width (Table 4). Of the literature reviewed there have been no other authors who have presented formulas which can be used for age estimation using viscerocranial measurements. The ZAD was measured in the coronal plane, while the ZALs and the mandibular width was measured in the transverse at the point where both landmarks could be identified. To test the reliability of the measurements 21 scans were randomly selected by an inter-observer and the measurements were replicated, inter-rater analysis was done by ICC. This showed a high degree of agreement between the intra- and inter-observers in this study for all the measurements, ZAD [1 (1–1, $p < 0.001$)], ZAL [right: 0.94 (0.88–0.97, $p < 0.001$); left: 0.97 (0.95–0.98, $p < 0.001$)] and mandible width [1 (1–1, $p < 0.001$)].

6. Conclusion

This study presented data of the viscerocranium in a South African cohort. The findings of this study highlighted the development and growth patterns of the viscerocranial regions (orbital, midfacial, nasal, maxillary and mandibular) according to age and sex using CT scans. This study identified which viscerocranial measurement has the strongest correlation to age and can be used for age estimation purposes utilising the formulas derived. The data from this study can be a useful addition to existing data on the skeletal development in South African subadults with African ancestry. Additionally, the findings from this study could be applied to viscerocranial surgery or forensic anthropology.

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Declaration of competing interest

There are no conflicts of interests to be declared.

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